THE SYSTEMATICS, DISTRIBUTION AND ASPECTS OF THE ECOLOGY OF THE FRESHWATER AMPHIPOD GENUS *PARAMELITA* (CRANGONYCTOIDEA: PARAMELITIDAE)

by

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CONTENTS

ABSTRACT

Freshwater amphipods in southern Africa are divided into three distinct elements. Five ingolfiellid species occur in Namibia, six *Sternophysinx* species are found in the northern Cape Province and Transvaal, while 12 *Paramelita* species are restricted to the south-western Cape. The crangonyctoid genus *Paramelita* was last reviewed in 1981 based mainly on a reassessment of museum material. Thus, large scale field surveys of these amphipods have not been undertaken since they were first described by Barnard in 1916 and 1927. In addition, little is known about the ecology of these amphipods.

This thesis examines the systematics, distribution and aspects of the ecology of *Paramelita*. After an extensive sampling programme in 1989 and 1990, several new species were discovered. Four new species, whose affinities with the known species were not immediately evident, were initially described, and later, after further analyses of morphological and genetic differentiation amongst *Paramelita* populations, eight other new species were recognised.

A phenetic analysis of the 24 *Paramelita* species revealed the existence of three distinct groups. The new genus *Afrocrangonyx* was proposed to accommodate seven of these species, whilst a single species was placed in the new genus *Aquadulcaris*. Sixteen species remained in *Paramelita*. Based on a cladistic analysis of morphological data, a fully resolved phylogenetic tree was obtained for *Afrocrangonyx*, but not for *Paramelita*, suggesting that the latter genus is not monophyletic.

A population of *P. nigroculus* in Window Stream, Table Mountain, was selected for a detailed ecological study. *P. nigroculus* occurred at high densities, and showed no seasonal breeding patterns. An investigation of thermal acclimation and tolerances to high temperatures in *P. nigroculus* individuals showed that their acclimation rates and lethal limits were similar to many other crustaceans.

P. nigroculus individuals feed mainly on allochthonous input. An investigation of the nature, timing and magnitude of this input indicated that litter was dominated by

abscissed leaves which fall throughout the year, peaking in summer. Leaf fall values and benthic detritus standing stocks were within the range reported for headwaters world wide. A study of leaf retention capabilities of two similar headwaters revealed that amphipod abundances were positively correlated with leaf retention characteristics. Leaf retention was strongly influenced by discharge, with increasing discharges resulting in decreases in retention and stream bed complexity. Thus the stream with higher discharges was less favourable for colonisation by amphipods.

Since *P. nigroculus* occurred at such high densities, it was expected to play a significant role in breakdown of detritus. Breakdown of three riparian leaf species in litter bags submerged in two streams was investigated. Litter bags were designed to allow amphipod access, yet retain fragments which resulted from physical abrasion and feeding activity. Breakdown was rapid, and the decomposition rate of one of the leaf species was the highest ever recorded for a riparian leaf species. Thus, it was concluded that where they occur at high densities, *Paramelita* individuals play a major role in the detritus dynamics of the stream.

INTRODUCTION

INTRODUCTION

The genus *Paramelita*

The amphipod genus *Paramelita* is a taxonomically isolated group of species restricted to the southwestern Cape region South Africa. This species group forms one of the three distinct elements of the freshwater amphipod fauna in southern Africa. The other two elements are the four described and one undescribed ingolfiellid species from northern Namibia (Griffiths, 1989; in press(a)) and the six species of *Sternophysinx* species of which four occur in the Transvaal, one in the northern Cape and one in Namibia (Griffiths, in press(b)). *Paramelita* first received attention when Barnard (1916) described four species collected from Table Mountain, placing them into the genus *Gammarus*. Barnard (1916) divided the four species into two groups; the white-eyed *G. capensis* and black-eyed *G. nigroculus*, which had the posterior margin of coxa 4 excavate, and *G. crassicornis* and *G. auricularius* which had poorly emarginate fourth coxal plates. Adult males of the latter two species were easily distinguishable by the stout antenna 2 in *G. crassicornis*, and the ear-like lobe on article 3 of the peduncle of antenna 2 and the subchelate nature of pereiopod 3 in *G. auricularius*.

In 1927, in addition to extending the known ranges of *P. nigroculus* and *P. capensis*, Barnard (1927) added a further six species and described another variety of *P. nigroculus*. *P. granulicornis, P. spinicornis, P. aurantius, P. kogelensis* and *P. seticornis* were all collected from various localities on the Hottentot Hollands Mountains, while *P. tulbaghensis* was found in a valley near Tulbagh. This valley was also colonised by *P. nigroculus* var. *persetosus*, a black-eyed amphipod with highly setose second antennae. All of the species described by Barnard (1916; 1927) were transferred to *Paramelita* by Schellenberg (1937). In 1973, Thurston added *P. barnardi* to the list when he described specimens collected from a cave on the Cape

Peninsula. Finally, in his revision of the freshwater amphipods of southern Africa, Griffiths (1981) described *P. flexa* from the Palmiet River near Grabouw. With the exceptions of *P. nigroculus* and *P. nigroculus* var. *persetosus*, all known paramelitids have unpigmented 'glistening' white eyes. Other than the brownish *P. nigroculus* and *P. auricularius*, and the orange *P. aurantius*, all of the species are off white in colour when alive.

Relationships amongst Paramelita species

Barnard (1927) commented on three main "evolutionary tendencies" within the genus - namely, differences in shape of coxa 4, 'thickening' of antenna 2 in males of many of the species, and development of secondary sexual characters on pereiopod 3. He noted that 'most' *Paramelita* species had distinctly concave hind margins of coxa 4, a condition well developed in *P. capensis* and *P. nigroculus*. Barnard (1927) also remarked that in contrast to the majority of gammarids, southern African species showed a marked tendency for antenna 2 in males to become distinctly thicker than antenna 1. To Barnard's (1927) knowledge, sexual adaptations of pereiopod 3 were unknown amongst gammarids. The abnormally shaped pereiopod 3 of *P. auricularius* was in contrast to this.

Although none of the above studies have attempted a phylogenetic tree for *Paramelita*, Barnard (1927) and Griffiths (1981) suggested relationships based on similarities in morphology. Barnard (1927) noted a 'strong resemblance' between *P. tulbaghensis* and *P. crassicornis*, while Griffiths (1981) added *P. flexa* to this group, distinguishing them by their enlarged peduncles of antenna 2, and the flexion between articles 4 and 5. Griffiths (1981) also linked *P. flexa* to *P. auricularius* because of the shared lobe on article 3 of antenna 2. Both Barnard (1927) and Griffiths (1981) have described *P. aurantius, P. granulicornis, P. kogelensis* and *P. seticornis* as "a closely

related group of species". In addition, *P. barnardi* and *P. capensis* have been described as being "similar" (Griffiths, 1981).

Relationships with other genera

When *Paramelita* was first erected by Schellenberg (1926), it was placed in the family Gammaridae. Later, Bousfield (1977) placed *Paramelita*, along with three Australian genera and *Falklandella* from the Falkland Islands, in the newly erected family Paramelitidae in the suborder Crangonyctoidea. Shortly afterwards, he (Bousfield, 1978) added *Sternophysinx* to this group, and later confirmed this (Bousfield, 1983) when he made other additions to the four families recognised by him as being members of Crangonyctoidea. Williams & Barnard (1988) have sharply criticised these rearrangements of the crangonyctoids, since Bousfield (1977, 1978, 1983) rarely rediagnosed the families following additions of other genera, and offered little justification for his rearrangements. Despite the need for a formal reassessment of the Crangonyctoidea, Williams & Barnard (1988) have accepted the crangonyctoid concept as a working model for their revision of freshwater amphipods of Australia, and have rediagnosed the family Paramelitidae. This resulted in the exclusion of *Sternophysinx* and *Falklandella*, and the inclusion of the following genera in the family: *Paramelita*, and the seven Australian genera *Protocrangonyx, Uroctena, Hurleya, Giniphargus, Austrogammarus, Austrocrangonyx* and *Antipodeus*.

Members of the family Paramelitidae are considered to be "plesiomorphic" crangonyctoids (Barnard & Barnard, 1983; Bousfield, 1983), since they have retained many primitive, or 'plesiomorphic' features of this amphipod suborder. These include the presence of sternal gills, a coxal gill on pereopod 7, and setae on the dorsal surface of the urosome. Crangonyctoids have a relict distribution; they dominate southern Australia, southern Africa and North America, but have largely been replaced in Europe by a wide variety of more advanced gammarid genera (Barnard & Barnard,

1983). The close affinity of southern African to Australian crangonyctoids suggests a Gondwanaland link between these two elements. Barnard & Barnard (1983) have noted that *Paramelita* is almost identical to *Austrogammarus*, differing only in the relative size of gnathopod 1. They reported that this appendage is of equal size to gnathopod 2 in *Austrogammarus*, but reduced in *Paramelita*. Williams & Barnard (1988) have suggested that *Paramelita* has strong affinities with *Uroctena*, and cite several similarities between the two genera, including the frequent "pediformity" of the second antennae in males of both genera. *Sternophysinx* is believed to be an 'apomorph' of *Paramelita*, and can be distinguished from the latter genus by the lack of a coxal gill on pereopod 7, an entire or notched telson, and reduced 'bladder-like' sternal gills (Griffiths, 1981; in press).

Distribution and Ecology

Other than the comments made by Barnard (1927) regarding habitat preferences and timing of breeding for some *Paramelita* species, nothing has been published regarding the ecology of these organisms. During his collection trips, Barnard (1927) noted that the amphipods generally occurred in small, often vegetated streams in preference to larger rivers, and that it appeared that larger specimens were correlated with stronger flowing perennial streams, while smaller species, particularly those with modified second antennae, were found in smaller trickles. In addition, he noted that the amphipods often occurred at very high densities.

Key questions and objectives

When Griffiths (1981) revised the genus *Paramelita* in 1981, his work was based chiefly on a reassessment of museum material, and few new specimens were collected. Large scale field surveys have not been undertaken since the time of Barnard (1916,

1927). Therefore, an extensive sampling programme for *Paramelita* specimens was undertaken in 1989 with several objectives in mind:

1. To describe any new species that were encountered,
2. To assess and quantify the amount of morphological and genetic differentiation amongst *Paramelita* species,
3. To determine the distribution patterns of *Paramelita* species,
4. To construct a new identification key for *Paramelita* species,
5. To determine the phylogenetic relationships between the species,
6. To select a suitable site for an in-depth study of the life history and reproductive biology of a representative species,
7. To determine the role of that population in the stream ecosystem in which it occurs.

To achieve these objectives, 20 key questions were formulated:

1. What undescribed forms occur, and how do they differ morphologically from the 12 previously known species of *Paramelita*?

2. Do any of these new species show the modifications of antenna 2 and pereopod 3 considered by Barnard (1927) to be important "evolutionary tendencies" within the genus *Paramelita*?

3. Are all the populations initially identified as members of a single widespread species, *P. capensis*, synonymous, or do some of them represent other, as yet, undescribed species?

4. Do the morphological differences amongst these populations coincide with genetic differences?

5. What is the extent of genetic differentiation amongst these populations, and how does it compare with that recorded for other amphipod species?

6. Are the groups of populations identified different enough to be considered as congeneric species?

7. Are the three phenotypic forms of *P. auricularius* collected members of a single highly variable species, or do these forms represent closely related, but distinctly different species?

8. Are the three forms of *P. crassicornis* collected members of a single variable species, or do they represent closely related, but distinctly different species?

9. Can genetic differentiation between the three *P. crassicornis* forms be related to geographical distribution of these forms?

10. Do the two morphological and genetic forms of *P. spinicornis* collected represent two different species?

11. Should all 24 species of *Paramelita* remain in a single genus, or should additional genera be recognised to accommodate morphological variation between groups of species?

12. How can new identification keys be constructed to aaccommodate the 12 new species described in this study?

13. What are the phylogenetic relationships between the South African paramelitid species?

14. What are the population densities, life history parameters and reproductive cycles of *P. nigroculus* in Window Stream, a small mountain headwater?

15. What were the rates of thermal acclimation and tolerances to lethal high temperatures of *P. nigroculus* individuals which enable them to survive extreme daily temperature fluctuations likely to be encountered in summer?

16. What is the nature, timing and magnitude of allochthonous input and what are benthic CPOM standing stocks in Window Stream on which *P. nigroculus* feed?

17. Could the differences in amphipod abundance in two headwaters be attributed to differences in their ability to retain leaf litter?

18. How is the retention of this leaf litter affected by changes in discharge in the two systems?

19. What is the best design for litter bags to be used to assess the affect of amphipod feeding on leaf decomposition?

20. What is the affect of feeding by *P. nigroculus* and *P. capensis* individuals on the decomposition of three riparian leaf species in two headwater streams?

The key questions outlined above are addressed in the 14 papers which form the bulk of this thesis. A brief resume of these papers follows.

Overview of the research

SECTION A: SYSTEMATICS

Paper 1. Four new species of *Paramelita* (Amphipoda: Crangonyctoidea) from South Africa. (*Annals of the South African Museum*, in press.)

This paper deals with the descriptions of four newly discovered forms, whose relationships with other *Paramelita* species were not immediately evident. In all four species, the males exhibited enlargement and thickening of antenna 2, and various modifications of pereopod 3, thus confirming Barnard's (1927) observations regarding "evolutionary tendencies" amongst *Paramelita* species.

Paper 2. Morphological and genetic differentiation of allopatric populations of a freshwater amphipod. (Submitted to *Journal of Zoology*, London.).

Two *Paramelita* species, *P. nigroculus* and *P. capensis* have been previously described as 'widespread' (Griffiths, 1981). For example, specimens collected in the present study from 17 streams as far north as Citrusdal ($32^{o}44'S$, $19^{o}02'E$) and as far east as Storms River ($34^{o}01'S$, $23^{o}55'E$) have been identified as *P. capensis*. However, preliminary observations of these populations revealed the existence of distinct morphological forms. In this paper the morphological differences amongst these supposed *P. capensis* populations are quantified, genetic differentiation of the populations is examined by means of gel electrophoresis, and evolutionary distinct lineages are identified. This study resulted in the recognition of five distinct lineages which were taken to represent separate species.

Paper 3. Further new species within the freshwater amphipod genus *Paramelita* (Crangonyctoidea: Paramelitidae) from South Africa. (Submitted to *Journal of Zoology*, London.).

Four of the 'new' species recognised in the previous chapter are described in this paper, the fifth group corresponded to the original *P. capensis* described by Barnard (1916) from Table Mountain.

Paper 4. A taxonomic reexamination of freshwater amphipods in the *P. auricularius* - *P. crassicornis* complex, with descriptions of three additional species. (Submitted to *Crustaceana*).

During the sampling programme in 1989, several forms of *P. auricularius* and *P. crassicornis* were collected. In this paper, the morphological and genetic differentiation between these populations are examined. The 16 populations studied were divided into seven distinct phenotypic forms, one of which was clearly recognisable as a new, undescribed species. Genetic variation between the remaining forms was examined by gel electrophoresis. It was concluded that the three forms of *P. auricularius* represented members of a single, morphologically variable species. The three forms previously identified as *P. crassicornis* were considered to be sufficiently different to warrant the recognition of two additional new species. The descriptions of all three new species recognised here are included in this paper.

Paper 5. **Morphological and genetic differentiation among populations of the freshwater amphipod *Paramelita spinicornis* Barnard (Crangonyctoidea: Paramelitidae), with a description of a new species.** (In preparation).

Six populations collected in 1989 were initially identified as ***P. spinicornis*** because of the 'tooth' on the enlarged fourth articles of antenna 2 (Barnard, 1927). However, two of the populations consisted of large amphipods with distinctly elongated second antennae, while the other four populations were smaller, and had short, strongly swollen second antennae. In this paper these morphological, as well as genetic differences between the two forms are quantified. The differentiation between the two forms was considered sufficient to warrant the recognition of a new species which is also described.

Paper 6. **A taxonomic revision of the family Paramelitidae (Crustacea: Amphipoda) from South African fresh waters.** (Submitted to *Annals of the South African Museum*).

Many of the *Paramelita* species were inadequately illustrated when they were first described (Barnard, 1916; 1927), and the discovery of new populations has allowed the study of morphological variation and extended the known ranges of these animals. The description of 12 additional new species has also made it necessary to include these species in new identification keys. Despite the fact that many *Paramelita* species do not fit the original diagnosis of the genus, all new paramelitid species from the south-western regions of South Africa have been included in *Paramelita* without rediagnosis of this genus. Since preliminary observations showed that morphologically and genetically similar groups could be identified within *Paramelita*, a phenetic analysis, based on morphological data was undertaken. As a result of this analysis, two additional new genera, *Afrocrangonyx* and *Aquadulcaris* are recognised and diagnosed.

In addition, the genus *Paramelita* is rediagnosed, all of the species are diagnosed and illustrated, distribution records are noted, and new keys are constructed.

Paper 7. Phylogenetic relationships among South African paramelitid amphipods (Crangonyctoidea: Paramelitidae) based on morphological variation. (In preparation).

In this paper, the phylogenetic relationships among freshwater species of two South African crangonyctoid amphipod genera, *Afrocrangonyx* and *Paramelita* are determined based on morphological variation. Three Australian paramelitid genera were chosen as outgroups for polarising the character states, and most parsimonious trees constructed by means of the HENNIG86 cladistic analyses software package. A fully resolved cladogram was calculated for *Afrocrangonyx*. Although the cladogram for *Paramelita* was not fully resolved, some monophyletic groups within the genus were identified. The phylogeny of each genus is related to geographical distribution of the species, and shortcomings in the data are also discussed.

SECTION B: ECOLOGY

Based on his experience in the field, Barnard (1927) noted that in those streams where they occur, *Paramelita* individuals are often found in very high numbers. Such would be the case when visiting Window Stream, a first order perennial stream draining the slopes of Table Mountain near Cape Town ($33^{o}59'S$, $18^{o}25'E$). This stream is dominated by the black-eyed *P. nigroculus*, one of the larger *Paramelita* species. Window Stream is easily accessible, is in pristine condition owing to its position in a protected national botanical garden, and therefore provided an ideal site for an in-depth ecological study of the *P. nigroculus* population.

Paper 8. Life history and reproductive biology of the mountain stream amphipod *Paramelita nigroculus*. (In preparation).

Population densities, and the life history and reproductive biology of *P. nigroculus* is the subject of this paper. Population densities were often exceptionally high, ranging from 264 to 12 227 individuals m^{-2}, with a mean density of 7 972 individuals m^{-2} during the summer months. Standing stocks varied from 0.37 to 24.32 g dry weight m^{-2}. Animals ranged in size from 1.0-11.0 mm, with mean sizes of 9.0-9.9 mm in females and 6.0-8.9 mm in males. Mature females were present all year round, and carried between 31 and-60 eggs in their brood pouches.

Paper 9. Thermal acclimation and tolerance to lethal high temperature in the mountain stream amphipod *Paramelita nigroculus* (Barnard). *Comparative Biochemistry and Physiology*. 89A (3): 425-431.

During summer, many small streams dry up or form a series of isolated pools. Such pools often support large numbers of *P. nigroculus* individuals, which are likely to encounter extreme daily temperature fluctuations. In this paper, the rates of thermal acclimation and tolerances to high temperatures of *P. nigroculus* were investigated using the Critical Thermal Maximum and LT_{50} methods. Acclimation rates were typical of most crustaceans, with a gain of resistance to high temperature, following transfer from 8.5 to 20°C being completed in 1-2 days. Loss of heat resistance took 3 days. The LT_{50} for 13.5°C acclimated animals ranged from about 300 min at 27°C to 4 min at 31°C.

Paper 10. Allochthonous input and retention in a small mountain stream, South Africa. *Hydrobiologia* 202: 135-146.

Preliminary observations in the field and laboratory indicated that these amphipods were 'shredders' (Cummins, 1974), feeding on coarse particulate organic matter (CPOM). The source of this detritus in Window Stream was predominantly in the form of abscissed leaves from riparian trees. The nature, timing and magnitude of this allochthonous input as well as benthic CPOM standing stocks in Window Stream are investigated in this paper. Total annual fall amounted to 426 g dry weight, of which abscissed leaves contributed 57%. Leaf litter fall peaked in summer, although standing stocks of CPOM showed no seasonal trend, ranging from 14-69 $g.m^{-2}.month^{-1}$. Leaf fall values and CPOM standing stocks were well within the range reported for low-order streams world wide.

Paper 11. Leaf litter retention and its implications for shredder distribution in two headwater streams. *Archiv fur Hydrobiologie* 120(3): 315-325.

In order for this detritus to be available to the amphipods, it needs to be retained on the stream bed. It follows, therefore, that streams which are efficient at organic retention would be favourable for amphipod colonisation, whilst poorly retentive streams would not encourage high amphipod numbers. Window Stream and Langrivier ($34^{o}00'S, 18^{o}55'E$) are headwaters with similar allochthonous inputs and CPOM standing stocks on the stream bed, yet Window Stream is dominated by amphipods, and Langrivier is characterised by little shredder activity. In this paper, the leaf litter retention abilities of these two streams are examined using a closed-system leaf release and recapture method. This study showed that Window Stream was clearly more retentive than Langrivier, and that amphipods were most abundant in riffles and backwaters, the features which had the greatest leaf trapping efficiencies.

Paper 12. The effect of discharge on leaf retention in two headwater streams. (Submitted to *Archiv fur Hydrobiologie*).

Since, with the exception of discharge, the two streams were similar in terms of their physical and chemical properties, the influence of discharge on leaf litter retention was further investigated in this paper. Leaf retention and stream bed complexity decreased markedly with an increase in discharge. Riffles decreased in trapping efficiency, whilst backwaters became even more retentive with increasing discharge. It was concluded that on an annual basis, Langrivier probably has a greater discharge than Window Stream, and that this results in lower retention capacities in this stream over the long term. Thus, Langrivier is not as suitable as Window Stream for colonisation by large numbers of amphipods.

Paper 13. The influence of different litter bag designs on the breakdown of leaf material in a small mountain stream. *Hydrobiologia* 183: 173-177.

Decomposition, or breakdown of leaf litter by invertebrates is usually measured either by means of 'leaf packs' or 'litter bags'. Although leaf packs are thought to better represent natural leaf accumulations, weight loss measured by this method represents losses due to biological consumption as well as physical fragmentation. Thus, if a measure of invertebrate ingestion is required, litter bags, which retain fragments, are more suitable. In this paper, leaves were confined in litter bags which allowed invertebrate access, yet retained fragments of leaves which resulted both from physical abrasion and from the feeding activity of the *P. nigroculus* individuals. The advantages of using bags of this design are discussed.

Paper 14. The effect of invertebrates on leaf breakdown in two woodland streams. (*Archiv fur Hydrobiologie*, accepted with revision).

As *P. nigroculus* individuals dominated the invertebrate community in Window Stream, they were expected to play a significant role in breakdown of CPOM. The breakdown of abscissed leaves of three riparian species was investigated in two headwater streams, and the results of this study are presented in this paper. Colonisation of the leaf material by amphipods was rapid, and the decomposition of *Cunonia* in litter bags which allowed invertebrate access was the highest ever recorded for a riparian leaf species. It was concluded that where shredders such as *Paramelita* amphipods occur in high numbers, they can be expected to have a marked effect on the rate of detritus decomposition.

Statement of responsibility

The above papers are the sole responsibility of the candidate although six of them are co-authored by either Profs C.L. Griffiths or B.R. Davies, the joint supervisors of the project. Exceptions are the four papers on which either C.D. Snaddon, J.A Buchanan, or K. Prochazka are the senior authors, or C.D. Snaddon is the junior author. The paper "Morphological and genetic differentiation among populations of the freshwater amphipod *Paramelita spinicornis* ....." was conceived jointly by the candidate and C.L. Griffiths, and closely supervised by the candidate while the initial analyses were undertaken by C.D. Snaddon who submitted the work as an honours project. The candidate subsequently revised the manuscript, reanalysed the data, and described the new species which was recognised as a result of the study. The papers, "Thermal acclimation and tolerance to .....", "Leaf litter retention and its implications .....", and "The effect of discharge on leaf retention ...." all resulted

from honours projects conceived and jointly supervised by the candidate and B.R. Davies, and conducted either by J.A. Buchanan, K. Prochazka and C.D. Snaddon. In all three cases, the candidate played a major role in data analyses and the preparation of the final manuscript for publication.

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PAPER 1

FOUR NEW SPECIES OF *PARAMELITA* (AMPHIPODA:CRANGONYCTOIDEA) FROM SOUTH AFRICA

By

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(With 8 figures)

ABSTRACT

Four new species of the endemic South African freshwater amphipod genus *Paramelita* are described from material collected in the south western Cape Province. Males of all four species exhibit enlargement and thickening of the second antenna and various modifications of pereopod 3, with two of the four having this limb fully subchelate. Morphological similarities between the four new species and the 12 previously known species of *Paramelita* are discussed.

CONTENTS

PAGE

INTRODUCTION

The first records of the freshwater amphipod genus *Paramelita* were those of Barnard (1916), who described four species collected from streams on Table Mountain, originally placing them in the genus *Gammarus*. Barnard (1927) added another six species to this list, and in 1937, Schellenberg transferred all of these species to the genus *Paramelita*. Two other species have subsequently been recognised by Thurston (1973), who described specimens collected from a cave on the Cape Peninsula, and Griffiths (1981), who described a new species from the Palmiet River near Grabouw.

In 1989, a research project was initiated to investigate the distribution patterns and phylogenetic relationships of the *Paramelita* species. Accordingly, an extensive sampling programme was undertaken to record distributions and to collect samples for the purposes of constructing a phylogenetic tree based on morphological and isozyme variation. This sampling programme has resulted in range extensions for many of the known species, and has also revealed several new undescribed forms. Some of these could be linked to existing species complexes, and will be discussed elsewhere. However, the relationships of four of the newly discovered taxa were not immediately evident, and are described below.

SYSTEMATICS

Superfamily CRANGONYCTOIDEA Bousfield, 1973

Family Paramelitidae Bousfield, 1977

Paramelita Schellenberg, 1926

Paramelita pinnicornis sp. nov.

Figs 1, 2

Material examined

Holotype. Male, 13,5 mm, SAM A40004, from a tributary of the Burgersbos River (34°01'S, 18°25'E) crossing Rhodes Drive, Constantia, on the Cape Peninsula. Collected by B.A. Stewart and Y. Dempster on 9 August 1989.

Paratypes. 14 males, 20 ovigerous females, SAM A40005, from the same sample as the type specimen.

Other material. This species has also been collected from Kenilworth Race Course (SAM A40008) on the Cape Peninsula and from two adjacent streams flowing into Koeelbaai in the Cape Hangklip area (SAM A40006 and SAM A40007) on the east coast of False Bay.

Etymology

From the Latin *pinna* (feather or plume) and *cornis* (horn), an allusion to the fin, or wing-like projections which are present on articles 5 of the second antenna.

Description (of holotype, male, 13,5 mm)

Body colour when alive grey tinged with pink. *Head* slightly shorter than pereon segments 1 and 2 combined, anterovental margin excavate to accommodate inflated article 1 of antenna 2, eyes glistening white when alive, difficult to discern in preserved material. *Antenna 1* 0,6 times length of body, setation sparse, articles 1 and 2 of peduncle subequal, each twice length of article 3, flagellum twice length of peduncle, 31-articulate, accessory flagellum 6-articulate, reaching past article 4 of primary flagellum. *Antenna 2* approximately the same length as antenna 1, but considerable stouter, peduncle sparsely to moderately setose, article 4 and 5 each about 2,7 times length of article 3, outer margin and tip of article 5 extended into an elongate triangular flange, flagellum 0,6 times length of enlarged peduncle, 16-articulate, sparsely setose. *Left mandible* with incisor bluntly 4-toothed, lacinia mobilis with four blunt teeth, six spinose accessory blades, molar strongly triturative, 3-articulate palp longer than body

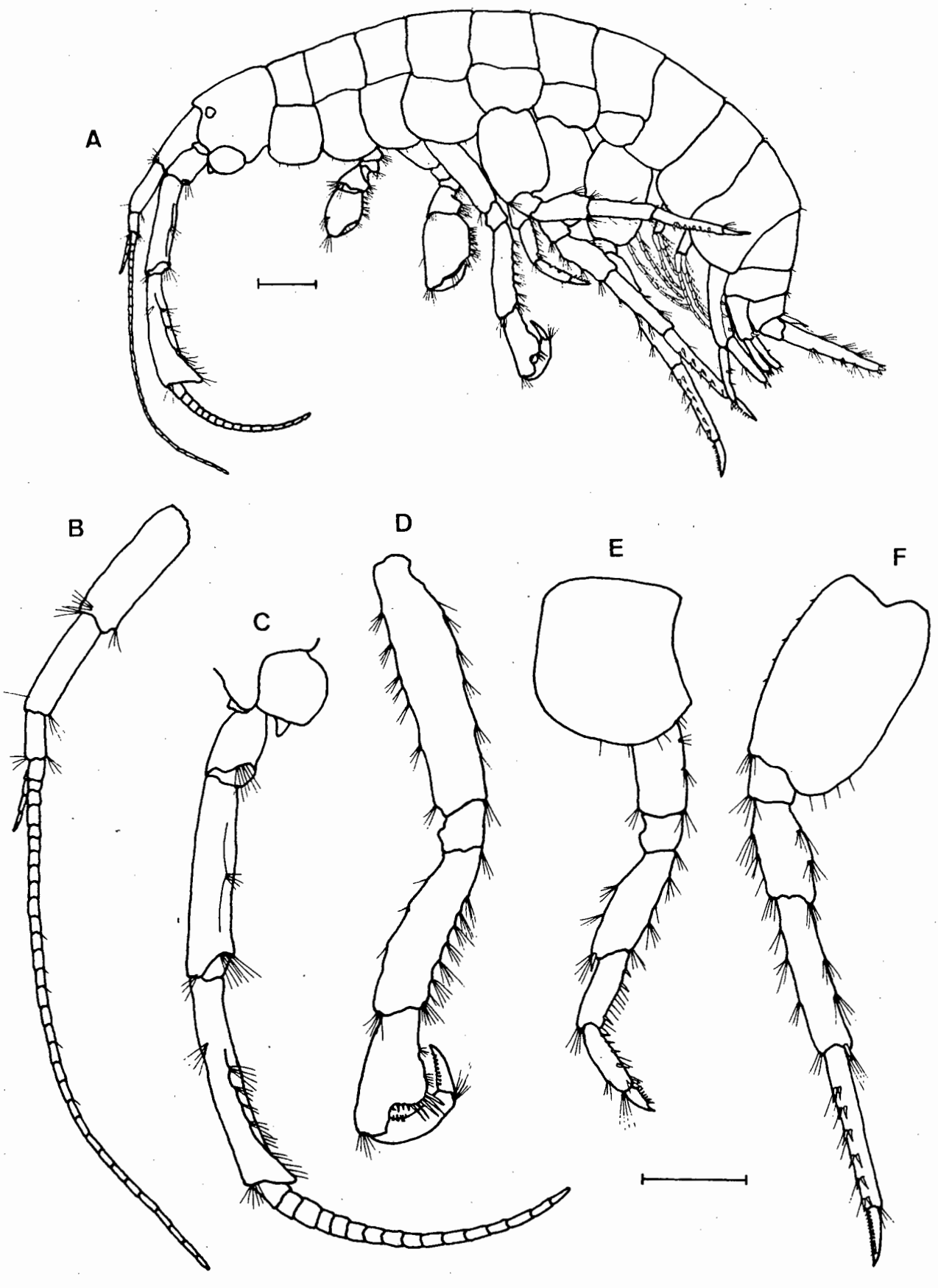

Fig. 1. *Paramelita pinnicornis* sp. nov., male, 13,5 mm. A. Lateral aspect. B. Antenna 1. C. Antenna 2. D. Pereopod 3. E. Coxa 4 and pereopod 4. F. Pereopod 7. Scale lines represent 1,0 mm.

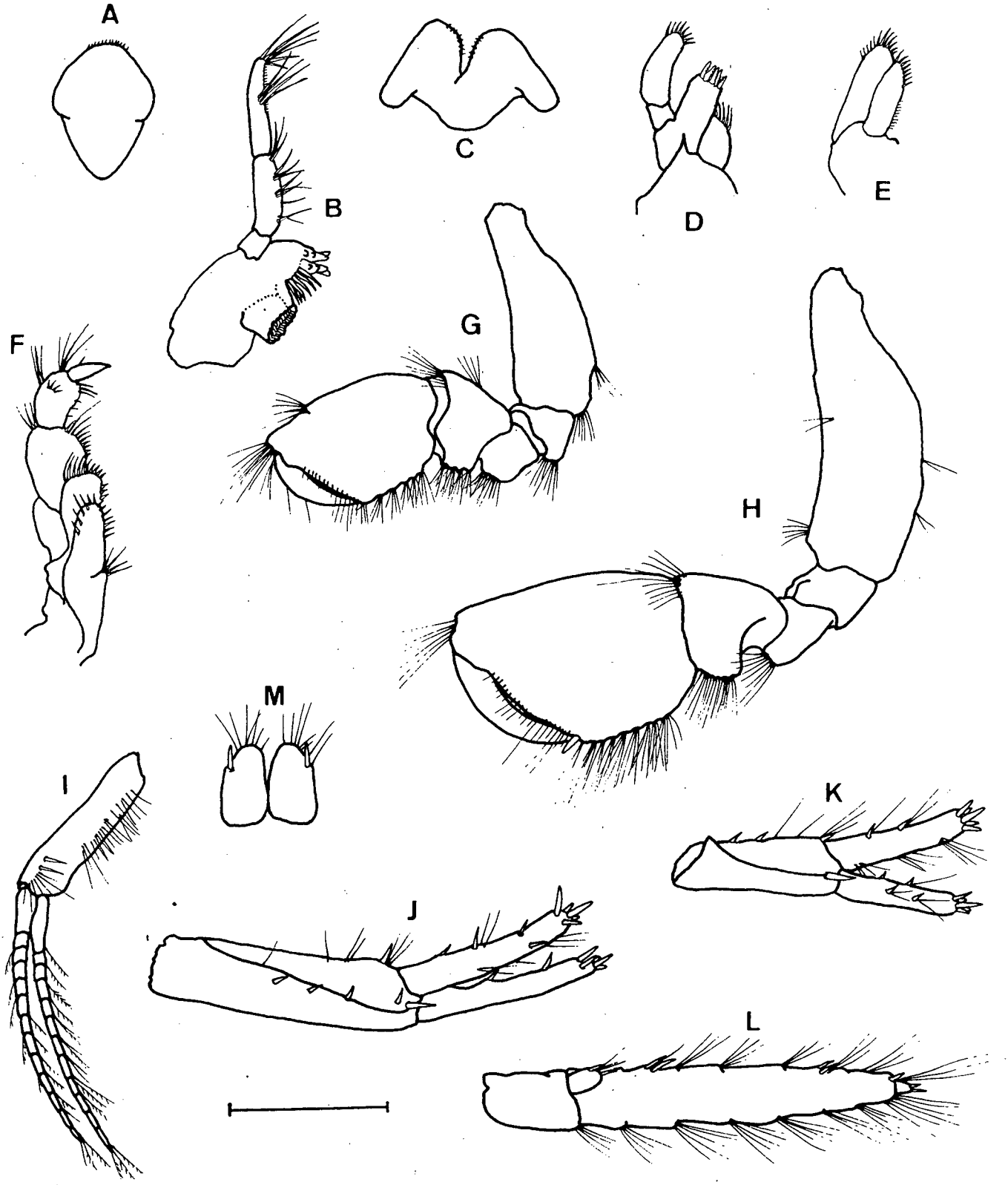

Fig. 2. *Paramelita pinnicornis* sp. nov., male, 13,5 mm. A. Upper lip. B. Left mandible. C. Lower lip. D. Maxilla 1. E. Maxilla 2. F. Maxilliped. G. Gnathopod 1. H. Gnathopod 2. I. Pleopod 3. J. Uropod 1. K. Uropod 2. L. Uropod 3. M. Telson. Scale lines represent 1,0 mm.

of mandible, article 1 as long as wide, article 2 3,5 times length of 1, with approximately 16 setae anteriorly, article 3 1,2 times length of 2, distal half lined with many short setae, six long apical setae present, tuft of four setae approximately 0,7 along length. *Right mandible*, incisor 3-toothed, lacinia mobilis bifurcate, three accessory blades. *Maxilla 1*, inner plate with five pectinate setae, inner margin pubescent, outer plate bearing two terminal rows each of about five stout serrated spines, palp exceeding outer plate, with eight apical spines. *Maxilla 2*, inner plate a little shorter and narrower than outer plate, proximally sparsely pubescent, both plates strongly setose terminally. *Maxilliped*, inner plate with many curved spinose setae, outer plate with approximately 10 stout, blunt spine-teeth on inner margin and 10 terminal curved spinose setae, palp article 2 the longest, 2 and 3 densely setose medially, 4 with four short setae on margin.

Pereon segments with very few dorsal setae, coxae 1-3 slightly deeper than corresponding segments, quadrate, sparsely setose ventrally, coxa 4 posteriorly excavate, slightly deeper than long, sparsely setose on ventral margin, coxae 5 and 6 longer than deep, bilobed, few setae ventrally, coxa 7 semicircular, smooth, segments 2-7 bearing 1 pair of coxal gills each, segment 2 with one, 3, 4, 5 and 7 with two, and 6 with four sausage-shaped sternal gills. *Gnathopod 1* subchelate, articles 5 and 6 together slightly longer than 2, article 6 approximately twice as long as 5, longer than wide, palm relatively straight, oblique, palmar angle with two long and three short spines, dactyl as long as palm. *Gnathopod 2* similar in structure to, but 1,4 times length and sturdier than 1, inner margin of article 2 bearing seven groups of strong spines, articles 5 and 6 combined longer than article 2, article 6 approximately twice as long as 5, slightly longer than wide, palm slightly convex, oblique, defined by four stout spines, dactyl as long as palm. *Pereopod 3* 1,4 times length of 4, articles 5 and 6 highly modified, 5 being posteriorly lobed, the lobe armed with five long and four shorter spines, article 6 folded back against lobed posterior margin of 5, bearing five short stout spines, dactyl stout, with eight short spinules. *Pereopod 4* unmodified,

article 5 with three posterior spines, article 6 with five pairs of posterior spines, dactyl with seven spinules. *Pereopod 5* basis posteriorly expanded, article 4 0,8 length of 5, 5 and 6 subequal in length, article 5 with three pairs of spines, article 6 with five pairs of spines, dactyl with 10 spinules. *Pereopods 6 and 7* similar in structure, bases expanded posteriorly, article 6 with six pairs of spines, dactyls each with 14 spinules anteriorly.

Pleon segments 1-3 sparsely setose dorsally, first pleonal epimeron rounded-quadrate, 2 and 3 quadrate, setose on posterior margin. Pleon segments 4-6 sparsely setose dorsally. *Uropod 1* extending slightly beyond 2, 1,1 length of uropod 3, rami equal, 0,7 length of peduncle, each ending in five spines. *Uropod 2* shorter, stouter than 1, rami subequal, each with five apical spines. *Uropod 3* exceeding 2 by 0,9 length of outer ramus, peduncle longer than broad, inner ramus reduced, 0,4 times length of peduncle, terminating in two spines and a few long setae, outer ramus three times length of peduncle, six groups of setae on inner and eight on outer margin, small second article ending in two spines. *Telson* as broad as long, deeply cleft, each lobe with one large subapical spine and several apical setae.

Remarks

P. pinnicornis sp. nov. adult males are clearly distinguished from other *Paramelita* species by the fin-like projections on the peduncle of antenna 2 and the claw-like structure of the distal end of pereopod 3. Antenna 2 in females is slender and shorter than 1, and pereopod 3, like 4, is not modified. In most other respects, the females resemble the males. Although the fin-like projections of the second antenna in males are unique, a 'claw-like' pereopod 3 is also found in *P. auricularius* Barnard, 1916 from Table Mountain, and in *P. andronyx* sp nov. from Kasteelsberg. Despite their superficial similarity, however, these structures are not homologous, and therefore not evidence of close affinities between these three species. In *P. pinnicornis* sp nov. the 'claw' is achieved by the folding back of article 6 against the lobed spiny posterior margin of article 5. In *P. andronyx* sp nov., however, it is article 4 which is strongly

protruded, with the right angle joint between articles 5 and 6 completing the claw. In *P. auricularius*, an elongated article 6 folds back against the lobed, swollen posterior margin of article 5, but this is of a quite different shape to the structure in *P. pinnicornis* sp nov. Coxa 4 in the latter two species is either quadrangular, or gently concave posteriorly; whereas in *P. pinnicornis* sp. nov. it is distinctly excavate posteriorly.

Paramelita magnicornis sp. nov.

Figs 3, 4

Material examined

Holotype. Male, 15,0 mm, SAM A40009, from a stream draining the Swartkop Mountains (34°14'S, 18°29'E) near Millers Point on the southern Cape Peninsula. Collected by B.A. Stewart and C.L. Griffiths on 30 November 1989.

Paratypes. 13 males and eight females, SAM A40010, from the same sample as the holotype.

Other material. This species has also been collected from the same stream as the holotype on another occasion (SAM A40013), a stream draining Chapman's Peak (SAM A40011 and A40015), a stream draining the Kalk Bay Mountains near Clovelly (SAM A40012) and Peck's Valley stream on Boyes Drive (SAM A40014 and A40016), all on the Cape Peninsula.

Etymology

From the Latin *magnus* (large), alluding to the swollen and elongated second antenna.

Description (of holotype, male, 15,0 mm)

Body colour when alive off white. *Head* shorter than pereon segments 1 and 2 together, anteroventral margin excavate to accommodate inflated article 1 of antenna 2, eyes glistening white when alive, difficult to discern when preserved. *Antenna 1*

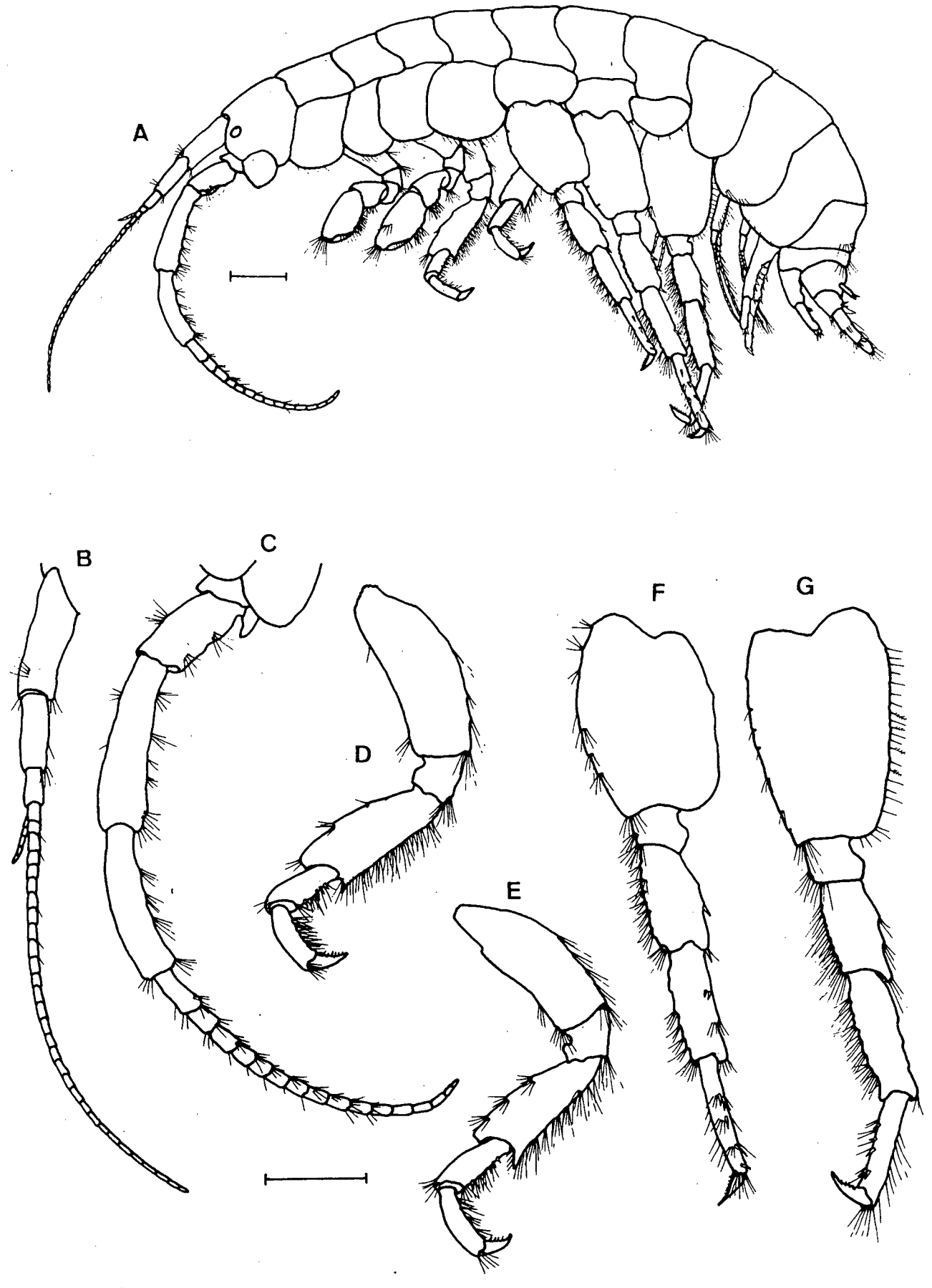

Fig. 3. *Paramelita magnicornis* sp. nov., male, 15,0 mm. A. Lateral aspect. B. Antenna 1. C. Antenna 2. D. Pereopod 3. E. Pereopod 4. F. Pereopod 5. G. Pereopod 7. Scale lines represent 1,0 mm.

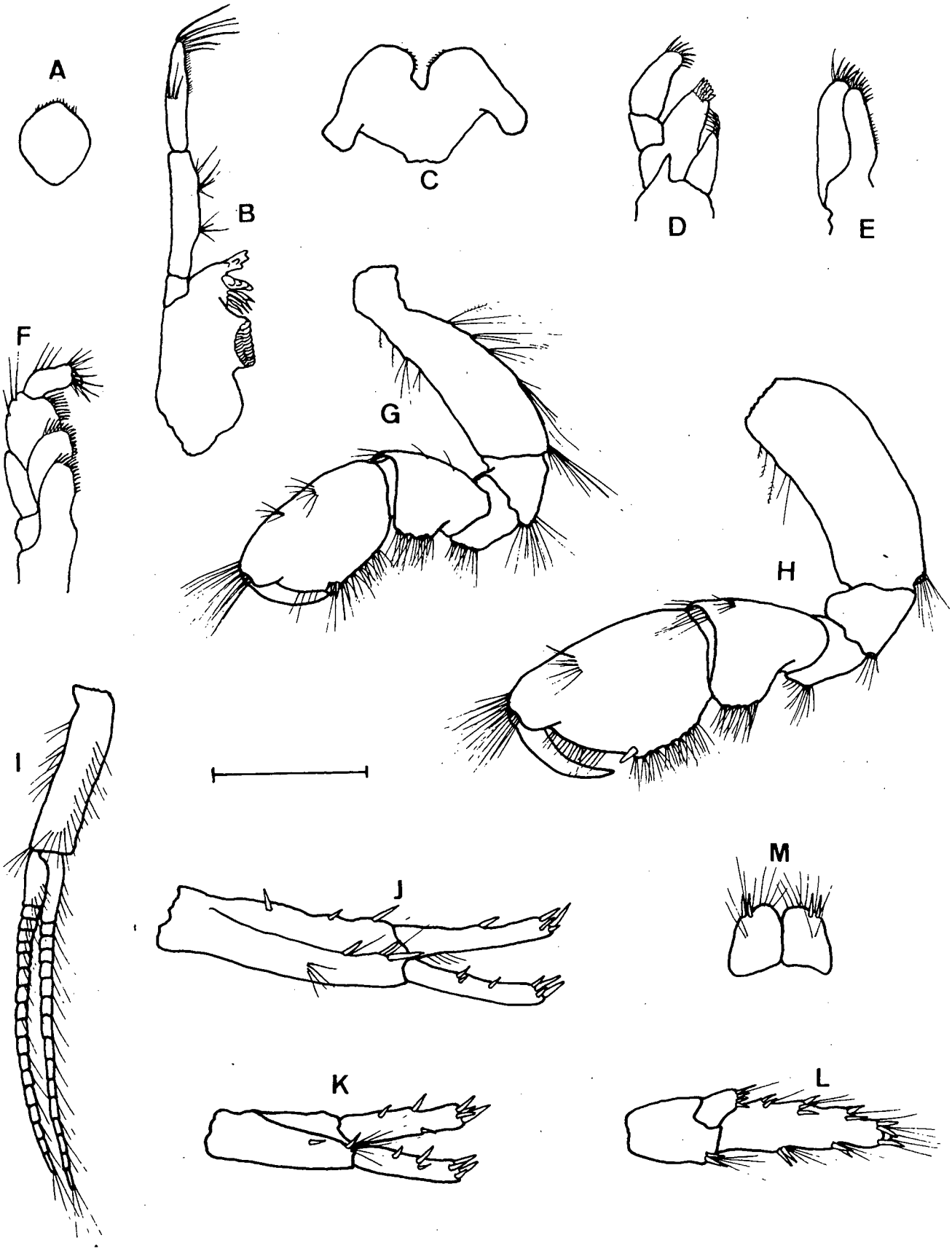

Fig. 4. *Paramelita magnicornis* sp. nov., male, 15,0 mm. A. Upper lip. B. Left mandible. C. Lower lip. D. Maxilla 1. E. Maxilla 2. F. Maxilliped. G. Gnathopod 1. H. Gnathopod 2. Pleopod 1. J. Uropod 1. K. Uropod 2. L. Uropod 3. M. Telson. Scale lines represent 1,0 mm.

relatively short, 0,4 length of body, setation sparse, articles 1 and 2 subequal, each twice length of 3, flagellum 1,7 times length of peduncle, 30-articulate, accessory flagellum 6-articulate, reaching to end of article 4 of flagellum. *Antenna 2* 1,2 times length of 1 and considerably stouter, peduncle moderately setose posteriorly, article 4 1,9 length of article 3, distally inflated, article 5 slightly shorter than article 4, flagellum 0,8 times length of peduncle, 16-articulate, moderately setose posteriorly. *Left mandible*, incisor, bluntly 5-toothed, lacinia mobilis with 4 blunt teeth, four accessory blades, molar strongly triturative, palp longer than body of mandible, article 1 as long as wide, article 2 five times length of article 1, with 10 strong setae anteriorly, article 3 slightly shorter than 2, distal half with comb of short setae, six long apical setae, tuft of setae half-way along length. *Right mandible*, incisor 4-toothed, lacinia mobilis bifurcate, two accessory blades. *Maxilla 1*, inner plate with 7 setae, inner margin pubescent, outer plate terminating in nine stout serrated spines, palp exceeding outer plate, with eight stout apical setae. *Maxilla 2*, inner plate a little shorter and narrower than outer plate, proximally pubescent, both plates strongly setose terminally. *Maxilliped*, inner plate with many curved spinose setae, outer plate with approximately seven stout blunt spine-teeth on inner margin and eight terminal curved setae, palp article 2 the longest, inner margin with row of strong curved setae, article 3 densely setose.

Pereon segments dorsally smooth, coxae 1-3 deeper than corresponding segments, quadrate, moderately setose ventrally, coxa 4 excavate posteriorly, approximately as deep as long, moderately setose ventrally, coxa 5 and 6 longer than deep, bilobed, coxa 5 moderately setose ventrally, coxa 6 with a few short setae and spinules, coxa 7 semicircular, setose ventrally, segments 2-7 bearing one pair of coxal gills each, segments 2 with one, 3, 4, 5 and 7 with two, and 6 with four sausage-shaped sternal gills. *Gnathopod 1* subchelate, article 2 bearing plumose setae on both anterior and posterior margins and two groups of spines on inner surface, articles 5 and 6 together longer than 2, article 6 1,7 times length of 5, longer than wide, palm gently convex,

oblique, with five palmar spines, dactyl as long as palm. *Gnathopod 2* similar to, but 1,2 length and sturdier than 1, article 2 with two groups of spines on inner margin and a few plumose setae on anterior margin, articles 5 and 6 together longer than article 2, article 6 1,7 times the length of 5, longer than wide, palm convex, distinctly oblique, defined by four stout spines, dactyl as long as palm. *Pereopod 3* approximately the same length as 4, article 4 anteriorly lobed over 5 and posterodistally protruded into a triangular tooth, articles 4, 5 and 6 densely setose posteriorly, dactyl with six spinules. *Pereopod 4* similar in structure to 3, article 4 posterodistally protruded into a distinct triangular tooth, dactyl with four spinules. *Pereopods 5, 6 and 7*, bases moderately expanded posteriorly, with some simple and plumose setae anteriorly and posteriorly, article 4 shorter than 5 and 6, article 5 and 6 approximately equal in length, article 5 with two groups, and 6 with four to five groups of spines posteriorly, articles 4, 5 and 6 moderately to densely setose anteriorly, dactyls of pereopods 5 and 7 with six spinules, and of pereopod 6 with seven spinules.

Pleon segments 1-3 with some dorsal setae, epimeral plates rounded to quadrate, ventrally setose. Pleon segments 4-6 moderately setose dorsally. *Uropod 1* extending a little beyond uropod 2, 1,5 length of uropod 3, rami subequal, 0,6 times length of peduncle, each ending in four spines. *Uropod 2* shorter than 1, inner ramus marginally longer than outer, each with four apical spines. *Uropod 3* relatively short, exceeding 2 by 0,7 length of outer ramus, peduncle longer than broad, inner ramus short, 0,6 length of peduncle and 0,3 times length of outer ramus, with four apical spines and one seta, outer ramus 2,4 times length of peduncle, three groups of spines and setae on inner and two on outer margin, second segment reduced, only 5% of length of first segment. *Telson* broader than long, deeply cleft, each lobe bearing two stout subapical spines, seven apical setae and two setae arising from the dorsal surface about half way along the length.

Remarks

P. magnicornis sp. nov. is most similar to the common and widely distributed *P. capensis* Barnard, 1916, with which it lives sympatrically in at least two known localities on the Cape Peninsula. Adult males of this newly described species are distinguished from *P. capensis* primarily by the swollen and elongate peduncle of antenna 2 and the 'spur-like' projections of the posterodistal apices of articles 4 of the first and second pereopods. The thickening and elongation of articles 4 and 5 of antenna 2 are also characteristic, these articles being noticeably more swollen distally than proximally. In females, antenna 2 is relatively slender and shorter than 1; similarly, pereopods 3 and 4 are unmodified. Coxa 4 in *P. magnicornis* sp. nov., as in *P. capensis*, is distinctly excavate.

Paramelita andronyx sp. nov.

Figs 5, 6

Material examined

Holotype. Male, 16,1 mm, SAM A40017, from a tributary of the Riebeek's River (33°22'S, 18°50'E), above the farm Waterval, on the slopes of Kasteelsberg, in the Malmesbury district. Collected by B.A. Stewart and P.A. Cook in September 1989.

Paratypes. 10 males, three females, SAM A40018, from the same locality as the type specimen.

Other material. A sample from a nearby farm, Wynkeldersberg (SAM A40019) is the only known other record of this species to date.

Etymology

From the Greek *aner* (man) and *onux* (claw), alluding to the claw-like structure of pereopod 3 in adult males.

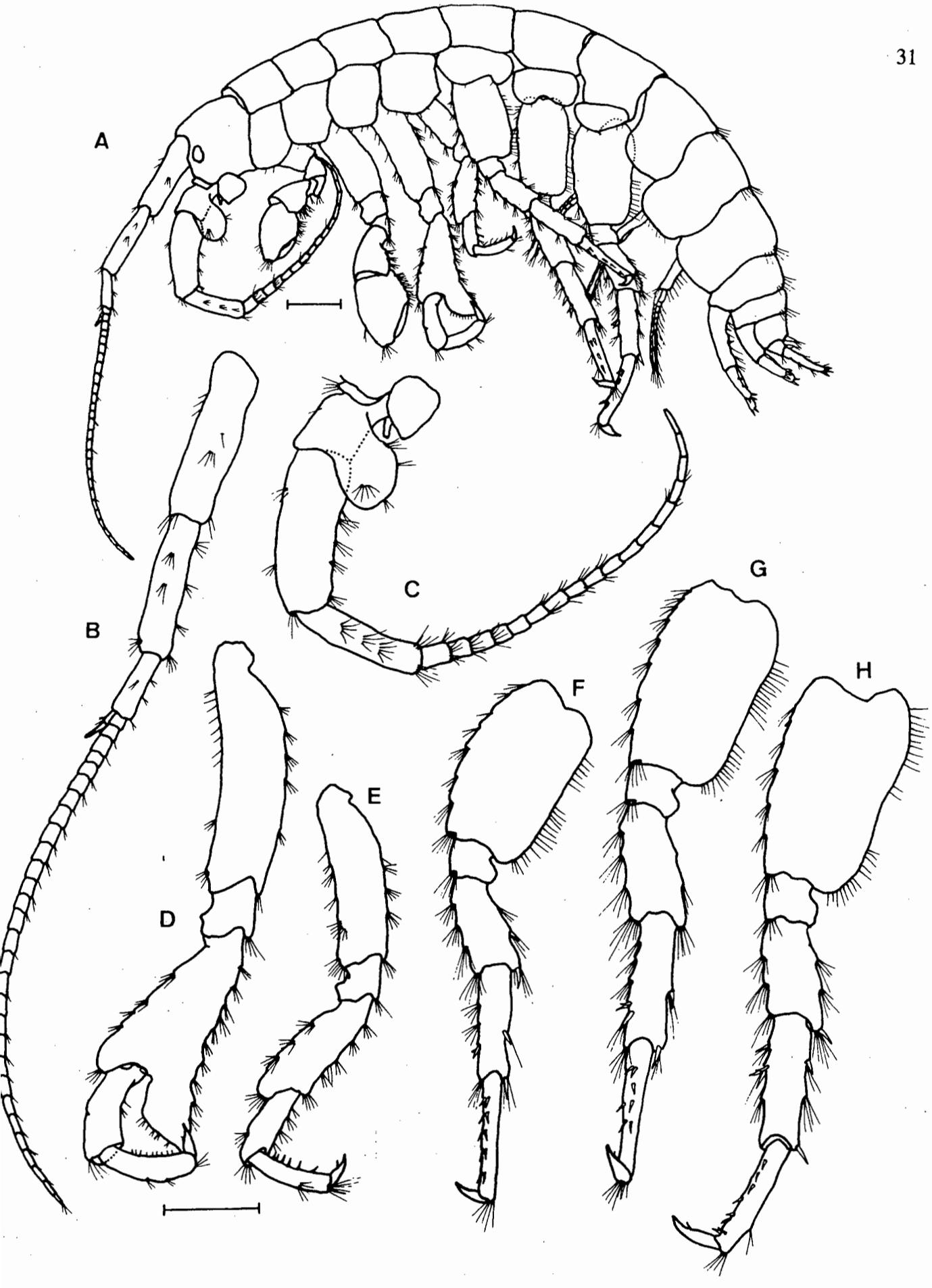

Fig. 5. *Paramelita andronyx* sp. nov., male, 16,1 mm. A. Lateral aspect. B. Antenna 1. C. Antenna 2. D. Pereopod 3. E. Pereopod 4. F. Pereopod 5. G. Pereopod 5. G. Pereopod 6. H. Pereopod 7. Scale lines represent 1,0 mm.

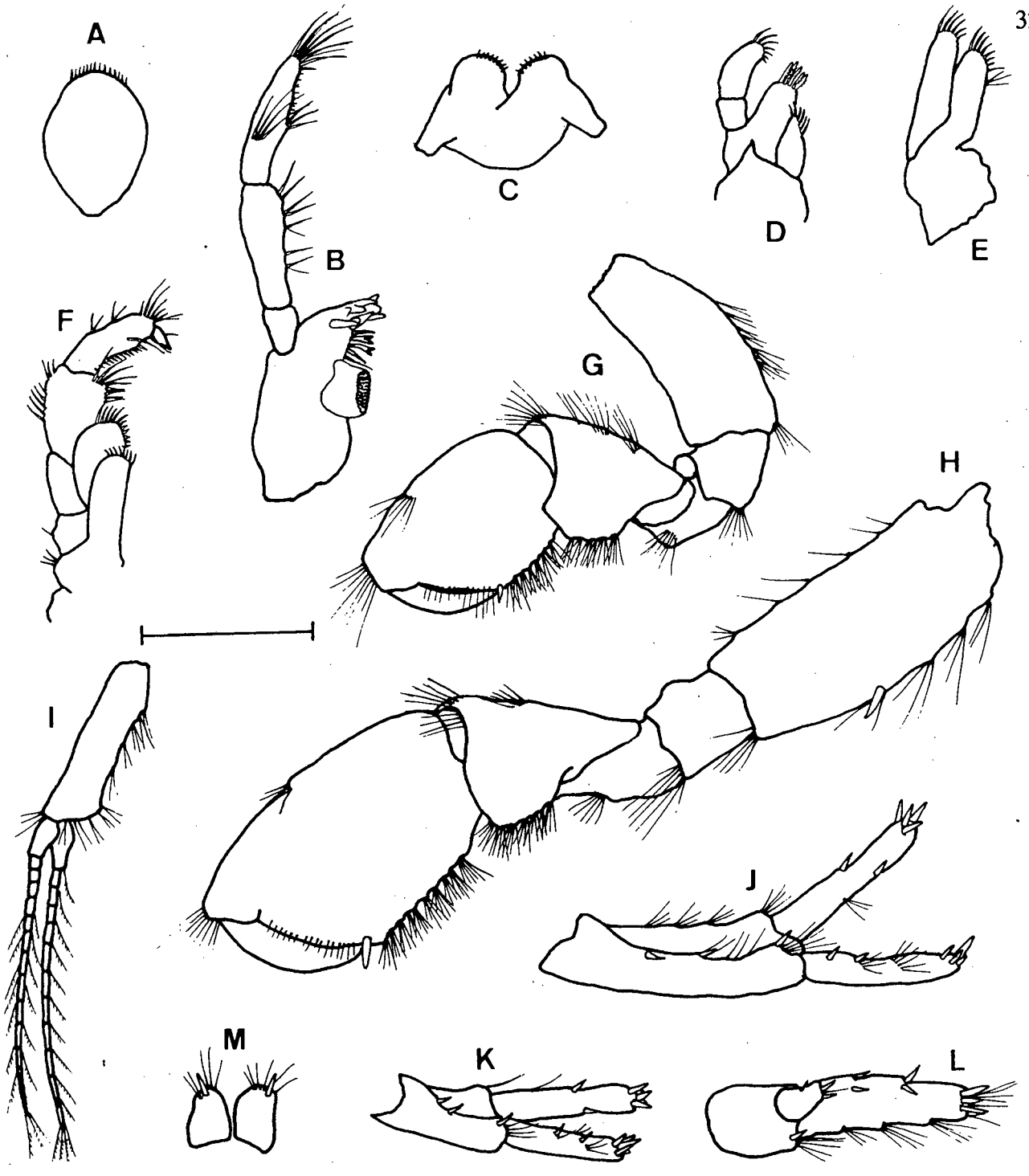

Fig. 6. *Paramelita andronyx* sp. nov., male, 16,1 mm. A Upper lip. B. Left mandible. C. Lower lip. D. Maxilla 1. E. Maxilla 2. F. Maxilliped. G. Gnathopod 1. H. Gnathopod 2. I. Pleopod 3. J. Uropod 1. K. Uropod 2. L. Uropod 3. M. Telson. Scale lines represent 1,0 mm.

Description (of holotype, male, 16.1 mm)

Body colour when alive whitish, tinged with pink. *Head* shorter than pereon segments 1 and 2 together, margin between eye lobe and post-antennal angle gently excavate to accommodate inflated article 1 of antenna 2, eyes glistening white when alive, invisible when preserved. *Antenna 1* relatively long, 0,6 length of body, sparsely setose, flagellum 1,5 times length of peduncle, 29-articulate, accessory flagellum 3-articulate, reaching to article 3 of flagellum. *Antenna 2* a little stouter and 0,8 times length of antenna 1, peduncle moderately setose, article 3 bearing a semicircular lobe posteriorly, article 4 three times length of 3, laterally swollen, article 5 0,8 times length of 4, flagellum 1,2 times length of peduncle, 17-articulate, moderately setose. *Left mandible*, incisor bluntly 5-toothed, lacinia mobilis with four blunt teeth, two bifurcate, one simple and one pectinate accessory blade, molar strongly triturative, palp longer than body of mandible, article 1 longer than wide, article 2 2,6 times length of 1, with nine setae anteriorly, article 3 approximately the same length as 2, distal half lined with short setae, nine long apical setae present, two tufts of setae about half way along length, *Right mandible*, incisor 4-toothed, lacinia mobilis bifurcate, four accessory blades. *Maxilla 1*, inner plate setose terminally, outer plate bearing eight serrate spines, palp exceeding outer plate, with six apical spines and three apical setae. *Maxilla 2*, inner plate shorter than outer, proximally pubescent, both plates strongly setose terminally. *Maxilliped*, inner plate with three spines and five curved setae, outer plate with eight stout spines on inner margin and seven terminal curved setae, palp article 3 as long as article 2, both articles strongly setose medially.

Pereon segments dorsally smooth, coxae 1-3 deeper than corresponding segments, quadrate, setose ventrally, coxa 4 only very slightly concave, deeper than long, setose on ventral margin, coxae 5 and 6 longer than deep, bilobed, bearing setae and a few spinules ventrally, coxa 7 semicircular, bearing short stout setae ventrally, segments 2-7 bearing one pair of coxal gills each, segment 2 with one, 3, 4, 5 and 7 with two, and segment 6 with four sausage-shaped sternal gills . *Gnathopod 1* subchelate, articles 5

and 6 together longer than article 2, inner posterior margin of article 2 with five stout spines, article 6 1,4 times length of article 5, longer than wide, palm slightly convex, gently oblique, with four palmar spines, dactyl as long as palm. *Gnathopod 2* similar to, but larger than, 1, articles 5 and 6 together longer than article 2, inner posterior margin of article 2 bearing five pairs of stout spines, article 6 1,6 times the length of 5, longer than wide, palm convex, slightly oblique, defined by four stout spines, dactyl as long as palm. *Pereopod 3* highly modified and 1,3 times length of 4, inner posterior margin of article 2 bearing five pairs of stout spines, articles 4, 5 and 6 modified to form a claw-like structure, article 4 posterodistally strongly projected, moderately setose, article 5 short and stout, bearing short spine-like setae posteriorly, article 6 bent at right angles to article 5, bearing a few short stout setae posteriorly, forming a claw with projection of article 4, dactyl with a single spinule. *Pereopod 4* unmodified, articles 4, 5 and 6 moderately setose and bearing some spines, dactyl with a single spinule. *Pereopods 5, 6 and 7*, bases slightly expanded posteriorly, bearing spinules and setae anteriorly and setae posteriorly, articles 4 and 5 moderately setose and bearing some groups of spines, articles 6 with five or six clusters of spines anteriorly, dactyls always with only a single spinule.

Pleon segments with a few setae along postero-dorsal margins, first epimeral plate rounded-quadrate, 2 and 3 quadrate, setose ventrally. Pleon segments 4-6 more heavily setose dorsally. *Uropod 1* extending to tip of uropod 2, 1,5 length of uropod 3, rami subequal, 0,8 times length of peduncle, both rami with some setae and spines along lateral margins, each ramus terminating in four spines. *Uropod 2* shorter than 1, inner ramus slightly longer than outer, 1,2 times length of peduncle, both rami with setae and spines laterally, each ending in five terminal spines. *Uropod 3* relatively short, exceeding uropod 2 by 0,6 length of outer ramus, peduncle longer than broad, inner ramus short, 0,6 length of peduncle and 0,3 length of outer ramus, terminating in two spines and a single seta, outer ramus approximately twice the length of peduncle, two groups of spines and setae on inner and three on outer margin, second article absent.

Telson broader than long, deeply cleft, each lobe with one large subapical spine and four to five apical setae.

Remarks

In addition to their uniquely subchelate first pereopods, *P. andronyx* sp. nov. males from Kasteelsberg are easily identified by the large semicircular lobe on the posterior margin of article 3 of antenna 2. In adult females, antenna 2 is more slender and shorter than 1, article 3 is not lobed, and an unmodified pereopod 3 resembles pereopod 4 in structure. In other respects, females are similar to males. *P. andronyx* sp. nov. males share a lobed article 3 (along with a distinctly swollen article 4) of antenna 2, with both *P. flexa* and *P. auricularius*, which also possesses a modified pereopod 3. The claw on pereopod 3 in *P. auricularius* is, however, formed from articles 5 and 6 only, not article 4. *P. flexa* is clearly distinguished from *P. andronyx* sp. nov. both by the shape of antenna 2 and by its unmodified pereopod 3. In addition to the swelling of article 4 of the second antenna, other features, such as the possession of only a single spinule on each dactyl, the poorly excavate coxa 4 and the loss of a second segment on the outer ramus of the third uropod suggest that *P. andronyx* sp. nov. might have affinities with *P. crassicornis* Barnard, 1916 and *P. tulbaghensis* Barnard, 1927.

Paramelita platypus sp. nov.

Figs 7, 8

Material examined

Holotype. Male, 12,8 mm, SAM A40020, from Fisherman's Kloof, a tributary of the Fernkloof River flowing through the Fernkloof Nature Reserve (34°24'S, 19°14'E) near Hermanus, Cape Province. Collected in September 1989 by B.A. Stewart and P.A. Cook.

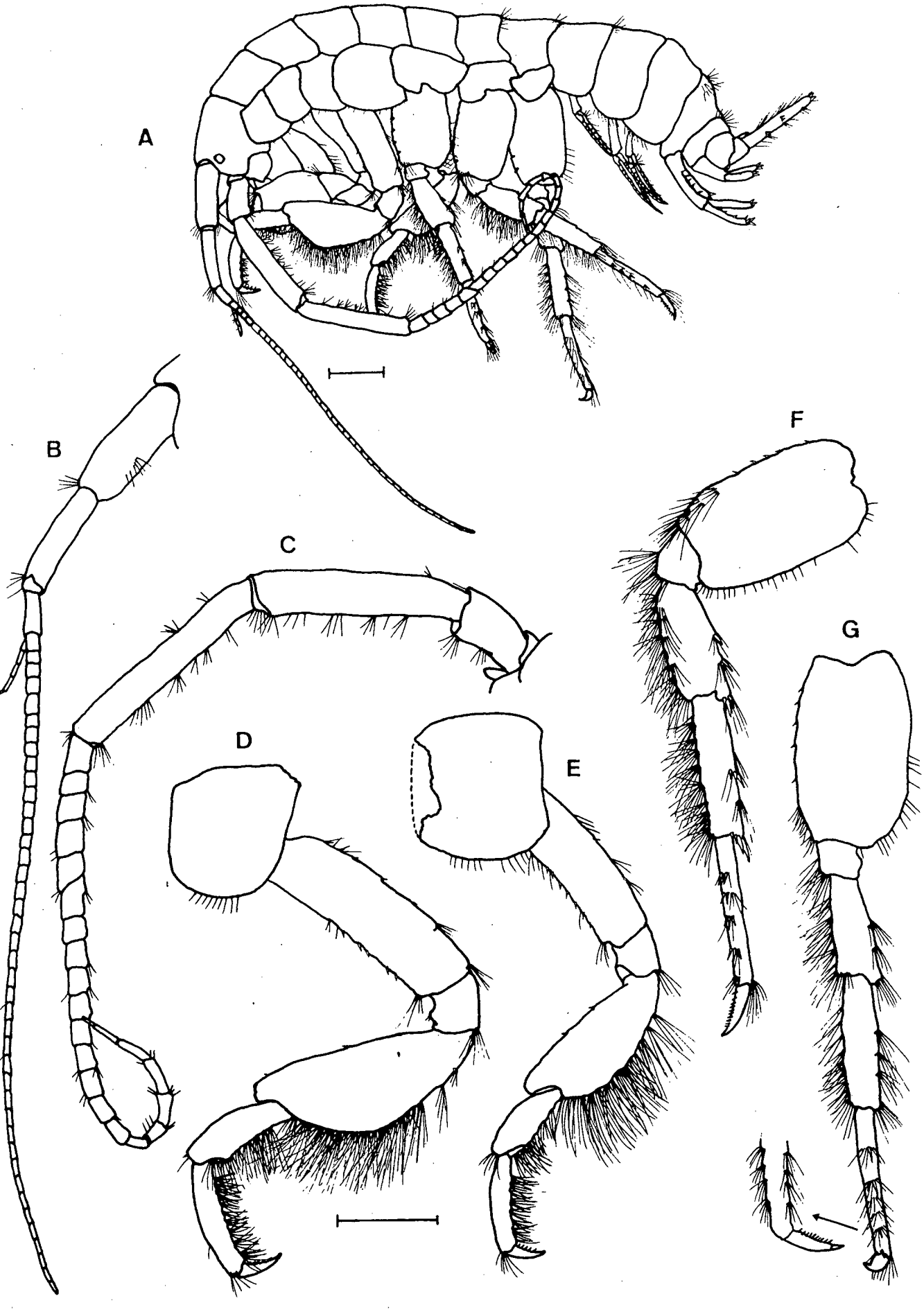

Fig. 7. *Paramelita platypus* sp. nov., male, 12,8 mm. A. Lateral aspect. B. Antenna 1. C. Antenna 2. D. Coxa 3 and pereopod 3. E. Coxa 4 and pereopod 4. F. Pereopod 6. G. Pereopod 7. Scale lines represent 1,0 mm.

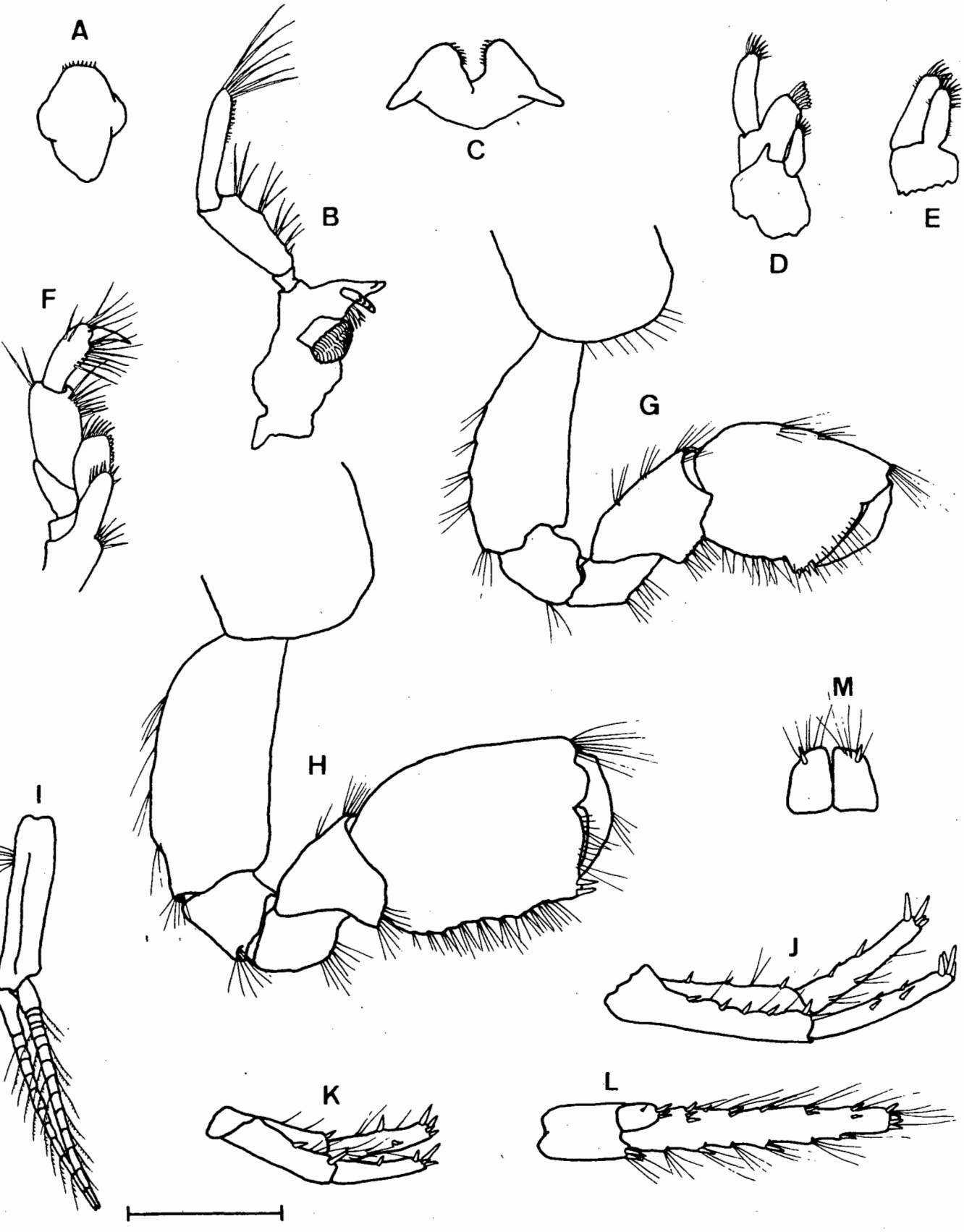

Fig. 8. *Paramelita platypus* sp. nov., male, 12,8 mm. A. Upper lip. B. Left mandible. C. Lower lip. D. Maxilla 1. E. Maxilla 2. F. Maxilliped. G. Gnathopod 1. H. Gnathopod 2. I. Pleopod 2. J. Uropod 1. K. Uropod 2. L. Uropod 3. M. Telson. Scale lines represent 1,0 mm.

Paratypes. Eight males, 12 females, SAM A40021, also from Fisherman's Kloof.

Other material. This species has also been collected from a stream near Stanford (SAM A40022).

Etymology

From the Greek *platùs* (broad) and *pous* (foot), alluding to the widened article 4 of pereopods 3 and 4.

Description (of holotype, male, 12,8 mm)

Body colour when alive orange to pink, eyes white when alive, invisible when preserved. *Head* considerably shorter than pereon segments 1 and 2 together, ventral margin excavate to accommodate inflated article 1 of antenna 2. *Antenna 1* relatively long, 0,7 times length of body, setation sparse, flagellum 2,2 length of peduncle, 41-articulate, accessory flagellum 5-articulate, reaching to article 5 of primary flagellum. *Antenna 2* approximately the same length as, but considerably stouter than, antenna 1, peduncle elongate, moderately setose, article 4 three times length of unmodified article 3, articles 4 and 5 equally long and relatively slender, lacking projections, flagellum 1,1 times length of enlarged peduncle, 22-articulate, sparsely setose. *Left mandible*, incisor with two blunt teeth, lacinia mobilis with four blunt teeth, three simple, and one bifurcate accessory blade, molar strongly triturative, palp longer than body of mandible, article 1 as long as wide, article 2 six times length of article 1, with approximately four groups of setae and one spine on anterior margin, article 3 1,3 times length of 2, distally lined with short setae and bearing six long apical setae, tuft of about four setae half way along length. *Right mandible*, incisor 3-toothed, lacinia mobilis bifurcate, three flattened spinose accessory blades. *Maxilla 1*, inner plate terminally setose, inner margin pubescent, outer plate terminating in about nine stout serrated spines, palp exceeding outer plate, with eight stout apical setae. *Maxilla 2*, inner plate shorter and narrower than outer plate, proximally pubescent, both plates strongly setose terminally. *Maxilliped*, inner plate with many curved spinose setae,

outer plate with about nine stout spine-teeth on inner margin and six terminal spinose setae, palp article 2 the longest, articles 2 and 3 densely setose medially.

Pereon segments with a few setae dorsally, coxae 1-3 slightly deeper than corresponding segments, quadrate, setose ventrally, coxa 4 virtually quadrate, only very slightly concave posteriorly, height and length subequal, setose ventrally, coxa 5 and 6 longer than deep, bilobed, setose ventrally, coxa 7 semicircular, setose ventrally, segments 2-7 bearing one pair of coxal gills each, segments 4, 5 and 7 with two, and segment 6 with four sternal gills. *Gnathopod 1* subchelate, articles 5 and 6 together longer than 2, article 6 1,6 times length of 5, longer than wide, palm slightly convex, palmar angle with two long and three short spines, dactyl as long as palm. *Gnathopod 2* similar in structure but larger than 1, articles 5 and 6 combined a little longer than 2, two pairs of short spines on inside of article 2, article 6 1,7 times length of 5, longer than wide, palm strongly convex, transverse, defining angle rectangular, bearing four strong spines, dactyl as long as palm. *Pereopod 3* enlarged, 1,2 times length of 4, article 2 with seven spinules on anterior, and eight spinules on posterior margin, article 4 greatly expanded laterally and lobed posteriorly, three spinules on anterior margin, articles 4, 5 and 6 densely setose posteriorly, dactyl with five spinules. *Pereopod 4* article 2 with nine anterior and five posterior marginal spinules, article 4 laterally expanded, although not quite as pronounced as in pereopod 3, with two small spinules on anterior margin, articles 4, 5 and 6 again densely setose posteriorly, dactyl bearing five spinules. *Pereopods 5, 6 and 7*, articles 2 moderately expanded posteriorly, with spinules and some setae anteriorly, setose posteriorly, articles 4 shorter than 5 and 6, bearing three groups of spines posteriorly, articles 5 and 6 subequal in length, articles 5 with three groups of spines and articles 6 with five groups of spines posteriorly, both 4 and 5 densely setose anteriorly, 6 moderately setose, dactyl of pereopod 5 with seven spinules, those of pereopods 6 and 7 with 10 spinules each.

Pleon segments 1-3 sparsely setose dorsally, epimeral plates rounded-quadrate, ventrally setose. Pleon segments 4-6 moderately setose dorsally. *Uropod 1* extending

slightly beyond 2,0,9 length of uropod 3, rami subequal, 0,8 times length of peduncle, each ending in four spines. *Uropod 2* shorter than 1, inner ramus slightly longer than outer, each with one dorsal and four apical spines. *Uropod 3* elongate, exceeding uropod 2 by 0,9 length of outer ramus, peduncle longer than broad, inner ramus short, 0.6 length of peduncle and only 0,2 length of outer ramus, with 3 apical spines, outer ramus about four times length of peduncle, six groups of spines and setae on each margin, second segment very reduced and only 4% of length of first segment. *Telson* broader than long, deeply cleft, each lobe bearing one stout subapical spine, four apical setae, two subapical setae, and two small plumose setae about one third the way along the outer margin.

Remarks

P. platypus sp. nov. males are unusual in two respects - the possession of extremely elongate and sturdy second antennae, and the wide, flattened articles 4 of pereopod 3, and to a lesser extent, pereopod 4. In females, antenna 2 is slender and shorter than antenna 1, articles 4 of pereopods 3 and 4 are not flattened, and the antennae and pereopods are only moderately setose. In other respects, the females resemble the males. In addition, all of the pereopods are markedly setose. Although several *Paramelita* species have elongated second antennae, none of the known species have males with the first two pereopods modified as in *P. platypus* sp. nov.. Coxa 4 in this species is only very slightly concave posteriorly, a condition found in several of the other *Paramelita* species such as *P. aurantius* Barnard, 1927, *P. granulicornis* Barnard, 1927, *P. crassicornis, P. auricularius* and *P. andronyx* sp. nov.

DISCUSSION

In his account of the 10 *Paramelita* species known to that time, Barnard (1927) commented on three "evolutionary tendencies" in the genus:- the thickening of the second antennae, modifications of pereopod 3, and variations in the shape of coxa 4.

All four species described here show unusual modifications of these features. Of the 12 previously known and the additional four species described here, at least 11 show some degree of enlargement, or 'pediformity', of the second antennae. This development is most marked in large adult males. The *Paramelita* species share this phenomenon with the Australian paramelitid genus *Uroctena* (Williams & Barnard, 1988). A 'claw-like' pereopod 3, found in three of the *Paramelita* species, has not been recorded in other paramelitids. This modification appears to have evolved more than once, and is probably a clasping organ used in reproduction. The shape of coxa 4 in *Paramelita* species varies from being strongly excavate posteriorly, such as in *P. capensis*, to being quadrate, as in the case in *P. granulicornis*. Although this coxal plate is deeply excavate in the most primitive Australian genus *Austrogammarus*, it is only 'weakly' emarginate in *Uroctena* (Williams & Barnard, 1988). The evolutionary trends within the *Paramelita* species, as well as the relationship of this genus with the Australian paramelitid genera will be the subject of a later study.

ACKNOWLEDGEMENTS

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PAPER 2

Morphological and genetic differentiation between allopatric populations of a freshwater amphipod

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(With 5 tables and 5 figures in the text)

Contents

Introduction

The genus *Paramelita* is a taxonomically isolated group of endemic freshwater amphipods found in the southwestern Cape, southern Africa. *Paramelita*, together with seven Australian genera, have been placed in the Family Paramelitidae (Williams & Barnard, 1988), members of which are considered to be 'plesiomorphic' crangonyctoids (Barnard & Barnard, 1983; Bousfield, 1983), since they have retained many primitive features of this amphipod suborder.

Although most *Paramelita* species have very 'narrow' distribution ranges, sometimes being known only from the type localities (Griffiths, 1981), two species, namely, *Paramelita capensis* Barnard and *Paramelita nigroculus* Barnard are reported to be widespread (Barnard, 1927; Griffiths, 1981). Thus, *P. capensis* has been described as being widely distributed from Clanwilliam, 200 km north, to Bredasdorp, 160 km east of Cape Town (Fig.1; Griffiths, 1981). This species lacks the modifications of antenna 2 and pereopod 3 found in many other paramelitid species, but is recognised by its relatively large size, unmodified antenna 2, oblique palm of gnathopod 2, deeply excavate coxal plate 4, and setose uropod 3 (Griffiths, 1981). *P. capensis* was first described in 1916 (Barnard, 1916), based on specimens collected from a stream draining Table Mountain (Fig. 1). Even within his original description, Barnard (1916) singled out three sets of specimens which did not correspond to his type specimens. These were specimens from Platteklip Stream (Fig. 1) with sharply pointed, as opposed to rounded antero-inferior angles on coxa 4; a form found at Buffels Bay (Fig. 1), distinguished by a stout antenna 2 and a generally densely setose pleon, and a smaller form dwelling on the Cape Flats (Fig. 1), distinguished by having the 6th joint of gnathopods 1 & 2 distinctly wider distally than proximally. Subsequently, Barnard (1927) described specimens identified as *P. capensis*, from the Cedarberg (Fig 1) as "smaller than the average size".

A more recent systematic sampling programme initiated in 1989 by the author confirmed Barnard's (1916, 1927) observations regarding the existence of distinct differences between populations which were identifiable, using the available key (Griffiths, 1981), as *P. capensis*. It was thus decided to quantify these morphological differences, to relate them to genetic variations and geographical patterns, and to identify evolutionary distinct lineages within the group which could subsequently result in the identification of new species.

The present study is the first to investigate genetic differentiation in a southern African amphipod species. Genetic variation was examined by investigating isozyme variation as determined by protein gel electrophoresis. Although the use of isozyme variation is not an exact reflection of the variation in the encoding DNA, it is a relative measure, and therefore a useful tool. In addition, Mendelian variation at individual loci can be detected. This is in contrast to the generally unknown genetic component of morphological variation (Grant, Dempster & Da Silva, 1988).

Electrophoresis of isozymes has been used to determine both the population genetics (Dickson *et al.*, 1979; Gooch & Hetrick, 1979; Bulnheim & Scholl, 1981a, 1982, 1986; Bulnheim, 1985; McDonald, 1985; Siegismund, 1985; Scheepmaker, 1987; Gooch, 1989) and the phylogenetic relationships (Bulnheim & Scholl, 1981b; Kolding & Simonsen, 1983; Siegismund, Simonsen & Kolding, 1985; Skadsheim & Siegismund, 1986) of some amphipod species. In addition, the technique has proved a useful taxonomic tool for distinguishing morphological similar amphipod species (e.g. Bulnheim & Scholl, 1980).

Materials and methods

Collection

Large samples (of at least 50 individuals each) were collected with handnets between April and June 1989 from the 17 localities shown in Fig. 1. All of the collection sites were small first-order mountain streams. Part of each sample was preserved in 70% alcohol, while the remaining animals were returned to the laboratory alive, where they were housed in small aquaria in a constant temperature chamber (10-14°C) until needed for electrophoresis. No live specimens could be collected from Grotto Stream, so that this population was included in the morphological, but not the electrophoretic analysis.

Morphological analyses

The five largest adult males in each preserved sample were selected for morphological analysis. Each animal was partially dissected using a stereo microscope, and 45 measurements taken by means of an eyepiece micrometer. In addition, the degree of setation and spination of the antennae, gnathopods, pereopods and uropods was noted. In preliminary analyses, performed with the aid of the programme NTSYS-pc (Rohlf, 1988), the data were treated in two different ways. Firstly, the data were log transformed, a correlation matrix between the variables was calculated, and this matrix was used in a Principal Components Analysis. The first three principal components calculated explained 95% of the total variance, with the first component accounting for 87% of this total. Populations were poorly grouped, and arranged along the first axis according to their sizes. The second component explained only 6%, and the third, only 2% of the variance. Secondly, the 45 'raw' measurements were standardised by subtracting the mean of each variable from each datum point and

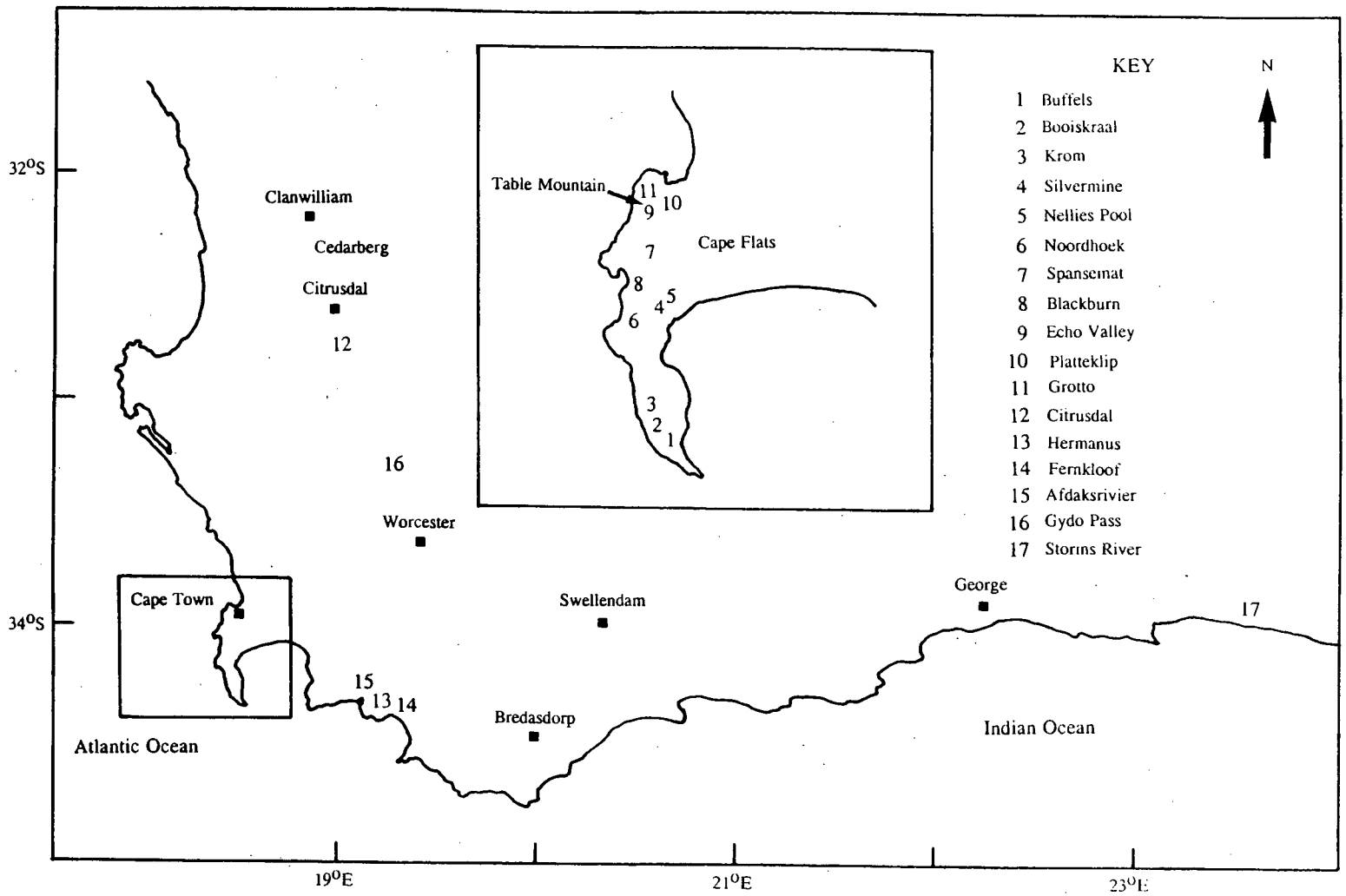

FIG. 1. Map of southwestern Cape, with Cape Peninsula inset, showing the 17 sampling sites.

dividing the difference by the standard deviation, and the resultant transformed data matrix used to compute Average Taxonomic Distance coefficients between all pairs of populations. Populations were subsequently clustered with the UPGMA clustering algorithm (Sneath & Sokal, 1973). It was evident, however, that the size, rather than the shape of the specimens played a significant role in the formation of these clusters. This was not surprising considering that the mean body length for all 17 populations ranged from 8.0 to 20.0 mm.

To correct for variable body size, the initial measurements were converted to bivariate ratios by dividing the length of each segment (of each limb) measured by its width (Table I). Despite some theoretical objections to using ratios (e.g. Atchley, Gaskin & Anderson, 1976), these dimensionless 'shape' variables do help to partially overcome the problems of size (Corruccini, 1977), and the use of ratios, particularly in taxonomic studies, is widely accepted (Dodson, 1978; Hills, 1978; Spivey, 1988). For example, ratios have been employed in morphometric analyses of population variation in several species of gastropods (e.g. Janson & Ward, 1985,), fish (Johnson, Ratkowsky & White, 1983), barnacles (Spivey, 1988) and muskrats (Pankakoski, Vaisanen & Nurmi, 1987). In the present study, ratios were only used after an equivalent operation with the raw data had been executed, as recommended by Dodson (1978). The ratios determined applied only to adults, and not juveniles, and were not statistically compared. None of the ratios were found to be linearly correlated with body length, and thus were all taxonomically useful (Abbott, Bisby & Rogers, 1985). These ratios were standardised, a matrix of dissimilarites was calculated, and the populations were clustered using the UPGMA cluster analysis algorithm.

In another approach, the morphological data were analysed by means of a stepwise discriminant functions analysis. The 45 measurements from each individual were log transformed, and this data matrix was analysed by means of the 7M programme in the BMDP Statistical Software package. Specimens for which there were missing data were excluded from the analysis. Discriminant analysis is a method for

TABLE I

Descriptions of ratios used in morphological analysis

Limb	Ratios
Antenna 1	Peduncle, article 1 length/width
	Peduncle, article 2 length/width
	Peduncle, article 3 length/width
Antenna 2	Peduncle, article 3 length/width
	Peduncle, article 4 length/width
	Peduncle, article 5 length/width
	Peduncle/flagellum length
Gnathopod 1	Article 5/6 length
	Article 6 length/width
Gnathopod 2	Article 5/6 length
	Article 6 length/width
Pereopod 3	Article 4 length/width
Pereopod 4	Article 4 length/width
Pereopod 5	Article 2 length/width
	Article 5 length/width
Pereopod 6	Article 2 length/width
	Article 5 length/width
Pereopod 7	Article 2 length/width
	Article 5 length/width
Uropod 3	Inner ramus/outer ramus length
	Peduncle/outer ramus length

testing preclassified groups. All of the specimens measured were thus grouped according to the clusters formed from the analysis of genetic data. Therefore, if the probability of correctly classifying individuals was sufficiently better than chance, we accepted the hypothesis that morphological differences existed between the genetic groups.

Electrophoretic analyses

The genetic variation at 10 allozyme loci, chosen without prior knowledge of their polymorphism, was examined by horizontal starch gel electrophoresis. Although as many as 25 enzymes were initially tested, only 10 isozyme loci were consistently scorable for all populations. Previous studies on amphipods have revealed that they do not electrophorese well (Dickson *et al.*, 1979; Gooch & Hetrick, 1979; Bulnheim & Scholl, 1981a), and, with the exception of Siegismund *et al.* (1985) who used 19 loci from 15 enzymes, Kolding & Simonsen (1983) who used 17 loci, and Skadsheim & Siegismund (1986) who examined 15 loci, very few loci have been used in earlier studies.

At least 20 individuals from each of 16 populations were analysed for each enzyme. Individuals from the Silvermine population were run on all gels as controls. Specimens were homogenised whole in 0.01 M Tris buffer, pH=8, and the homogenate centrifuged at 2500 g for 5 min. Filter paper (Schleicher & Schuell # 470 and Whatmans #3) wicks were dipped into the supernatant of the samples, and then inserted into a horizontal starch gel (13%). All gels were run at 4°C in a constant temperature chamber. The following buffer systems were used: (A) Tris-borate-EDTA buffer (Markert & Faulhaber, 1965), (B) N-(3- aminopropyl)-morpholine-citrate buffer (Clayton & Tretiak, 1972) and (C) Tris-citrate-lithium hydroxide-borate buffer (Ridgeway, Sherburne & Lewis, 1970). Gels were run for between 2.5 and 3.5 h at a constant current of 50 mA. Gels were divided into five slices, and the sites of

enzymatic activity stained using specific chemical reagents applied in agar overlays (Shaw & Prasad, 1970, Harris & Hopkinson, 1976). The enzymes stained for, and the buffer systems used are listed in Table II. When comparing the populations, the electrophoretic mobility of all electromorphs was measured relative to the most frequent electromorphs in the Silvermine population. The most common electromorph at each locus was designated the value of 100.

All numerical analyses were performed using the programme BIOSYS-1 (Swofford & Selander, 1981). Allele and genotype frequencies were computed. Genotype frequencies were tested to see whether they were in Hardy-Weinberg equilibrium. Average heterozygosity (H) per locus for each population was calculated using Nei's (1978) unbiased estimator. The percentage of polymorphic loci in each population was determined where a locus was considered polymorphic if the frequency of the most common allele did not exceed 0.95. Where alleles were shared by populations, contingency table analysis (Chi - squared) was used to test for significant allele frequency differences among populations. The average unbiased genetic identity (I) and distance (D) among the populations were calculated from the allele frequencies according to Nei (1978), and these were used to construct a dendrogram using the UPGMA clustering algorithm (Sneath & Sokal, 1973).

Results

Morphological analyses

Cluster analysis

The populations examined grouped into five distinct clusters, with the population from Gydo Pass (Fig. 1), forming a distinct group ('cluster 5') on its own (Fig. 2). Amphipods from this population were easily distinguished by the slender

TABLE II

Enzymes investigated and buffer systems used

Enzyme	Abbreviation	E.C. No.	Buffer
Arginine kinase	ARK	2.7.3.3	C
Aspartate amino-transferase	GOT	2.6.1.1	B
Diaphorase	DIA	1.6.2.2	C
Esterase	EST	3.1.1.1	A
Glucose-phosphate isomerase	GPI	5.3.1.9	C
Glyceraldehyde-phosphate dehydrogenase	GAP	1.2.1.12	B
Leucine amino-peptidase	LAP	3.4.11.-	A
Peptidase (glycyl-leucine as substrate)	GL	3.4.11.-	A
Peptidase (leucyl tyrosine as substrate)	LT	3.4.11.-	C
Phosphoglucomutase	PGM	2.7.5.1	A

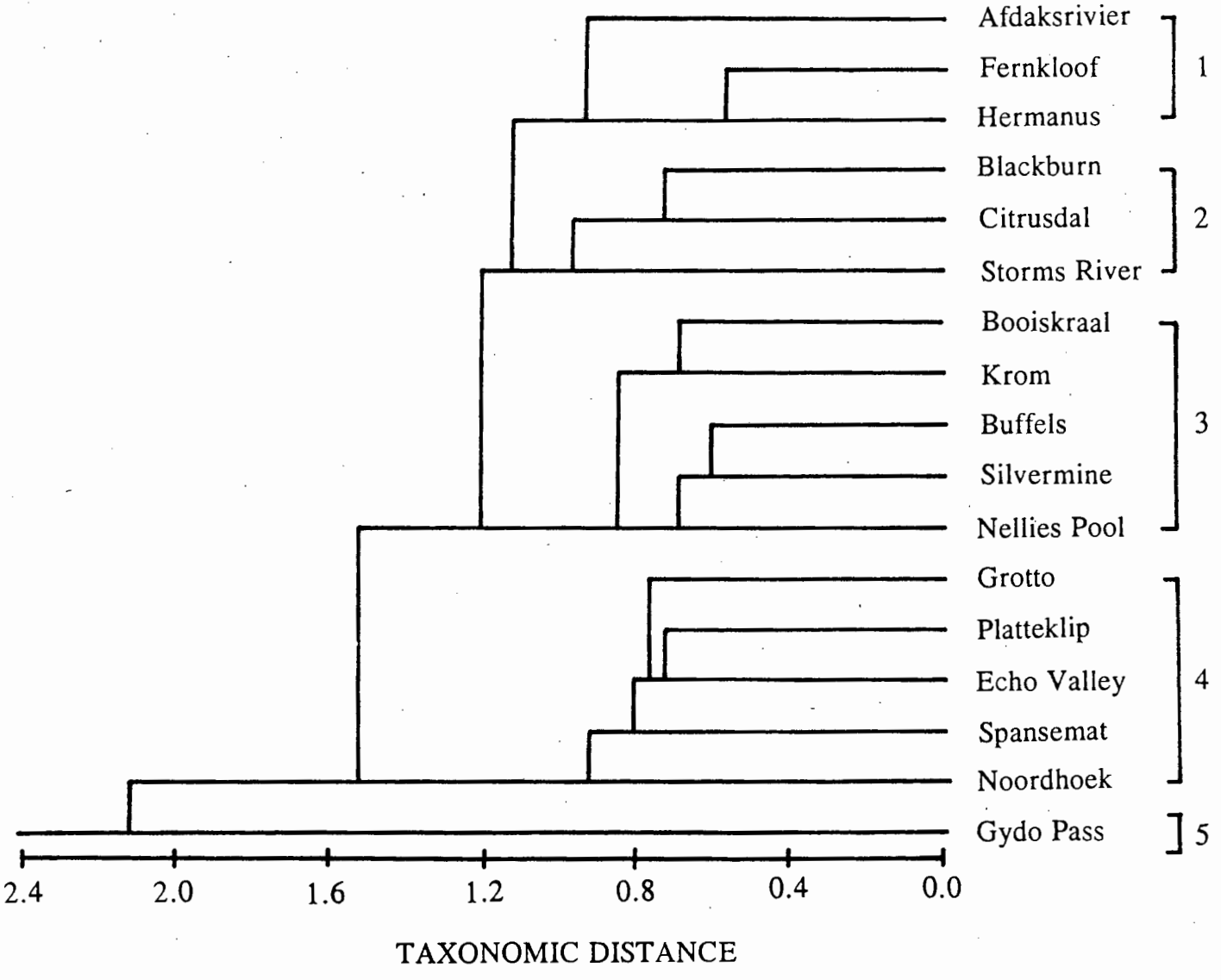

FIG. 2. Dendrogram generated using a matrix of dissimilarities (taxonomic distances) and the UPGMA cluster algorithm, based on morphological data.

nature of the peduncles of their first antennae (Fig. 3(a)) and the pereopods, particularly the second articles of pereopods 5-7 (Fig. 3e-g). They also possessed a rudimentary second segment on the outer ramus of the third uropod, and dense 'combs' of setae on the second antennae and pereopods. Additional features identifying specimens from Gydo Pass included flagella and peduncles of equal length in the second antennae (Fig. 3c) and relatively long fifth segments in the gnathopods.

All the populations collected from streams on the southern part of the Cape Peninsula (Fig. 1), with the exception of Noordhoek, formed a distinct group shown as 'cluster 3' on Fig. 2. Adults in these populations were large, darkly pigmented and setose, with the largest males having antenna 2 exceeding antenna 1 in length. This was due to the enlargement of the peduncle, rather than elongation of the flagella, a phenomenon shared with specimens from 'cluster 1' (Fig. 3c). Other attributes shared by specimens of cluster 3 included broad peduncular segments in antenna 1 (Fig. 3a), distinctly wide pereopods (Fig. 3e-g), relatively long and wide sixth segments of the gnathopods, with article 5 forming only 44- 52% of the length of article 6 in gnathopod 2, Fig. 3d), and a relatively short outer ramus in uropod 3 (Fig. 3h).

Populations from the northern Cape Peninsula similarly clustered together to form a distinct group identified as cluster 4. This group included some of the largest specimens encountered in the study, and was generally characterised by long, slender antennae (Fig. 3a&b), moderately wide second segments in pereopods 5-7 (Fig. 3f) but slender fifth articles in these limbs (Fig. 3g), and a relatively long outer ramus in uropod 3 (Fig 3h).

Specimens from Afdaksrivier, Fernkloof and nearby Hermanus on the southern Cape coast (Fig. 1) were all morphologically similar (Fig. 2). These 'cluster 1' populations were distinguished from 'cluster 2' populations (Citrusdal, Storms River and Blackburn), to which they were most closely linked, by the possession of stout third articles and generally enlarged peduncles in antenna 2 (Fig. 3b & c), a lower

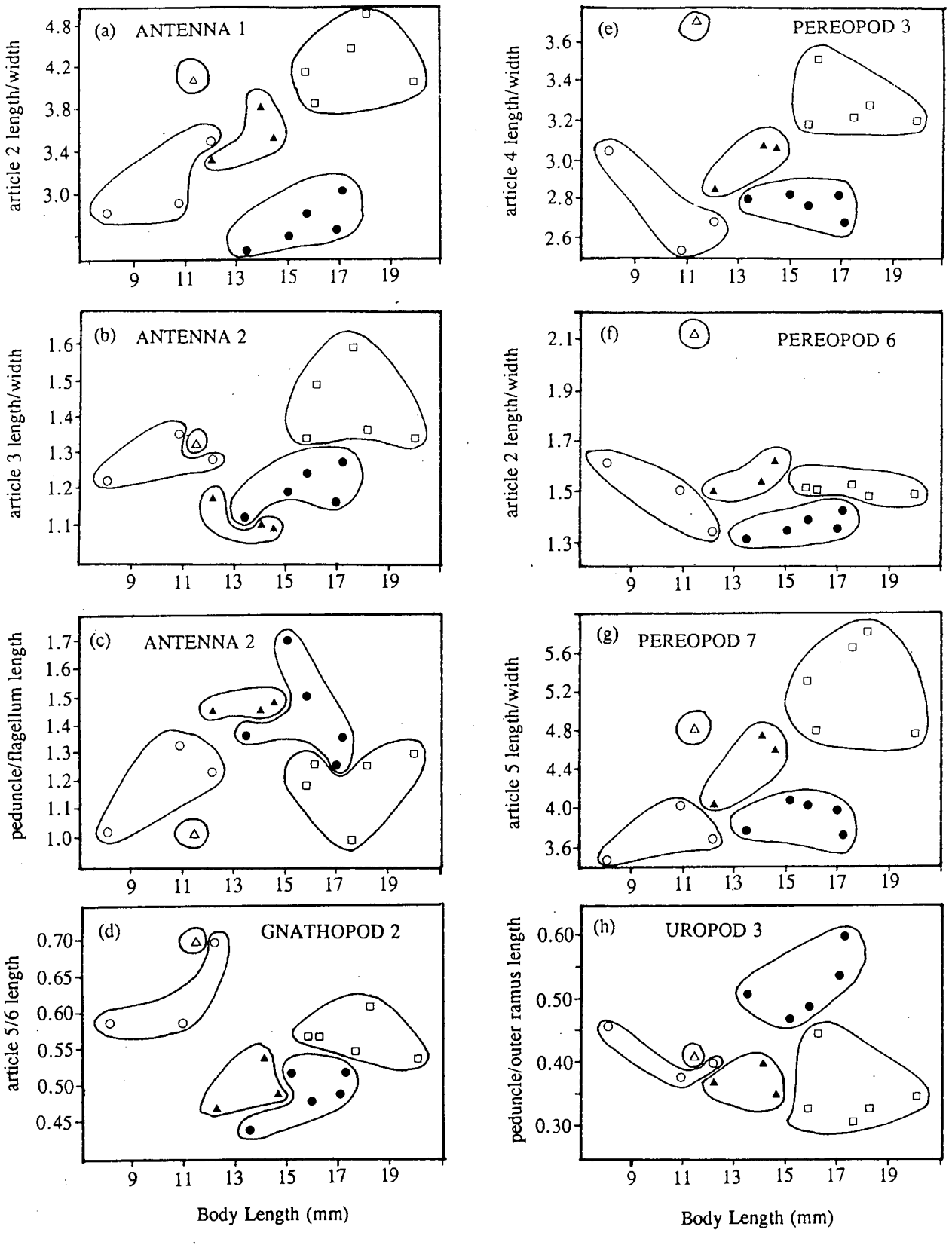

FIG. 3. Scatterplots of body length versus various morphological ratios for the 17 populations. Mean values are plotted. Solid triangles, cluster 1 populations; open circles, cluster 2 populations; solid circles, cluster 3 populations; squares, cluster 4 populations; open triangle, cluster 5 population. See Fig. 2 for cluster numbers.

article 5 to 6 ratio for gnathopod 2 (Fig. 3d), and moderately slender fifth articles in pereopods 5-7 (Fig. 3g).

Discriminant analysis

The discriminant functions analysis showed that each genetic 'cluster', or group (Fig. 5), was phenotypically distinct from the other clusters (Fig. 4). Canonical variables 1 and 2 together accounted for 85% of the total dispersion, and canonical variables 1, 2 and 3 together, 95%. With these functions, 86.4% of the specimens known to belong to cluster A, 95.2% known to belong to cluster B, and 100% known to come from clusters C, D and E were correctly classified. The probability that individuals would be correctly reassigned to their original cluster was an average of 1.000 for five of the six cluster A populations. For Noordhoek specimens, this probability was an average of 0.784. All specimens from the cluster B populations, Blackburn Ravine and Spansemat, had a probability of 1.000 of being correctly assigned, whilst those from Citrusdal, Platteklip Gorge,Grotto and Echo Valley had a mean probability of 0.962 of being correctly classified as belonging to cluster B. All individuals from Gydo Pass (cluster C) and Storms River (cluster E) were 100% certain of being reassigned to their original clusters. Specimens from Afdaksrivier, Fernkloof and Hermanus (cluster D) had a probability of 0.996 of being correctly reassigned.

The most important discriminating variables were the width of article 2 and the length of article 3 in antenna 1, the length and width of article 3, and the length of article 4 in antenna 2, the lengths of articles 5 & 6 in gnathopod 1, the length of the peduncle in uropod 2, and the lengths of the first and second articles of the outer rami of uropod 3.

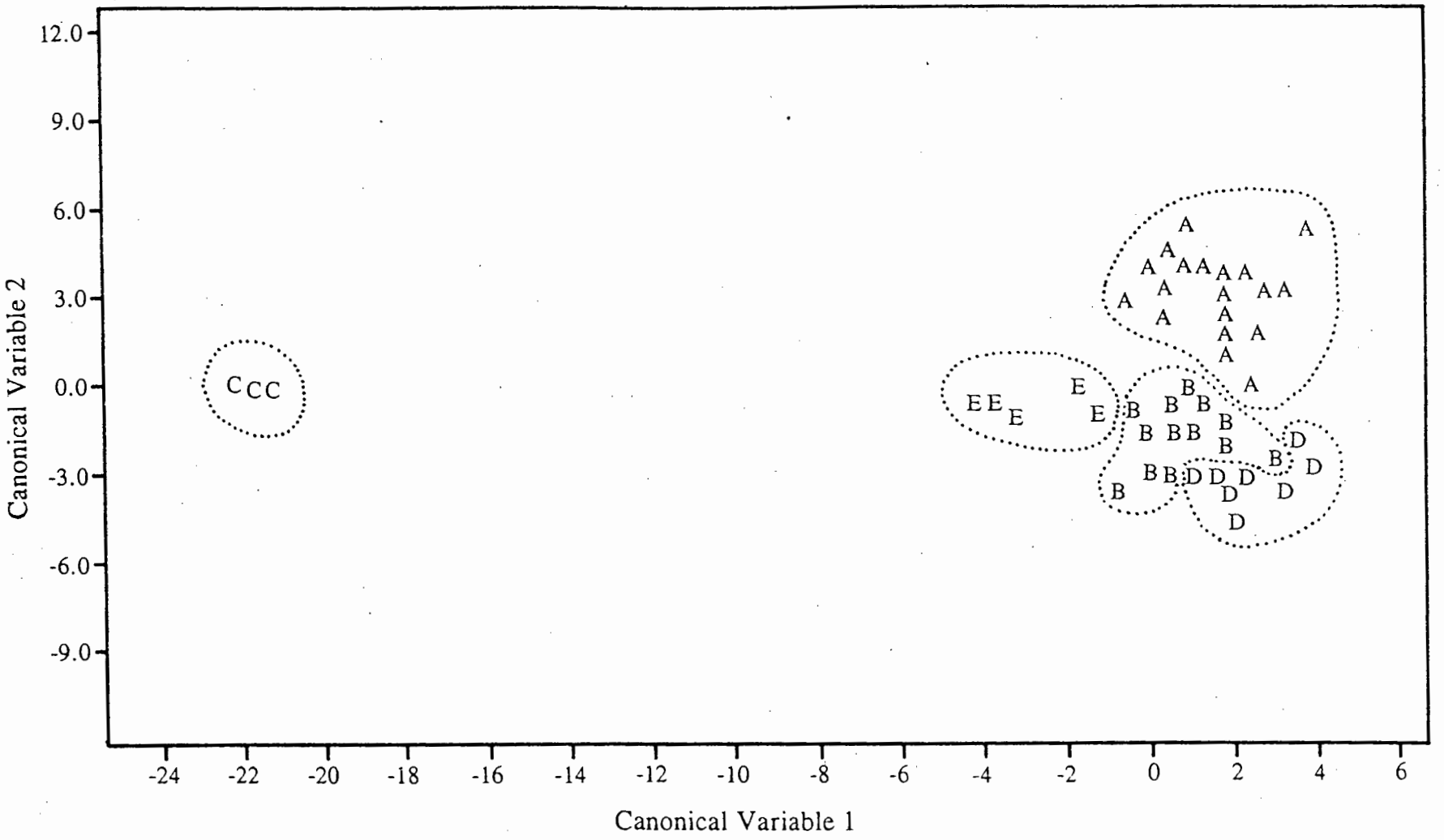

FIG. 4. Position of individuals from the 17 populations in the projection of the first two canonical variables generated from discriminant function analysis. Dotted lines enclose individuals within each group.

Electrophoretic analyses

Allele and Genotype frequencies

The allele frequencies for the 10 loci and 16 populations are given in Table III. There were no loci which were monomorphic for the same allele for all populations, and the number of alleles per locus ranged from three in DIA to 11 in GPI. Within any one population, the maximum number of alleles per locus was four for GPI in Noordhoek specimens and for PGM in Blackburn. Of the 69 alleles encountered for all loci and populations, 15 (22%) were recorded at frequencies below 0.01, and were considered 'rare'. Out of 56 cases of polymorphisms for all loci and populations, 12 (21%) were found to be out of Hardy-Weinberg (Chi-square, $p<0.05$). Genotype frequencies which were not in Hardy-Weinberg (due in all cases to a lack of heterozygotes) were for PGM in four populations, GL and DIA in two populations each, and EST, GPI, LAP and LT in one population each.

Cluster analysis and Diagnostic alleles

Cluster analysis (Fig. 5) of Nei's (1978) genetic identities between all pairs of populations (Table IV) revealed five main clusters, or groups. The mean identity within 'cluster A' which consisted of six populations, was 0.765+ 0.167. This group was joined to 'cluster B' at a level of 0.310. The five populations in 'cluster B' had a mean identity value of 0.639+0.152 between each other. The Gydo Pass population formed a group of its own (cluster C), and joined clusters A and B at a level of 0.179. The Hermanus and Fernkloof populations were genetically very similar (I=0.997), and linked to Afdaksrivier at a level of 0.460. These three populations (cluster D) were joined to the Storms River population (cluster E) at a level of 0.199.

TABLE III

Distribution of allele frequencies at 10 loci in 16 populations. N = sample size.

Locus	Silvermine	Noordhoek	Buffels	Booiskraal	Krom	Nellies Pool	Platteklip	Spansemat	Hermanus	Afdaksrivier	Gydo Pass	Blackburn	Citrusdal	Stormsriver	Fernkloof	Echo V
ARK																
N	121	45	45	28	29	32	63	43	20	17	18	22	20	20	15	15
75	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000
86	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	0.000	0.000	1.000	1.000	1.000	0.000	0.000	1.000
92	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	0.000	0.000	0.000	0.000	1.000	0.000
100	0.996	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
112	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
DIA																
N	78	25	40	23	29	31	31	38	20	15	18	12	20	15	15	15
87	0.000	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
100	1.000	0.980	1.000	1.000	1.000	1.000	0.097	0.092	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000
102	0.000	0.000	0.000	0.000	0.000	0.000	0.903	0.908	1.000	1.000	1.000	0.000	1.000	1.000	1.000	1.000
EST																
N	108	45	38	33	33	31	65	41	20	17	18	22	20	15	15	15
87	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.971	0.000	0.000	1.000	0.000	0.000	0.000
93	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.029	0.000	0.000	0.000	0.000	0.000	0.000
96	0.153	0.000	0.974	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
100	0.847	1.000	0.026	0.000	0.000	1.000	1.000	1.000	0.000	0.000	1.000	1.000	0.000	0.367	0.000	0.000
105	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.633	0.000	0.000
110	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000

Locus	Silvermine	Noordhoek	Buffels	Booiskraal	Krom	Nellies Pool	Platteklip	Spansemat	Hermanus	Afdaksrivier	Gydo Pass	Blackburn	Citrusdal	Stormsriver	Fernkloof	Echo V
GL																
N	92	20	38	33	33	31	42	41	20	17	18	22	20	19	15	15
45	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.026	0.000	0.000
61	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.132	0.000	0.000
69	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.842	0.000	0.000
87	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000
90	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.559	0.000	0.000	0.00	0.000	0.000	0.000
94	0.005	0.000	0.013	0.030	0.045	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
97	0.027	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
100	0.940	0.000	0.961	0.970	0.955	0.968	0.881	1.000	0.000	0.441	0.000	1.000	1.000	0.000	0.000	1.000
104	0.016	0.000	0.000	0.000	0.000	0.000	0.024	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
110	0.000	0.000	0.026	0.000	0.000	0.032	0.095	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
125	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000
GOT																
N	96	40	43	28	31	26	48	38	15	12	13	18	15	15	10	15
78	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
100	0.990	0.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
118	0.000	0.988	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000
123	0.000	0.013	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
125	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	0.000	0.000	0.033	0.000	1.000	0.000
130	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.028	0.967	0.000	0.000	0.000
140	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000
145	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	0.000	0.000	0.000	0.972	0.000	0.000	0.000	1.000
GPI																
N	110	45	40	25	26	29	60	40	15	12	15	20	15	18	15	15
38	0.000	0.000	0.000	0.000	0.000	0.000	0.092	0.000	0.000	0.000	0.000	0.100	0.000	1.000	0.000	1.000
55	0.000	0.000	0.000	0.000	0.000	0.000	0.908	0.000	0.000	1.000	0.000	0.900	0.867	0.000	0.000	0.000
63	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.000	0.000
75	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.967	0.000	0.000	0.000	0.000	0.000	1.000	0.000
76	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.133	0.000	0.000	0.000
80	0.000	0.044	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
90	0.000	0.133	0.000	0.000	0.038	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
97	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000
100	0.141	0.800	1.000	1.000	0.962	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
104	0.859	0.022	0.000	0.000	0.000	0.966	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
109	0.000	0.000	0.000	0.000	0.000	0.034	0.000	0.000	0.000	0.000	0.000	0.000	0.00	0.000	0.000	0.000

Locus	Silvermine	Noordhoek	Buffels	Booiskraal	Krom	Nellies Pool	Platteklip	Spansemat	Hermanus	Afdaksrivier	Gydo Pass	Blackburn	Citrusdal	Stormsriver	Fernkloof	Echo V
LAP																
N	100	34	30	23	20	18	44	35	20	17	10	22	15	20	15	15
85	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000
90	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	0.000
95	0.000	0.838	0.000	0.000	0.175	0.000	0.864	0.943	0.000	0.000	0.000	0.000	1.000	0.000	0.000	1.000
100	1.000	0.162	1.000	1.000	0.825	1.000	0.125	0.057	1.000	0.000	0.000	0.000	0.000	1.000	1.000	0.000
107	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
GAP																
N	56	10	18	8	11	17	20	25	20	7	18	20	15	13	10	15
70	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	1.000	0.000	0.000	0.000
83	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.975	0.000	0.077	0.000	0.000
86	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.769	0.000	0.000
89	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.154	0.000	0.000
100	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
110	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	1.000	1.000
122	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000
PGM																
N	105	44	43	33	33	31	60	36	20	17	18	19	19	20	15	15
82	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.500	0.000	0.184	0.000	0.000	0.000	0.000
90	0.010	0.557	0.035	0.000	0.000	0.000	0.000	0.000	0.000	0.412	0.000	0.105	0.000	0.000	0.067	0.000
93	0.010	0.432	0.895	0.242	0.333	0.016	0.075	0.014	0.775	0.088	1.000	0.658	0.421	0.000	0.933	0.167
100	0.962	0.011	0.070	0.682	0.500	0.984	0.908	0.986	0.225	0.000	0.000	0.053	0.579	0.800	0.000	0.833
104	0.019	0.000	0.000	0.076	0.167	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.175	0.000	0.000
110	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.000
LT																
N	50	3	10	3	9	17	18	13	15	17	13	22	20	15	15	15
80	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.700	0.000	0.000
85	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.045	0.000	0.300	0.000	0.000
90	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.933	0.000	0.000	0.000	0.000	0.000	0.933	0.000
95	0.020	0.000	0.000	0.000	0.000	0.029	0.000	0.000	0.067	0.412	0.000	0.000	0.000	0.000	0.067	0.000
97	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.588	0.000	0.000	0.000	0.000	0.000	0.000
100	0.980	1.000	1.000	1.000	1.000	0.971	1.000	1.000	0.000	0.00	0.000	0.955	1.000	0.000	0.000	1.000
112	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000

TABLE IV

Matrix of Nei's (1978) coefficients of genetic identity (above diagonal) and genetic distance (below diagonal) between the 16 populations. Calculations are based on 10 loci.

POPULATION	Silvermine	Noordhoek	Buffels	Booiskraal	Krom	Nellies P	Platteklip	Spansemat	Hermanus	Afdaks	Gydo P	Blackburn	Citrusdal	Storms R	Fernkloof	Echo V
Silvermine	*****	.570	.768	.838	.822	.996	.411	.408	.131	.049	.089	.413	.269	.241	.107	.288
Noordhoek	.562	*****	.582	.554	.585	.555	.323	.315	.055	.031	.258	.366	.225	.064	.065	.208
Buffels	.265	.541	*****	.958	.970	.721	.238	.229	.179	.058	.094	.377	.252	.121	.189	.224
Booiskraal	.177	.591	.043	*****	.996	.788	.294	.292	.142	.051	.025	.336	.264	.178	.128	.269
Krom	.196	.536	.031	.004	*****	.773	.298	.296	.130	.052	.035	.350	.283	.148	.122	.280
Nellies Pool	.004	.588	.327	.238	.258	*****	.420	.417	.129	.049	.103	.420	.265	.242	.104	.283
Platteklip	.889	1.129	1.434	1.225	1.212	.868	*****	.901	.248	.370	.313	.644	.658	.259	.224	.802
Spansemat	.895	1.156	1.474	1.232	1.216	.875	.104	*****	.331	.261	.297	.541	.575	.240	.306	.791
Hermanus	2.031	2.906	1.721	1.953	2.038	2.051	1.394	1.107	*****	.461	.183	.056	.160	.250	.997	.242
Afdaksrivier	3.007	3.468	2.855	2.985	2.952	3.021	.996	1.342	.774	*****	.119	.291	.382	.122	.459	.273
Gydo Pass	2.418	1.356	2.368	3.692	3.344	2.275	1.160	1.215	1.700	2.128	*****	.279	.253	.152	.196	.220
Blackburn	.885	1.004	.975	1.089	1.049	.868	.441	.614	2.877	1.234	1.277	*****	.448	.070	.066	.445
Citrusdal	1.312	1.491	1.378	1.330	1.264	1.327	.419	.553	1.833	.961	1.376	.803	*****	.170	.151	.588
Storms River	1.422	2.753	2.116	1.724	1.912	1.417	1.350	1.428	1.388	2.105	1.883	2.663	1.773	*****	.226	.373
Fernkloof	2.239	2.734	1.664	2.058	2.105	2.261	1.497	1.183	.003	.779	1.630	2.718	1.892	1.489	*****	.222
Echo Valley	1.247	1.572	1.498	1.313	1.275	1.261	.221	.234	1.419	1.299	1.515	.810	.532	.987	1.507	*****

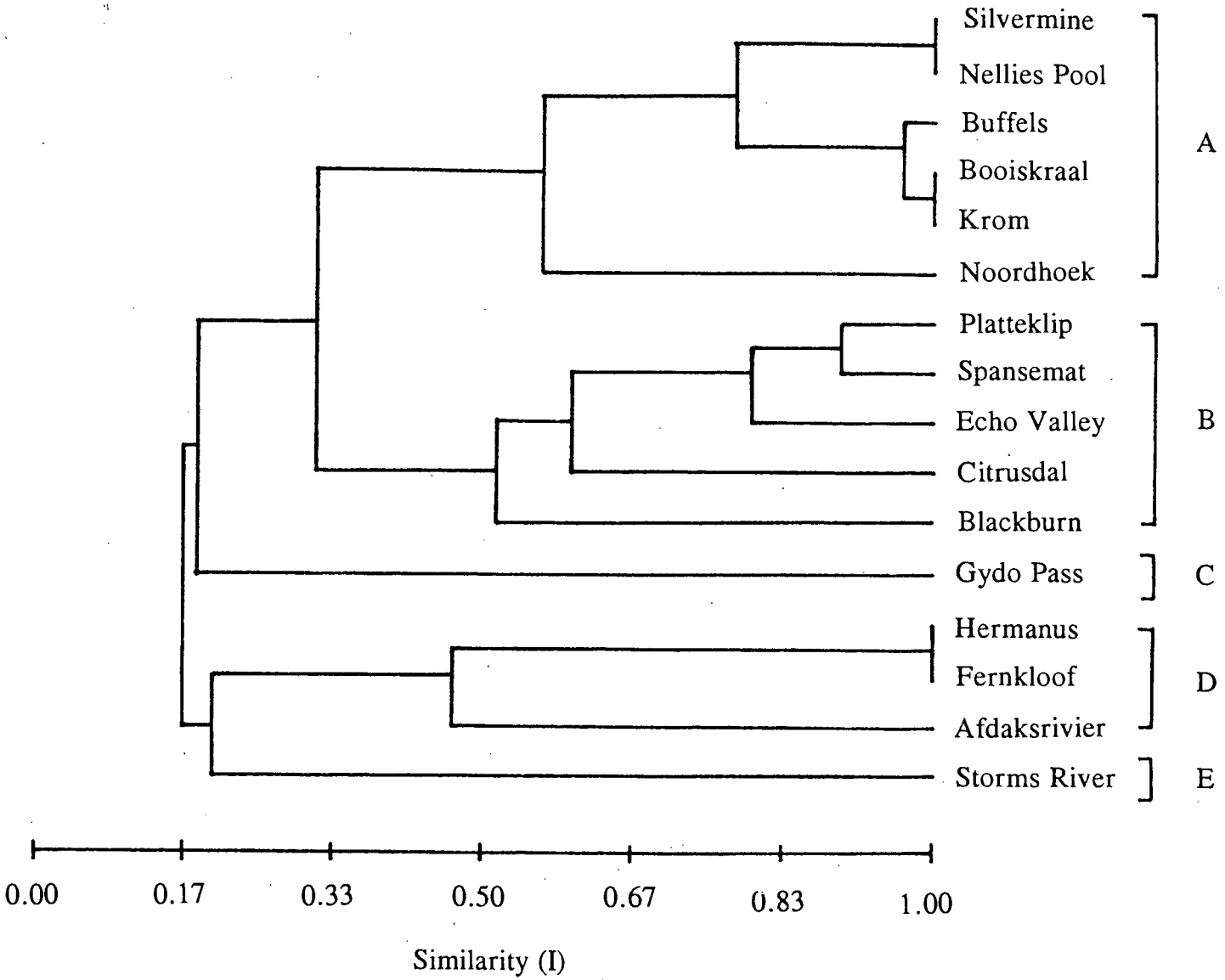

FIG. 5. Dendrogram generated using Nei's (1978) genetic identities and the UPGMA cluster algorithm, based on isozyme data.

All of the loci were highly differentiated among populations (Contingency Table Analysis, $p<0.05$), and several alleles, which could be regarded as 'cluster specific', were identified as playing a major role in the formation of the five main groups. The allele ARK^{100} was fixed for five of the cluster A populations, and in the sixth population, Silvermine, occurred at a very high frequency ($I=0.996$). Although not present in Noordhoek, GOT^{100} was fixed in Buffels, Booiskraal, Krom and Nellies, and ocurred at a frequency of 0.990 in Silvermine. The allele GAP^{100} was fixed for all of the six cluster A populations. Several other alleles, also specific to cluster A, e.g. GL^{94}, GPI^{90}, GPI^{100} and GPI^{104} occurred at low to high frequencies in at least two to five of the six populations.

Only one allele, GOT^{145}, could be regarded as 'diagnostic' of cluster B populations. Specimens from Echo Valley, Platteklip Gorge and Spansemat were fixed for this allele, whilst GOT^{145} was common (frequency of 0.972) in the Blackburn population. The allele GOT^{145} was absent in the Citrusdal population, where the 'population specific' allele, GOT^{130} was the most common allele, occuring at a frequency of 0.967.

Four alleles, GL^{125}, GPI^{97}, GAP^{122} and LT^{112} were fixed for specimens from Gydo Pass, making this a clearly distinct population. Although all three populations of cluster D were fixed for ARK^{92}, only Hermanus and Fernkloof specimens possessed EST^{110} and GL^{87}, which were fixed in both, and LT^{90}, which occurred at a frequency of 0.993 in both. LT^{97} (frequency of 0.588) was diagnostic of Afdaksrivier. The Storms River population was clearly defined by several diagnostic alleles - specimens were monomorphic for ARK^{75} and GOT^{140}, whilst GL^{45}, GL^{61}, GL^{69}, GAP^{86}, GAP^{89} and LT^{80}, occurring at various frequencies, were characteristic of this population.

Genetic variation within populations

Genetic variation within the populations (and clusters) was examined by calculating average heterozygosities (H), the mean number of alleles per locus, and the percentage loci which were polymorphic (Table V). For cluster A populations, H values varied from 0.022 (Nellies Pool) to 0.119 (Noordhoek), and the mean number of alleles varied from 1.3 (Booiskraal) to 2.2 (Silvermine), whilst between 0 (Nellies Pool) and 30% (Silvermine and Noordhoek) of loci were polymorphic. Average heterozygosities were relatively low (0.021 (Echo Valley) to 0.097 (Platteklip Gorge)) in cluster B populations, with the mean number of alleles ranging from 1.1 (Echo Valley) to 1.8 (Platteklip Gorge) and the percentage polymorphic loci from 10 to 50%. All loci were monomorphic in Gydo Pass specimens. In contrast, the highest average heterozygosity recorded was for Storms River, where H was 0.193, the mean number of alleles was 1.8, and 50% of the loci were polymorphic. Average heterozygosities at Hermanus (H=0.055) and at Fernkloof (H=0.026) were low relative to that at Afdaksrivier (H=0.166), although there were similar numbers of alleles per locus (1.2-1.5), and 20-30% of the loci were polymorphic for these cluster D populations. Average heterozygosities between the populations were not statistically compared because of the relatively small number of loci examined.

Discussion

On the whole, morphological differences between the 17 populations coincided with genetic differentiation, and this in turn, could be related to the geographical distribution of the populations. Both the dendrogram produced from the morphological data (Fig. 2), and that generated from the genetic data (Fig. 5) identified five groups, four of which showed a high degree of coincidence. These four groups were the populations from the southern Cape Peninsula, the northern Cape Peninsula, the

TABLE V

Average heterozygosities (H), the mean number of alleles per locus, and the percentage loci which were polymorphic in each of the 16 populations.

Cluster	Population	H (unbiased)	Mean no. alleles per locus	Percent polymorphic
A	Silvermine	0.076	2.2	30
	Noordhoek	0.119	1.8	30
	Buffels	0.032	1.5	10
	Booiskraal	0.054	1.3	10
	Krom	0.108	1.5	20
	Nellies Pool	0.022	1.4	0
B	Platteklip	0.970	1.8	50
	Spansemat	0.031	1.3	20
	Blackburn	0.091	1.7	20
	Citrusdal	0.081	1.3	20
	Echo Valley	0.029	1.1	10
C	Gydo Pass	0.000	0.0	0
D	Hermanus	0.055	1.3	20
	Afdaksrivier	0.166	1.5	30
	Fernkloof	0.026	1.2	20
E	Stormsriver	0.193	1.8	50

Hermanus region, and the single population from Gydo Pass, north of Ceres. Five of the six populations collected in the southern Cape Peninsula were both morphologically (Fig. 2) and genetically (Fig. 5) similar. Although specimens from the other southern Cape Peninsula site, Noordhoek shared some alleles with these populations, including 'diagnostic' alleles such as ARK^{100} and GAP^{100}, these animals were, in terms of 'shape', more similar to those collected from localities on the northern part of the Cape Peninsula. As a result, this population was included with the northern Peninsula, or Table Mountain populations in Fig. 2, and there was only a 78% chance of correctly assigning specimens (based on morphology) from this population to their 'genetic cluster'. Three populations from the northern Peninsula were morphologically (Fig. 2) and genetically similar (Fig. 5). Discriminant functions analysis showed that the Blackburn and Citrusdal populations had a good chance (96-100%) of being correctly classified as belonging to this genetic group (cluster B), despite the fact that these populations did not group with these northern Cape Peninsula populations in the dendrogram based on ratios. The relative geographical isolation of the Storms River population (Fig. 1), which was 440 km east of the other populations, was matched by its genetic isolation. These animals were the smallest encountered in the study, and although similar in shape to the specimens from Blackburn on the Cape Peninsula and Citrusdal from the Cedarberg area (Fig. 2), were easily assigned to a separate genetic group. The nearest populations geographically to the Storms River population, Afdaksrivier, Hermanus and Fernkloof, formed a distinct morphological and genetic group. Finally, the slender, setose animals from the isolated Gydo Pass population were easily distinguished both morphologically and genetically.

A high coincidence of morphological and genetic differentiation has not always been the case in other studies. Scheepmaker (1987) examined genetic variation in three morphological forms of the freshwater amphipod, *Gammarus stupendus*, in Europe, and found no correlation between morphological and genetic variation. They therefore supported Gooch and Hetrick's (1979) conclusions drawn from their study of

forms of *G. minus*, in North America, that the forms were independently evolved phenotypes, and not genetically 'distinct' species.

The question arises, are the genetic groups in the present study 'different enough' to be considered as congeneric species? Thorpe (1982) concluded from his analysis of the frequency of different values of Nei's (1978) I for different taxonomic levels for 2664 pairs of taxa that the critical level for I values distinguishing between species and genera is about 0.35. His analysis showed that about 85% of I values between congeneric species exceeded 0.35, while between conspecific populations, 98% of I values exceeded 0.85. Hedgecock, Tracey and Nelson (1982) reported a mean identity for congeneric crustacean species of 0.59 for 40 comparisons between crustacean species. In our study, five distinct clusters were linked below an I value of 0.35, suggesting that these clusters be considered, at the least, as separate species.

Unfortunately, in many studies on genetic divergence in amphipods, authors have based their estimations of I values on very few, usually polymorphic loci (Gooch & Hetrick, 1979; Bulnheim & Scholl, 1981a; Bulnheim, 1985; Bulnheim & Scholl, 1986; Scheepmaker, 1987; Gooch, 1989). This deliberate exclusion of monomorphic loci accentuates differences between populations and species, and makes these indices not directly comparable with the range of I values characteristic of species differentiation (Thorpe, 1982). Based on 17 loci, Kolding and Simonsen (1983) calculated mean genetic identities of 0.376 to 0.892 between five species of *Gammarus* collected from estuarine and marine localities in Europe. Working on the same species of *Gammarus*, and an additional *Chaetogammarus* species, Siegismund *et al.* (1985) calculated I values based on 19 loci between conspecific populations ranging from 0.966 to 1.000. The two "most closely related" species, *G. zaddachi* and *G. salinus* , were linked at an I value of approximately 0.8 (read from the dendrogram), and this pair was linked to *G. oceanicus* at a value of approx. 0.6. The I values between these three species and the remaining three were all below 0.35. Skadsheim & Siegismund (1986) examined 15 loci in six additional species to those of Siegismund *et al* (1985),

and calculated I values ranging from 0.85 between *G. zaddachi* and G. *salinus*, to as low as 0 between *G. pulex* and four of the other gammarid species. In a study on freshwater amphipods, Dickson *et al.* (1979) found that I values between populations of the cave-dwelling amphipod, *Crangonyx antennatus* fell between 0.858 and 0.968, a range "typical" of conspecific populations (Thorpe, 1982). Vainola and Varvio (1989) collected populations of three species of the "glacial relict" amphipod genus, *Pontoporeia* from North America and Europe, and calculated genetic identity estimates between the species in the range of 0.06 to 0.23. They found that the differentation among populations of the same species was only "slight" compared with the interspecific variation, and identified no diagnostic alleles. The genetic identities reported by these authors between the three species are extremely low, and fall into the range found among different genera of the family Gammaridae (e.g. Skadsheim and Siegismund, 1986). Thus it appears that the genetic identities obtained in our study between clusters A to E are similar to those obtained between other amphipod species.

The practical application of the "biological species concept" (Mayr, 1963) often meets with difficulties (McKitrick and Zink, 1988), and frequently requires arbitrary decisions on the specific status of disjunct allopatric populations, such as those in this study. This is because it is often difficult to tell whether allopatric forms would interbreed or not given the opportunity. The existence of diagnostic alleles in each of our five 'genetic clusters' suggests that they are reproductively isolated, since no gene flow exists between these groups (Bock, 1986). Bock (1986) has suggested that this lack of gene flow, and not necessarily lack of reproduction capacity between members of different species, is used for practical taxonomic work, and has offered a slightly different definition for the biological species concept, which emphasizes the genetic isolation of species. The lack of gene flow can probably be attributed to the fact that these amphipods are restricted to small tributaries in separate drainage basins, and, therefore, have little potential for dispersal. In addition, amphipods, like other

peracarids, brood their young, and have no migratory larval stage. Barnard & Barnard (1983) have suggested that these factors can lead to a high degree of endemism and speciation in freshwater amphipods.

The fact that in small populations, polymorphic loci are likely to drift to fixation, due both to the absence of gene flow and to drastic reductions in population size (Futuyuma, 1986), is clearly illustrated by the Gydo Pass specimens. All of the loci were monomorphic in these animals. The locality in question is a small first-order stream, where many amphipods die in the dry summer period when the surface water ceases to flow. In contrast, 50% of the loci in Storms River specimens were polymorphic, and an average heterozygosity of 0.193 was recorded. These animals can be found in several small perennial headwaters within a single, large catchment. In their study of heterozygosity in populations of *G. minus*, Gooch & Hetrick (1979) also reported high heterozygosity in an area where small surface streams were numerous. Average heterozygosities ranged from 0.022 to 0.166 in the other populations, and were similar to figures obtained in other studies of amphipods. For example, Siegismund *et al.* (1985) recorded average heterozygosities of 0.009 in *Chaetogammarus marinus* to 0.099 in *G. salinus*, Gooch & Hetrick (1979) found values ranging from 0.116 to 0.254 in three forms of *G. minus*, and Dickson *et al* (1979) calculated values of 0.058 to 0.161 for six populations of the freshwater amphipod *Crangonyx antennatus*.

The author wishes to thank Charles, Roberta, Melinda and Matthew Griffiths, Peter and Melinda Cook, Yvonne and Allan Dempster and John Allen for their assistance in the field. Yvonne Dempster offered valuable advice in the electrophoresis laboratory, Tim Crowe and Carlos Villacastin-Herrero assisted with data analysis, Kim Prochazka helped with the preparation of tables, and Charles Griffiths commented on the manuscript. Part of the funds which supported this project was awarded by the Inland Water Ecosystems section of the South African National Scientific Programme,

CSIR, and the remainder, by the FRD, Special Programme on S A Rivers, directed by Prof. B.R. Davies.

Summary

The morphological and isozyme variation in 17 populations of the freshwater amphipod genus *Paramelita* was investigated to see whether morphological differences were genetically based. On the whole, morphological and genetic differentiation coincided, and could be related to geographical distribution. Five distinct genetic groups, separated at Nei's (1978) genetic identity values below 0.35, and possessing diagnostic alleles, were identified. Discriminant functions and cluster analyses confirmed that these genetic clusters were phenotypically distinct. It was concluded that these groups were sufficiently different to warrant the recognition of five species, four of which were new, and as yet, undescribed.

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PAPER 3

Further new species within the freshwater amphipod genus *Paramelita* (Crangonyctoidea: Paramelitidae) from South Africa.

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(With 9 figures in the text)

Four new species within the crangonyctoid amphipod genus *Paramelita* are described from material collected from small streams in the south-western Cape, South Africa. Two of the species are large, with robust second antennae which exceed the first in length, especially in adult males. The third species is recognised by its slender pereopods and its densely setose second antennae, and the fourth by its small size and relatively short, slender, unmodified second antennae. All of the species have an unmodified pereopod 3, and lack teeth, spines, ridges or lobes on antenna 2, features common to many of the known *Paramelita* species. Morphological similarities between the four new species and 16 previously known species of *Paramelita* are discussed.

Contents

Introduction

When Griffiths (1981) revised the crangonyctoid genus *Paramelita* in 1981, there were 12 known species, two of which were described as widespread. In particular, *Paramelita capensis* (Barnard, 1916) was described as being "widely distributed from Clanwilliam in the north to Bredasdorp in the east" (Fig. 1). In the course of an extensive sampling programme of mountain streams in the south western Cape during 1989, a number of new species were discovered (Stewart & Griffiths, in press), and at the same time, it was noted that different 'forms' of '*P. capensis*' appeared to exist. Although these forms all shared features such as an excavate coxa 4, unmodified pereopod 3, and an oblique palm in gnathopod 2, and all lacked lobes, teeth or spines on antenna 2, it was clear that they differed in several other respects, such as setation and body proportions. This has resulted in an in-depth study of the morphological and genetic differentiation within the *P. capensis* group (Stewart, in prep), and consequently, the recognition of five distinctly different forms, four of which were recognised as new species, and whose descriptions form the subject of this paper. The fifth group corresponded to the original *P. capensis* described by Barnard (1916).

Materials and methods

Specimens were collected with handnets from small headwater streams at the localities shown in Fig. 1. Part of each sample was preserved in 70% alcohol, while the remaining amphipods were returned to the laboratory and held alive in a 12^{o}C constant temperature room until needed for electrophoresis. All drawings were made by means of a camera lucida attached either to a Wild dissecting or compound microscope. Measurements were made by means of an eyepiece micrometer. Specimens (always the holotype) were partially dissected to facilitate the illustration of the limbs and mouthparts.

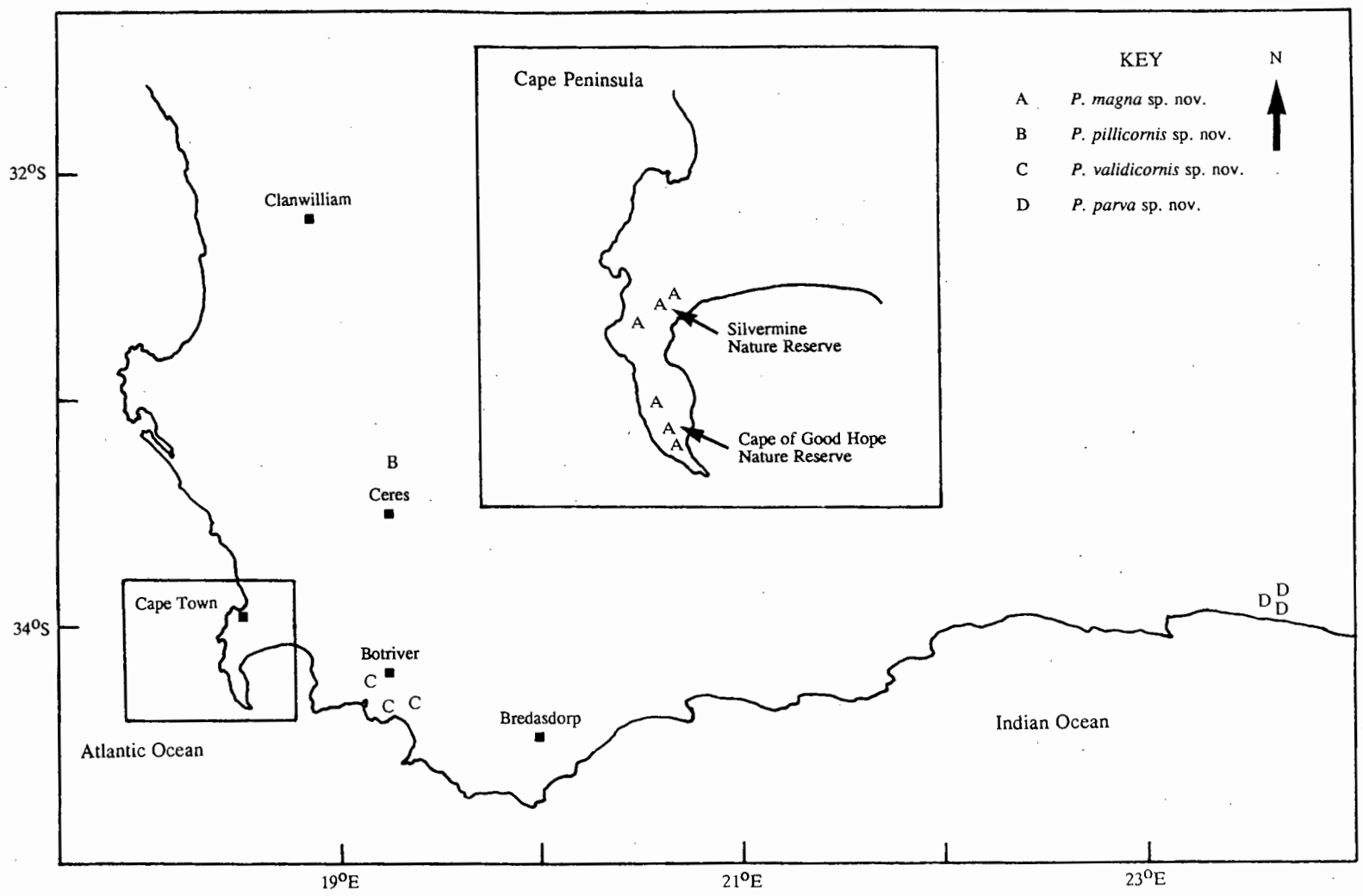

FIG. 1. Map of the southwestern Cape, with Cape Peninsula inset, showing the localities where *Paramelita magna* sp. nov., *P. pillicornis* sp. nov., *P. validicornis* sp.nov. and *P.parva* sp. nov. were collected.

Systematics

Superfamily CRANGONYCTOIDEA Bousfield, 1973

Family Paramelitidae Bousfield, 1977

Paramelita Schellenberg, 1926

Paramelita magna sp. nov.

(Figs 2, 3)

Material. (Holotype): SAM A40208; collected by B.A. Stewart and P.A. Cook from a tributary of the Krom River flowing through the Cape of Good Hope Nature Reserve (34°13'S, 18°26'E); 15 April 1989; male; 22.3 mm.

(Paratypes): SAM A40209; 11 males and 11 females from the same sample as the holotype.

(Other material): SAM A40210; collected by B.A. Stewart and P.A. Cook from the Booiskraal River in the Cape of Good Hope Nature Reserve; 15 April 1989. SAM A40211; collected by B.A. Stewart and P.A. Cook from the Buffels River in the Cape of Good Hope Nature Reserve; 15 April 1989. SAM A40212; collected by C.L. Griffiths from Nellies Pool in the Silvermine Nature Reserve; 29 October 1989. SAM A40213; collected by B.A. Stewart and P.A. Cook from a tributary of the Silvermine River in the Silvermine Nature Reserve; 15 April 1989. SAM A40214; collected by B.A. Stewart and Y. Dempster from a stream in Noordhoek valley; 28 April 1989.

Description. Body colour grey-brown when alive. Head shorter than pereon segments 1 and 2 combined, anteroventral margin excavate to accomodate inflated article 1 of antenna 2, eyes glistening white when alive, invisible when preserved.

Antenna 1 half length of body, sparsely setose, article 1 of peduncle 1.3 times length of 2, 2.6 times length of 3, flagellum 1.7 times length of peduncle, 38-articulate, accessory flagellum 8-articulate, reaching past article 5 of primary flagellum. Antenna 2 about 10% longer and considerably stouter than 1, peduncle

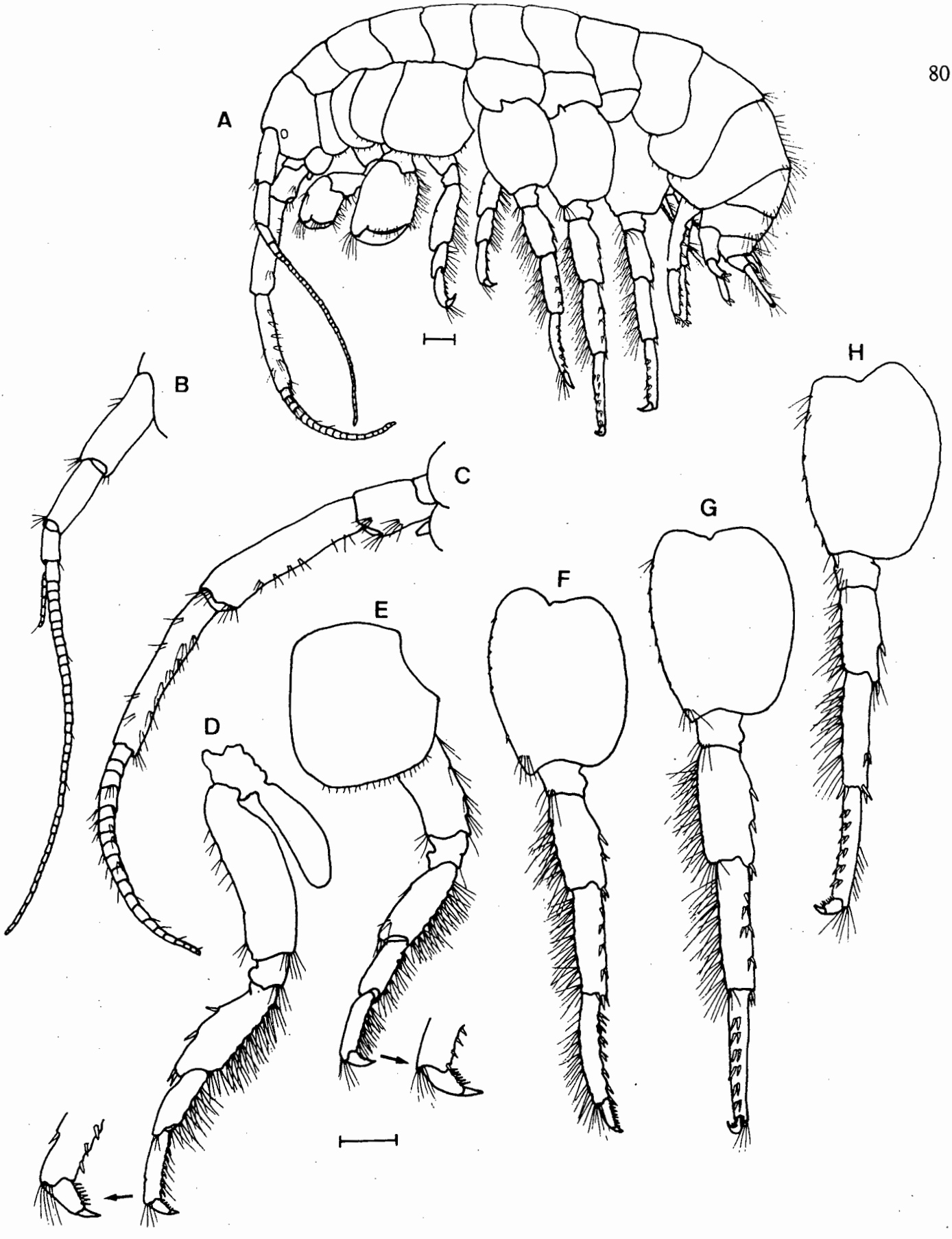

FIG. 2. *Paramelita magna* sp. nov., male, 22.3 mm. A. Lateral aspect. B. Antenna 1. C. Antenna 2. D. Pereopod 3. E. Pereopod 4 and coxa 4. F. Pereopod 5. G. Pereopod 6. H. Pereopod 7.

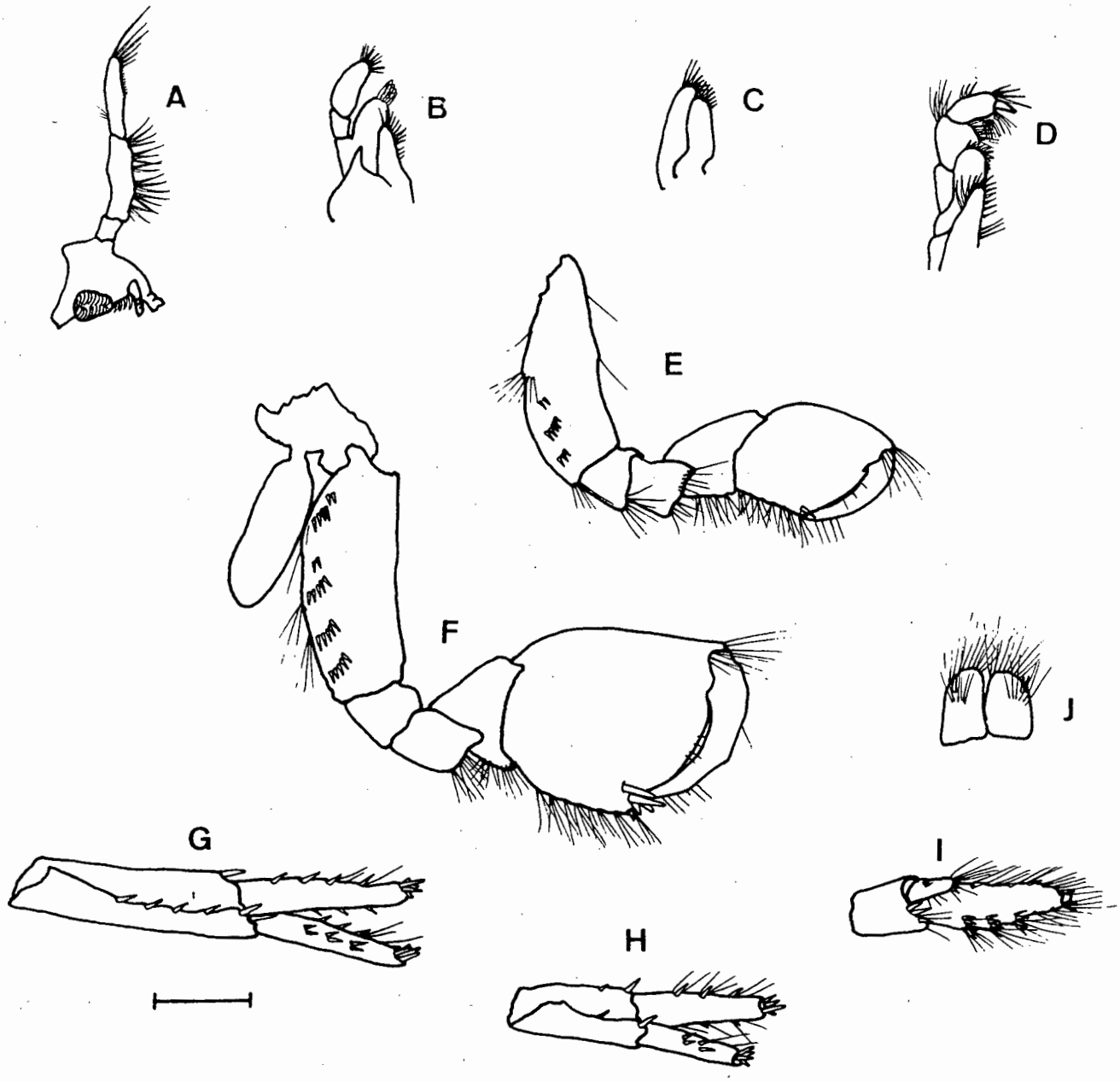

FIG. 3. *Paramelita magna* sp. nov., male, 22.3 mm. A. Left mandible. B. Maxilla 1. C. Maxilla 2. D. Maxilliped. E. Gnathopod 1, medial view. F. Gnathopod 2, medial view. G. Uropod 1. H. Uropod 2. I. Uropod 3. J. Telson.

moderately setose, articles 4 and 5 subequal, about 2.8 times length of article 3, flagellum 0.6 times length of enlarged peduncle, 20-articulate, moderately setose.

Left mandible with incisor bluntly 4-toothed, lacinia mobilis with four blunt teeth, four flattened spinose accessory blades, molar strongly triturative, 3-articulate palp longer than body of mandible, article 1 longer than wide, article 2 3.6 length of 1, with several setae anteriorly, article 3 equal to 2, distal half lined with short setae, several long apical setae present, tuft of setae approximately 0.3 times along length. Right mandible incisor 4-toothed, lacinia mobilis bifurcate, three accessory blades. Maxilla 1, inner plate densely setose on inner margin and apex, outer plate bearing two apical rows each of 5-6 stout serrate spines, palp bi-articulate, exceeding outer plate, with about 10 apical setae. Maxilla 2, inner plate shorter than outer, inner margin pubescent, both plates strongly setose terminally. Maxilliped, inner plate with many curved stout setae, outer plate with approximately 15 stout blunt spine-teeth on inner margin and about 10 terminal curved spinose setae, palp articles 2 and 3 densely setose.

Pereon segments dorsally smooth, coxae 1-3 approximately quadrate, setose ventrally, coxa 4 posteriorly excavate, slightly deeper than long, setose on ventral margin, coxa 5 and 6 longer than deep, bilobed, with some setae ventrally, coxa 7 semicircular, sparsely setose ventrally, segments 2-7 bearing one pair of coxal gills each, segments 2, 3, 4, 5 and 7 with two, and 6 with four sausage-shaped sternal gills.

Gnathopod 1 subchelate, article 2 with three rows of spines on medial surface, articles 5 and 6 together slightly longer than 2, article 6 1.2 times length of 5, longer than wide, palm convex, oblique, palmar angle with five spines, dactyl as long as palm. Gnathopod 2 similar in structure to, but 1.2 times length and sturdier than 1, medial margin of article 2 with six groups of flattened spines, articles 5 and 6 together longer than 2, article 6 2.2 times length of 5, widening distally, palm convex, gently oblique, defined by five strong spines, dactyl as long as palm. Pereopod 3 1.1 times length of 4, unmodified, articles 4, 5, and to a lesser extent 6, strongly setose posteriorly, article 4 with four groups of strong spines, article 6 with six groups of spines, dactyl with six spinules. Pereopod 4 similar in structure to 3, article 2 with four groups of small spines medially, articles 4, 5 and 6 strongly setose posteriorly,

article 6 with seven groups of spines, dactyl with six spinules. Pereopod 5, basis strongly expanded posteriorly, article 2 with small spinules on anterior margin, article 4 shorter than 5 and 6, article 6 slightly longer than 5, article 5 with two groups, and 6 with seven groups of spines, articles 4 and 5, and to a lesser extent, 6, densely setose anteriorly, dactyl with nine spinules. Pereopods 6 and 7 similar in structure to 5, dactyl of 6 with 10, and of 7 with nine spinules.

Pleon segment 1 sparsely, and 2 and 3 strongly setose dorsally, epimeral plates rounded to quadrate, ventrally sparsely setose. Pleon segments 4-6 strongly setose dorsally. Uropod 1 extending a little beyond 2, rami subequal, 0.8 times length of peduncle, inner ramus with eight and outer with three single and three pairs of marginal spines, each ending in five spines. Uropod 2 shorter than 1, inner ramus longer than outer, with six marginal spines, outer with two single and two pairs of marginal spines, each ramus ending in five spines. Uropod 3 relatively short, about 9% body length, exceeding 2 by about 0.5 length of outer ramus, peduncle longer than broad, inner ramus short, 0.8 length of peduncle and 0.3 times length of outer ramus, with two subapical and three apical spines and many apical setae, outer ramus 2.4 times length of peduncle, approximately five groups of spines on inner and six on outer margin, densely setose, second segment small, only 6% length of first. Telson broader than long, deeply cleft, right lobe bearing a single stout subapical spine, left lobe with two, both with several apical and dorsal setae.

Distribution. Southern Cape Peninsula, Cape Province, occurring in small streams draining hilly or mountainous areas (Fig. 1).

Etymology. The specific epithet is derived from the Latin *magnus*, meaning large, and is in reference to the exceptionally large size of the adults at maturity.

Females. Adult females, which reach a smaller size than males, are similar to males, except that the second antenna is shorter than the first, and is moderately slender.

Paramelita pillicornis sp. nov.

(Figs 4, 5)

Material. (Holotype): SAM A40214; collected by C.L. Griffiths from a tributary of Waboomsrivier on Gydo Pass (33°14'S, 19°20'E); 24 September 1989; male, 10.8 mm.

(Paratypes): SAM A40215; six males and two females collected by C.L. Griffiths and B.A. Stewart from the same locality as the holotype; 12 October 1990.

Description. Body colour orange-brown when alive. Head shorter than pereon segments 1 and 2 combined, anteroventral margin excavate to accommodate inflated article 1 of antenna 2, eyes glistening white when alive, difficult to discern in preserved material.

Antenna 1 0.7 times length of body, setation sparse, article 1 1.3 times length of 2, 3.2 times length of 3, flagellum 2.2 times length of peduncle, 33-articulate, accessory flagellum 4-articulate, reaching to end of article 3 of primary flagellum. Antenna 2 80% length of 1, stouter, peduncle densely setose posteriorly, article 4 2.4 times length of 3, article 5 1.1 times length of 4 and 2.7 times length of 3, flagellum stout, as long as peduncle, 16-articulate, densely setose posteriorly.

Incisor of left mandible with five blunt teeth, lacinia mobilis with four blunt teeth, five spinose accessory blades, molar strongly triturative, 3-articulate palp longer than body of mandible, article 1 as long as wide, article 2 4.0 times length of 1, with approximately 10 setae anteriorly, article 3 as long as 2, distal half lined with many short setae, several long apical setae present. Right mandible incisor 4-toothed, lacinia mobilis bifurcate, three accessory blades. Maxilla 1 inner plate with numerous apical setae, inner margin pubescent, outer plate with about 10 stout serrate spines, palp bi-articulate, exceeding outer plate, with eight apical stout setae. Maxilla 2, inner plate shorter than outer, proximally sparsely pubescent, both plates strongly setose terminally. Maxilliped, inner plate with many curved stout setae, outer plate with approximately 15 stout spine-teeth on inner margin and about 10 terminal curved setae, palp article 2 the longest, 2 and 3 densely setose on inner margin.

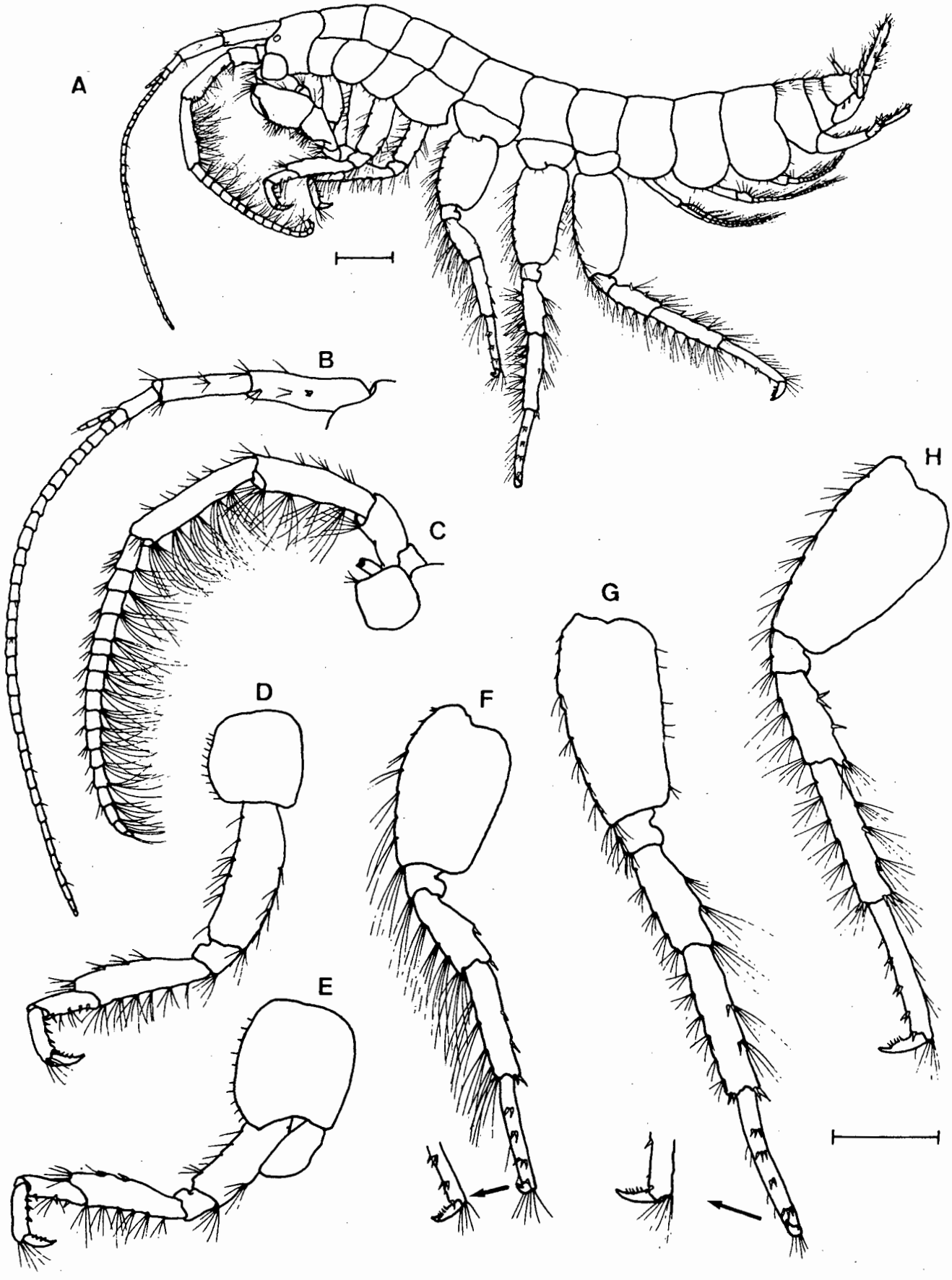

FIG. 4. *Paramelita pillicornis* sp. nov., male, 10.8 mm. A. Lateral aspect. B. Antenna 1. C. Antenna 2. D. Pereopod 3. E. Pereopod 4 and coxa 4. F. Pereopod 5. G. Pereopod 6. H. Pereopod 7.

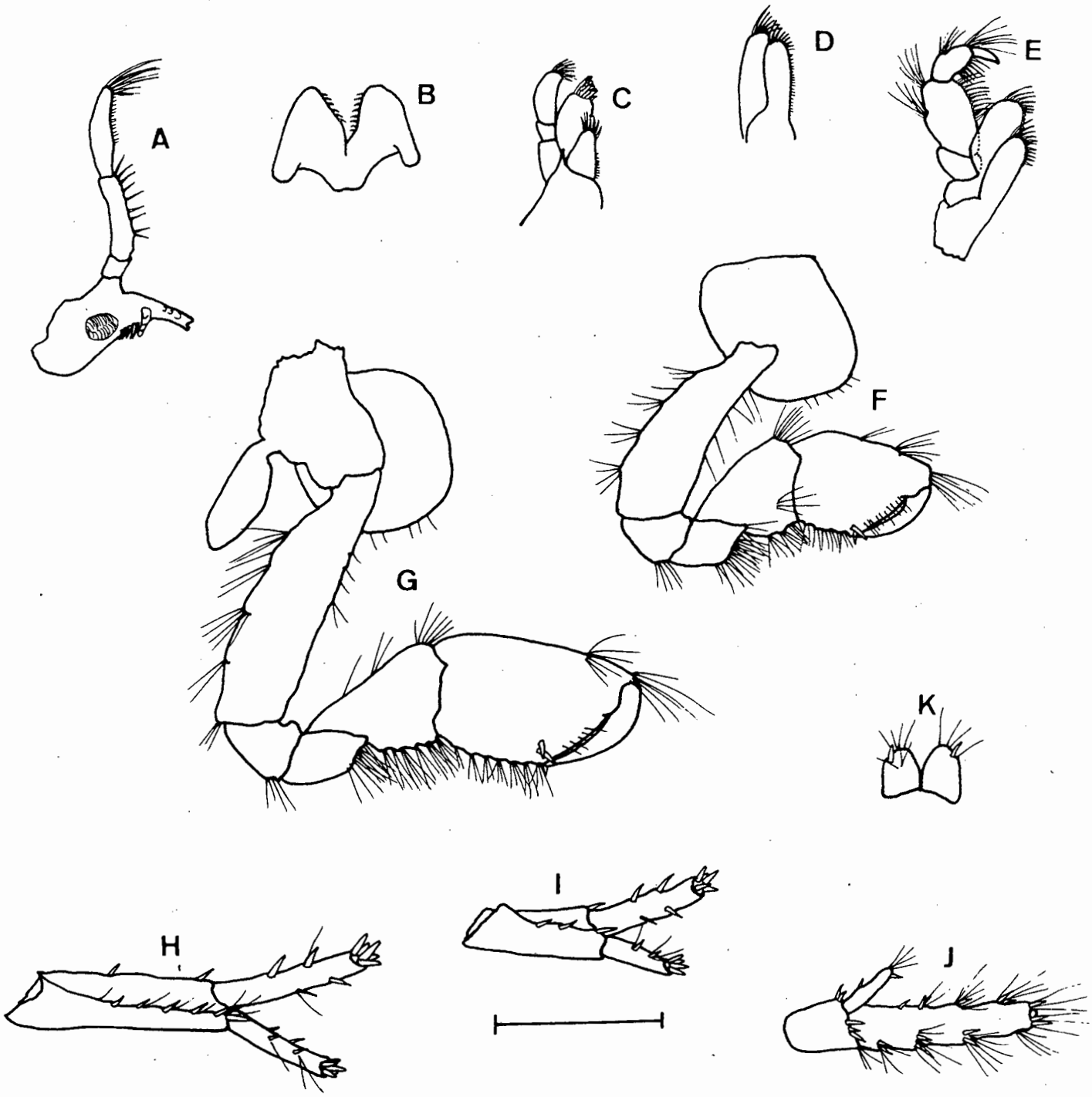

FIG. 5. *Paramelita pillicornis* sp. nov., male, 10.8 mm. A. Left mandible. B. Lower lip. C. Maxilla 1. D. Maxilla 2. E. Maxilliped. F. Gnathopod 1, medial view. G. Gnathopod 2, medial view. H. Uropod 1. I. Uropod 2. J. Uropod 3. K. Telson.

Pereon segments dorsally smooth, coxae 1-3 rounded-quadrate, sparsely setose ventrally, coxa 4 posteriorly excavate, as deep as long, setose on ventral margin, coxae 5 and 6 longer than deep, bilobed, with some setae ventrally, coxa 7 semicircular, sparsely setose ventrally, segments 2-7 bearing one pair of coxal gills each, segment 2 with one, 3, 4, 5 and 7 with two, and 6 with four sausage-shaped sternal gills.

Gnathopod 1 subchelate, articles 5 and 6 together longer than 2, article 6 1.2 times length of 5, longer than wide, palm slightly convex, oblique, with three spines at defining angle, dactyl as long as palm. Gnathopod 2 similar in structure to, but 1.3 times length and larger than 1, articles 5 and 6 combined longer than 2, article 6 1.3 times length of 5, longer than wide, palm convex, distinctly oblique, with three spines at defining angle, dactyl as long as palm. Pereopod 3 approximately the same length as 4, unmodified, moderately setose, articles 4, 5 and 6 with four groups of spines each, dactyl with three spinules. Pereopod 4 similar in structure to 3, moderately setose and spinose, dactyl with three spinules. Pereopod 5 basis moderately expanded, pereopods 6 and 7 with bases poorly expanded, all pereopods densely setose, articles 5 with four to five groups of spines, dactyl of pereopod 5 with four, and those of pereopods 6 and 7 with five spinules each.

Pleon segments 1-3 sparsely setose dorsally, epimeral plates rounded-quadrate, setose ventrally. Pleon segments 4-6 moderately setose dorsally. Uropod 1 extending slightly beyond 2, rami subequal, 0.8 times length of peduncle, each with four marginal spines and 2-3 setae, each ending in five spines. Uropod 2 shorter than 1, inner ramus longer than outer ramus, with four marginal spines, outer ramus with three spines and four setae, both ending in five spines. Uropod 3 moderately long, about 12% body length, exceeding 2 by about half of the length of the outer ramus in the undissected animal, peduncle longer than broad, inner ramus reduced, approximately the same length as peduncle and 0.4 times length of outer ramus, terminating in one spine and four setae, with a single spine half way along length, outer ramus 2.8 times length of peduncle, with four groups of setae and spines each on inner and outer margins, rudimentary second segment surrounded by terminal spines. Telson broader than long, deeply cleft, each lobe with one large subapical spine and four apical setae.

Distribution. This species is known only from the type locality on Gydo Pass, north of Ceres.

Etymology. The specific epithet is from the Latin *pillus*, meaning hair, and *cornus*, meaning horn, and is in reference to the dense setal brushes present on the posterior surface of antenna 2.

Females. With the exception of the second antenna, which is slender and markedly shorter than the first, females are similar to males.

Paramelita validicornis sp. nov.

(Figs 6, 7)

Material. (Holotype): SAM A40216; collected by B.A. Stewart and P.A. Cook from a small stream flowing into Kleinriviervlei in the grounds of the Hermanus Yacht Club (34°25'S, 19°22'E); 23 July 1989; male; 13.8 mm.

(Paratypes): SAM A40217; 10 males and three females from the same sample as that of the holotype.

(Other material): SAM A40218; collected by C.L. Griffiths from a tributary of the Afdaksrivier; 12 January 1989. SAM A40219; collected by B.A. Stewart and P.A. Cook from a stream in the Fernkloof Nature Reserve near Hermanus; 22 July 1990.

Description. Body colour when alive grey-white. Head as long as pereon segments 1 and 2 combined, anteroventral margin excavate to accommodate inflated article 1 of antenna 2, eyes white when alive, difficult to discern in preserved specimens.

Antenna 1 0.7 times length of body, sparsely setose, article 1 of peduncle 1.2 times length of 2, 3.4 times length of 3, flagellum 2.5 times length of peduncle, 45-articulate, accessory flagellum 6-articulate, reaching to end of article 4 of primary flagellum. Antenna 2 0.8 times length of, and considerably stouter than 1, peduncle sparsely setose, articles 4 and 5 considerably enlarged and expanded, article 4 1.1

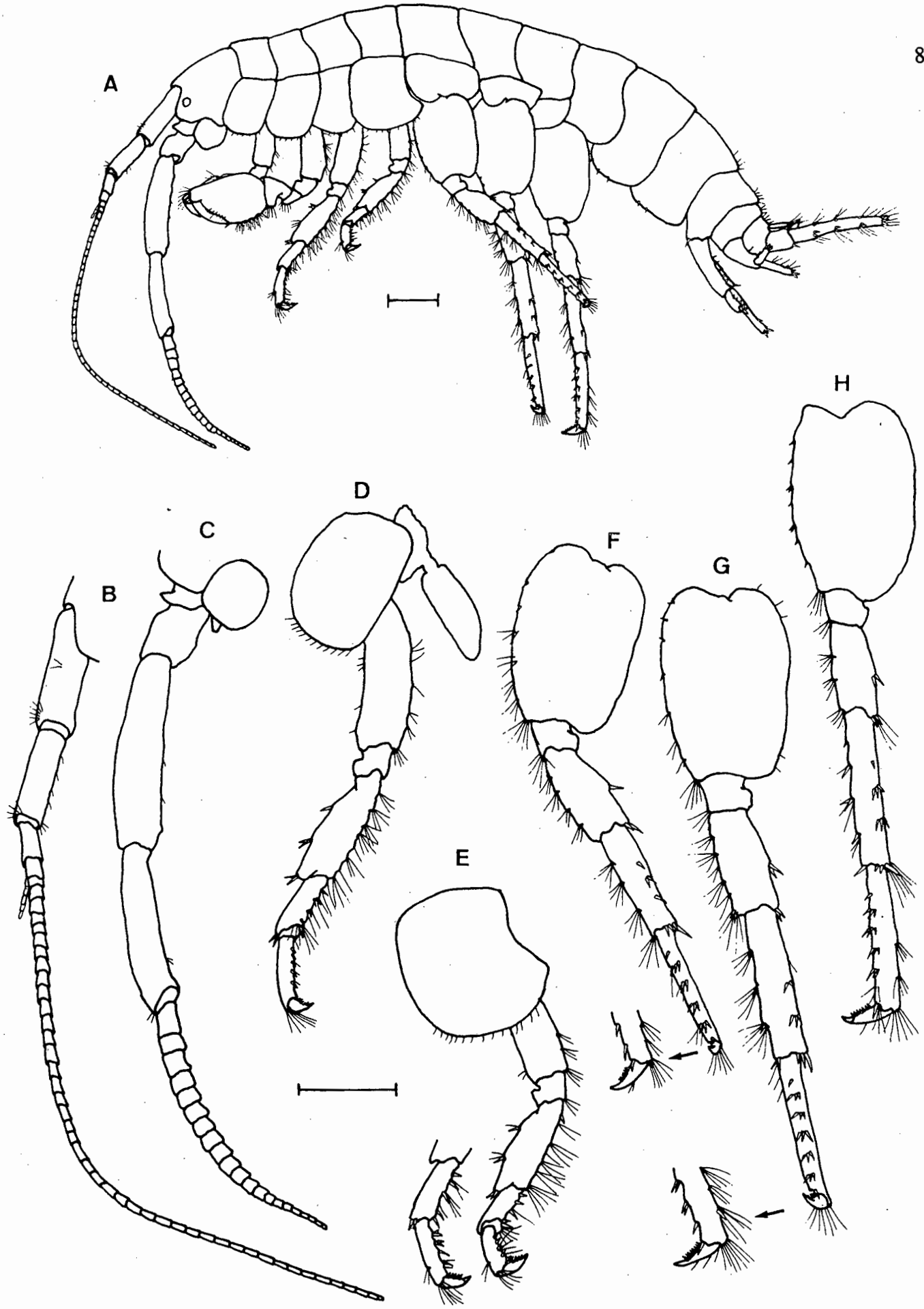

FIG 6. *Paramelita validicornis* sp. nov., male, 13.8 mm. A. Lateral aspect. B. Antenna 1. C. Antenna 2. D. Pereopod 3. E. Pereopod 4 and coxa 4. F. Pereopod 5. G. Pereopod 6. H. Pereopod 7.

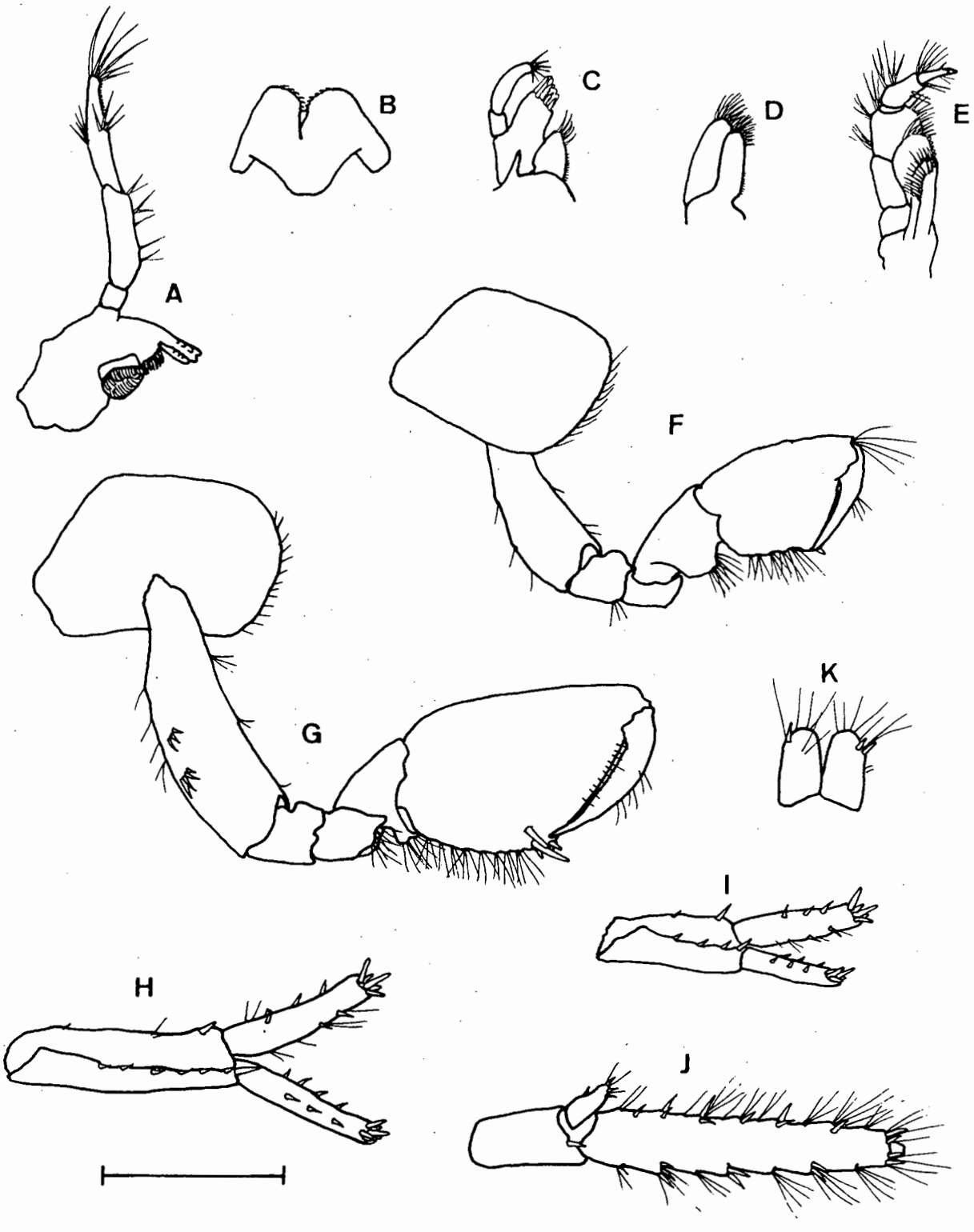

FIG. 7. *Paramelita validicornis* sp. nov., male, 13.8 mm. A. Left mandible. B. Lower lip. C. Maxilla 1. D. Maxilla 2. E. Maxilliped. F. Gnathopod 1, medial view. G. Gnathopod 2, medial view. H. Uropod 1. I. Uropod 2. J. Uropod 3. K. Telson.

length of 5 and 3.6 length of 3, flagellum 0.7 times length of enlarged peduncle, with 21 broad, flattened articles, sparsely setose.

Incisor of left mandible with five blunt teeth, lacinia mobilis with four blunt teeth, three simple and two bifurcate accessory blades, molar strongly triturative, 3-articulate palp longer than body of mandible, article 1 as long as wide, article 2 4.6 length of 1, with eight setae along anterior margin, article 3 slightly longer than 2, distal half pubescent, with six long apical setae, with two tufts of setae approximately half way along length. Right mandible incisor 3-toothed, lacinia mobilis bifurcate, three accessory blades. Maxilla 1, inner plate proximally pubescent, with five apical setae, outer plate bearing 10 stout serrate spines, palp bi-articulate, exceeding outer plate, with seven apical setae. Maxilla 2, inner plate shorter than outer, inner margin with many short setae, both plates strongly setose terminally. Maxilliped, inner plate with many setae on apex and distally on margin, outer plate with 11 blunt spine-teeth on inner margin and seven terminal curved spinose setae, palp articles 2 and 3 moderately setose, dactyl with three setae on inner margin.

Pereon segments dorsally smooth, coxae 1-3 quadrate, setose ventrally, coxa 4 posteriorly excavate, deeper than long, setose ventrally, coxa 5 and 6 longer than deep, bilobed, with a few spinules on ventral margin, coxa 7 semicircular, segments 2-7 bearing one pair of coxal gills each, segments 2 with one, 3, 4, 5 and 7 with two, and 6 with four sausage-shaped sternal gills.

Gnathopod 1 subchelate, articles 5 and 6 together longer than 2, article 6 1.5 times length of 5, longer than wide, palm slightly convex, distinctly oblique, with four spines at defining angle, dactyl as long as palm. Gnathopod 2 similar in structure to, but 1.2 times length and much sturdier than 1, article 2 with two groups of spines medially, articles 5 and 6 together longer than 2, article 6 2.3 times length of 5, longer than wide, palm convex, distinctly oblique, defined by five long spines, dactyl as long as palm. Pereopod 3 1.3 times length of 4, unmodified, moderately setose, article 6 with five groups of spines, dactyl with five spinules. Pereopod 4 similar in structure to 3, moderately setose, article 6 with four groups of spines, dactyl with five spinules. Pereopod 5, basis moderately expanded, article 2 with some spinules and setae

anteriorly, articles 4, 5 and 6 moderately setose and spinose, article 4 shorter than 5 and 6, article 6 with six groups of spines, dactyl with six spinules. Pereopods 6 and 7 similar in structure to 5, dactyl of 6 with eight, and of 7 with seven spinules.

Pleon segments 1 and 2 dorsally smooth, 3 sparsely setose dorsally, epimeral plates rounded to quadrate, with some spinules and a few setae ventrally. Pleon segments 4-6 moderately setose dorsally. Uropod 1 extending beyond 2, 0.8 times length of 3, rami subequal, 0.7 times length of peduncle, inner ramus with five marginal spines and eight setae, outer with seven marginal spines, lacking setae, both rami ending in five spines. Uropod 2 shorter than 1, inner ramus slightly longer than outer, with six marginal spines and eight setae, outer with six marginal spines, asetose, each ramus with five terminal spines. Uropod 3 relatively long, about 18% body length, exceeding 2 by most of the length of the outer ramus in the undissected animal, peduncle longer than broad, inner ramus short, 0.6 length of peduncle and 0.2 length of outer ramus, with one subapical and three apical spines and three apical setae, outer ramus 3.3 length of peduncle, approximately six groups of spines on inner and three on outer margin, moderately setose, second segment small, only 5% of length of first segment. Telson a little broader than long, deeply cleft, left lobe bearing two, and right a single stout subapical spine, both with a few subapical and apical setae.

Distribution. Known from as far west as Botrivier, to as far east as Bredasdorp, occurring in streams draining mountainous areas (Fig. 1).

Etymology. The specific epithet is derived from the Latin *validus*, meaning strong or powerful, and *cornus*, meaning horn, and alludes to the stout second antennae of the adult males.

Females. Females, which reach a smaller size than males at maturity, are similar to males, with the exception that antenna 2 is only moderately stout and always markedly shorter than antenna 1.

Paramelita parva sp. nov.

(Figs 8, 9)

Material. (Holotype): SAM A40226; collected by B.A. Stewart, P.A. Cook and J.C. Allen from a tributary of the Storms River (34°01'S, 23°55'E); 6 September 1989; male; 8.7 mm.

(Paratypes): SAM A40227; 11 males and 11 females from the same sample as that of the holotype.

(Other material): SAM A40228, SAM A40229, SAM A40230 and SAM A40231; all collected by B.A. Stewart, P.A. Cook and J.C. Allen from various tributaries of the Storms River; 6 September 1988.

Description. Body colour white when alive. Head shorter than pereon segments 1 and 2 combined, anteroventral margin excavate to accommodate inflated article 1 of antenna 2, eyes white when alive, invisible in preserved material.

Antenna 1 0.7 times length of body, sparsely setose, article 1 of peduncle 1.5 times length of 2, 2.3 times length of 3, flagellum 2.7 times length of peduncle, 36-articulate, accessory flagellum 5-articulate, reaching to middle of article 4 of primary flagellum. Antenna 2 0.6 times length of 1, relatively slender, sparsely to moderately setose, articles 4 and 5 subequal, not enlarged, twice length of 3, flagellum 1.1 times length of peduncle, 18-articulate.

Left mandible, incisor with three blunt teeth, lacinia mobilis with four blunt teeth, five spinose accessory blades, molar strongly triturative, 3-articulate palp longer than body of mandible, article 1 longer than wide, article 2 2.8 times length of 1, with about eight setae anteriorly, article 3 the same length as 2, distal half lined with many short setae, about 10 long apical setae present, tuft of setae approximately half way along length. Right mandible, incisor 3-toothed, lacinia mobilis bifurcate, three accessory blades. Maxilla 1, inner plate with five setae on apex, inner margin proximally pubescent, outer plate with 10 stout serrate spines, palp bi-articulate, exceeding outer plate, with six apical setae. Maxilla 2, inner plate shorter than outer

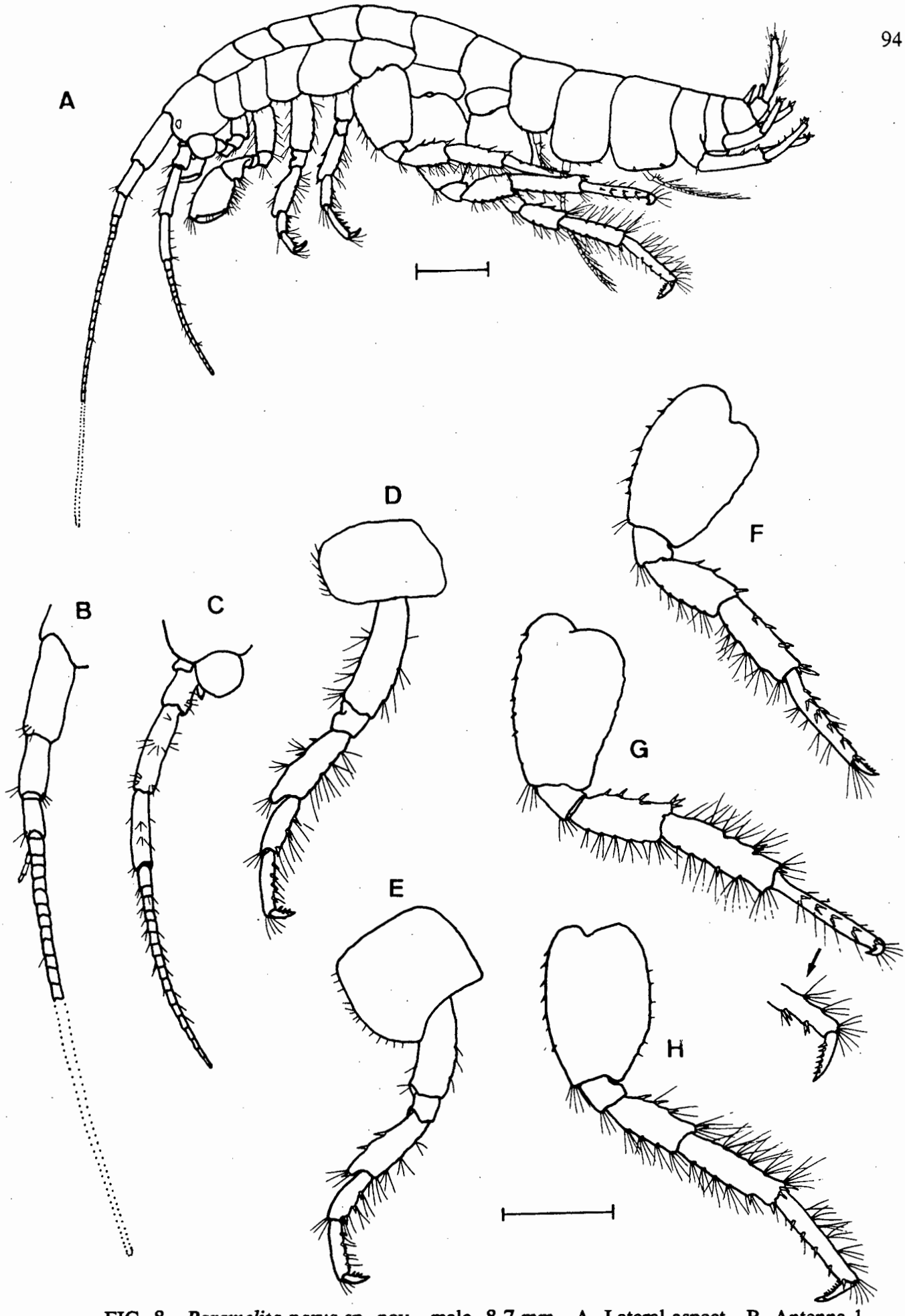

FIG. 8. *Paramelita parva* sp. nov., male, 8.7 mm. A. Lateral aspect. B. Antenna 1. C. Antenna 2. D. Pereopod 3 and coxa 3. E. Pereopod 4 and coxa 4. F. Pereopod 5. G. Pereopod 6. Pereopod 7.

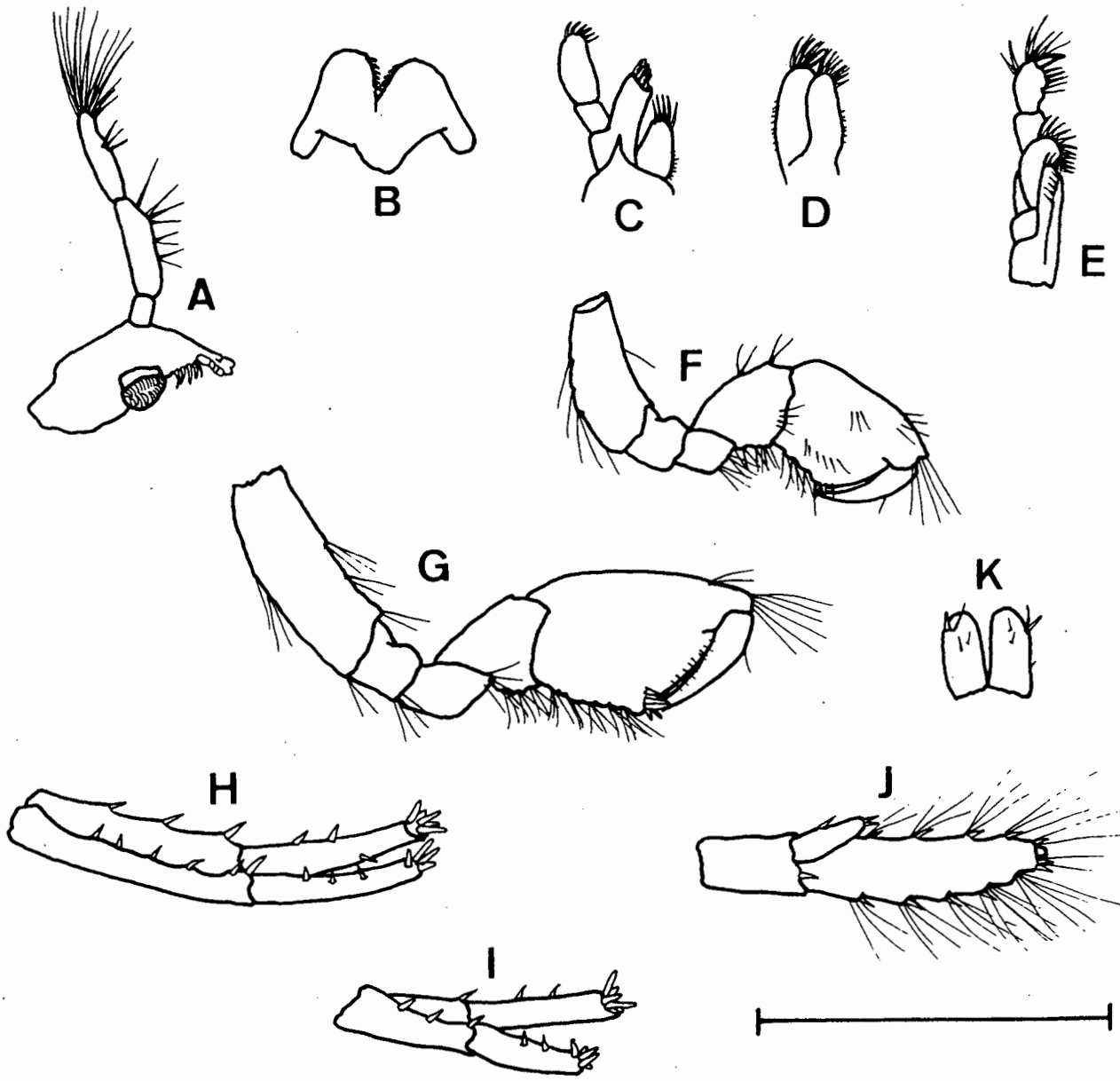

FIG. 9. *Paramelita parva* sp. nov., male, 8.7 mm. A. Left mandible. B. Lower lip. C. Maxilla 1. D. Maxilla 2. E. Maxilliped. F. Gnathopod 1, medial view. G. Gnathopod 2, medial view. H. Uropod 1. I. Uropod 2. J. Uropod 3. K. Telson.

plate, inner margin pubescent, both plates strongly setose terminally. Maxilliped, inner plate with about 12 curved setae, outer plate with several spine-like setae in inner margin and about eight terminal curved setae, palp articles 2 and 3 densely setose.

Pereon segments with no setae dorsally, coxae 1-3 quadrate, setose ventrally, coxa 4 posteriorly excavate, setose ventrally, coxa 5 and 6 longer than deep, bilobed, with very few setae, coxa 7 semicircular, smooth, segments 2-7 bearing one pair of coxal gills each, segments 2 with one, 3, 4, 5 and 7 with two, and 6 with four sausage-shaped sternal gills.

Gnathopod 1 subchelate, articles 5 and 6 together longer than 2, article 6 1.4 times length of 5, longer than wide, palm convex, gently oblique, with four spines at defining angle, dactyl as long as palm. Gnathopod 2 similar in structure to, but longer and sturdier than 1, medial surface of article 2 not spinose, articles 5 and 6 together longer than 2, article 6 1.7 times length of 5, palm convex, moderately oblique, with four defining spines, dactyl as long as palm. Pereopod 3 slightly longer than 4, unmodified, moderately setose, article 6 with four groups of spines posteriorly, dactyl with three spinules. Pereopod 4 similar in structure to 3, articles 4, 5 and 6 moderately setose, article 6 with five groups of spines, dactyl with three spinules. pereopod 5 moderately expanded posteriorly, article 2 with several spinules on anterior margin, with some short setae on posterior margin, articles 4 and 5 moderately setose and spinose, article 6 with five groups of spines, dactyl with four spinules. Pereopods 6 and 7 similar in structure to 5, dactyl of 6 with six, and that of 7 with eight spinules.

Pleon segments 1-3 dorsally smooth, epimeral plates quadrate, with spinules on ventral margins. Pleon segments 4-6 with no setae dorsally. Uropod 1 extending a little beyond 2 in undissected animal, rami subequal, 0.8 times length of peduncle, inner ramus with four, and outer with six marginal spines, each ending with five spines. Uropod 2 shorter than 1, inner ramus only slightly longer than outer, both rami with four marginal and five terminal spines, both lacking setae. Uropod 3 relatively long, about 15% body length, exceeding 2 by about 80% of the length of the outer ramus in the undissected animal, peduncle longer than broad, inner ramus short, 0.9 length of peduncle and 0.3 length of outer ramus, with one subapical and two apical

spines, outer ramus 2.7 times length of peduncle, four groups of spines and setae on inner and five on outer margins, second segment small, only 3% length of first. Telson as broad as long, each lobe with one subapical spine, one stout subapical seta and a few short setae on dorsal surface.

Distribution. Storms River catchment in the eastern Cape, occurring in several small streams flowing through natural forested areas.

Etymology. The specific epithet is derived from the Latin *parvus*, meaning small, and is in reference to the small size of the animals at maturity.

Females. Females are morphologically similar to males.

Discussion

Of the 24 known species of *Paramelita*, 11 are easily distinguished by either the possession of lobes, ridges or teeth on antenna 2, and/or by the possession of a modified pereopod 3, where either article 4 is weakly to strongly posterodistally protruded or greatly expanded laterally, or article 5 is either posteriorly lobed or has teeth-like spines. Of the remaining 13 species, one is distinguished by black eyes, two have article 4 of antenna 2 strongly laterally swollen with article 5 bent at right angles to 4, and two have coxa 4 quadrate, or only slightly emarginate posteriorly. The remaining eight species, which include the four new species described here, as well as *P. capensis*, *P. kogelensis*, *P.barnardi* and *P. seticornis*, all lack lobes, teeth or ridges on antenna 2, and have an unmodified pereopod 3 and a distinctly excavate coxa 4.

Two of the new species, *P. magna* sp. nov. and *P. validicornis* sp. nov. are easily distinguished from the other six by the possession of large, robust second antennae which exceed the first in length in adult males. Although initially identified as *P. capensis*, these two species are clearly separated from *P. capensis*. In addition to the differences in the relative lengths of antenna 2, the latter species has markedly slender, long antennae and limbs, and a relatively long flagellum in antenna 2. The limbs and antennae of *P. validicornis* and *P. magna* are markedly broader and sturdier than those of *P. capensis*, and in *P. validicornis*, the enlarged peduncle in antenna 2

always exceeds the sturdy flattened flagellum in length. In turn, *P. magna* and *P. validicornis* are also easily separated from each other. In *P. magna* sp. nov., article 1 of antenna 1 is broad, the articles of the pereopods are markedly laterally expanded, and the outer ramus of uropod 3 is distinctly short. In *P. validicornis* sp. nov., article 1 of antenna 1 is not as broad, the pereopods are only moderately wide, and the outer ramus of uropod 3 is distinctly long. There is also no overlap in the geographical ranges of the two species. *P. magna* is confined to the southern Cape Peninsula, while the nearest known population of *P. validicornis* is located more than 70 km south-east of the Cape Peninsula.

In addition to its densely setose antenna 2 and pereopods, *P. pillicornis* sp. nov. is easily recognised by the slenderness of article 4 in pereopod 3 and of articles 2 in pereopods 5-7. This species is most similar to *P. seticornis* Barnard, 1927, with which it shares several features, the most obvious being the setose antenna 2, a rudimentary second segment in the outer ramus of uropod 3, and a moderately excavate coxa 4. However, in *P. seticornis*, antenna 2 is markedly stout, with the enlarged peduncle exceeding the short flagellum in length, and articles 2 of pereopods 5-7 are wider than those in *P. pillicornis* sp. nov. The peduncle and flagellum of antenna 2 are of equal length in *P. pillicornis* sp. nov. These two species are also geographically isolated from each other, with the Gydo Pass population situated over 120 km away from the nearest known *P. seticornis* population.

P. parva sp. nov. is most easily identified by its small size at maturity and its short, unmodified antenna 2. Specimens of *P. capensis* are considerably larger than those of *P. parva* sp. nov., while the presence of setae on uropod 2, and the relatively sparsely setose uropod 3 in *P. kogelensis* distinguishes this species from *P. parva* sp. nov. *P. barnardi* is also characterised by the possession of a poorly setose uropod 3. *P. parva* sp. nov. is by far the most isolated of all the known *Paramelita* species, its nearest white-eyed neighbour being *P. validicornis* sp. nov., which occurs over 400 km west of the Storms River catchment.

We are indebted to our families, Yvonne Dempster, and John Allen for their invaluable help in the collection of specimens. Part of the funds which supported this project were awarded by the Inland Water Ecosystems section of the South African National Scientific Programme, CSIR, and the remainder, by the Foundation for Research Development, Special Programme on South African Rivers, directed by Prof. B.R. Davies.

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PAPER 4

A TAXONOMIC REEXAMINATION OF FRESHWATER AMPHIPODS IN THE *PARAMELITA AURICULARIUS* - *P. CRASSICORNIS* COMPLEX, WITH DESCRIPTIONS OF THREE ADDITIONAL SPECIES

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RESUME

Cette étude porte sur les différences morphologiques et génétiques existant entre les populations d'amphipodes d'eau douce appartenant aux groupes *Paramelita auricularius* and *P. crassicornis*. Les seize populations étudiées ont été divisée en sept formes phénotypiques distinctes, dont une est indubitablement une nouvelle espèce n'ayant pas encore été décrite. Quant aux autres formes phénotypiques, les variations portant sur les isozymes ont été investiguées au moyen de gels électrophorétiques. Il en fut déduit que trois des formes phénotypiques du groupe *P. auricularius* ne sont en fait que des variations morphologiques d'une seule espèce. Les trois formes préalablement identifiées sous le nom de *P. crassicornis* sont suffisement différente pour être reconnue comme formant deux nouvelles espèces. La description des trois nouvelles especes mentionnees plus haut est inclue dans cette étude.

INTRODUCTION

Paramelita is a genus of crangonyctoid amphipods found only in the south-western Cape region of South Africa. Of the 12 species recognized when the group was last reviewed by Griffiths (1981), two, namely, *P. crassicornis* Barnard and *P. auricularius* Barnard were considered to be narrowly endemic, occurring only in streams draining the top of Table Mountain (fig. 1). During an extensive collection programme in 1989 when over 100 populations of *Paramelita* were sampled from localities all over the south-western Cape, several additional populations representing, or allied to these two species were collected. *P. auricularius*-like forms were collected from a locality on the top of Table Mountain as well as from a stream draining Constantiaberg, some 11 km south of the original type locality, whilst three populations of *P. crassicornis*-like forms were sampled from the top of Table Mountain, and six populations from localities between 4.4 and 13.1 km from the type locality.

At Echo Valley (fig. 1), where *P. crassicornis* and *P. auricularius* occurred in sympatry, the two species were clearly differentiated. The ear-like lobe on article 3 of antenna 2, the extraordinary condition of pereopod 3, and the grey colour of *P. auricularius* males made them easy to distinguish from *P. crassicornis* males, whose most characteristic features were their swollen second antennae and white colour.

The two 'apomorphic' *P. auricularius* features, the lobe on antenna 2 and the structure of pereopod 3 varied considerably between the two *P. auricularius* populations collected. Thus, it was difficult to assess whether or not these populations were members of a single highly variable species, or whether they represented a pair of closely related, but distinctly different species. Similarly, different forms existed amongst those populations allied to *P. crassicornis*. Adult males from the populations collected from streams in the Silvermine area possessed a 'tooth' on article 5 of antenna 2, a feature not found in any of the other populations. Although males from the Table Mountain populations possessed stout teeth-like spines on article 5 of pereopod 3, it

was only in two populations collected 4.4-5.0 km from the top of Table Mountain that article 6 of this pereopod folded back against the toothed posterior margin of article 5 to form a 'claw-like' structure. One of the populations consisted of specimens which were clearly morphologically distinct. Although adult males possessed the lobe on article 3 of antenna 2 characteristic of *P. auricularius*, the claw-like structure in pereopod 3 was quite distinct, with article 4 (not 5 as in *P.auricularius*) posterodistally protruded to form a long 'spur', and article 5 swollen and bent, but not lobed. The aims of this paper are to identify and illustrate the different forms within the *P. auricularius* and *P. crassicornis* groups, to quantify the genetic differentiation between these forms using gel electrophoresis, to assess whether or not any of these forms warrant consideration as new species, and, to describe any new species recognised.

MATERIALS AND METHODS

a) Collection. - Populations within the *P. auricularius* - *P. crassicornis* group, or related forms were collected using small handnets from 12 small mountain streams in the Cape Peninsula during 1989 and 1990 (fig. 1). A proportion of each sample (preferably of at least 50-100 individuals) was immediately preserved in 70% alcohol, while the remaining animals were returned to the laboratory where they were held in a 12^{o}C coldroom until needed for electrophoresis. No live material could be obtained from the Manganese Mine site, consequently this population was not included in the electrophoretic study. Additional preserved material of *P. auricularius* and *P. crassicornis* was obtained from the collections of the South African Museum, and where possible, these populations were included in the morphological analysis.

b) Morphology. - At least five adult males from each of 16 populations were examined using a Wild stereo microscope. The morphological differentiation of antenna 2 and pereopod 3 were noted for each population, and the different forms

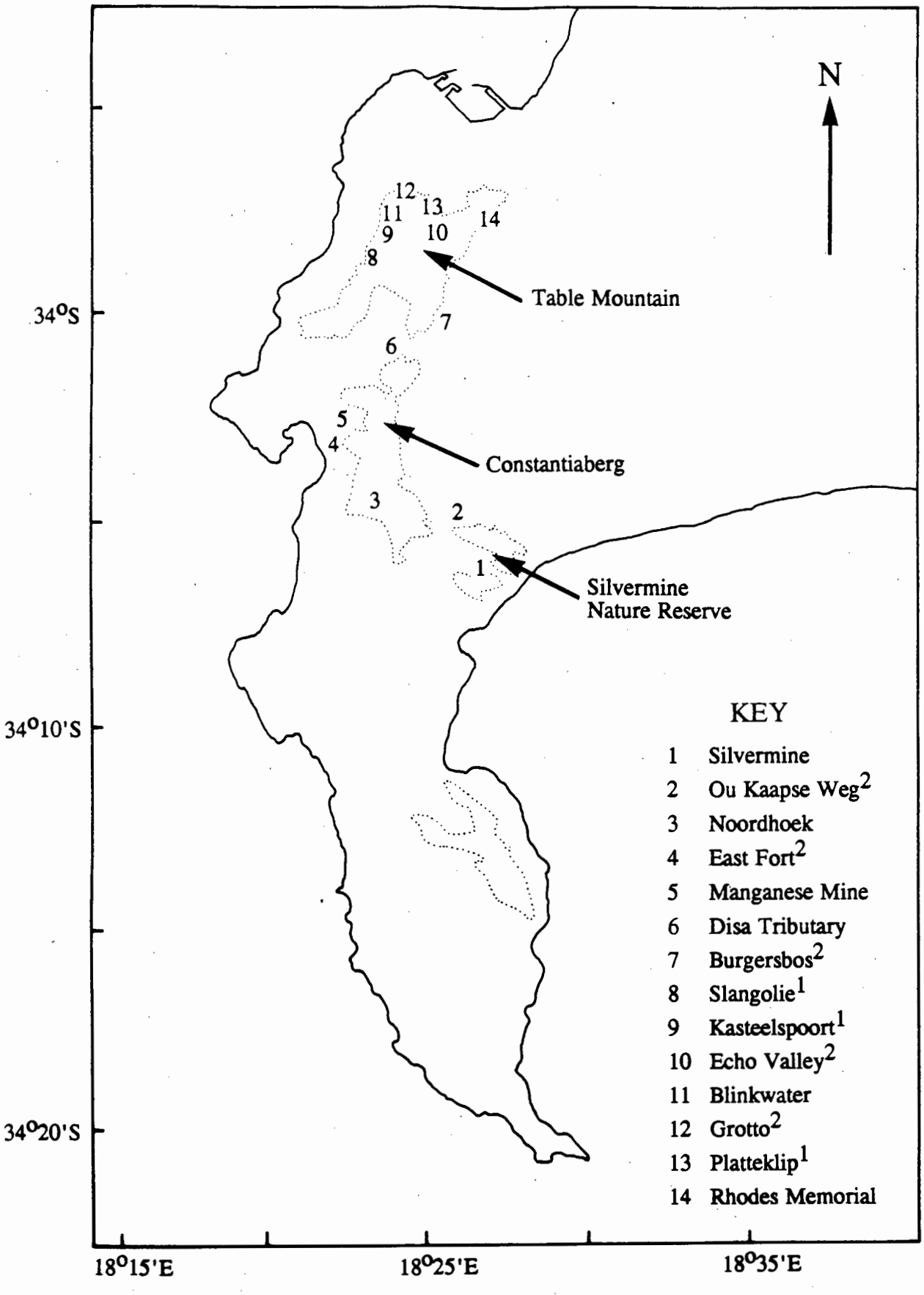

Fig. 1. Map of the Cape Peninsula showing the localities where either *Paramelita auricularis*, *P. crassicornis* or related forms were collected. ([1]), preserved material from the South African Museum; ([2]), populations used for electrophoresis. The dotted lines represent the 500m contour.

illustrated by means of a camera lucida attached either to a dissecting or compound microscope.

c) Electrophoresis. - Between 10 and 54 amphipods from each of two *P. auricularius* and four *P. crassicornis* allied populations were electrophoresed on horizontal starch gels using the methods outlined in Stewart (in prep.). Of 25 enzymes initially screened, only seven could be consistently scored for all six populations. Dickson et al. (1979) have suggested that amphipods do not electrophorese well because of inhibitory enzymes from the hepatopancreas during homogenization. The enzymes were separated using three buffer systems:

(1) a discontinuous tris-citrate-borate-lithium hydroxide buffer system; gel buffer pH 8.7, electrode pH 8.0 (Ridgeway et al., 1970),

(2) a continuous citrate-(N-(3-aminopropyl)-morpholine) buffer system; gel and electrode buffer pH 6.1 (Clayton & Tretiak, 1972),

(3) and, a continuous tris-borate-EDTA buffer system; gel and electrode buffer pH 8.6 (Markert & Faulhaber, 1965).

The enzymes stained for, and the buffer systems used, are listed in Table I. The BIOSYS-1 software programme of Swofford & Selander (1981) was used for data analysis. Allele frequencies were computed, and these used to calculate Nei's (1978) index of unbiased genetic identity between each pair of populations. From these data, the populations were clustered using the UPGMA algorithm (Sneath & Sokal, 1973).

RESULTS

a) Morphology. - Based on differentiation in the form of antenna 2 and pereopod 3, the 12 populations examined were grouped into seven distinct forms (Table II). In *P. auricularius*, considerable variation in the degree of enlargement of article 5 of pereopod 3 (fig. 2) and the size of the lobe on article 3 of antenna 2 (fig. 3) existed, so that three forms (A-C) were recognised. Three forms (D-F) within the *P.*

TABLE I

Enzymes investigated and buffer systems used

Enzyme	Abbreviation	E. C. No.	Buffer
Arginine kinase	ARK	2.7.3.3	1
Aspartate amino-transferase	GOT	2.6.1.1	2
Diaphorase	DIA	1.6.2.2	1
Glucose-phosphate isomerase	GPI	5.3.1.9	1
Glyceraldehyde-phosphate dehydrogenase	GAP	1.2.1.12	2
Peptidase (glycyl-leucine as substrate)	GL	3.4.11.-	3
Peptidase (leucyl tyrosine as substrate)	LT	3.4.11.-	1
Phosphoglucomutase	PGM	2.7.5.1	3

TABLE II

Features used to distinguish various forms of *Paramelita auricularius*, *P. crassicornis* and related new species, and the localities from which they were collected.

Form	Description	Population names
P. auricularius		
A	Antenna 2, article 3 enormously lobed posteriorly; pereopod 3, article 5 expanded posteriorly with a proximal tooth bearing a stout spine	Echo Valley
B	Antenna 2, article 3 moderately lobed posteriorly; pereopod 3, article 5 not expanded, but proximally lobed, with a single stout spine	East Fort
C	Antenna 2, article 3 moderately lobed posteriorly; pereopod 3, article 5 with a rectangular-shaped lobe, bearing a single stout spine	Table Mountain (lectotype)
P. crassicornis		
D	Antenna 2, articles 3 and 4 swollen, not lobed or toothed; pereopod 3, article 5 with one to four stout spine-teeth, article 6 not folded to form a "claw"	Kasteelspoort, Platteklip, Rhodes Memorial, Grotto, Blinkwater, Echo Valley, Slangolie
E	Antenna 2, articles 3 and 4 swollen, not lobed or toothed; pereopod 3, article 5 with 1 - 4 stout spine-teeth, article 6 folded to form a "claw"	Burgersbos, Disa Tributary
F	Antenna 2, articles 3 and 4 swollen, article 5 posterodistally protruded to form a "tooth"; pereopod 3, article 5 with two pairs of spines, article 6 not folded to form a "claw"	Ou Kaapse Weg, Silvermine, Noordhoek
Related new species		
G	Antenna 2, article 3 strongly swollen and bearing a large lobe, article 4 also laterally expanded; pereopod 3, article 4 posterodistally protruded into a long "spur", article 5 swollen and arched, article 6 folded to form a "claw"	Manganese Mine

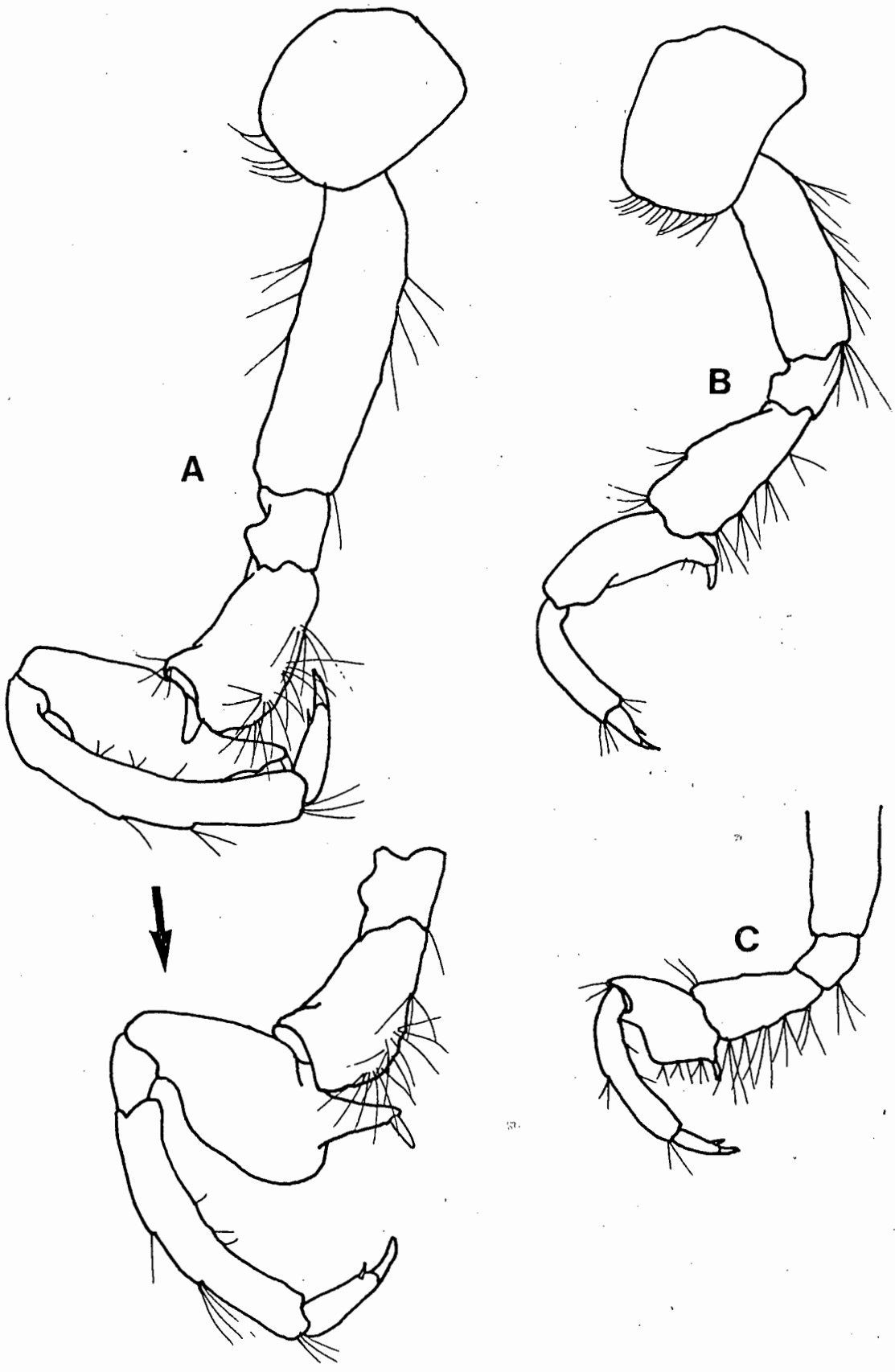

Fig. 2. Pereopod 3 in *Paramelita auricularius* forms. A. Form A, from Echo Valley. B. Form B, from East Fort. C. Form C, the lectotype from Table Mountain.

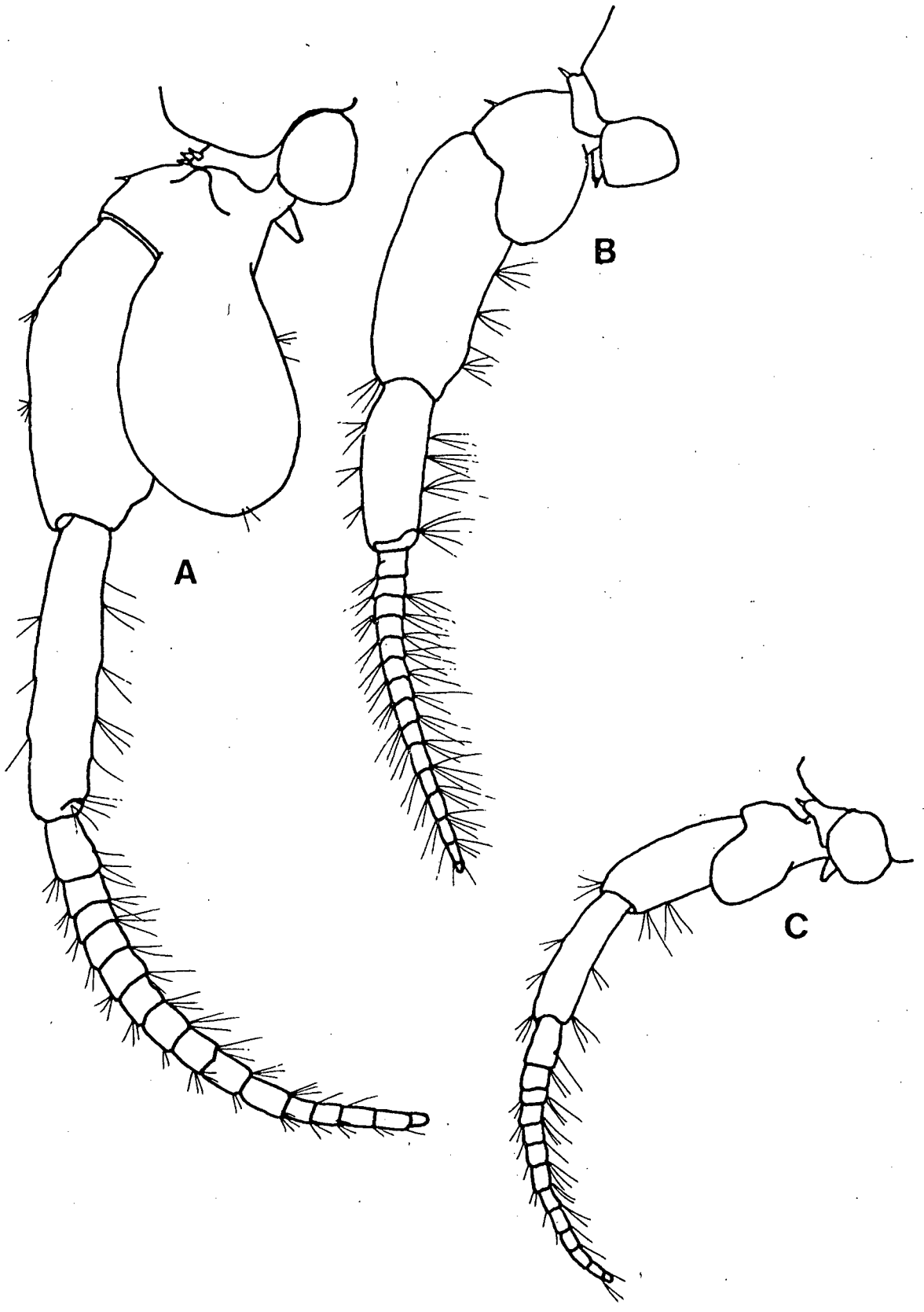

Fig. 3. Antenna 2 in *Paramelita auricularius* forms. A. Form A, from Echo Valley. B. Form B, from East Fort. C. Form C, lectotype from Table Mountain.

crassicornis group were also identified on the basis of differences in the structure of pereopod 3 (fig. 4). Seven populations collected from various localities on the slopes of Table Mountain had amphipods with article 5 on pereopod 3 with 1-4 stout spine-teeth, and article 6 attached normally to article 5 (form D). Two other populations from 4-5 km south of Table Mountain were similar in form, except that article 6 of pereopod 3 was folded back against the posterior margin of 5 to form a 'claw-like' structure (form E). A single geographically isolated population from the southern Cape Peninsula was characterised by the presence of a 'tooth' on article 5 of antenna 2 (fig. 5), and a lack of spines-teeth on article 5 of pereopod 3 in males (form F; fig. 4). Specimens from Manganese Mine (form G) were considered different enough from both *P. auricularius* and *P. crassicornis* to warrant the immediate recognition of a new, undescribed species. In this form, article 3 of antenna 2 is strongly lobed as in *P. auricularius* (fig. 5), and article 4 of pereopod 3 is posterodistally protruded to form a remarkably elongate 'spur', with article 6 folded to complete the claw-like structure (fig. 4). A description of this newly discovered species is included below.

b) Electrophoresis. - The allele frequencies for the seven loci are given in Table III. All six populations were monomorphic for the ARK and DIA loci. The total number of alleles for the remaining five enzymes ranged from three in GAP to seven in GL and LT. The maximum number of alleles at a single locus within any one population was five for GL in Grotto specimens. None of the enzymes was consistently polymorphic for all populations. Specimens of *P. auricularius* from Echo Valley were fixed for GL^{55}, LT^{68} and GAP^{95}. The LT^{68} allele was also fixed in *P. auricularius* specimens from East Fort, as were the GAP^{78} and PGM^{93} alleles. The alleles LT^{104}, GAP^{95}, GOT^{50} and PGM^{90} were all fixed in the Ou Kaapse Weg population, whilst *P. crassicornis* specimens from Grotto were monomorphic for GAP^{95} and GOT^{78}, and those from Burgersbos were fixed for GAP^{78} and GOT^{78}. Only ARK and DIA were monomorphic in the *P. crassicornis* specimens from Echo Valley.

TABLE III

Allele frequencies at seven loci for the six populations studied.

Locus	Populations					
	P. auricularius		*P. crassicornis*			
	Echo Valley	East Fort	Echo Valley	Grotto	Burgersbos	Ou Kaapse Weg
ARK						
75	1.000	1.000	1.000	1.000	1.000	1.000
DIA						
102	1.000	1.000	1.000	1.000	1.000	1.000
GAP						
78	0.000	1.000	0.000	0.000	1.000	0.000
95	1.000	0.000	0.962	1.000	0.000	1.000
100	0.000	0.000	0.038	0.000	0.000	0.000
GL						
42	0.000	0.000	0.050	0.036	0.906	0.000
55	1.000	0.900	0.067	0.089	0.094	0.000
64	0.000	0.100	0.850	0.839	0.000	0.000
79	0.000	0.000	0.033	0.018	0.000	0.000
90	0.000	0.000	0.000	0.018	0.000	0.000
100	0.000	0.000	0.000	0.000	0.000	0.050
112	0.000	0.000	0.000	0.000	0.000	0.950
GOT						
50	0.000	0.000	0.000	0.000	0.000	1.000
78	0.974	0.900	0.976	1.000	1.000	0.000
100	0.026	0.100	0.024	0.000	0.000	0.000
LT						
51	0.000	0.000	0.069	0.034	0.933	0.000
64	0.000	0.000	0.000	0.017	0.000	0.000
68	1.000	1.000	0.034	0.034	0.067	0.000
77	0.000	0.000	0.845	0.845	0.000	0.000
81	0.000	0.000	0.052	0.052	0.000	0.000
100	0.000	0.000	0.000	0.017	0.000	0.000
104	0.000	0.000	0.000	0.000	0.000	1.000
PGM						
84	0.000	0.000	0.000	0.021	0.027	0.000
90	0.579	0.000	0.029	0.042	0.014	1.000
93	0.421	1.000	0.882	0.917	0.865	0.000
100	0.000	0.000	0.088	0.021	0.095	0.000

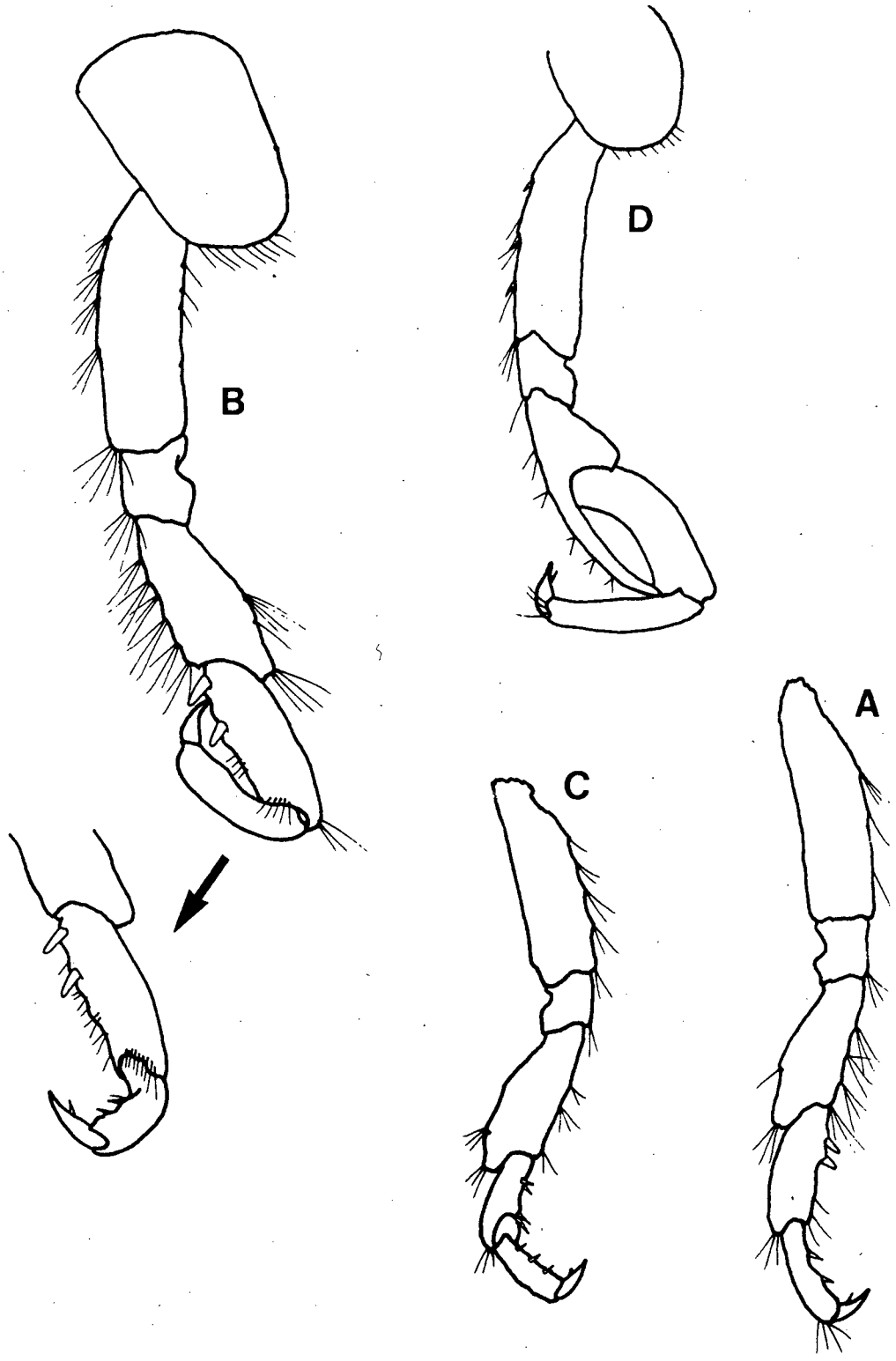

Fig. 4. Pereopod 3 in *Paramelita crassicornis* and related forms. A. Form D, from Echo Valley. B. Form E, from Burgersbos. C. Form F, from Ou Kaapse Weg. D. Form G, from Manganese Mine.

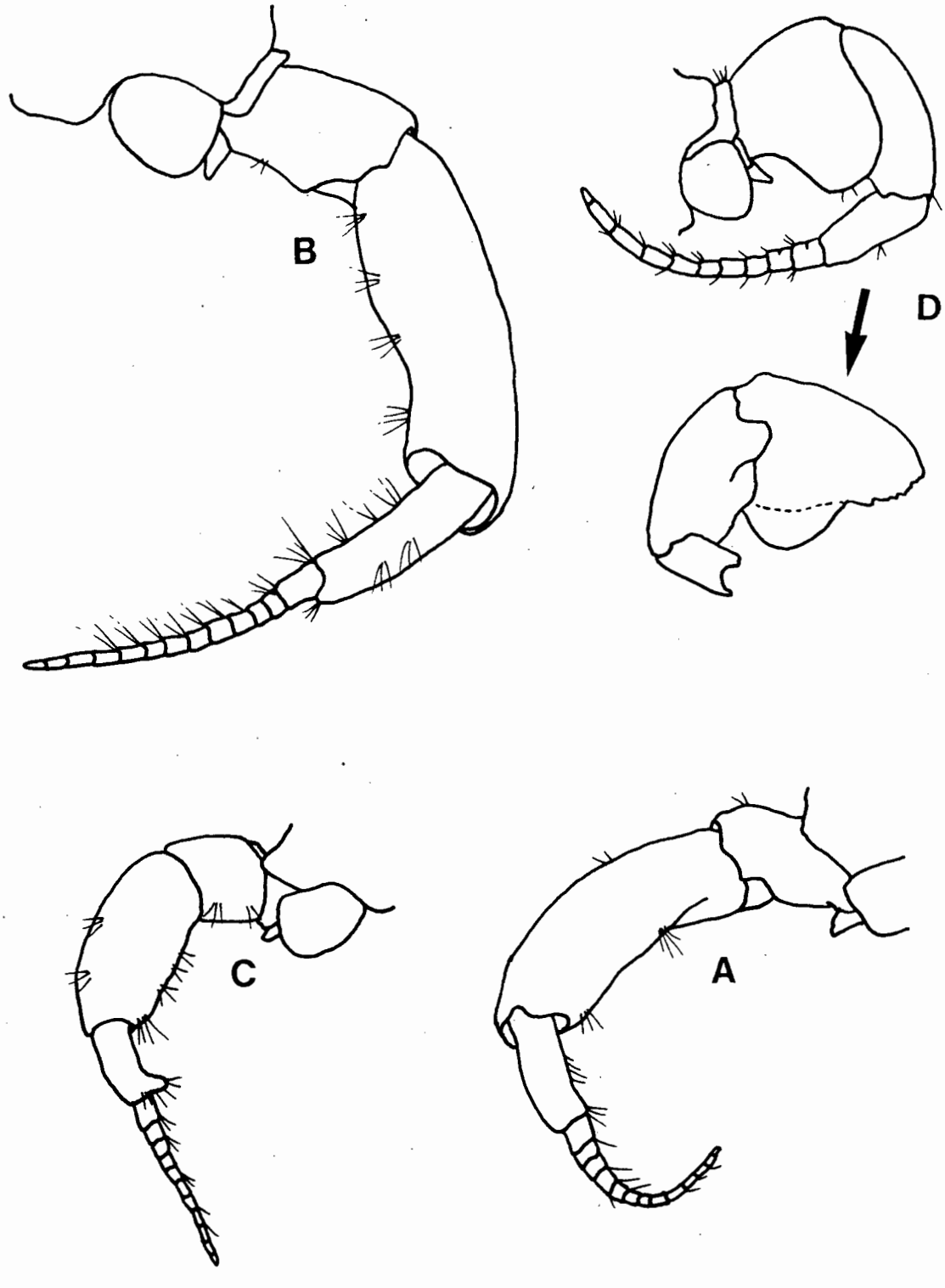

Fig. 5. Antenna 2 in *Paramelita crassicornis* and related forms. A. Form D, from Echo Valley. B. Form E, from Burgersbos. C. Form F, from Ou Kaapse Weg. D. Form G, from Manganese Mine.

Nei's (1978) genetic identities (Table IV) were calculated from the allele frequency data (Table III), and these data used to construct a dendrogram (fig. 6). The six populations separated into four distinct clusters, with the two *P. crassicornis* populations from Table Mountain, Echo Valley and Grotto, showing no genetic differentiation (I = 1.000), and the two *P. auricularius* populations, Echo Valley and East Fort, separating at an I value of 0.797. The unusual *P. crassicornis* form from Burgersbos with its claw-like pereopod 3 was genetically distinct from the *P. crassicornis* form on Table Mountain, separating at an I value of 0.630. The Ou Kaapse Weg population, characterised by the possession of a tooth on antenna 2, was joined to the other five populations at an I value of 0.411. This relatively low I value was due to the presence of several diagnostic alleles in the Ou Kaapse Weg population. Thus, GL^{100}, GL^{112}, LT^{104} and GOT^{50} were not found in any of the other five populations. Although specimens from Burgersbos did not possess any diagnostic alleles, allele frequency differences existed for three alleles between Burgersbos and the two Table Mountain populations of *P. crassicornis*. Thus, GL^{42}, GAP^{78} and LT^{51} were common in Burgersbos animals (frequencies of 0.906-1.000), but rare in the Grotto and Echo Valley populations (0.000-0.069). Three alleles, GL^{79}, LT^{77} and LT^{81} occurred in the Grotto and Echo Valley populations of *P. crassicornis*, but were absent in the other four populations. The two *P. auricularius* populations did not contain any alleles diagnostic of the species. However, GL^{55} and LT^{68}, common in *P. auricularius* specimens (frequencies of 0.900-1.000), were rare in the other populations in which they occurred (0.034-0.094).

DISCUSSION

In the *P. crassicornis* forms, the results showed an interesting correlation between geographical separation and morphological and genetic differentiation. Although the two *P. crassicornis* populations on Table Mountain used in the isozyme

TABLE IV

Matrix of Nei's (1978) genetic identities between populations of *Paramelita auricularius* (1 & 5) and *P. crassicornis* (2, 3, 4 & 6).

	Population	1 Echo Valley	2 Burgersbos	3 Echo Valley	4 Grotto	5 East Fort	6 Ou Kaapse Weg
1	Echo Valley	***	0.544	0.702	0.709	0.797	0.537
2	Burgersbos		***	0.616	0.609	0.753	0.302
3	Echo Valley			***	1.000	0.621	0.461
4	Grotto				***	0.624	0.463
5	East Fort					***	0.296
6	Ou Kaapse Weg						***

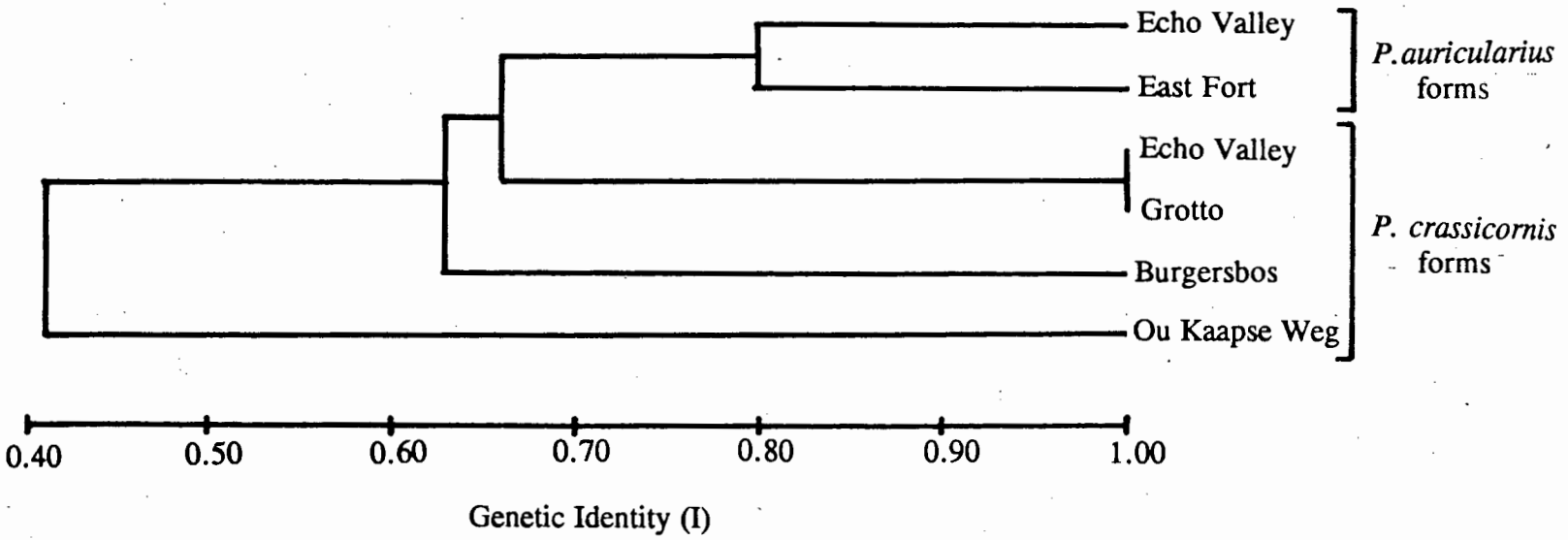

Fig. 6. Dendrogram of the six populations generated using Nei's (1978) genetic identities and the UPGMA clustering algorithm

study occurred in separate streams some 900 m apart, these tributaries were ultimately connected, thus allowing the two populations to mix, resulting in a lack of morphological and genetic differentiation (form D).

Specimens of form E of *P. crassicornis* collected from a tributary of the Burgersbos River, 4.4 km from Table Mountain, were clearly genetically differentiated from those of Grotto and Echo Valley, despite the lack of diagnostic alleles. It is interesting to note that the two *P. auricularius* populations investigated in the electrophoretic study also lacked alleles diagnostic to the species. Although form E animals were morphologically similar to those on Table Mountain, the nature of attachment of article 6 to 5 in pereopod 3 was unique in this form. This apomorphic condition in pereopod 3, along with the distinct genetic differentiation of these animals from *P. crassicornis* form D, suggests that form E should be considered a new, undescribed species.

The relative geographic isolation of form F from the Silvermine River on the Ou Kaapse Weg road, 13.1 km from Table Mountain, is reflected in their strong differentiation from the 'original' *P. crassicornis* form D. These animals are morphologically distinct, possess at least four known diagnostic alleles, and join the other *P. crassicornis* and *P. auricularius* forms investigated at a relatively "low" I value. There is thus strong evidence supporting the recognition of this form as a new species.

Despite the fact that the two *P. auricularius* populations investigated in the isozyme study occurred in different catchments 10 km apart, these animals were poorly genetically differentiated, suggesting that they represent two forms of a single, morphologically variable species.

The different degrees of genetic differentiation in populations of *P. crassicornis* and *P. auricularius* forms raises the question as to what constitutes a species. On the one hand, relatively little morphological differentiation in *P. crassicornis* forms was accompanied by strong genetic variation. On the other hand, what appeared to be

significant changes in the shape of article 5 in pereopod 3 in *P. auricularius* forms was not paralleled by large genetic changes. There are many examples in the literature where relatively small morphological changes have been accompanied by a much greater degree of divergence at the molecular level. For example, Hedgecock (1979) demonstrated the existence of morphologically "cryptic" species of barnacles using electrophoresis, and Bulnheim & Scholl (1980) used allele frequency differences in selected polymorphic enzymes to demonstrate that the morphologically similar amphipod species, *Gammarus zaddachi* and *G. salinus* were indeed sparate species. On the other hand, variation in morphology in some amphipod species has not always coincided with genetic variation. Although three distinct phenotypic forms of the freshwater amphipod *G. stupendus* have been identified, Scheepmaker (1987) failed to demonstrate that these forms were distinct species or subspecies.

In all of these studies, as well as our own, a decision had to be made as to whether the variation observed between related populations was 'sufficient' to warrant the recognition of more than one species. In an ideal situation where a species is considered as a group "of interbreeding natural populations that are reproductively isolated from other such groups", as defined by the biological species concept (Mayr, 1970), breeding experiments would provide the answer. If the animals are unable to reproduce, then the populations in question can be considered separate species. In many cases, however, breeding experiments which are often time consuming and demand constant, stable laboratory conditions, are impractical. The taxonomist is usually faced with a limited number of preserved specimens providing him with clues as to morphological variation, and if he is fortunate, live material to examine genetic variation by, for example, gel electrophoresis.

One option would be to use the criterion of a lack of gene flow between different populations, rather than the lack of reproduction, as the theoretical basis for practical taxonomic work (Bock, 1986). With an absence of gene flow, the populations of an ancestral species would form separate phyletic lineages, and undergo independent

phyletic changes. Thus, the presence of diagnostic alleles and the apomorphic 'tooth' on antenna 2 in specimens of form F from Ou Kaapse Weg demonstrate the lack of gene flow between this population and other forms in the *P. crassicornis* group. Similarly, the apomorphic condition of pereopod 3 in form E, not found in specimens of form D, and the sharp discontinuities in allele frequencies in three of the isozymes examined, suggests that there is an absence, or considerable reduction of gene flow between these forms. The poor genetic differentiation of the two *P. auricularius* populations studied suggests that there is either gene flow between these populations, despite their relative geographical isolation, or that similar selective forces are acting in the two populations, resulting in similar genetic varation.

Further indirect evidence of the specific status of forms E and F is provided by the genetic identities (Nei, 1978) between these populations and those from the top of Table Mountain. The I values (0.609-0.616) between forms D and E were similar to those between the sympatric populations at Echo Valley of *P. crassicornis* and *P. auricularius* (I = 0.702). Form F, with I values of 0.296-0.537 between others in the study, was clearly even more distant from form D of *P. crassicornis*. These I values fall well within the range calculated by Thorpe (1982) for distinguishing species. Thorpe (1982) has suggested that if allopatric populations of dubious status have genetic identities below about 0.85, it is doubtful that they represent populations of a single species, and should rather be considered as separate species. With the exception of the I value (I = 1.000) between the Echo Valley and Grotto populations of *P. crassicornis*, all of the I values on the dendrogram (fig. 6) fall below this figure. However, because of the relatively low number of loci used to calculate these values (seven loci), a conservative approach must be adopted in the application of Thorpe's (1982) 'rule of thumb'. Thus, the value of 0.797 between the two *P. auricularius* forms, A and B, is considered to be too high to warrant specific recognition, therefore, these populations are considered to be conspecific.

Thus, in this study, we have concluded that the seven forms in the *P. auricularius* - *P. crassicornis* group represent five species. These are the original *P. auricularius* (Barnard, 1916; 1927), and *P. crassicornis* (Barnard, 1916), and three new species, *P. dentata* sp. nov., *P. marunguis* sp. nov. and *P. pheronyx* sp. nov., the descriptions of which follow.

DESCRIPTION OF SPECIES

Paramelita dentata sp. nov. (figs 7, 8)

Material examined. - Holotype. Male, 6.9 mm, SAM A40244, collected from a tributary of the Sandvlei River on the road, Ou Kaapse Weg, Cape Peninsula (34°6'S, 18°26'E). Paratypes. Nine males, six females, 55 juveniles, SAM A40245, from the same locality as the holotype. Other material. SAM A40249, from a tributary of the Silvermine River, Cape Peninsula.

Etymology. - From the Latin *dentatus*, meaning toothed, alluding to the characteristic tooth on article 5 of antenna 2 in adult males.

Male holotype, description. - Body colour, white when alive. Head shorter than pereon segments 1 and 2 combined, anteroventral margin excavate to accomodate inflated article 1 of antenna 2, eyes glistening white when alive, invisible in alcohol.

Antenna 1 half length of body, peduncle sparsely setose, article 1 1.5 length of 2 and 3.5 length of 3, flagellum strongly setose posteriorly, 1.2 times length of peduncle, 20-articulate, accessory flagellum with four articles, reaching past article 3 of primary flagellum.

Antenna 2 0.6 length of 1, peduncle sparsely setose, articles 3 and 4 strongly laterally swollen, article 4 2.5 length of 3 and 2.1 length of 5, article 5 short, with a distinct, conical posterodistal tooth, flagellum 0.6 length of swollen peduncle, sparsely setose, 11-articulate.

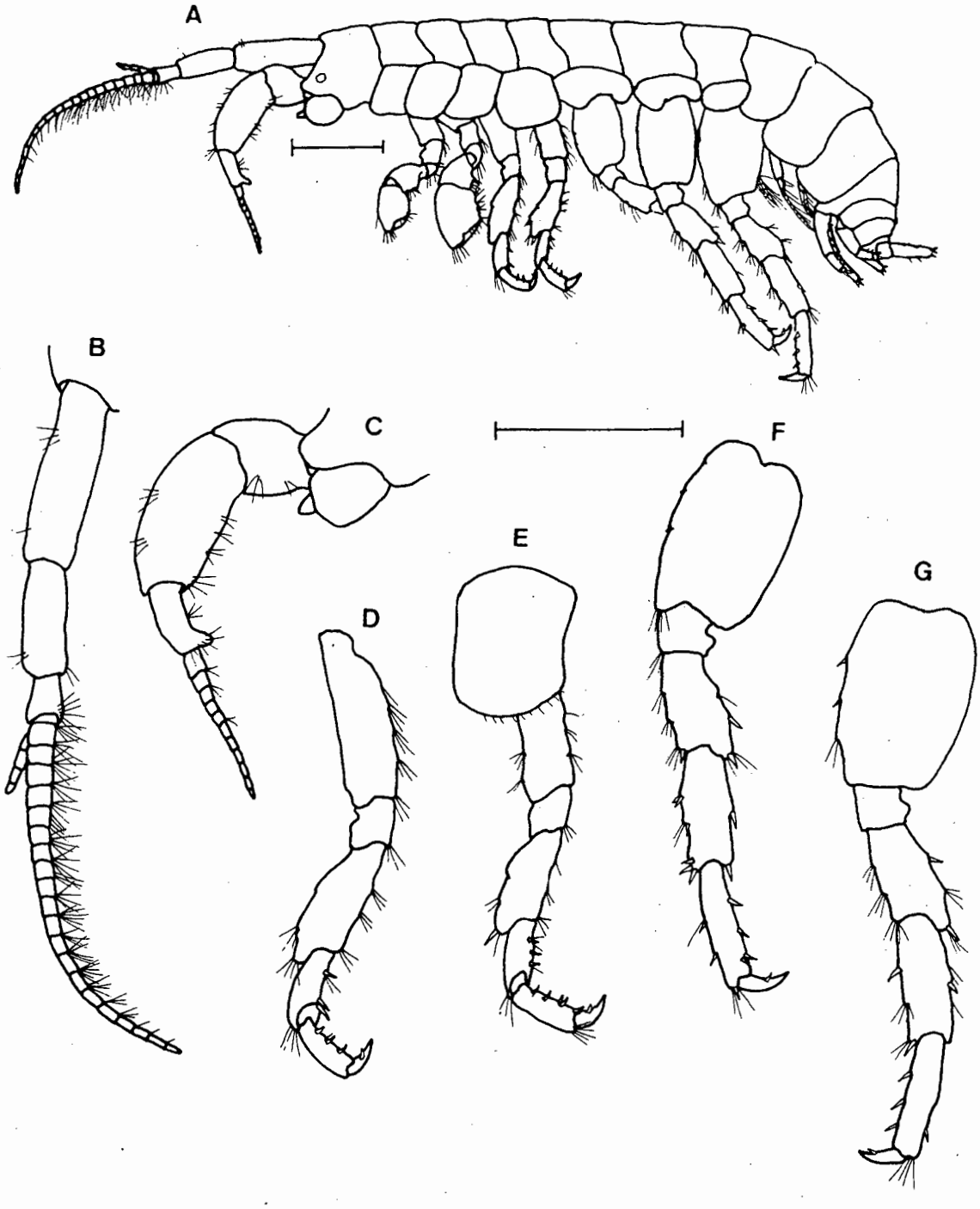

Fig. 7. *Paramelita dentata* sp. nov., male, 6.9 mm. A. Lateral aspect. B. Antenna 1. C. antenna 2. D. Pereopod 3. E. Pereopod 4 and coxa 4. F. Pereopod 6. G. Pereopod 7. Scale line represents 1 mm.

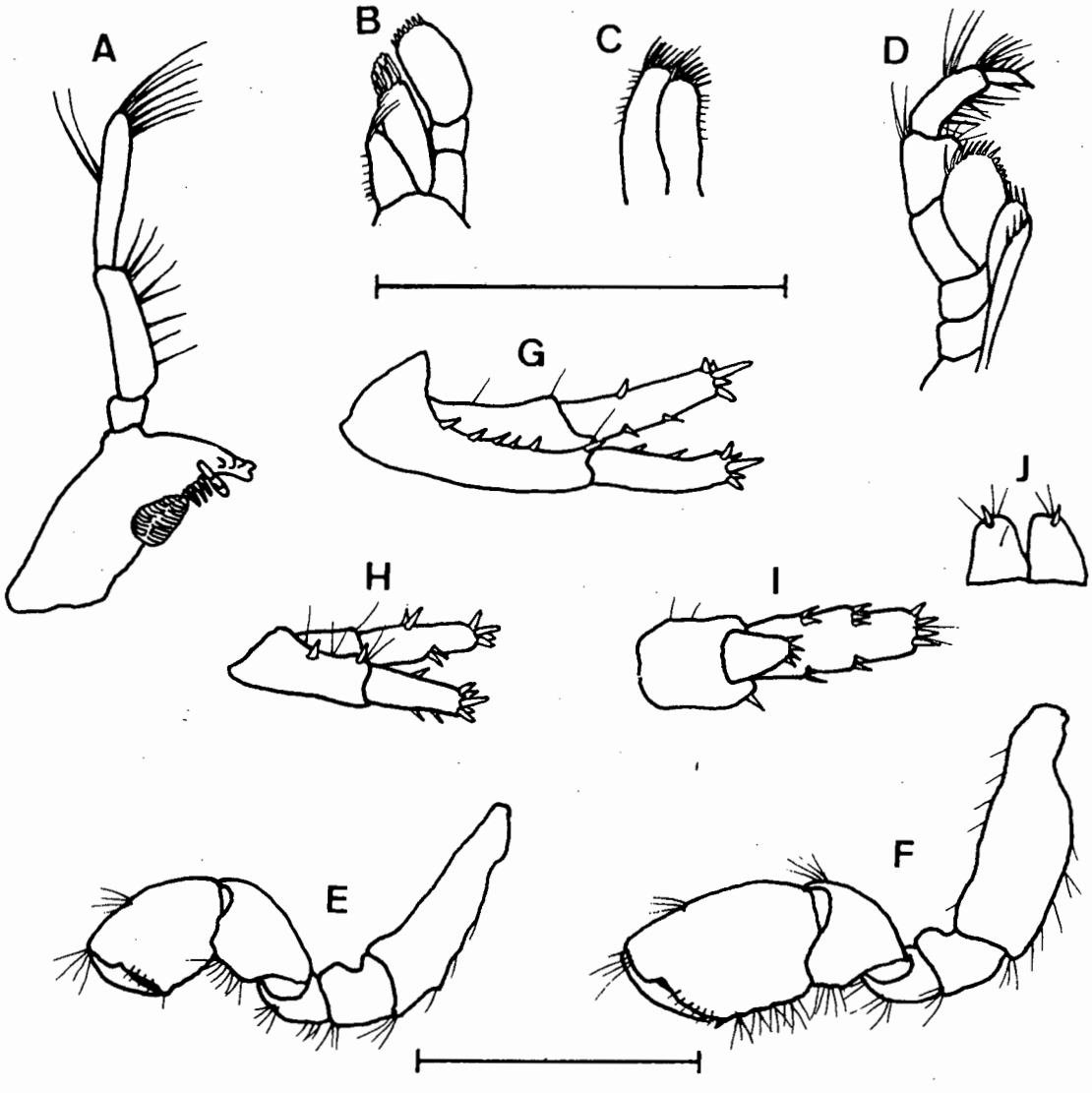

Fig. 8. *Paramelita dentata* sp. nov., male, 6.9 mm. A. Left mandible. B. Right maxilla. C. Left maxilla. D. Maxilliped. E. Gnathopod 1. F. Gnathopod 2. G. Uropod 1. H. Uropod 2. I. Uropod 3. J. Telson. Scale line represents 1 mm.

Left mandible, incisor with five blunt teeth, lacinia mobilis with three blunt teeth, four accessory blades, molar strongly triturative, 3-articulate palp much longer than body of mandible, article 1 about as long as wide, article 2 4.2 length of 1, with eight setae anteriorly, article 3 10% longer than 2, with eight long apical setae and one tuft of setae 60% along length.

Incisor of right mandible with four blunt teeth, lacinia mobilis bifurcate, with four accessory blades.

Maxilla 1, inner plate with numerous short setae on medial surface, five longer setae on apex, outer plate bearing two rows of five serrate spines, bi-articulate palp exceeding outer plate, with six stout setae in left maxilla 1 and six spine-teeth in right maxilla 1.

Maxilla 2, inner plate shorter and narrower than outer, both plates with margins distally pubescent and apices strongly setose.

Maxilliped, inner plate with six setae and one spine terminally, outer plate with about 10 stout spine-teeth on inner margin and eight terminal curved setae, palp articles 2 and 3 densely setose medially.

Pereon segments dorsally smooth, coxae 1-3 quadrate, setose ventrally, coxa 4 slightly emarginate posteriorly, deeper than long, setose ventrally, coxae 5 and 6 longer than deep, bilobed, sparsely setose ventrally, coxa 7 semi-circular, sparsely setose ventrally. Segments 2-7 bearing one pair of coxal gills each, segment 2 with one, 3, 4, 5 and 7 with two, and 6 with four sausage shaped sternal gills.

Gnathopod 1 subchelate, articles 5 and 6 together longer than 2, article 6 about the same length as 5, longer than wide, palm slightly convex, transverse, with three defining spines, dactyl as long as palm.

Gnathopod 2 similar in structure to, but larger than 1, article 2 not spinose medially, articles 5 and 6 combined longer than 2, article 6 1.6 length of 5, longer than wide, palm convex, moderately oblique, with four defining spines, dactyl as long as palm.

Pereopod 3 unmodified, moderately setose, article 5 with two groups and 6 with three groups of spines, dactyl with a single spinule.

Pereopod 4 similar in structure and length to 3, moderately setose, article 5 with three, and 6 with four groups of spines posteriorly, dactyl with a single spinule.

Pereopod 5 sparsely setose, article 2 moderately expanded, with spinules and setae on anterior margin and setae on posterior margin, article 5 with three groups of spines anteriorly and one group posteriorly, article 6 with two groups of spines anteriorly and one group posteriorly, dactyl with a single spinule. Pereopods 6 and 7 similar in structure to but longer than 5, article 6 with four groups of spines, dactyl with a single spinule.

Pleon segments 1-3 dorsally smooth, epimeral plates rounded to quadrate, sparsely setose ventrally. Pleon segments 4-6 sparsely setose dorsally.

Uropod 1 relatively short, not extending past 2 in undissected animal, peduncle with four setae on medial margin and three setae and six spines on outer margin, rami subequal, inner ramus with three, and outer with four marginal spines, both ending in five terminal spines. Uropod 2 shorter than 1, peduncle with six setae and two spines, inner ramus only slightly longer than outer, with two pairs of marginal spines, outer ramus with three marginal spines, both rami terminating in five spines.

Uropod 3 relatively short, 8% body length, peduncle as long as wide, inner ramus short, 0.7 length of peduncle and 0.4 length of outer ramus, with four apical and one subapical spine, outer ramus twice length of peduncle, with three groups of spines on inner and two on outer margin, second segment rudimentary.

Telson broader than long, left lobe with two spines and a single seta, right lobe with one subapical spine and three apical setae and a dorsal seta.

Remarks. - Adult males of *P. dentata* sp. nov. are most easily recognised by a strongly swollen article 4 in antenna 2, the possession of a tooth on article 5 of this antenna, a poorly emarginate coxa 4, a rudimentary second segment on the outer ramus of uropod 3, and the possession of only a single spinule on the dactyls of pereopods 3-

7. In females, the peduncular segments of antenna 2 are not strongly swollen or toothed, and the gnathopods are noticeably smaller than those of the males. Two other *Paramelita* species have 'teeth' on the peduncle of antenna 2, but these species are easily distinguished from *P. dentata* sp. nov. In *P. spinicornis* Barnard, 1927, article 4 of antenna 2 always has a distal, forwardly directed tooth, while article 5 in certain populations terminates in a small tooth. Coxa 4 is distinctly, but shallowly excavate, the outer ramus of uropod 3 has a small, but distinct second segment, and the dactyls of pereopods 3-7 have 4-8 spinules. In *P. odontophora* Stewart & Griffiths, 1991, the peduncle of antenna 2 is extremely elongate, and it is article 4, and not 5, which has a subterminal, posterodistal tooth. In addition, coxa 4 is distinctly excavate, the outer ramus of uropod 3 has a small, but distinct second segment, and the dactyls of pereopods 3-7 have 4-13 spinules.

Paramelita marunguis sp. nov. (figs 9, 10)

Material examined. - Holotype. Male, 10.8 mm, SAM A40224, collected from a tributary of the Burgersbos River on Rhodes Drive, Cape Peninsula (33°59'S, 18°25'E). Paratypes. 22 males, 15 females, SAM A40246, from the same sample as the holotype. Other material. SAM A40221, from a tributary of the Disa River on Rhodes Drive.

Etymology. - From the Latin *mas*, meaning male, and *unguis*, meaning claw, alluding to the claw-like structure of pereopod 3 in adult males.

Male holotype, description. - Body colour white when alive. Head as long as pereon segments 1 and 2 combined, anteroventral margin excavate to accomodate inflated article 1 of antenna 2, eyes white when alive, difficult to discern in preserved material.

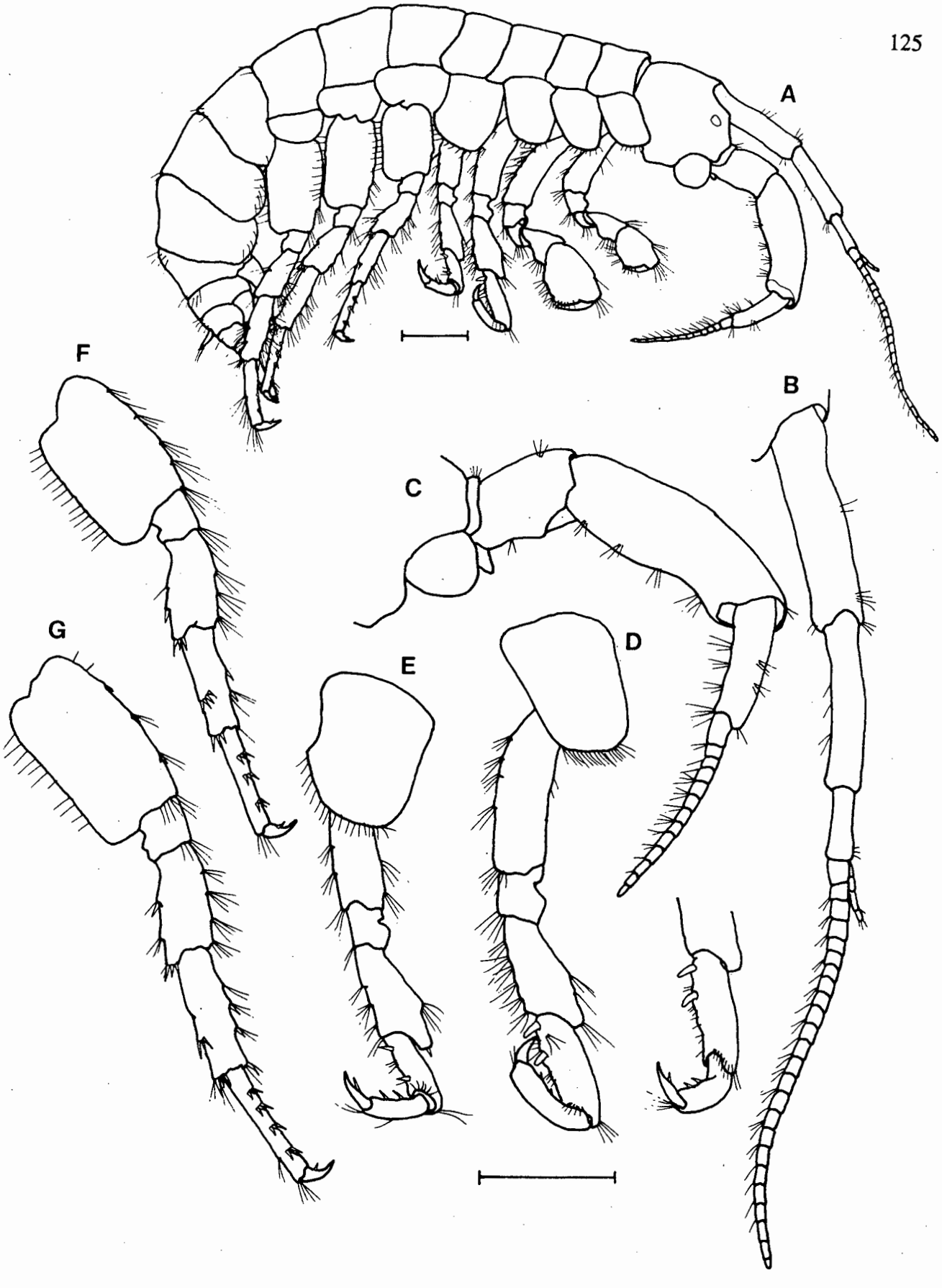

Fig. 9. *Paramelita marunguis* sp. nov., male, 10.8 mm. A. Lateral aspect. B. Antenna 1. C. Antenna 2. D. Pereopod 3 and coxa. E. Pereopod 4 and coxa 4. F. Pereopod 5. G. Pereopod 6. Scale line represents 1 mm.

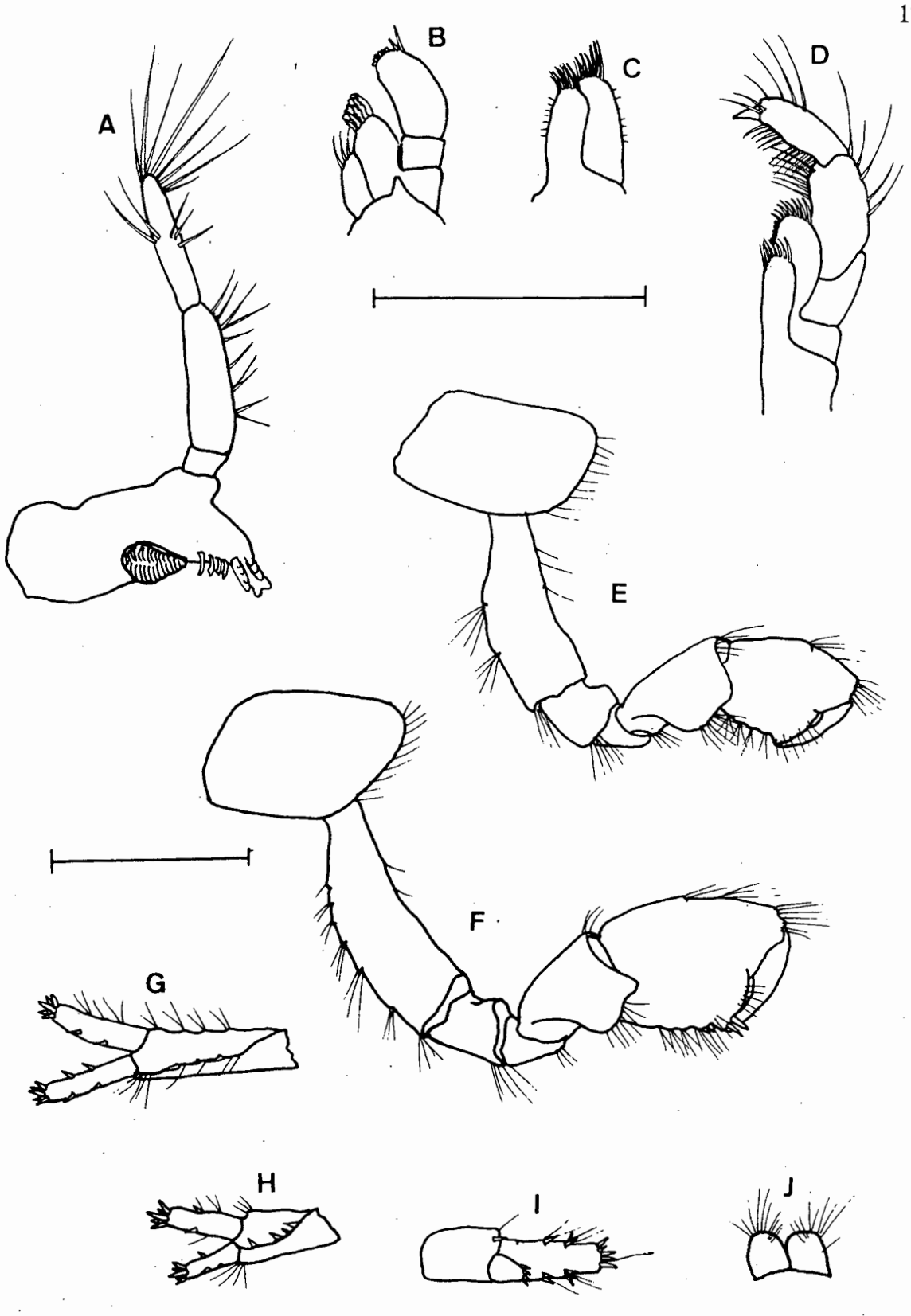

Fig. 10. *Paramelita marunguis* sp. nov., male, 10.8 mm. A. Left mandible. B. Maxilla 1. C. Maxilla 2. D. Maxilliped. E. Gnathopod 1. F. Gnathopod 2. G. Uropod 1. H. Uropod 2. I. Uropod 3. J. Telson. Scale line represents 1 mm.

Antenna 1 0.6 length of body, peduncle sparsely setose, article 1 1.2 length of 2 and 2.8 length of 3, flagellum moderately setose, 0.9 length of peduncle, 24-articulate, accessory flagellum with four articles, reaching past article 3 of primary flagellum.

Antenna 2 0.8 length 0f 1, peduncle sparsely setose, article 3, and particularly 4, strongly laterally swollen, article 4 1.9 length of 3 and 1.8 length of 5, flagellum half length of enlarged, swollen peduncle, moderately setose, 13-articulate.

Left mandible, incisor with five blunt teeth, lacinia mobilis with four blunt teeth, four accessory blades, molar strongly triturative, 3-articulate palp longer than body of mandible, article 1 wider than long, article 2 6.0 length of 1, with 10 setae anteriorly, article 3 similar in length to 2, with eight long apical setae and two tufts of setae half way along length.

Right mandible, incisor with four blunt teeth, lacinia mobilis bifurcate, with two accessory blades.

Maxilla 1, inner plate with five long setae, outer plate terminating in 10 serrate spines, 2-articulate palp with one seta, one spine and six spine-teeth on apex.

Maxilla 2, inner plate shorter than outer, both plates with margins distally pubescent and apices strongly setose.

Maxilliped, inner plate with eight terminal setae, outer plate with about 10 spine-teeth on inner margin and eight curved setae on apex, palp articles 2 and 3 densely setose medially.

Pereon segments dorsally smooth, coxae 1-3 quadrate, strongly setose ventrally, coxa 4 only very slightly emarginate posteriorly, setose ventrally, coxae 5 and 6 longer than deep, bilobed, with a few setae and spinules on ventral margin, coxa 7 semicircular. Segments 2-7 bearing a pair of coxal gills each, segment 2 with one, 3, 4, 5 and 7 with two, and 6 with four sausage-shaped sternal gills.

Gnathopod 1 subchelate, articles 5 and 6 together longer than 2, article 6 10% longer than 5, longer than wide, palm convex, transverse, with three defining spines, dactyl as long as palm.

Gnathopod 2 similar in structure to, but larger than 1, articles 5 and 6 together slightly longer than 2, article 6 1.5 length of 5, longer than wide, palm convex, transverse, with three spines at defining angle and two shorter spines on posterior margin, dactyl as long as palm.

Pereopod 3 moderately to densely setose posteriorly, articles 5 and 6 modified, article 5 with two large teeth on posterior margin, article 6 bent backwards against toothed posterior margin of 5, with two stout spines, dactyl with a single spinule.

Pereopod 4 unmodified, moderately setose, article 5 with two stout spines posteriorly, article 6 with two spines posteriorly, dactyl with a single spinule.

Pereopod 5 moderately setose, article 2 with tufts of short setae anteriorly, lined with longer setae posteriorly, article 4 with two groups of spines, 5 with two groups of spines anteriorly and two posteriorly, 6 with three groups of spines anteriorly, dactyl with a single spinule. Pereopods 6 and 7 similar in structure to, but longer than 5, dactyls each with a single spinule.

Pleon segments 1-3 sparsely setose dorsally, epimeral plates rounded to quadrate, setose ventrally. Pleon segments 4-6 moderately setose dorsally.

Uropod 1, peduncle with 17 setae and three spines, rami subequal, inner ramus with five setae and two marginal spines, outer ramus with four marginal spines, each ramus ending in five spines.

Uropod 2 shorter than 1, peduncle with four setae and four spines, inner ramus longer than outer, with three setae and two spines on margins, outer ramus with three marginal spines, each ramus ending in five spines.

Uropod 3 relatively short, 8% body length, peduncle a little longer than wide, inner ramus short, 0.5 length of peduncle and 0.3 length of outer ramus, with four apical spines, outer ramus 1.5 length of peduncle, with two groups of spines and setae each on inner and outer margins, second segment rudimentary.

Telson broader than long, each lobe with 8-10 apical and subapical setae, lacking spines.

Remarks. - *P. marunguis* sp. nov. is clearly a close relative of *P. crassicornis*, and adult males of this species can be distinguished from those of *P. crassicornis* by the claw-like structure of pereopod 3. Distinguishing the females of these two species is more difficult; females of *P. marunguis* sp. nov. are similar to those of *P. crassicornis*, having antenna 2 shorter and only moderately shorter than 1, and pereopod 3 unmodified.

Paramelita pheronyx sp. nov. (figs 11, 12)

Material examined. - Holotype. Male, 7.3 mm, SAM A40247, collected from a stream draining the slopes of Constantiaberg, near an old disused mine (34°03'S, 18°22'E). Paratypes. 10 males, 10 females, SAM A40248, from the same sample as the holotype.

Etymology. - From the Greek *pherein*, to carry, and *onyx*, a claw or spur, alluding to the long 'spur' on article 4 of pereopod 3 in males.

Male holotype, description. - Body colour white when alive. Head shorter than pereon segments 1 and 2 together, anteroventral margin excavate to accommodate inflated article 1 of antenna 2, eyes white when alive, invisible in preserved material.

Antenna 1 half length of body, peduncle sparsely setose, article 1 1.5 length of 2 and 5.0 length of 3, flagellum sparsely setose, 1.5 length of peduncle, 19-articulate, accessory flagellum 3-articulate, reaching past article 3 of primary flagellum.

Antenna 2 0.8 length of 1, peduncle sparsely setose, article 3 extremely swollen and enlarged, with a large lobe posteriorly, article 4 laterally swollen, with a lobe on medial surface at point of attachment with 3 , 1.4 length of 3 and 1.9 length of 5, flagellum 0.7 length of enlarged, swollen peduncle, sparsely setose, 10-articulate.

Left mandible, incisor with four blunt teeth, lacinia mobilis with three teeth, one accessory blade, molar strongly triturative, 3-articulate palp longer than body of mandible, article 1 longer than wide, article 2 3.0 length of 1, with a few setae

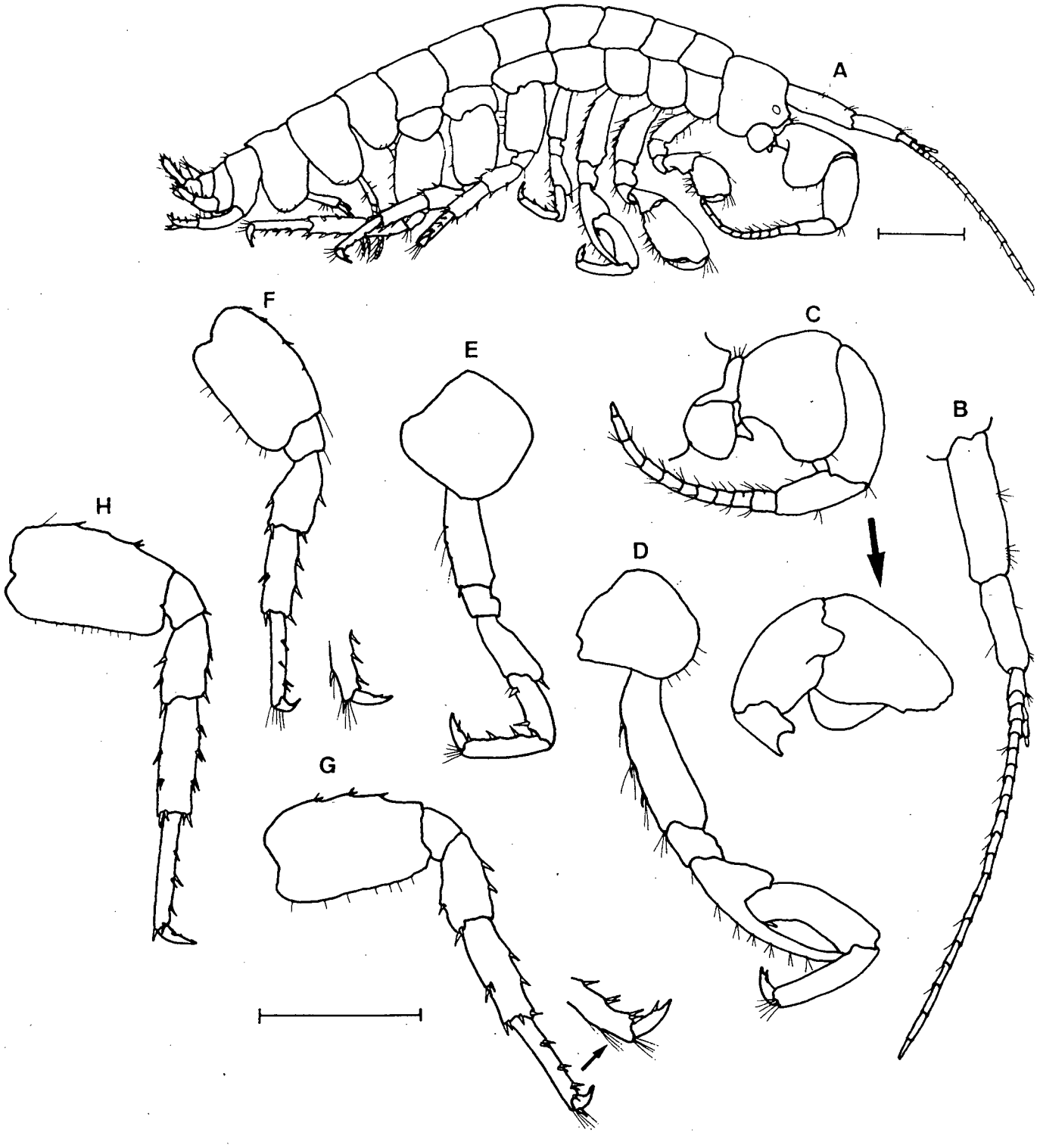

Fig. 11. *Paramelita pheronyx* sp. nov., male, 7.3 mm. A. Lateral aspect. B. Antenna 1. C. Antenna 2, lateral and medial view. D. Pereopod 3 and coxa 3. E. Pereopod 4 and coxa 4. F. Pereopod 5. G. Pereopod 6. H. Pereopod 7. Scale line represents 1 mm.

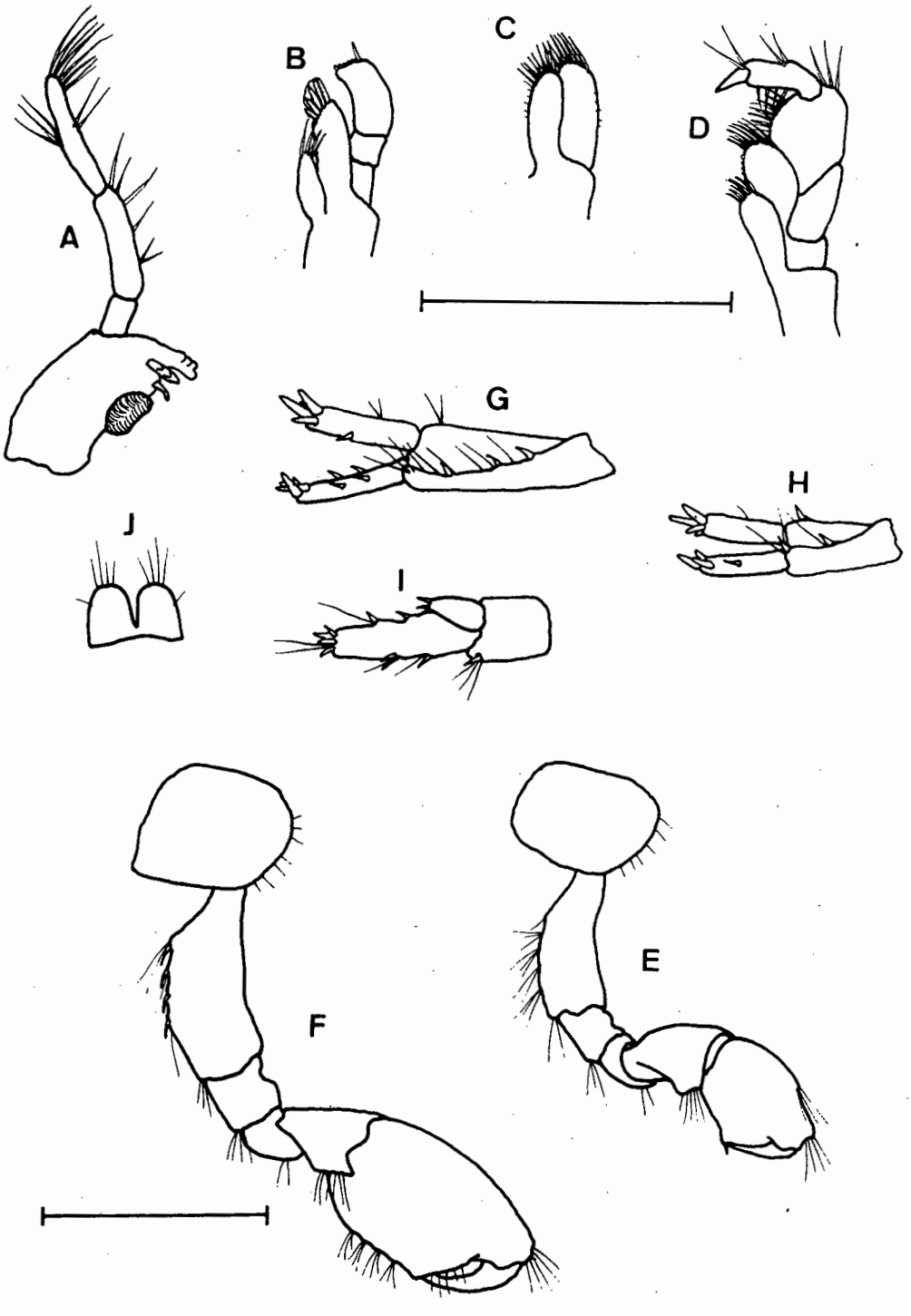

Fig. 12. *Paramelita pheronyx* sp. nov., male, 7.3 mm. A. Left mandible. B. Maxilla 1. C. Maxilla 2. D. Maxilliped. E. Gnathopod 1. F. Gnathopod 2. G. Uropod 1. H. Uropod 2. I. Uropod 3. J. Telson. Scale line represents 1 mm.

anteriorly, article 3 1.2 length of 2, with eight long apical setae and two tufts of setae half way along length.

Incisor of right mandible with four blunt teeth, lacinia mobilis bifurcate, with three accessory blades.

Maxilla 1, inner plate with four setae on apex, outer plate with 10 serrate spines, palp exceeding outer plate, with six spine-teeth and one spine.

Maxilla 2, inner plate shorter and narrower than outer, both plates with margins distally pubescent and apices strongly setose.

Maxilliped, inner plate with seven setae, outer plate with about 10 short, stout setae on inner margin and eight terminal curved setae, palp article 2 and 3 densely setose medially.

Pereon segments dorsally smooth, coxae 1-3 quadrate, setose ventrally, coxa 4 with only a very slight emargination posteriorly, as deep as long, setose ventrally, coxae 5 and 6 longer than deep, bilobed, coxa 7 semicircular. Segments 2-7 bearing a pair of coxal gills each, segment 2 with one, 3, 4, 5 and 7 with two, and 6 with four sausage shaped sternal gills.

Gnathopod 1 subchelate, article 2 sparsely spinose medially, articles 5 and 6 together longer than 2, article 6 1.3 length of 5, longer than wide, palm convex, transverse, with two defining spines, dactyl as long as palm.

Gnathopod 2 similar in structure to, but larger than 1, article 2 strongly spinose on posterior margin, article 5 and 6 together much longer than 2, article 6 1.9 length of 5, longer than wide, palm convex, transverse, with three defining spines, dactyl as long as palm.

Pereopod 3 sparsely to moderately setose, article 2 strongly spinose on posterior margin, articles 4 and 5 highly modified, article 4 short, widening distally, with a long, narrow posterodistal projection, article 5 elongate and enlarged, curved, bearing a stout spine at point of attachment with 4, article 6 lacking spines, dactyl with a single spinule.

Pereopod 4 unmodified, sparsely setose, article 2 with a posterodistal spine, articles 4 and 5 with two groups of spines each, dactyl with a single spinule.

Pereopod 5 sparsely setose, article 2 moderately expanded, article 2 with spinules on anterior margin and a few setae on posterior margin, article 4 with four groups of spines, 5 with three groups of spines anteriorly and two posteriorly, article 6 with four groups of spines anteriorly and a single spine posterodistally, dactyl with one spinule. Pereopods 6 and 7 similar in structure to, but longer than 5, dactyls each with a single spinule.

Pleon segments 1-3 dorsally smooth, epimeral plates rounded, sparsely setose ventrally. Pleon segments 4-6 sparsely setose dorsally.

Uropod 1 of moderate length, peduncle with two setae on medial margin and nine setae and four spines on outer margin, rami subequal, inner ramus with one spine and one seta on margins, outer ramus with four marginal spines, each ending in four terminal spines.

Uropod 2 shorter than 1, peduncle with one spine on medial margin and three setae and two spines on outer margin, rami approximately subequal, inner ramus with a single marginal seta, outer ramus with one marginal spine, each ending in four spines.

Uropod 3 relatively short, 8% body length, peduncle slightly longer than wide, inner ramus short, 0.7 length of peduncle and 0.4 length of outer ramus, with three apical spines, outer ramus twice length of peduncle, with three groups of spines on inner, and two groups on outer margin, second segment rudimentary.

Telson broader than long, each lobe with four apical and one lateral seta, lacking spines.

Remarks. - Females of *P. pheronyx* sp. nov. lack the lobe on antenna 2, and have an unmodified pereopod 3. The only other *Paramelita* species which has both a lobe on article 3 of antenna 2, and a projection on article 4 of pereopod 3 in males is *P. andronyx* Stewart & Griffiths, 1991 described from Kasteelsberg, 75 km north of the Cape Peninsula. These species are also similar in other respects. Coxa 4 in both is

only slightly emarginate posteriorly, the second segment on the outer ramus of uropod 3 is absent or rudimentary, and the dactyls of pereopods 3-7 have a single spinule each. However, *P. pheronyx* sp. nov. and *P. andronyx* can be separated by the differences in form of the projections on article 4. In *P. pheronyx*, article 4 of pereopod 3 is short and posterodistally protruded into a long, narrow 'spur', whereas in *P. andronyx*, article 4 is long, and posterodistally protruded into a triangular shaped lobe.

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PAPER 5

MORPHOLOGICAL AND GENETIC DIFFERENTIATION AMONG POPULATIONS OF THE FRESHWATER AMPHIPOD *PARAMELITA SPINICORNIS* (AMPHIPODA:CRANGONYCTOIDEA), WITH DESCRIPTION OF A NEW SPECIES

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SUMMARY

Morphological and genetic differentiation between populations of freshwater amphipods initially identified as *Paramelita spinicornis* were examined. Morphological data were analysed by means of cluster and discriminant functions analyses, while genetic differentiation was determined using starch gel electrophoresis. The seven populations studied divided into two distinct groups, one of which was clearly recognisable as a new species, and is described here. The other group corresponded to *P. spinicornis* originally decribed by Barnard (1927).

INTRODUCTION

The endemic South African crangonyctoid genus, *Paramelita* has been placed, along with seven other Australian genera, in the family Paramelitidae (Bousfield, 1977; Williams & Barnard, 1988). Twelve species and one variety of this freshwater genus had been recorded from streams in the south-western Cape Province by 1981, when the group was last reviewed (Griffiths, 1981). With the exception of two 'widespread' species, most of these species have restricted distributions. *Paramelita spinicornis* was first described by Barnard (1927), who collected it from localities in the Hottentot Holland Mountains to Swellendam in the south-western Cape. During an extensive

collection programme in 1989-1990, over 100 populations of *Paramelita* were sampled, resulting in the recognition of 11 new species to date (Stewart & Griffiths, 1991a,b,c). Six of these populations were initially identified, using the available key (Griffiths, 1981), as *P. spinicornis*. Although adult males from all these samples possessed either a stout or swollen article 4 with its characteristic 'tooth' in antenna 2, two morphological forms seemed to be evident. Two of the populations consisted of large individuals with distinctly elongated second antennae, while those from the four remaining sites were considerably smaller, and had relatively shorter, strongly swollen second antennae.

It was decided, therefore, to quantify these morphological differences, to determine whether they coincided with genetic differentiation, and to decide if genetic differentiation was sufficient to warrant the recognition of a new species.

Genetic differentiation was examined by means of starch gel electrophoresis. This method has proved useful in past studies in the recognition of sibling species and its use in the study of genetic differentiation in amphipods has gained impetus in recent years, resulting in the resolution of many taxonomic uncertainties, especially within the family Gammaridae. For example, Bulnheim & Scholl (1980) were able to show that the morphologically similar euryhaline amphipods *Gammarus zaddachi* and *G. salinus* were indeed genetically distinct. More recently, Scheepmaker et al (1988) used the method to assess genetic differentiation and the possible existence of subspecies in *G. ibericus* and *G. gauthieri* populations. Scheepmaker & Van Dalfsen (1989) used gel electrophoresis to assess the taxonomic status of *G. fossarum* and *G. caparti*, while Lop & Oliver (1989) used allozyme data to sort out taxonomic problems amongst four species of the *Echinogammarus berilloni*-group, and Scheepmaker (1990) attempted to clear up some of the confusion which exists regarding the systematic position of species in the *G. pulex*-group with electrophoretic data.

MATERIALS AND METHODS

a) Collection.- Populations of *P. spinicornis* were collected from six streams in the south-western Cape between February 1989 and September 1990 (fig. 1). All of the streams were shallow, and within two to three kilometers of the coast, with the exception of the Orchard stream site near Grabouw. The amphipods were collected using small fine-mesh hand nets. They were transported back to the laboratory in closed plastic bottles with a layer of detritus from the stream bed, and were held in a 12°C coldroom. A selection of adult male individuals from each sample was preserved in alcohol for morphological analysis, and at least 30 individuals from each locality were freshly frozen at -70°C until needed for gel electrophoresis. No live specimens were collected from Fernkloof (fig. 1), consequently, this population was not included in electrophoresis.

Additional preserved material of *P. spinicornis* was obtained from the South African Museum. The localities from which these samples were collected are also shown in fig. 1. Of all the preserved material examined, only one sample (SAM A6053) from the Riviersonderend mountains, possessed enough adult males to be included in the morphological analysis.

b) Morphological analysis.- Five adult males were chosen from each population (only three individuals from Palmiet) for the morphometric analysis. The largest males were selected to minimise intrapopulation variation. Males exhibited the swollen article 4 of antenna 2 with its tooth diagnostic of *P. spinicornis*. Each animal was partially dissected, and 42 measurements were taken using a stereo microscope and an eyepiece micrometer.

Three approaches were adopted to analyse the morphological data. In preliminary analyses of the means of each measurement for each population, performed with the aid of the computer progamme NTSYS-pc (Rohlf, 1988), the raw measurements were standardised by subtracting the mean of each variable from each datum point. Taxonomic distance coefficients were then computed from this standardised matrix. A dendrogram was constructed using the unweighted pair group method (UPGMA) based

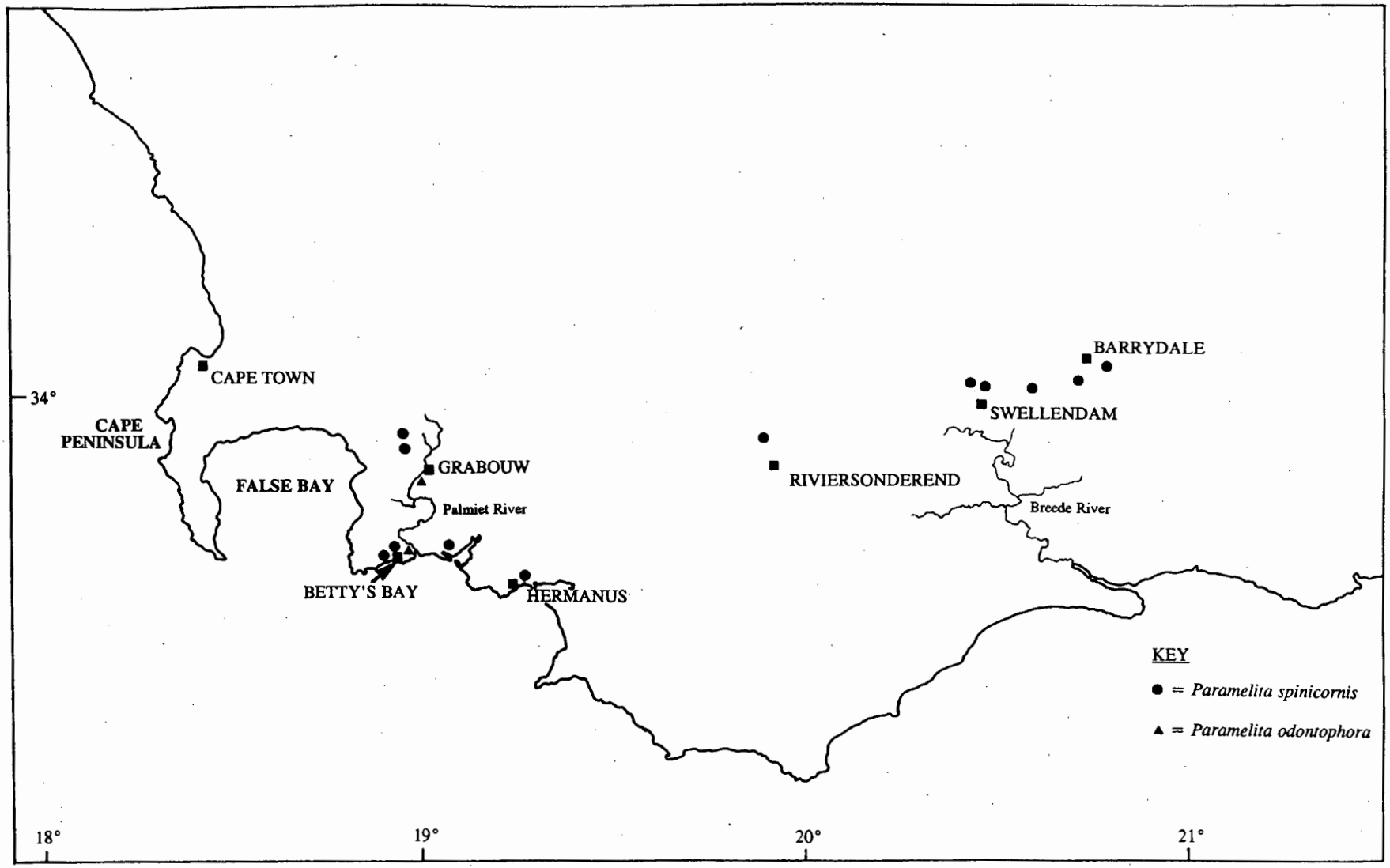

Fig. 1. Map of the south-western Cape showing the localities where *Paramelita spinicornis* specimens have been collected.

on these coefficients (Sneath & Sokal, 1973). The advantage of this method was that all information contained in each measurement was retained; the disadvantage was the inevitable influence of body size that ranged from 5.3 mm (Betty's Bay) to 9.0 mm (Palmiet).

In a second approach, intended to eliminate the influence of body size, the raw measurements were converted to ratios by dividing the length of each segment measured by its width (Table I). These ratios were then plotted against body size to yield scatterplots, and breaks in distribution of the points were taken to represent changes in character states. Abbott et al. (1985) have recommended that this "natural" method of determining character states is superior to dividing the range into an arbitrary number of equal divisions. The clusters of points were coded, and these codes formed the working characters from which similarity coefficients could be calculated. Qualitative features such as setation and the presence of a spine or lobe were similarly coded. Simple Matching Coefficients (Sneath & Sokal, 1973) were calculated by dividing the number of matches between two populations by the total number of characters. From these coefficients a dendrogram using UPGMA was constructed.

In a third approach, the raw measurements for all specimens were analysed by means of a stepwise discriminant functions analysis. The 42 measurements from each individual were log transformed, and these data analysed by means of the 7M programme in the BMDP Statistical Package. All of the specimens were grouped according to the clusters formed from the analysis of the genetic data.

c) Electrophoretic analysis.- Whole animals were homogenised in Tris 0.1 M (pH 8.0) buffer. The ratio of fresh weight to buffer was approximately 1:1. The homogenate was centrifuged in a microcentrifuge at 2500 g for about 10 minutes. Filter paper wicks were dipped into the supernatant and inserted into horizontal starch gels (13% hydrolysed potato starch). To enable interpopulation comparisons some individuals from each population were interspersed across each gel. In addition, *Paramelita capensis* (Barnard, 1916) individuals from a population found on the Cape Peninsula were used as a reference population. The gels were run at a constant current

TABLE I

A list of ratios used in the calculation of Simple Matching Coefficients

Limb	Ratio
Antenna 1	article 1 length/width
	article 2 length/width
	article 3 length/width
Antenna 2	article 3 length/width
	article 4 length/width
	article 5 length/width
Gnathopod 1	article 5 length/width
	article 6 length/width
Gnathopod 2	article 5 length/width
	article 6 length/width
Pereopod 3	article 2 length/width
	article 4 length/width
	article 5 length/width
Pereopod 4	article 2 length/width
	article 4 length/width
	article 5 length/width
Pereopod 5	article 2 length/width
Pereopod 6	article 2 length/width
Pereopod 7	article 2 length/width

of 50 mA for 2.5 hours (buffer 1) and 3.5 hours (buffers 2 and 3) at 4°C. The following buffer systems were used:

(1) a discontinous tris-citrate-borate-lithium hydroxide buffer system; gel buffer pH 8.7, electrode buffer pH 8.0 (Ridgeway et al., 1970),

(2) a continuous citrate-(N-(3-aminopropyl)-morpholine) buffer system; gel and electrode buffer pH 6.1 (Clayton & Tretiak, 1972),

(3) a continuous tris-borate-EDTA buffer system; gel and electrode buffer pH 8.6 (Markert & Faulhaber, 1965).

After electrophoresis, the gels were sliced into four or five slices and stained at room temperature. Both agar overlays and liquid baths were used following the procedures of Shaw & Prasad (1970) and Harris & Hopkinson (1976). In total, 15 loci were scored from 14 enzyme assays (Table II). In scoring the enzymes, the main allele for each locus of the reference population was assigned the value of 100, and the electrophoretic mobility of all other alleles scored relative to this value. When more than one locus occurred, the faster travelling locus was numbered 1.

To assess the genetic variation within and between the populations, several parameters were calculated using the BIOSYS-1 program of Swofford & Selander (1989). Allele and genotype frequencies were computed at each locus. Deviations from Hardy-Weinberg equilibrium was investigated by comparing the observed heterozygosity at each locus with that expected under Hardy-Weinberg conditions. Significant differences in allele frequencies were investigated between populations by means of a contingency table analysis. Genetic differentiation was also investigated by calculating Nei's (1978) index of unbiased genetic identity. From these data, a cluster analysis using UPGMA was performed. The percentage of loci that were polymorphic was calculated using the 95% criterion, that is, where the most common allele is less than 0.95. This criterion was chosen due to the small sample sizes in this study.

TABLE II

Enzymes investigated and buffer systems used

Enzyme	Abbreviation	E.C. Number	Buffer
Aldehyde oxidase	AO	1.2.3.1	3
Arginine phosphate kinase	ARK	2.7.3.3	1
Diaphorase	DIA	1.6.2.2	1
Esterase	EST	3.1.1.1	3
Glyceraldehyde phosphate dehydrogenase	GAP	1.2.1.12	2
Peptidase (Glycyl leucine as substrate)	GL	1.1.1.47	3
Glutamate oxalacetate transaminase	GOT	2.6.1.1	2
Glucose phosphate isomerase	GPI	5.3.1.9	3
Glutamate pyruvate transaminase	GPT	2.6.1.2	3
Hexokinase	HEX	2.7.1.1	3
Leucine amino peptidase	LAP	3.4.11.-	3
Peptidase (Leucyl tyrosine as substrate)	LT	3.4.11.-	1
Mannose phosphate isomerase	MPI	5.3.1.8	3
Phosphoglucomutase	PGM	2.7.5.1	3

RESULTS AND DISCUSSION

a) Morphology.- The seven populations examined in the morphological study grouped together into two distinct clusters in the analysis of both the 'raw' measurements (fig. 2a) and of the ratios (fig. 2b). Thus, two groups could be clearly distinguished in terms of both size and shape. Amphipods from the cluster consisting of the Betty's Bay, Harold Porter, Lamloch, Fernkloof and Riviersonderend populations were easily identified by several features. These included the possession of a lobe on the medial ventral margin, and a lobe and forwardly directed tooth on the terminal end of article 4, the presence of a triangular projection at the end of article 5, and a relatively short article 3 of antenna 2 (fig.4). In addition, article 3 of antenna 2 was strongly swollen, and article 2 of pereopods 5-7 relatively wide (fig.3). The classification function that best discriminated this group from the Orchard and Palmiet Valley individuals was calculated by means of discriminant functions analysis and was as follows: y = 14.87 (antenna 2, article 4 width) + 166.65 (gnathopod 2, article 6 length) + 125.00 (antenna 2, article 3 length) - 21.41. All of the individuals in this group were reassigned to this group with a posterior probability of 1.000,

Individuals from Orchard and Palmiet lacked proximal ventral lobes on article 4 of the peduncle of antenna 2; a terminal 'spine' on article 5 of antenna 2 was similarly absent. The 'tooth' on article 4 of the second antennae in these animals was always subterminal, and was usually about 0.2 mm from the end of this article (Fig. 4). In addition, article 5 of antenna 2 was 'trumpet-shaped', rather than rectangular and not as strongly swollen in this group, article 3 of antenna 2 was relatively long, and article 2 in pereopods 5-7 was relatively long and slender in comparison with those in the other *P. spinicornis* populations (fig. 3). The classification function that best discriminated these specimens from those in the other main cluster was as follows: y = 308.17 (gnathopod 2, article 6) - 175.34 (antenna 2, article 4 width) + 543.40 (antenna 2, article 3 length) - 83.33. Again, all the specimens were correctly reassigned to their original group.

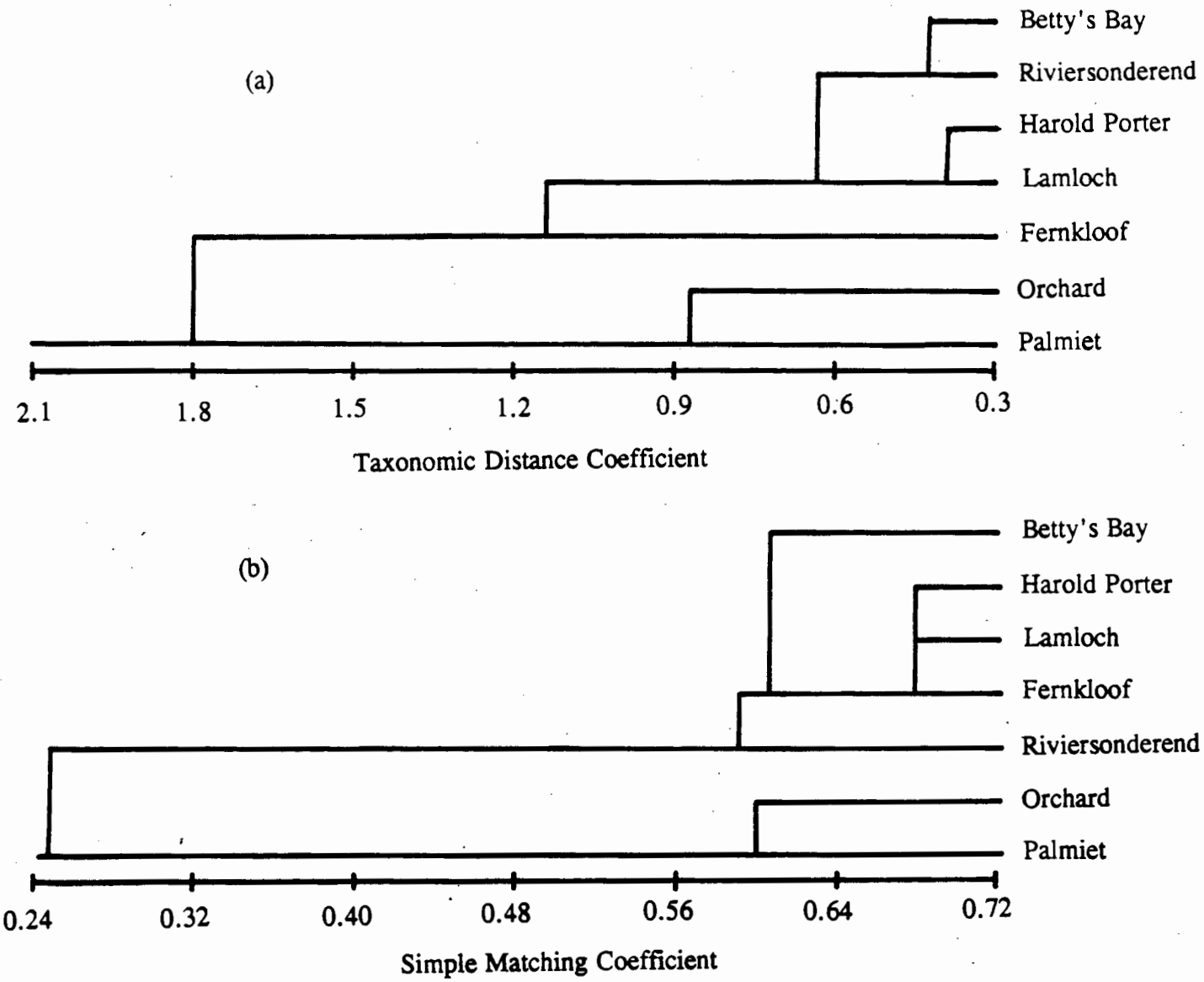

Fig. 2. (a) Dendrogram generated using a matrix of Taxonomic Distance Coefficients and the UPGMA cluster algorithm. (b) Dendrogram from a matrix of Simple Matching Coefficients and UPGMA.

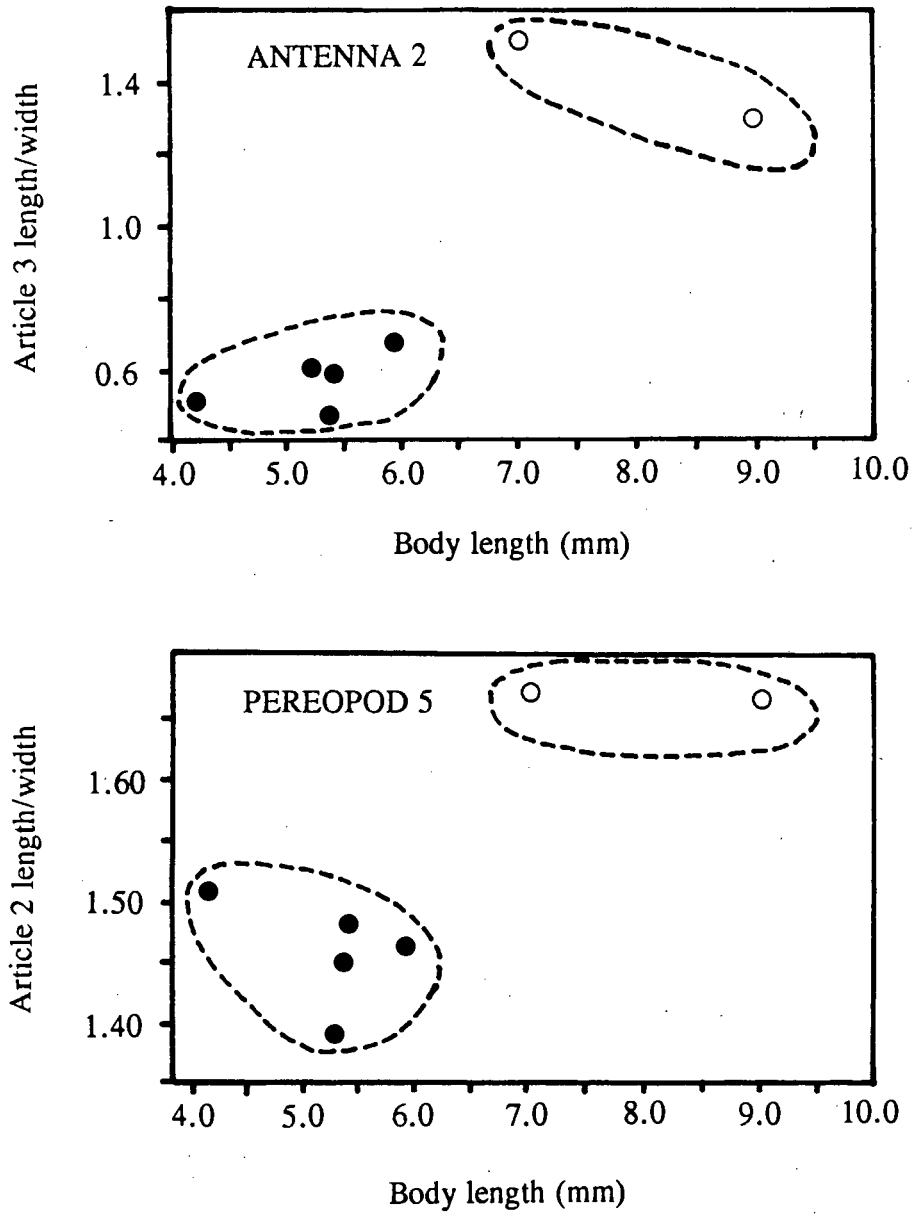

Fig. 3. Scatterplots of body length versus article 3 length/width of antenna 2 and article 2 length/width of pereopod 5 for seven populations. Open circles, Orchard and Palmiet. Solid circles, Betty's Bay, Riviersonderend, Harold Porter, Fernkloof and Lamloch.

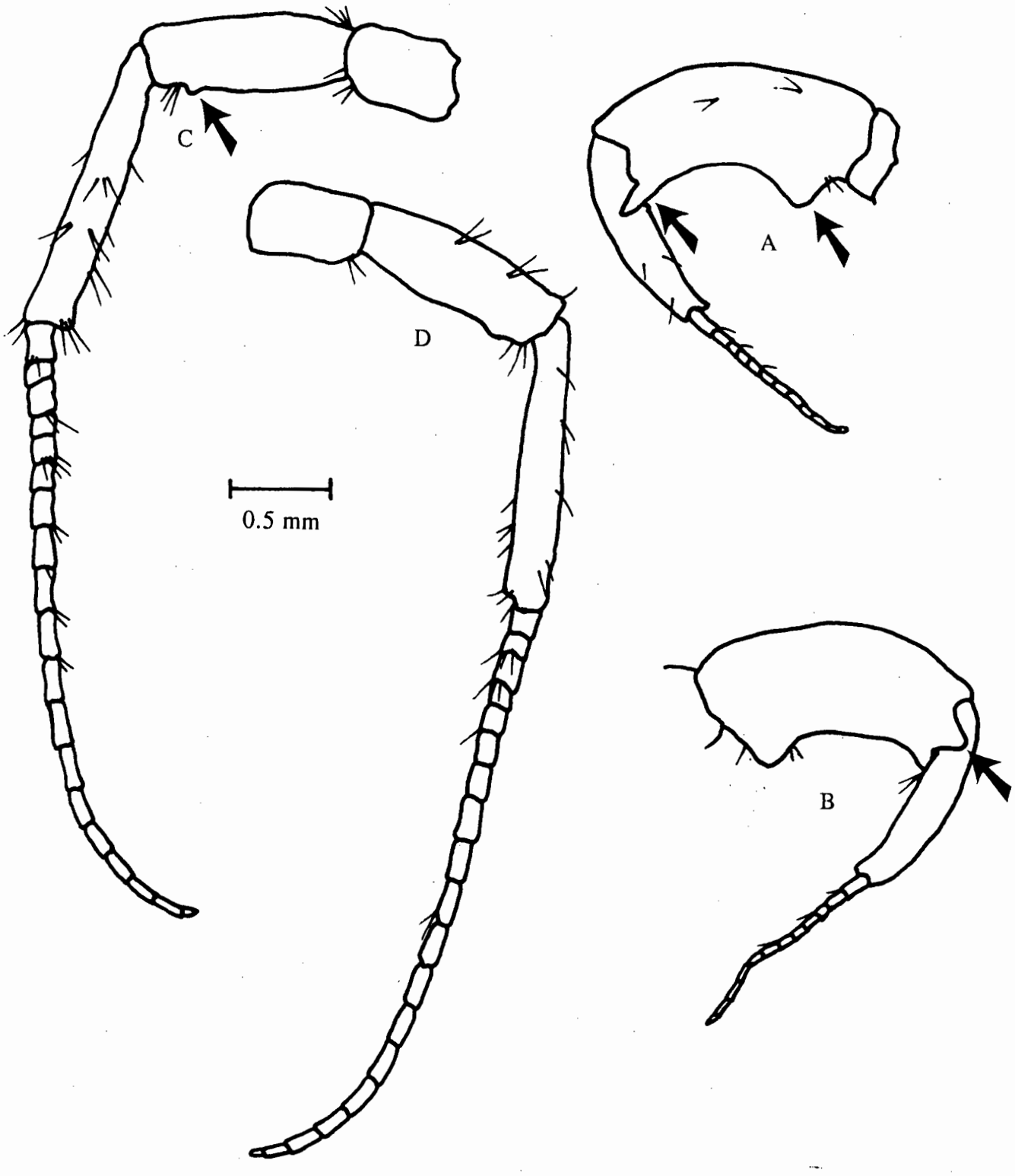

Fig. 4. Articles 3, 4 and 5 of peduncle of antenna 2 in specimens from (A) Betty's Bay, lateral view, (B) medial view, and (C) Orchard, lateral view, (D) medial view.

b) Electrophoretic analysis.- The allele frequencies for the 14 polymorphic loci are given in Table III. The AO_I locus was monomorphic for all populations. In the polymorphic loci, the total number of alleles encountered ranged from two in ARK, DIA and EST, to as many as eight in PGM. Within any one population, the maximum number of alleles per locus was four for GPI in Betty's Bay, GPT in Harold Porter and MPI and PGM in Orchards. The mean number of alleles per locus varied from 1.67 (Harold Porter and Lamloch) to 2.00 (Palmiet), and only PGM was consistently polymorphic for all populations. Most of the loci exhibited low levels of polymorphism, and alleles such as AO_{II}^{100}, AO_{II}^{127}, ARK^{74}, DIA^{100}, EST^{96}, GAP^{100}, GL^{95}, GL^{100}, GOT^{103}, HEX^{103}, GPT^{125} and LAP^{103} were fixed in at least one to four of the five populations.

Loci showing greater polymorphism and heterogeneity of frequencies were the GL, GPI, GPT, MPI and PGM loci. The allele GL^{100} varied in frequency from 0.050 (Harold Porter) to 1.000 (Orchard). The Betty's Bay population was monomorphic for the GL^{95} allele, which was also the most common allele in the Harold Porter population. There was similar variation at the GPI locus. The Betty's Bay, Harold Porter and Lamloch populations had the GPI^{35} allele at a high frequency, while GPI^{38} was the common allele at Orchard and Palmiet. The allele GPT^{125} occurred at highest frequency in four of the populations; GPT^{100} was most common in the Harold Porter population (0.750). Other rare alleles in this population were GPT^{167} and GPT^{42}, also shared by the Orchard and Palmiet populations respectively. The MPI locus was highly polymorphic. In the Harold Porter, Palmiet and Lamloch populations, the most common allele occurred at high frequencies of 1.000 (MPI^{105}), 0.800 (MPI^{99}) and 0.900 (MPI^{105}) respectively. Amongst the Betty's Bay and Orchard populations, five alleles occurred at varying frequencies; only MPI^{105} and MPI^{99} were common between them. At the PGM locus, eight alleles were noted. In the Betty's Bay and Harold Porter populations, PGM^{86} was the most common. The allele PGM^{100} occurred at highest frequency in the Orchard population and PGM^{89} was most common in the Palmiet and Lamloch populations. Contingency table analysis (Table IV) showed

TABLE III

Allele frequencies at each locus for all populations; N=sample size, H=heterozygosity per locus.

POPULATION:		BETTY'S BAY	ORCHARD	HAROLD PORTER	PALMIET	LAMLOCH
LOCUS:						
AO1	100	1.000	1.000	1.000	1.000	1.000
	N	10	10	10	4	6
	H	0.000	0.000	0.000	0.000	0.000
AO2	127	0.000	1.000	1.000	0.733	1.000
	100	1.000	0.000	0.000	0.000	0.000
	45	0.000	0.000	0.000	0.267	0.000
	N	19	20	15	15	12
	H	0.000	0.000	0.000	0.391	0.000
ARK	74	0.975	1.000	1.000	1.000	1.000
	62	0.025	0.000	0.000	0.000	0.000
	N	20	20	20	15	12
	H	0.049	0.000	0.000	0.000	0.000
DIA2	107	0.000	0.000	0.000	0.267	0.389
	100	1.000	1.000	1.000	0.733	0.611
	N	20	20	20	15	9
	H	0.000	0.000	0.000	0.391	0.475
EST	106	0.000	0.000	0.000	0.067	0.167
	96	1.000	1.000	1.000	0.933	0.833
	N	20	20	20	15	12
	H	0.000	0.000	0.000	0.124	0.278
GAP	107	0.000	0.000	0.275	0.167	0.000
	100	1.000	1.000	0.725	0.833	0.958
	97	0.000	0.000	0.000	0.000	0.042
	N	15	15	20	15	12
	H	0.000	0.000	0.399	0.278	0.080
GL	116	0.000	0.000	0.000	0.000	0.708
	105	0.000	0.000	0.000	0.100	0.000
	100	0.000	1.000	0.050	0.700	0.000
	95	1.000	0.000	0.950	0.000	0.000
	88	0.000	0.000	0.000	0.200	0.292
	N	15	20	20	15	12
	H	0.000	0.000	0.090	0.460	0.410
GOT	125	0.075	0.000	0.000	0.000	0.000
	103	0.925	0.950	1.000	0.900	1.000
	100	0.000	0.000	0.000	0.100	0.000
	81	0.000	0.050	0.000	0.000	0.000
	N	20	20	20	15	12
	H	0.139	0.095	0.000	0.180	0.000
GPI	70	0.150	0.000	0.000	0.000	0.000
	35	0.675	0.000	0.575	0.067	0.833
	32	0.000	0.000	0.225	0.000	0.000
	28	0.000	1.000	0.000	0.900	0.000
	18	0.025	0.000	0.000	0.000	0.000
	11	0.000	0.000	0.000	0.033	0.167
	0	0.150	0.000	0.200	0.000	0.000
	N	20	18	20	15	12
	H	0.499	0.000	0.579	0.184	0.278
GPT	167	0.000	0.118	0.050	0.000	0.000
	125	1.000	0.882	0.150	0.800	1.000
	100	0.000	0.000	0.750	0.000	0.000
	42	0.000	0.000	0.050	0.200	0.000
	N	20	17	20	15	8
	H	0.000	0.208	0.410	0.320	0.000
HEX	103	0.875	0.925	1.000	0.900	0.875
	100	0.000	0.050	0.000	0.100	0.125
	95	0.125	0.025	0.000	0.000	0.000
	N	20	20	20	15	12
	H	0.219	0.000	0.000	0.180	0.219
LAP	104	0.000	0.000	0.050	0.000	0.000
	103	0.900	0.950	0.900	0.867	1.000
	100	0.100	0.050	0.050	0.133	0.000
	N	20	20	20	15	9
	H	0.180	0.185	0.185	0.231	0.000
LT1	119	0.100	0.000	0.000	0.000	0.000
	106	0.000	0.000	0.000	0.000	0.833
	104	0.250	0.053	0.000	0.000	0.000
	100	0.650	0.947	1.000	0.733	0.000
	90	0.000	0.000	0.000	0.267	0.167
	N	20	19	20	15	12
	H	0.505	0.100	0.000	0.391	0.278
MPI	115	0.000	0.000	0.000	0.000	0.100
	111	0.000	0.342	0.000	0.000	0.000
	109	0.158	0.000	0.000	0.000	0.000
	105	0.474	0.342	1.000	0.200	0.900
	99	0.368	0.079	0.000	0.800	0.000
	89	0.000	0.237	0.000	0.000	0.000
	N	19	19	15	15	10
	H	0.615	0.000	0.000	0.320	0.180
PGM	110	0.000	0.000	0.000	0.033	0.000
	103	0.000	0.025	0.000	0.000	0.000
	100	0.000	0.875	0.000	0.000	0.000
	93	0.000	0.000	0.025	0.000	0.125
	89	0.000	0.050	0.000	0.967	0.583
	86	0.850	0.000	0.975	0.000	0.292
	60	0.000	0.050	0.000	0.000	0.000
	54	0.150	0.000	0.000	0.000	0.000
	N	20	20	20	15	12
	H	0.255	0.299	0.049	0.064	0.559

TABLE IV

Contingency chi-square analysis at each locus; D.F. =degrees of freedom.

LOCUS	*CHI-SQUARE*	*D.F.*	*P*
AO2	*197.01*	*8*	*0.000*
ARK	*3.37*	*4*	*0.498*
DIA	*43.24*	*4*	*0.000*
EST	*17.81*	*4*	*0.001*
GAP	*28.83*	*18*	*0.000*
GL	*311.53*	*16*	*0.000*
GOT	*31.42*	*12*	*0.002*
GPI	*228.80*	*24*	*0.000*
GPT	*142.69*	*12*	*0.000*
HEX	*22.14*	*8*	*0.005*
LAP	*10.56*	*8*	*0.228*
LT1	*214.62*	*16*	*0.000*
MPI	*178.93*	*20*	*0.000*
PGM	*330.96*	*28*	*0.000*
TOTAL	*1761.91*	*172*	*0.000*

that there were highly significant differences in allele frequencies between the five populations (chi-square, $p < 0.001$) at all but two of the loci (ARK and LAP).

Certain alleles were diagnostic of the populations in which they occurred. For example, AO_{II}^{45}, GOT^{100} and GL^{105} occurred only in Palmiet individuals, usually at low frequencies. Although LT_I^{106} and GL^{116} were common, and GAP^{97}, rare, in the Lamloch population, they were absent in the other populations. The diagnostic alleles, ARK^{62}, GPI^{18}, GOT^{125}, LT^{119}, MPI^{109} and PGM^{54} occurred at low frequencies in the Betty's Bay population, whilst LAP^{104}, and the more common GPT^{100} were characteristic of the specimens from Harold Porter. Amphipods from Orchards did not share the alleles PGM^{103}, PGM^{100}, PGM^{60}, MPI^{89}, MPI^{111} and GOT^{81} with the other populations.

Based on the allele frequency data, genetic identities between each pair of populations were calculated (Table V), and a dendrogram constructed (Fig. 5). The five populations separated into two distinct groups, and this result was consistent with the cluster analysis of the morphological data. The group consisting of the Orchard and Palmiet individuals split off from the rest (Betty's Bay, Harold Porter, Riviersonderend and Lamloch) at a genetic identity value of 0.73. The Lamloch population separated from the rest of its group at a relatively low similarity coefficient (0.76), compared with the similarity between the other populations. Two diagnostic alleles contributed to the formation of these two groups. The allele GPI^{28}, which occurred at high frequencies in Orchard (frequency of 1.000) and Palmiet (0.900) were absent in the other populations. Similarly, Orchard was monomorphic for GL^{100}, and this allele occurred at a frequency of 0.700 in specimens from Palmiet. This allele was not present in two of the other populations, and occurred at a very low frequency (0.050) in the third population.

Heterozygosity at each locus, as calculated from the allele frequencies (Table 3), varied greatly between loci and between populations. No apparent pattern was noticeable. Overall heterozygosity was low. In only a few isolated cases was heterozygosity greater than 0.500. However, three of the populations (Betty's Bay, Palmiet and Lamloch) were heterozygous to varying degrees at more than 50% of the

TABLE V

Similarity matrix using Nei's index of genetic similarity.

	BETTY'S BAY	*ORCHARD*	*HAROLD POR*	*PALMIET*	*LAMLOCH*
BETTY'S BAY	*1.000*				
ORCHARD	*0.714*	*1.000*			
HAROLD PORTER	*0.839*	*0.746*	*1.000*		
PALMIET	*0.722*	*0.887*	*0.715*	*1.000*	
LAMLOCH	*0.749*	*0.733*	*0.768*	*0.780*	*1.000*

TABLE VI

Percentage polymorphism and mean heterozygosity over all the loci, for each population.

POPULATION	*% POLYMORPHISM*	*MEAN HETEROZYGOSITY*
Betty's Bay	*47*	*0.164*
Orchard	*47*	*0.105*
Harold Porter	*33*	*0.114*
Palmiet	*80*	*0.234*
Lamloch	*67*	*0.183*

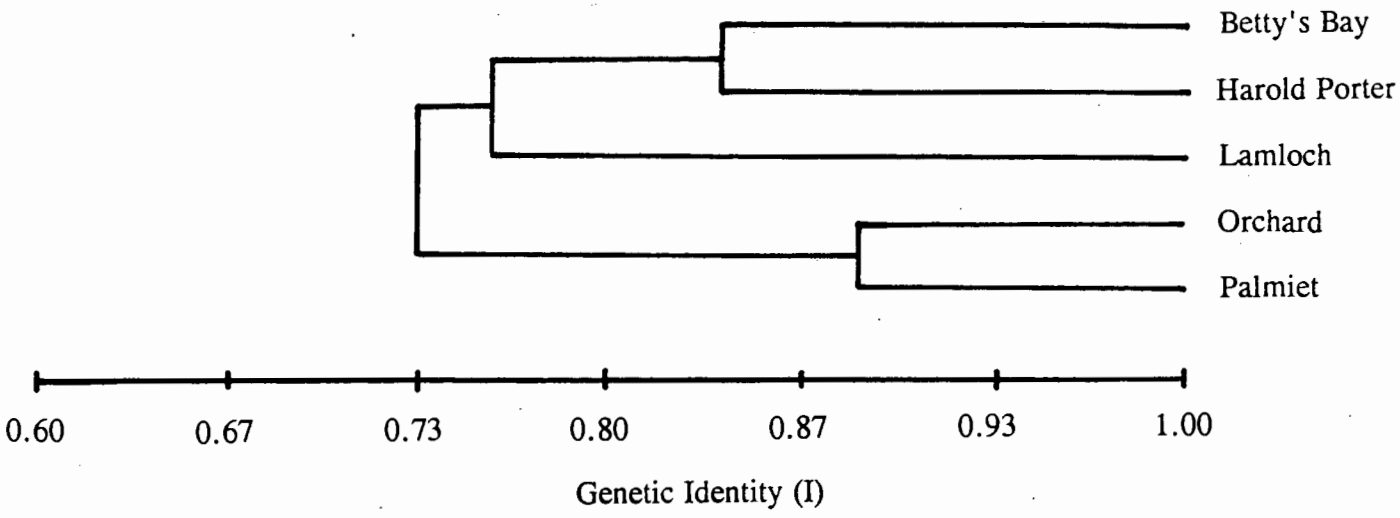

Fig. 5. Dendrogram generated from a matrix of Nei's (1978) Genetic Identities and the UPGMA cluster algorithm.

loci. At a few of the loci, most populations had intermediate or low levels of heterozygosity (i.e. the GPI, LAP, LT_I and PGM loci). The percentage of loci that were polymorphic (Table VI) also showed no clear geographic variation. The Palmiet and Lamloch populations had high percentages of polymorphism, and this corresponded to high mean heterozygosities.

It is thus apparent from both the morphological and electrophoretic data that the seven populations investigated were clearly divided into two distinct groups. Morphological differences were not only as a result of differences in body size - the specimens could be distinguished easily by shape and by the presence or absence of lobes and 'teeth' on the second antennae. Although not as marked as the morphological differences, distinct genetic differentiation also existed between the two main groups. Two 'diagnostic' alleles, GPI^{128} and GL^{100}, were characteristic of the Orchard and Palmiet Valley specimens, and were either absent, or occurred at a very low frequency in the other populations, thus indicating either little, or no gene flow between the two main clusters. In addition, significant differences in allele frequencies existed for several enzymes between the populations.

Thorpe (1982) has concluded that I values distinguishing between species usually exceeds 0.35, and that within species (i.e. between conspecific populations), 98% of I values exceed 0.85. It would, therefore, not be unreasonable to assume that the two main clusters in our study represents two morphological and genetically distinct species, separated at an I value of 0.73.

A reexamination of the *P. spinicornis* holotype and paratypes (Barnard, 1927) revealed that specimens from Betty's Bay, Harold Porter, Lamloch, Fernkloof and Riviersonderend are representatives of the described species *P. spinicornis*. The individuals collected from Palmiet Valley and Orchard therefore constitute members of a new undescribed species, the description of which follows.

DESCRIPTION OF NEW SPECIES

Paramelita odontophora sp. nov. (figs 6, 7)

Material examined. - Holotype. Male, 11.1 mm, SAM A40240, from a tributary of the Palmiet River near Elgin (34°09'S, 19°01'E). Paratypes. 20 males, 15 females, SAM A40241, from the same locality as the holotype. Other material. SAM A40250, from another tributary of the Palmiet River near Kleinmond.

Etymology. - From the Greek, *odontophoros*, meaning tooth-bearing, an allusion to the tooth on article 4 of antenna 2.

Male holotype, description. - Body colour when alive grey-brown. Head as long as pereon segments 1 and 2 combined, anteroventral margin excavate to accomodate inflated article 1 of antenna 2, eyes glistening white when alive.

Antenna 1 0.8 times body length, setation sparse, articles 1 and 2 of peduncle subequal, each approximately twice length of article 3, flagellum 2.3 times length of peduncle, 36-articulate, accessory flagellum 5-articulate, reaching to article 4 of primary flagellum. Antenna 2 elongate, as long as but stouter than 1, peduncle moderately setose, article 4 twice length of 3, with a tooth ventrally near distal end, article 5 1.2 times length of 4, widening distally, flagellum 1.3 times length of peduncle, 22-articulate, sparsely setose.

Left mandible with incisor bluntly 3-toothed, lacinia mobilis with four blunt teeth, four spinose flattened accessory blades, molar strongly triturative, 3-articulate palp much longer than body of mandible, article 1 slightly longer than wide, article 2 3.7 times length of 1, with approximately 13 setae anteriorly, article 3 0.9 times length of article 2, distal half lined with many short setae, six long apical setae present, with two tufts of setae approximately half way along length. Incisor of right mandible 3-toothed, lacinia mobilis bifurcate, one long and two shorter accessory blades.

Inner plate of maxilla 1 setose terminally, outer plate bearing two terminal rows each of about five stout serrate spines, palp exceeding outer plate, with seven stout

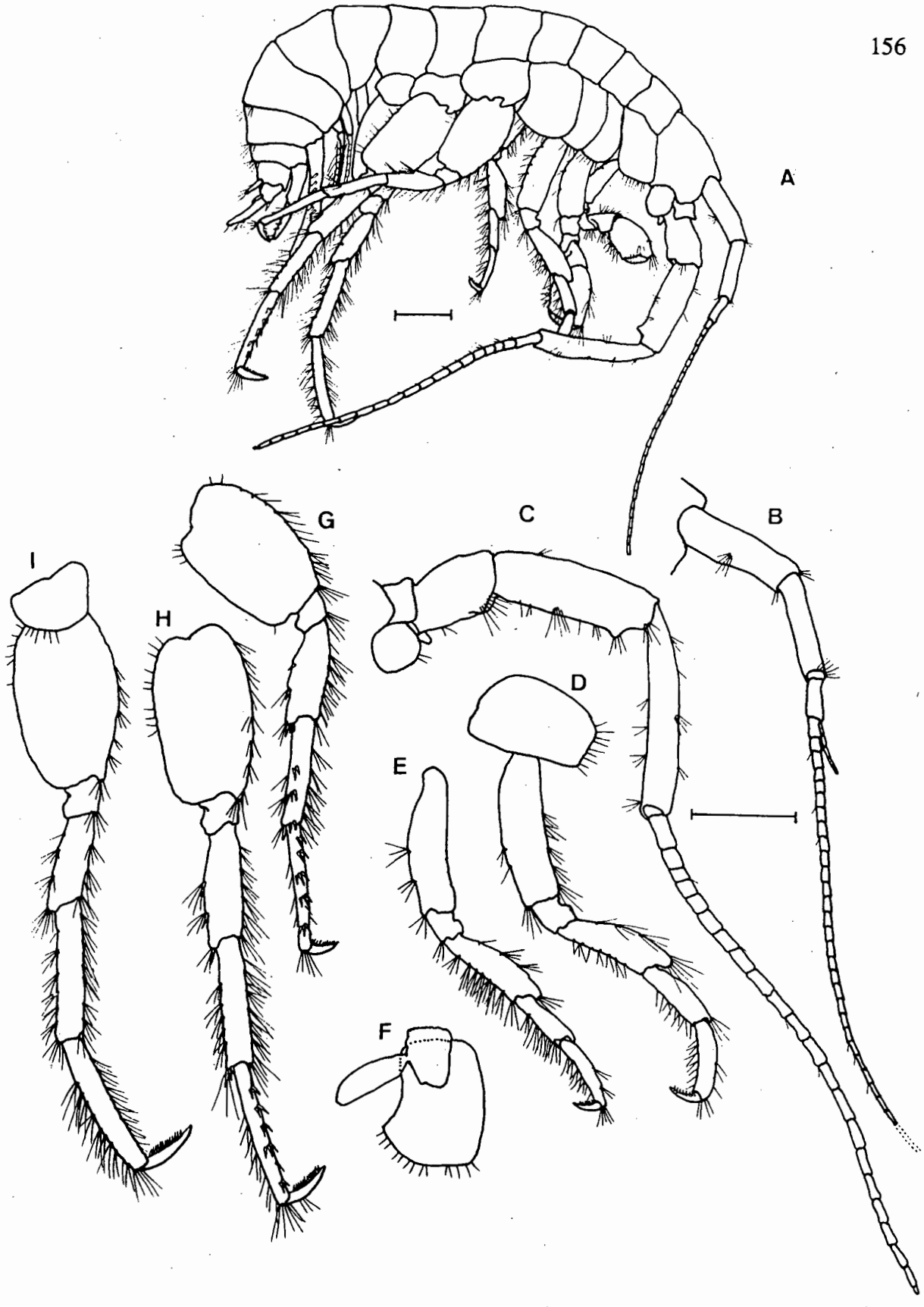

Fig. 6. *Paramelita odontophora* n. sp., male, 11.1 mm. (A) Lateral aspect, (B) Antenna 1, (C) Antenna 2, (D) Coxa 3 and pereopod 3, (E) Pereopod 4, (F) Coxa 4, (G) Pereopod 5, (H) Pereopod 6 and (I) Pereopod 7.

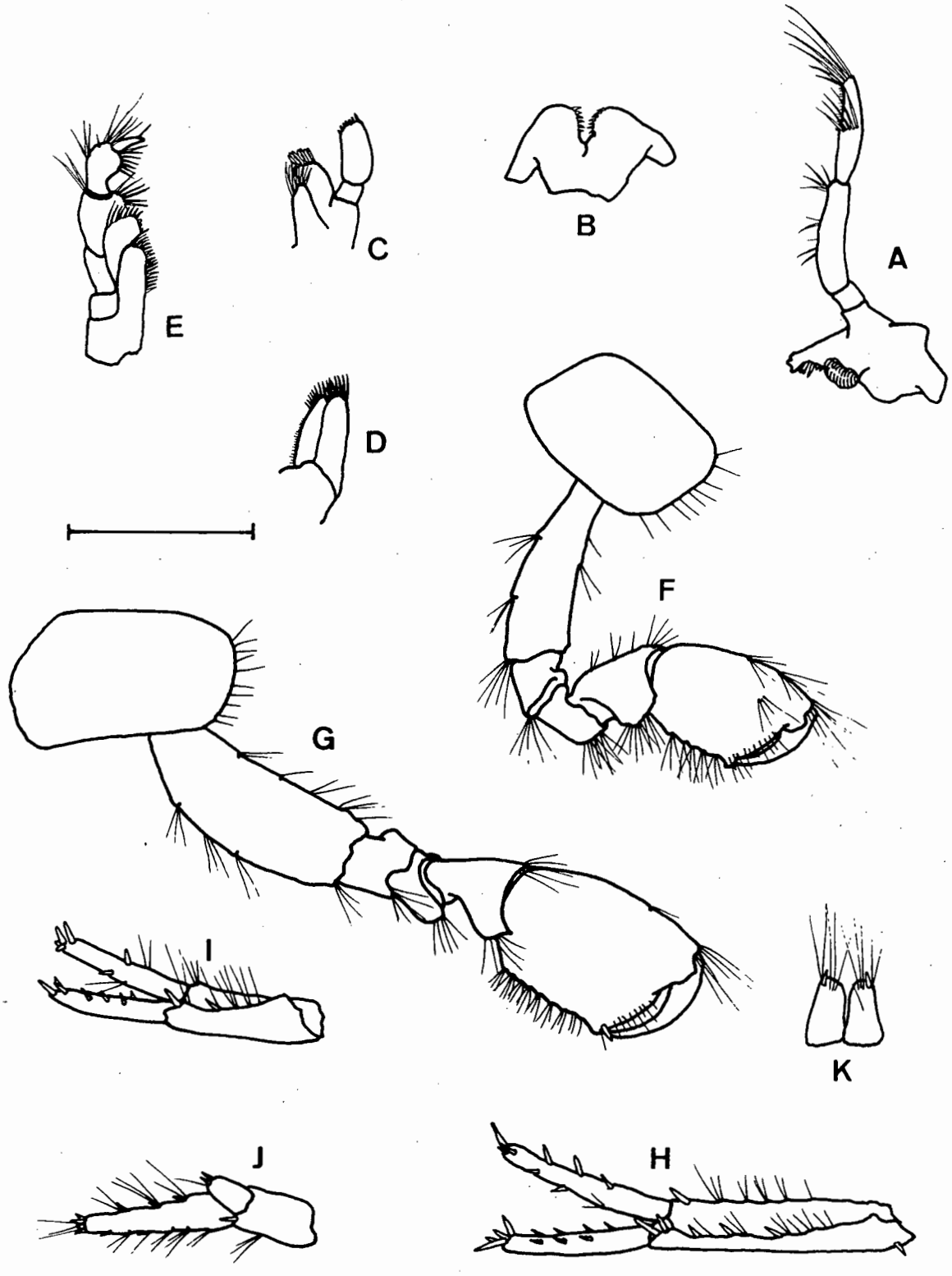

Fig. 7. *Paramelita odontophora* n. sp., male, 11.1 mm. (A) Right mandible, (B) Lower lip, (C) Maxilla 1, (D) Maxilla 2, (E) Maxilliped, (F) Gnathopod 1, (G) Gnathopod 2, (H) Uropod 1, (I) Uropod 2, (J) Uropod 3, (K) Telson.

apical teeth. Maxilla 2, inner plate, a little shorter and narrower than outer plate, inner plate pubescent, both plates strongly setose terminally.

Maxilliped inner plate, with many curved setae, outer plate with approximately eight stout blunt spine-teeth on inner margin and eight terminal curved setae, palp articles 2 and 3 densely setose medially.

Pereon segments dorsally smooth, coxae 1-3 deeper than corresponding segments, quadrate, setose ventrally, coxa 4 posteriorly excavate, deeper than long, setose ventrally, coxae 5 and 6 longer than deep, bilobed, setose ventrally, coxa 7 semicircular, setose ventrally. Segments 2-7 with one pair of coxal gills each, segment 2 with one, 3, 4, 5 and 7 with two, and 6 with four sausage shaped sternal gills.

Gnathopod 1 subchelate, articles 5 and 6 together longer than article 2, article 6 1.5 times of 5, longer than wide, palm gently convex, slightly oblique, with four palmar spines, dactyl as long as palm. Gnathopod 2 similar in structure to, but larger than 1, inner margin of article 2 bearing four groups of spines, articles 5 and 6 combined longer than 2, article 6 twice as long as 5, longer than wide, palm convex, slightly oblique, with four palmar spines, dactyl as long as palm.

Pereopod 3 slightly longer than 4, moderately setose, dactyl with seven spinules. Pereopod 4 similar in structure to 3, articles 4, 5 and 6 densely setose posteriorly, dactyl with six spinules.

Bases of pereopods 5, 6 and 7 moderately expanded posteriorly, with some setae anteriorly and posteriorly, articles 4 shorter than 5 and 6, articles 5 slightly shorter than 6, pereopods 5 and 6, articles 4 and 5, with three groups each, and 6 with five to six groups of spines, pereopods 5-7, articles 4, 5 and 6 moderately to densely setose, dactyl of pereopod 5 with 11 spinules, and of 6 and 7, with 13 spinules.

Pleon segments 1-3 sparsely setose dorsally, epimeral plates rounded to quadrate, ventrally setose. Pleon segments 4-6 moderately setose dorsally, uropod 1 extending beyond 2, peduncle setose, rami approximately subequal, 0.8 times length of peduncle, each with 6-7 marginal and four terminal spines. Uropod 2 shorter than 1, peduncle setose, rami approximately subequal, inner ramus with four marginal setae, each ramus with 2-6 marginal and four terminal spines. Uropod 3 relatively short,

exceeding 2 by 0.4 length of outer ramus, peduncle longer than broad, inner ramus short, 0.6 times length of peduncle and 0.3 times length of outer ramus, with two apical spines and one seta, outer ramus 1.9 times length of peduncle, three groups of spines and setae on inner and five on outer margin, second segment reduced, only 4.4% of length of first segment. Telson deeply cleft, each lobe with one spine and six apical and subapical setae and about five dorsal setae.

Remarks. - Female specimens are similar morphologically to the males, except for the possession of more slender, unmodified second antennae. Adult males of *P. odontophora* sp. nov. are clearly distinguished by their elongated, enlarged second antennae with the subterminal tooth on article 4. *P. spinicornis* Barnard, 1927 is also identified by the possession of toothed second antennae, but in this species, the tooth on article 4 is terminal and forwardly directed. In addition, article 4 of antenna 2 in *P. spinicornis* possesses a medial lobe, article 5, which is rectangular, often possesses a terminal spine, and article 3 of antenna 2 is considerably shorter than the swollen and enlarged article 4. The larger *P. odontophora* individuals are characterised by a relatively long article 3 in antenna 2 and a trumpet-shaped fifth article which lacks spines or teeth.

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PAPER 6

A TAXONOMIC REVISION OF THE FAMILY PARAMELITIDAE (CRUSTACEA: AMPHIPODA) FROM SOUTH AFRICAN FRESH WATERS.

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(With 26 figures)

ABSTRACT

Twenty-four species and one variety belonging to three paramelitid genera, *Paramelita*, *Afrocrangonyx* gen. nov. and *Aquadulcaris* gen. nov. are diagnosed and illustrated. Distribution records are updated, and new keys for the identification of these species are provided. Morphological similarities between the species are discussed.

CONTENTS

PAGE

INTRODUCTION

South African freshwater amphipods first received attention when Barnard (1916) described four species from Table Mountain, Cape Peninsula, placing them in the genus *Gammarus*. In 1926, Schellenberg erected the genus *Paramelita* to accommodate the new species *P. ctenodactyla*, but a year later, this species was listed by Barnard (1927) as a synonomy for "*G. capensis*". Although it is clear that Barnard (1927) had seen Schellenberg's (1926) paper, he did not recognise, or discuss the validity of *Paramelita*. Instead, he extended the known ranges of two of his "*Gammarus*" species and added a further six new species and one variety. In 1937, Schellenberg again argued that the South African freshwater species assigned to *Gammarus* were sufficiently different from those of the palaeartic and neartic regions to warrant the recognition of a new genus, *Paramelita*. Thus, when Thurston (1973) described a new cave dwelling species, he placed it into this genus, recognising the transfer of the South African freshwater "*Gammarus*" species to *Paramelita*. In 1981, a further new *Paramelita* species was added to the list (Griffiths, 1981).

More recent collections in 1989 and 1990, have almost doubled the number of known species of *Paramelita*. Four species, whose affinities with the known species were not immediately evident, were initially described by Stewart & Griffiths (1991a), and later, after further morphological and isozyme analysis, eight other new species were added to the growing number of known *Paramelita* species (Stewart & Griffiths, 1991b, c; Stewart & Snaddon, 1991). Thus, since Griffiths (1981) revised the genus in 1981, 12 new species have been added, bringing the total of known *Paramelita* species to 24.

Despite the fact that many of the *Paramelita* species do not fit the original diagnosis of the genus, Schellenberg (1937) did not extend his diagnosis, nor did Griffiths (1981) comment on, or rediagnose the genus in his revision. Barnard & Barnard (1983) did provide a rediagnosis of the genus in their study of freshwater

amphipods of the world, but their diagnosis contains some inaccuracies, and also does not adequately accommodate species recently described. Thus, a relatively large assemblage of morphologically variable paramelitid species from the south-western regions of South Africa have all been included in a single, poorly diagnosed genus. Initial cladistic analysis (Stewart, in prep.) has confirmed that *Paramelita*, as it is presently composed, is not monophyletic.

The closest relatives to *Paramelita* are the 21 Australian crangonyctoid species placed in seven genera in the family Paramelitidae (Williams & Barnard (1988). In contrast to the situation in South Africa, these genera were all rediagnosed or described in a recent, detailed account of the Australian crangonyctoids by Williams & Barnard (1988). There is some dispute as to how closely related *Sternophysinx* from the northern parts of South Africa is to the *Paramelita* species. Bousfield (1983) added this genus to the Paramelitidae, but Williams & Barnard (1988) did not recognise its inclusion in the family in their revision of Australian crangonyctoids.

In addition to the obvious need for a revision of the status of the genus *Paramelita*, several of the known species have been collected from new localities. Moreover, when many of the earlier species were first described by Barnard (1916, 1927), they were not adequately illustrated. This paper thus provides an updated morphological analysis and review of the genus *Paramelita*, describes two new genera, *Afrocrangonyx* and *Aquadulcaris*, and contains illustrations and a diagnosis of each of the 24 South African paramelitid species, as well as notes on their distribution patterns, characteristic features, and similarities with other species. New keys based on adult males, which best show the taxonomically important features of each species, are also included.

MATERIALS AND METHODS

Specimens were obtained both from collections made by the author mainly during 1989 and 1990 and from the collections of the South African Museum. Specimens of both sexes were examined from all populations collected to assess the level of morphological variability. When necessary, specimens were partially dissected to facilitate measurement and illustration of the limbs. Drawings were made with the aid of a camera lucida attached either to Wild dissecting or compound microscopes. Morphological similarity between species was investigated phenetically by calculating a matrix of Simple Matching Coefficients based on 29 variables. A cluster analysis based on this matrix was performed by means of the UPGMA algorithm. This analysis was performed with the aid of the NTSYS-pc computer programme (Rholf, 1989).

PHENETIC ANALYSIS

A phenetic analysis of the 24 known species of *Paramelita* confirmed the existence of morphological distinct groups within the species (Fig. 1). Six of the species fell into a relatively 'tight' and distinct cluster (cluster A), and were easily distinguished from the remaining species by a combination of characteristic features such as the possession of a sparsely setose urosome and a poorly emarginate coxa 4, the presence of only a single spinule on the dactyls of pereopods 3 and 4, and with the exception of one population of *P. auricularius*, on the dactyls of pereopods 5-7, a strongly swollen article 4 in antenna 2, and the absence of a second segment on the outer ramus of uropod 3. In his original diagnosis of *Paramelita*, Schellenberg (1926) described dactyls with "a row of spinules", and also alluded to the second segment on the outer ramus of uropod 3 as "well formed". It is proposed that this group be recognised as belonging to a new genus, *Afrocrangonyx* gen. nov., the description of which follows. This new genus is almost certainly most closely allied to the Australian

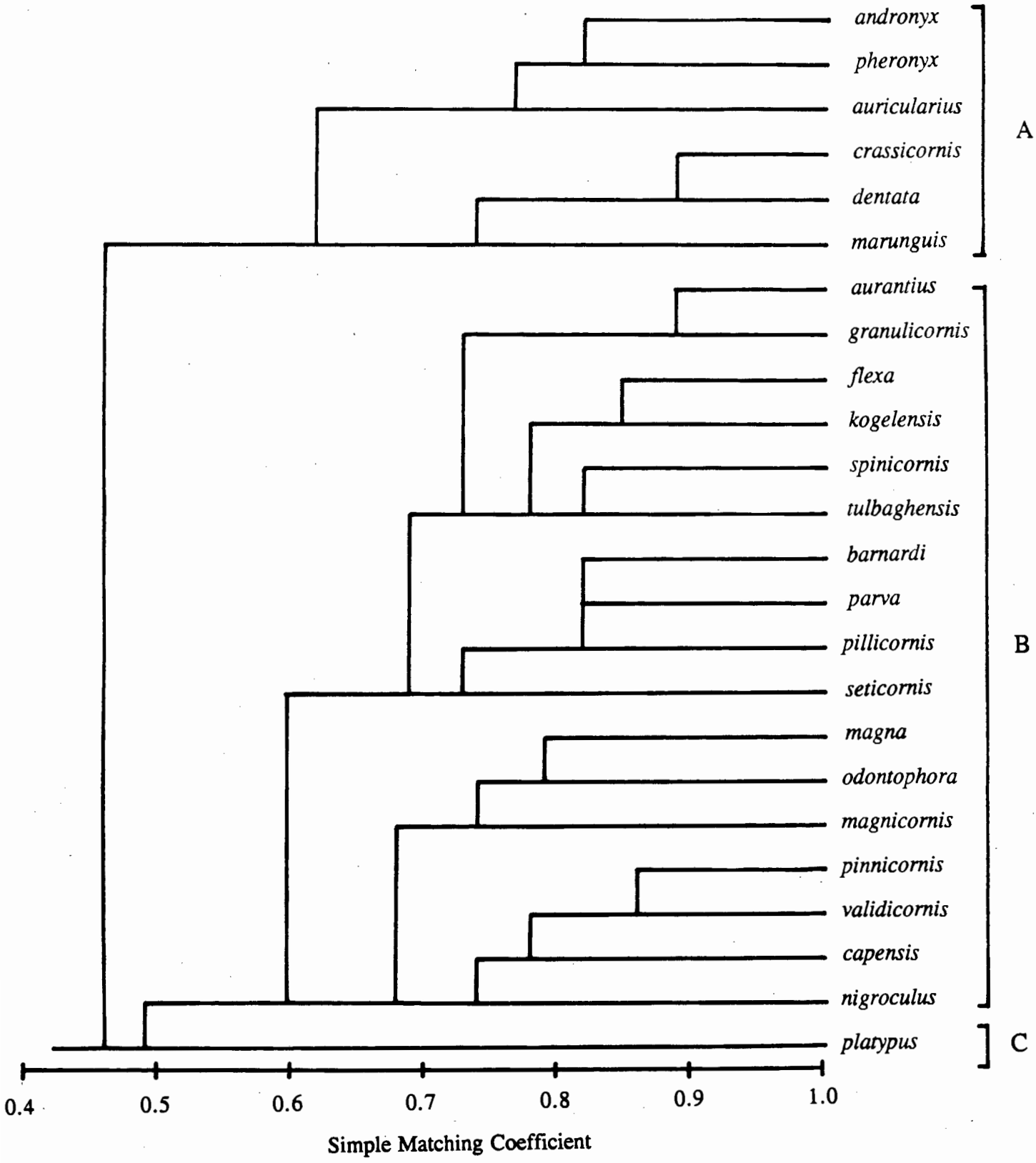

Fig. 1. Result of cluster analysis of *Paramelita*.

genus, *Uroctena*, which also contains members with "pediform" second antennae and poorly emarginate fourth coxal plates.

The only other species which have article 4 of antenna 2 markedly laterally swollen are *P. tulbaghensis* and *P. spinicornis*. The former species is in fact very similar to the 'cluster A' species, and shares features such as a moderately emarginate (rather than distinctly excavate) coxa 4 and the absence of an outer segment on the outer ramus of uropod 3. It differs, however, in the possession of more than one spinule (2-4) on the dactyls of the pereopods. If the characters concerned with the number of spinules on the dactyls are removed, and the remaining data set reanalysed, *P. tulbaghensis* is grouped with the other species considered to be members of the new genus *Afrocrangonyx*. Preliminary analysis of electrophoretic data (Stewart, in prep.) supports this inclusion of *P. tulbaghensis* in *Afrocrangonyx* n. gen.

The 'pediformity' of antenna 2 in *P. spinicornis* is more likely to represent an example of convergent evolution. This species has an excavate, rather than a poorly emarginate coxa 4, 3-4 spinules on the dactyls of pereopods 3-4 and 5-8 on pereopods 5-7, and a distinct, but small second article on the outer ramus of uropod 3. It is therefore, more likely that lateral swelling of article 4 of the peduncle of antenna 2 has occurred more than once in different lineages, and does not suggest that *P. spinicornis* and the *Afrocrangonyx* species are related.

Sixteen species (excluding *A. tulbaghensis*) grouped together to form 'cluster B'. This cluster was clearly divisible into five smaller, but distinct 'subclusters'. Like the *Afrocrangonyx* species (cluster A), *P. aurantius* and *P. granulicornis* are characterised by the possession of a poorly emarginate coxa 4, and the absence of a second article on the outer ramus of uropod 3. However, these species have multispinose dactyls on the pereopods, and the peduncle of antenna 2 is never markedly laterally swollen. With the exception of *P. seticornis*, all of the remaining 'cluster B' species have an excavate coxa 4 and a distinct second segment on the outer ramus of uropod 3. Although it is possible that these 16 species should be considered as belonging to more than one

genus, it is proposed that until further, detailed analysis is undertaken, they remain in the genus *Paramelita*.

The unique combination of character states in *P. platypus* is reflected in the position of this species in the phenogram ('cluster C'). This species is characterised by the possession of an almost quadrate coxa 4, a strongly convex palm with a palmar tooth in gnathopod 2, lateral expansion of article 4 in both pereopods 3 and 4, and a small, but distinct second segment on the outer ramus of uropod 3. With the exception of *P. magnicornis*, which has article 4 of pereopod 4 posterodistally protruded, this is the only species which has this limb modified. Since this species is so unlike the others, it is proposed that it be placed in a monospecific genus, *Aquadulcaris* gen. nov.

SYSTEMATICS

Key to the South African paramelitid genera

1 Pereopod 3, article 4 greatly expanded laterally (Fig. 9E) . . . . . *AQUADULCARIS*

Pereopod 3, article 4 either posterodistally protruded (Figs 2E, 7E) or normal (Figs 4E, 8E, etc), never greatly expanded laterally. 2

2 Antenna 2, article 4 always laterally swollen (Figs 2C, 3C, 4C etc); pereopods 3 & 4, dactyls with 1-2 spinules, pereopods 5-7, dactyls with 1-4 spinules. . . . . *..AFROCRANGONYX*

Antenna 2, article 4 sometimes elongate and stout (Figs 16C, 17C, 19C, etc), but rarely laterally swollen; pereopods 3 & 4, dactyls with 2-8 spinules, pereopods 5-7, dactyls with 3-14 spinules . . . . . *.PARAMELITA*

Afrocrangonx gen. nov.

Type species. *Paramelita crassicornis* (Barnard, 1916: 207-209, pl. 27, figs 24-25.).

Diagnosis

Eyes white. Antenna 1 longer than 2, peduncle sparsely setose, article 1 1,2-1,5 length of article 2, 3,0-3,8 longer than wide, flagellum sparsely to densely setose, 16-30 articulate, accessory flagellum 3-5 articulate. Antenna 2 sparsely setose, peduncle either shorter or longer than flagellum, article 4 laterally swollen, 1,9-2,7 longer than wide, semicircular lobe on article 3 present or not, flagellum 8-20 articulate. Gnathopod 2, article 2 medially spinose or not, palms slightly to markedly convex, transverse to slightly oblique. Pereopod 3 modified or not, article 4 either posterodistally protruded or not, article 5 lobed or not, often bearing 1-4 teeth-like spines. Coxa 4, posterior margin transverse to slightly emarginate. Pereopods 3 and 4, dactyls usually with a single spinule, sometimes with two, pereopods 5-7, usually with one, but occasionally with two spinules. Segments 2-7 with 1-4 sausage-shaped sternal gills, coxal gill 7 present. Uropod 3, second segment of outer ramus rudimentary to absent.

Etymology

From the Latin, *africus*, meaning African, and the Greek, *krangon*, meaning shrimp.

Key to Afrocrangonyx species

1 Pereopod 3 unmodified (Figs 5E, 8E) 2

Pereopod 3 modified, either article 4 posterodistally protruded to form a lobe or spur (Figs 2E, 7E), or article 5 posteriorly lobed (Fig. 3E), or with 1-4 teeth-like spines (Figs 4E, 6E). 3

2 Antenna 2, article 5 lacking teeth (Fig. 8C). . . . . *A. tulbaghensis*

Antenna 2, article 5 with a posterodistal tooth (Fig. 5C). . . . . *A. dentata*

3 Antenna 2, article 3 bearing a semicircular lobe (Figs 2C, 3C, 7C). 4

Antenna 2, article 3 lacking a lobe 6

4 Pereopod 3, article 4 not posterodistally protruded, article 5 usually posteriorly lobed, always bearing a tooth-like spine (Fig. 3E) . . . . . *A. auricularius*

Pereopod 3, article 4 posterodistally protruded (Figs 2E, 7E). 5

5 Pereopod 3, article 4 short, posterodistally protruded into a long, narrow 'spur', antenna 2, article 3 strongly swollen and enlarged (Fig. 7A, C, E). . . . . *A.pheronyx*

Pereopod 3, article 4 long, posterodistally protruded into a triangular shaped lobe, antenna 2, article 3 moderately swollen (Fig. 2A, C, E) . . . . . *A. andronyx*

6 Pereopod 3, article 5 attached normally to article 6 (Fig. 4E) . . . . . *A. crassicornis*

Pereopod 3, article 5 attached at right angles to article 6 to form a 'claw' (Fig. 6E). . . . . *A. marunguis*

Afrocrangonyx andronyx (Stewart & Griffiths, 1991)

Fig. 2A-J

Paramelita andronyx Stewart & Griffiths, 1991a: 30-35, figs 5,6.

Material examined

Types. Holotype, SAM A40017, paratypes, SAM A40018, from a tributary of the Riebeek's River, above the farm Waterval, Kasteelsberg.

Other material. SAM A40019, from a stream above the farm Wynkeldersberg, draining the slopes of Kasteelsberg.

Diagnosis

Eyes white. Antenna 1 sparsely setose, flagellum about 20-30 articulate, accessory flagellum 3-5 articulate. Antenna 2 shorter than 1, moderately setose, article 3 of peduncle bearing a semicircular lobe posteriorly and article 4 laterally swollen in males, flagellum 13-20 articulate. Coxa 4 slightly emarginate posteriorly. Gnathopod 2, article 2 strongly spinose medially, palm slightly oblique, with 3-5 defining spines. Pereopod 3 moderately setose, modified in males, article 2 strongly spinose medially, article 4 posterodistally projected into a large lobe, article 5 short and stout, article 6 bent at right angles to article 5, dactyl with a single spinule. Pereopods 4-7 sparsely to moderately setose, unmodified, dactyls each with a single spinule. Uropods 1 and 2, peduncle spinose and setose, rami usually with marginal spines and setae and apical spines. Uropod 3, inner ramus 0.3 length of outer, apically spinose, sometimes with a seta, outer ramus with marginal and apical spines, sparsely to moderately setose, second segment rudimentary or absent. Telson deeply cleft, each lobe with one spine and 4-5 setae.

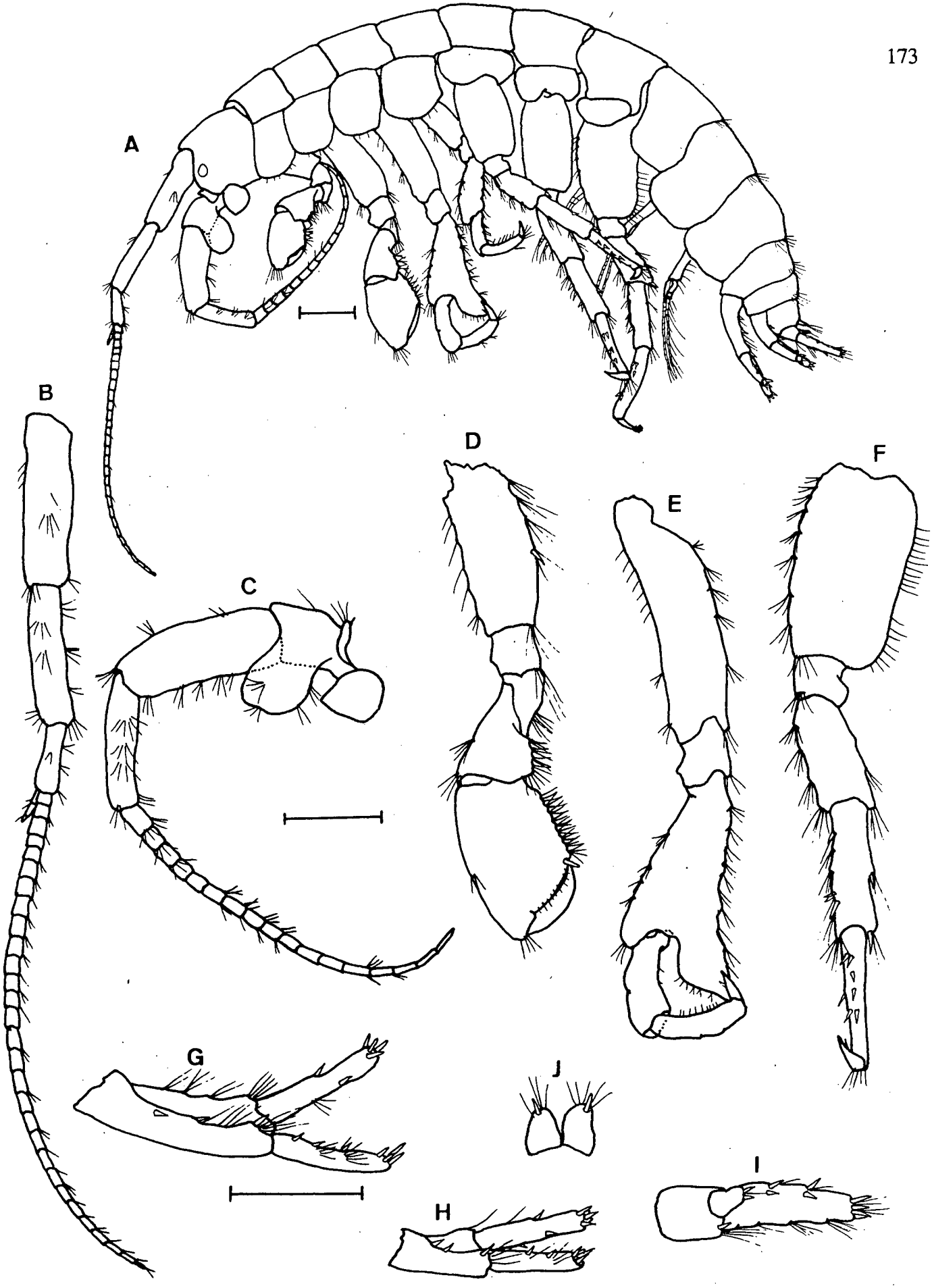

Fig. 2. *Afrocrangonyx andronyx*, male, 16,1 mm. A. Lateral aspect. B. Antenna 1. C. Antenna 2. D. Gnathopod 2. E. Pereopod 3. F. Pereopod 6. G. Uropod 1. H. Uropod 2. I. Uropod 3. J. Telson. Scale lines represent 1 mm.

Remarks

One of three species with article 3 of antenna 2 lobed and pereopod 3 modified to form a 'claw', *P. andronyx* is easily distinguished by the manner in which the claw-like structure is achieved.

Distribution

From streams draining the slopes of Kasteelberg, north of Malmesbury (Fig. 26).

Afrocrangonyx auricularius (Barnard, 1916)

Fig. 3A-J

Gammarus auricularius Barnard, 1916: 209-210, pl. 27 (figs 26-28); 1927: 169-170.
Paramelita auricularis (Barnard) Thurston, 1973: 166.
Paramelita auricularius (Barnard) Griffiths, 1981: 82-85, fig. 3A-C.

Material examined

Types. Lectotype and paratypes, SAM A2599, top of Table Mountain, Cape Peninsula.

Other material. SAM A2634, A2962, A3882, A4559, A5907, A40251, all from various localities on the top of Table Mountain. SAM A40252, from a stream draining Constantiaberg, Cape Peninsula.

Diagnosis

Eyes white. Antenna 1 sparsely setose, flagellum 20-25 articulate, accessory flagellum 3-4 articulate. Antenna 2 moderately setose, shorter than 1, article 3 with a posterodistal lobe extending forward to middle of swollen article 4 in males, flagellum 8-13 articulate. Coxa 4, posterior margin transverse to very slightly emarginate. Gnathopod 2, palm transverse, with 1-3 defining spines. Pereopod 3 sparsely to

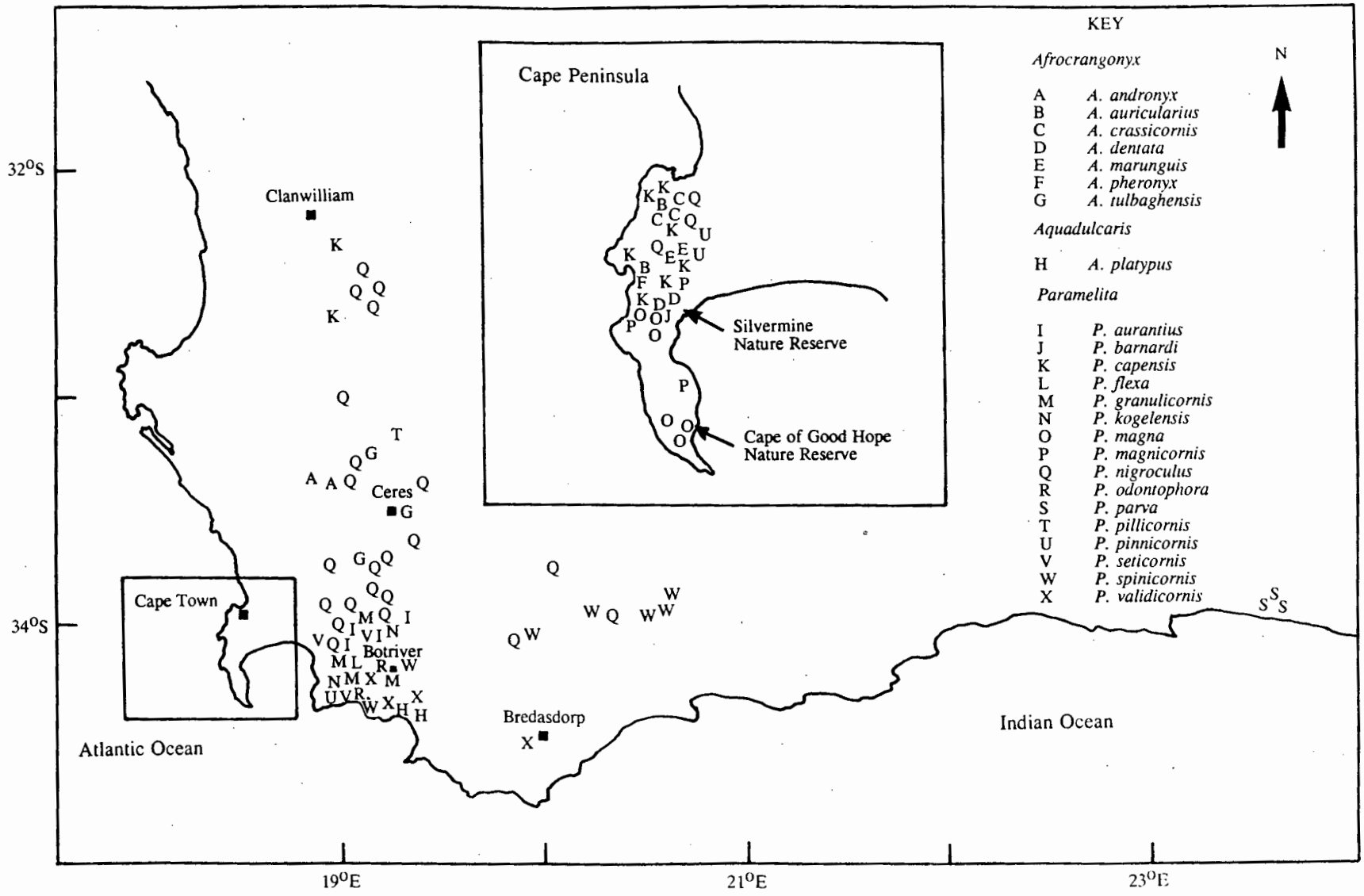

Fig. 26. Map of south-western Cape, showing distribution of paramelitid species.

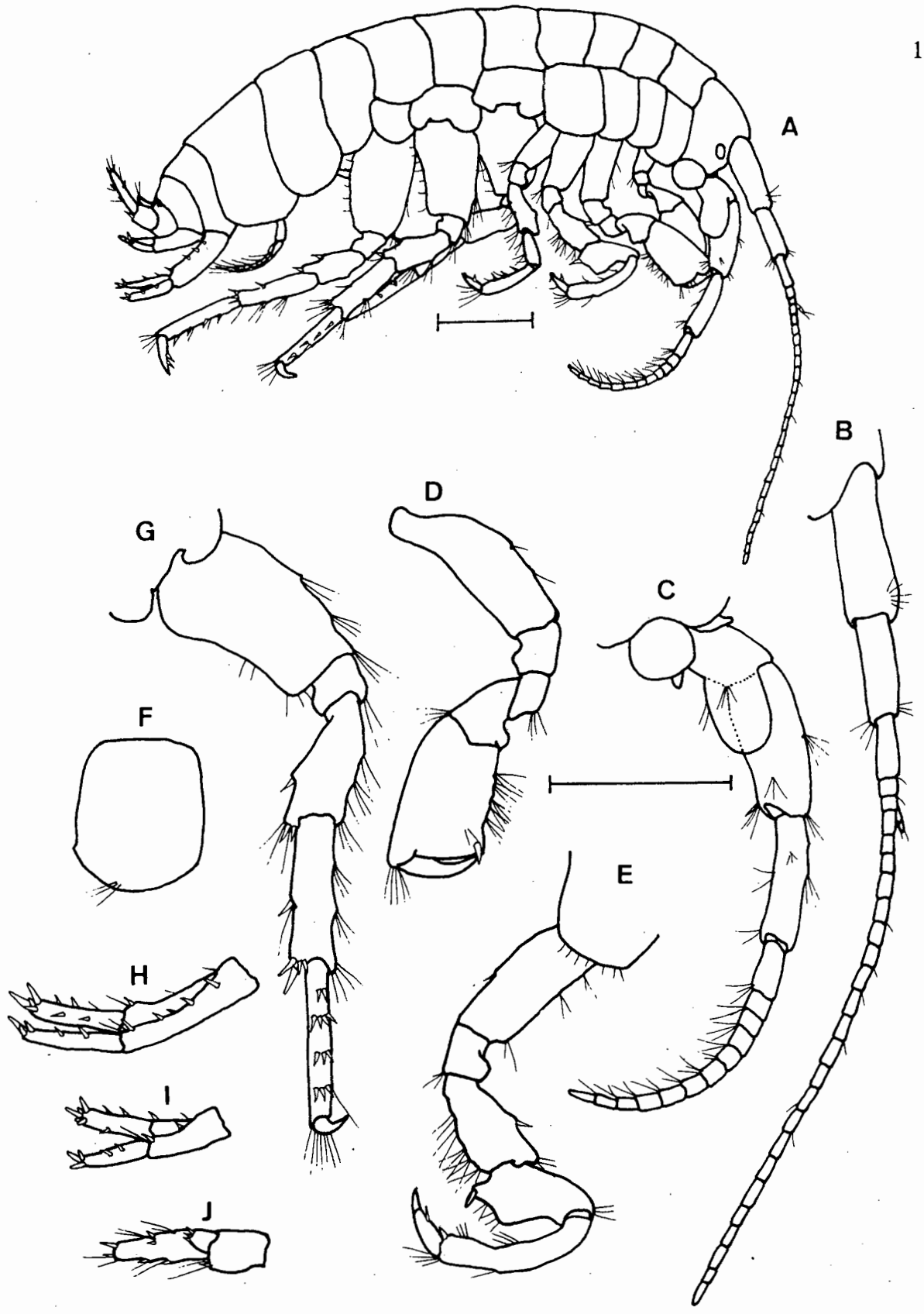

Fig. 3. *Afrocrangonyx auricularius*, male, 6,7 mm. A. Lateral aspect. B. Antenna 1. C. Antenna 2. D. Gnathopod 2, medial view. E. Pereopod 3. F. Coxa 4. G. Pereopod 6. H. Uropod 1. I. Uropod 2. J. Uropod 3. Scale lines represent 1 mm.

moderately setose, modified in males, article 4 widened distally, article 5 with a rectangular lobe and a single tooth-like spine posteriorly, article 6 elongate and arched and folded back against the lobed, posterior margin of 4, dactyl with a single spinule. Pereopod 4 sparsely to moderately setose, unmodified, dactyl with one spinule. Pereopods 5-7 sparsely to moderately setose, dactyls with 1-2 spinules. Uropod 1, peduncle spinose, sometimes with 1-2 setae, rami subequal, with marginal and apical spines, inner ramus sometimes with a single seta. Uropod 2, peduncle spinose and setose, inner ramus slightly longer than outer, both rami with marginal and apical spines, lacking setae. Uropod 3, inner ramus about 0.3 length of outer, apically spines, outer ramus with marginal and apical spines, sparsely setose, second segment rudimentary to absent. Telson deeply cleft, each lobe with about 6-8 apical setae, lacking spines.

Remarks

This species most closely resembles *P. andronyx* and *P. pheronyx*, but can be distinguished from these species by the manner in which the claw-like structure is achieved. Article 5 of this pereopod can vary from having a small projection, to having a large, rounded to rectangular shaped lobe posteriorly.

Distribution

Cape Peninsula, in streams draining the upper slopes of Table Mountain in the north to Constantiaberg in the south (Fig. 26).

Afrocrangonyx crassicornis (Barnard, 1916)

Fig. 4A-J

Gammarus crassicornis Barnard, 1916: 207-209, pl. 27, figs 24-25.

Paramelita crassicornis (Barnard) Thurston, 1973: 166. Griffiths, 1981: 85-86, fig. 3E-G.

Material examined

Types. Lectotype and paratypes, SAM A3031, from Table Mountain.

Other material. SAM A3865 and A40220, Grotto Ravine, Table Mountain. SAM A3864, Platteklip Gorge. SAM A3881, Slangolie. SAM A4368 and A4368, other unknown localities on Table Mountain. SAM A4868, Stinkwater. SAM A40222, Blinkwater. SAM A40223, Echo Valley. SAM A40225, Rhodes Memorial.

Diagnosis

Eyes white. Antenna 1 sparsely to moderately setose, flagellum 19-25 articulate, accessory flagellum with 3-5 articles. Antenna 2 moderately setose, peduncle articles 3, and to a greater extent, 4, strongly swollen in males, normal in females, flagellum 10-13 articulate. Coxa 4, posterior margin slightly emarginate. Gnathopod 2, palm transverse to slightly oblique, with 2-4 defining spines. Pereopod 3, article 5 in males usually with 1-4 stout, teeth-like spines, dactyl with one spinule. Pereopods 4-7 sparsely to moderately setose, unmodified, dactyls each with a single spinule. Uropod 1, peduncle spinose and setose, rami subequal, with marginal and apical spines, inner ramus usually with a few setae. Uropod 2, peduncle spinose and setose, inner ramus longer than outer, both with marginal and apical spines, lacking setae. Uropod 3, inner ramus 0.3 length of outer, apically spinose, outer ramus with marginal and apical spines, sparsely setose, second segment rudimentary to absent. Telson deeply cleft, each lobe with a few setae but no spines.

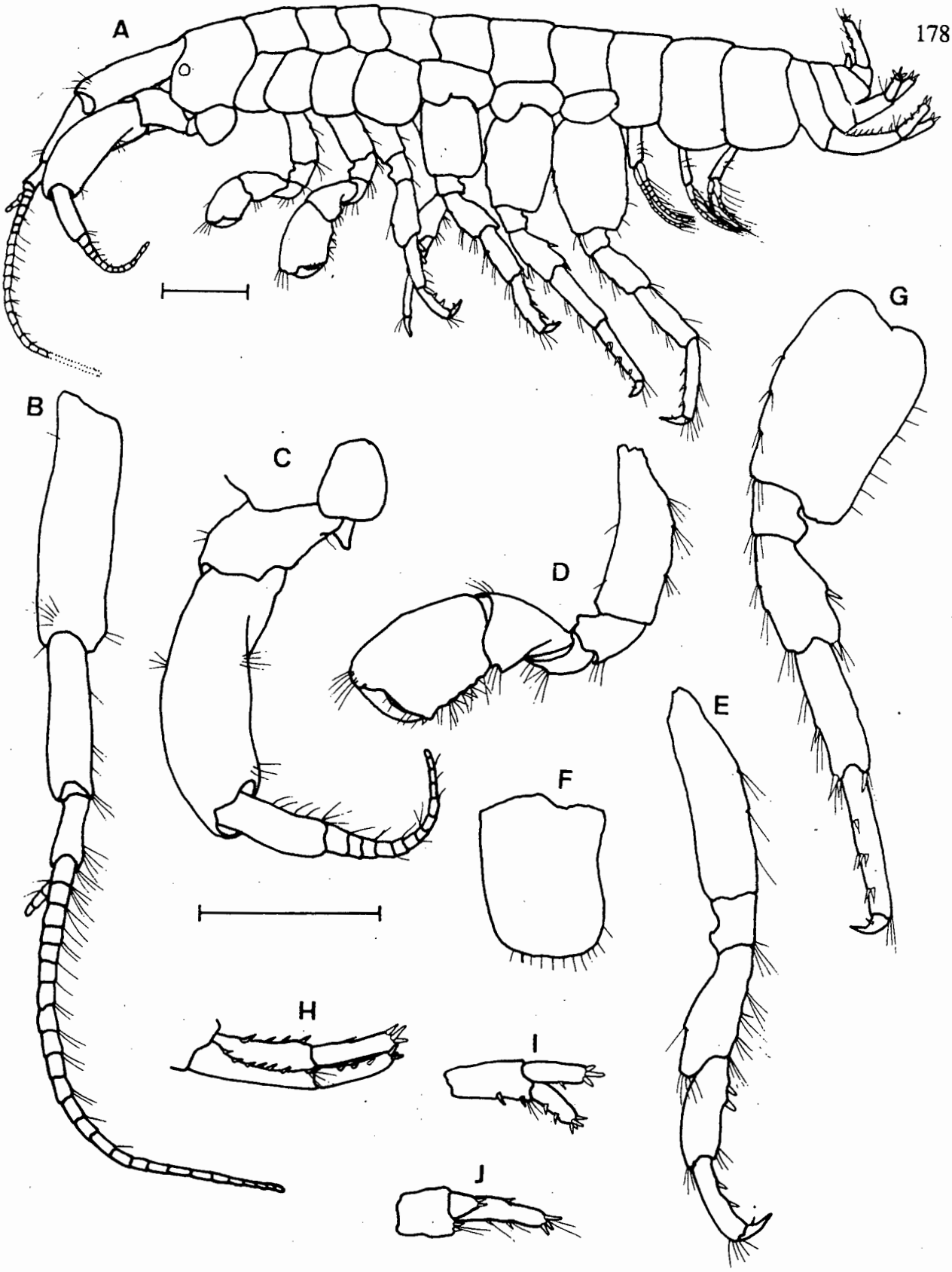

Fig. 4. *Afrocrangonyx crassicornis*, male, 6,9 mm. A. lateral aspect. B. Antenna 1. C. Antenna 2. D. Gnathopod 2. E. Pereopod 3. F. Coxa 4. G. Pereopod 6. H. Uropod 1. I. Uropod 2. J. Uropod 3. Scale lines represent 1 mm.

Remarks

The swollen peduncle in antenna 2, poorly emarginate coxa 4, absence of a second segment on the outer ramus of uropod 3, and the presence of only a single spinule on the dactyls of the pereopods suggests a strong link between this species and *P. marunguis*, *P. dentata*, *P. auricularius*, *P. andronyx* and *P. tulbaghensis*.

Distribution

Endemic to streams draining the upper slopes of Table Mountain (Fig. 26).

Afrocrangonyx dentata (Stewart & Griffiths, 1991)

Fig. 5A-J

Paramelita dentata Stewart & Griffiths, 1991c: 119-124, figs 7, 8.

Material examined

Types. Holotype, SAM A40244, paratypes, SAM A40245, from a tributary of the Sandvlei River on Ou Kaapse Weg, Cape Peninsula.

Other material. SAM A40249, from a tributary of the Silvermine River, Cape Peninsula.

Diagnosis

Eyes white. Antenna 1, peduncle sparsely setose, flagellum moderately to densely setose posteriorly, 18-21 articulate, accessory flagellum 3-5 articulate. Antenna 2 shorter than 1, sparsely setose, articles 3 and 4 of peduncle strongly laterally swollen and enlarged and article 5 with a posterodistal tooth in adult males, flagellum 9-11 articulate. Coxa 4 slightly emarginate posteriorly. Gnathopod 2, palm transverse to slightly oblique, with 3-4 defining spines. Pereopods 3-7 sparsely setose, unmodified, dactyls each with a single spinule. Uropods 1 and 2, peduncles spinose and setose, rami with marginal and apical spines, lacking setae. Uropod 3, inner ramus

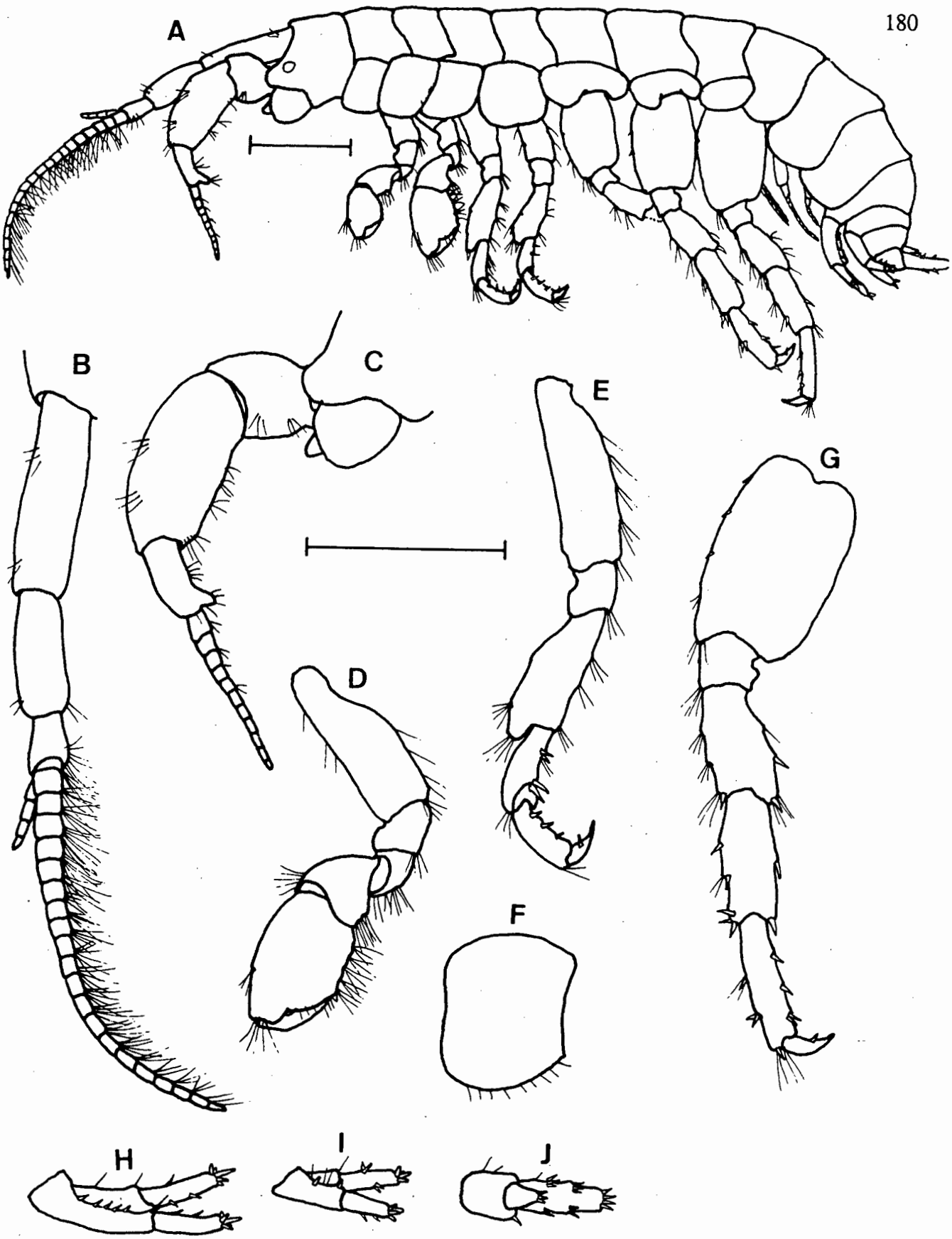

Fig. 5. *Afrocrangonyx dentata*, male, 6,9 mm. A. Lateral aspect. B. Antenna 1. C. Antenna 2. D. Gnathopod 2. E. Pereopod 3. F. Coxa 4. G. Pereopod 6. H. Uropod 1. I. Uropod 2. J. Uropod 3. Scale lines represent 1 mm.

0.3-0.4 length of outer, apically spinose, lacking setae, outer ramus with marginal and apical spines, poorly setose, second segment rudimentary to absent. Telson deeply cleft, each lobe with 1-2 spines and 1-3 setae.

Remarks

Although obviously related to *A. crassicornis*, *A. dentata* is distinguished from it by the presence of a posterodistal tooth on article 5 of the peduncle of antenna 2. *P. spinicornis*, with its swollen article 4 of antenna 2 and occasional tooth on article 5 is also superficially similar, but this species can be distinguished by the possession of multispinose dactyls, an excavate coxa 4, and the presence of a small, but distinct second article on the outer ramus of uropod 3.

Distribution

Known from small streams draining the Kalk Bay Mountains, as well as Chapman's Peak Mountains above Noordhoek, Cape Peninsula (Fig. 26).

Afrocrangonyx marunguis (Stewart & Griffiths, 1991)

Fig. 6A-J

Paramelita marunguis Stewart & Griffiths, 1991c: 124-129, figs 9, 10.

Material examined

Types. Holotype, SAM A40224, and paratypes, SAM A40246, from a tributary of the Burgersbos River, Cape Peninsula.

Other material. SAM A40221, from a tributary of the Disa River, Cape Peninsula.

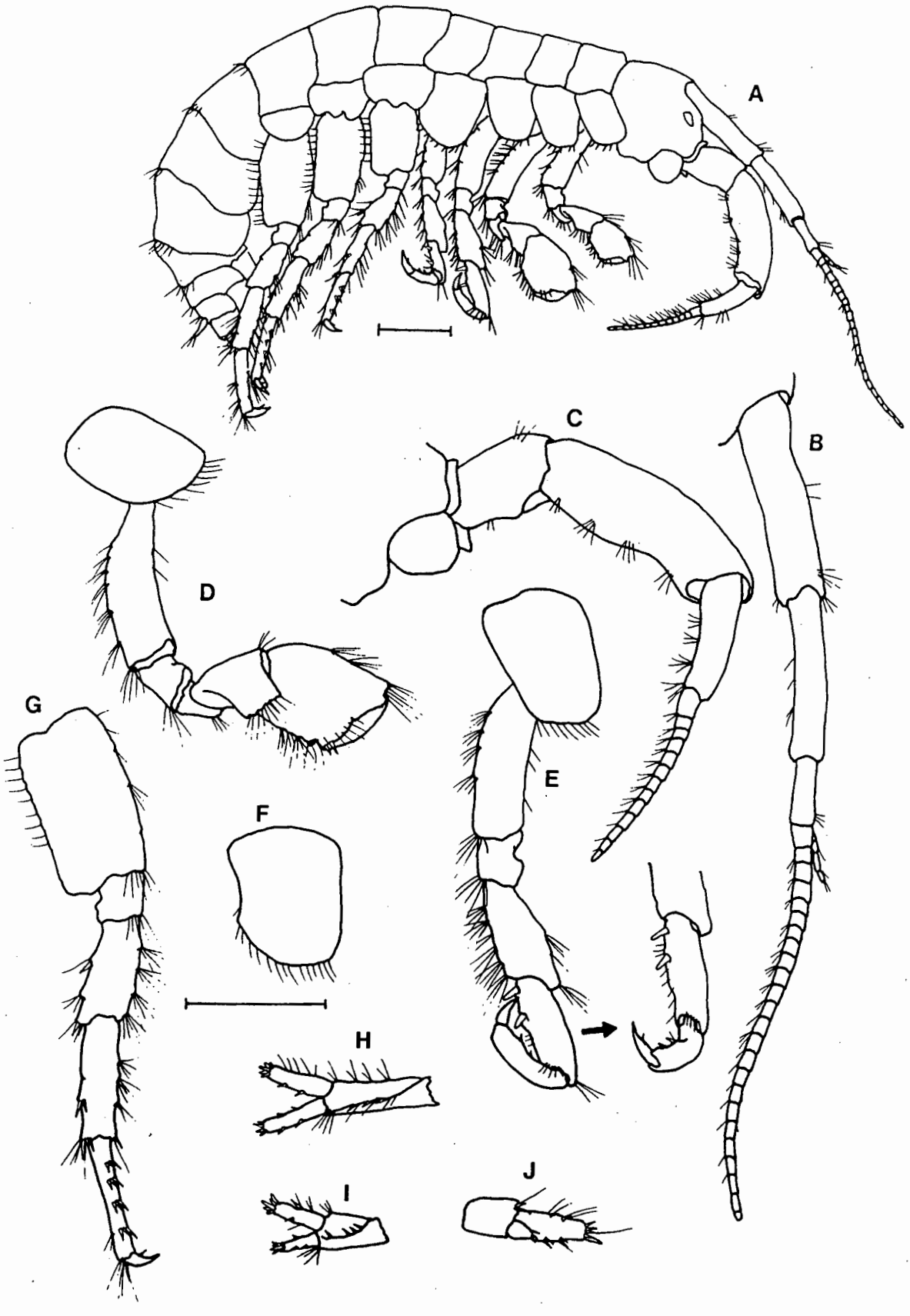

Fig. 6. *Afrocrangonyx marunguis*, male, 10,8 mm. A. Lateral aspect. B. Antenna 1. C. Antenna 2. D. Gnathopod 2. E. Pereopod 3. F. Coxa 4. G. Pereopod 6. H. Uropod 1. I. Uropod 2. J. Uropod 3. Scale lines represent 1 mm.

Diagnosis

Eyes white. Antenna 1 sparsely to moderately setose, flagellum 24-26 articulate, accessory flagellum 4 articulate. Antenna 2 shorter than 1, sparsely to moderately setose, articles 3 and 4 laterally swollen and enlarged in adult males, flagellum with 12-15 articles. Coxa 4, posterior margin weakly emarginate posteriorly. Gnathopod 2, palm transverse and markedly convex, with 3-5 spines. Pereopod 3 moderately to densely setose posteriorly, articles 5 and 6 modified in males, article 5 with two large teeth on posterior margin, article 6 bent backwards against toothed, posterior margin of 5, with two spines, dactyl with one spinule. Pereopods 4-7 unmodified, moderately setose, dactyls each with a single spinule. Uropods 1 and 2, peduncle spinose and setose, inner rami with marginal spines and setae and apical spines, outer rami with marginal and apical spines, lacking setae. Uropod 3, inner ramus 0.3 length of outer, apically spinose, outer ramus with marginal and apical spines, sparsely setose, second segment rudimentary. Telson deeply cleft, each lobe with 8-10 setae.

Remarks

This species is very similar to *A. crassicornis*, from which it is distinguished by the 'claw-like' nature of pereopod 3. In *A. marunguis*, article 6 of this limb is attached 'at right angles' to article 5, while in *A. crassicornis*, these articles are attached normally.

Distribution

This species has so far been collected from two streams draining the southern most parts of Table Mountain, Cape Peninsula (Fig. 26).

Afrocrangonyx pheronyx (Stewart & Griffiths, 1991)

Fig. 7A-J

Paramelita pheronyx Stewart & Griffiths, 1991c: 129-134, figs 11, 12.

Material examined

Types. Holotype, SAM A40247, paratypes, SAM A40248, from a stream draining the slopes of Constantiaberg, Cape Peninsula.

Diagnosis

Eyes white. Antenna 1 sparsely setose, flagellum 16-21 articulate, accessory flagellum 3-4 articulate. Antenna 2 sparsely setose, shorter than 1, articles 3 and 4 of peduncle strongly swollen and enlarged in males, article 3 with a large lobe posteriorly, flagellum with 10-14 articles. Coxa 4, posterior margin slightly emarginate. Gnathopod 2, article 2 strongly spinose medially, palm transverse, with 3-4 defining spines. Pereopod 3 sparsely setose, article 2 medially setose, articles 4 and 5 modified in males, article 4 short, widening distally, with a long, narrow posterodistal projection, article 5 elongate and enlarged, curved, bearing a stout spine at point of attachment with 4, dactyl with one spinule. Pereopods 4-7 sparsely setose, unmodified, dactyls each with a single spinule. Uropods 1 and 2, peduncle spinose and setose, inner rami with marginal spines and setae, outer rami with marginal spines, lacking setae, all rami with apical spines. Uropod 3, inner ramus 0.3-0.4 length of outer, apically spinose, outer rami with marginal and apical spines, sparsely setose, secong segment rudimentary to absent. Telson deeply cleft, each lobe with 4-5 setae, lacking spines.

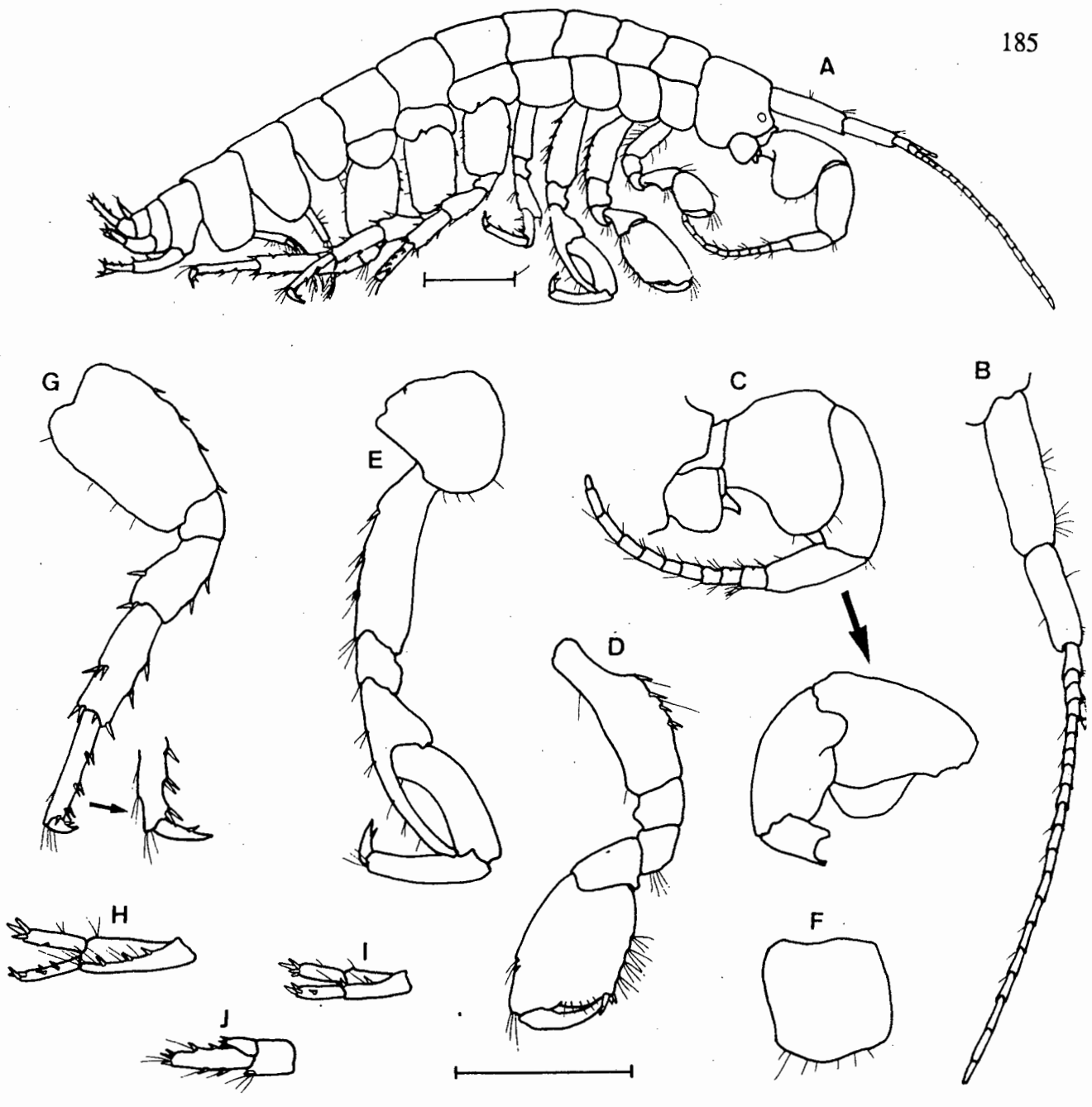

Fig. 7. *Afrocrangonyx pheronyx*, male, 7,2 mm. A. Lateral aspect. B. Antenna 1. C. Antenna 2, lateral and medial views. D. Gnathopod 2, medial view. E. Pereopod 3. F. Coxa 4. G. Pereopod 6. H. Uropod 1. I. Uropod 2. J. Uropod 3. Scale lines represent 1 mm.

Remarks

The 'spur-like' projection on article 4 of pereopod 3 makes *A. pheronyx* unmistakable. *A. andronyx* also has a projection on this article, but in this specie, the projection is wide and triangular shaped. Both species have a lobe on article 3 of the peduncle of antenna 2.

Distribution

This species is known only from the type locality, a stream draining the southern slopes of the Constantiaberg, above the Hout Bay Hotel (Fig. 26).

Afrocrangonyx tulbaghensis (Barnard, 1927)

Fig. 8A-J

Gammarus tulbaghensis Barnard, 1927: 170-171, pl. 10. (figs 5, 15).

Paramelita tulbaghensis (Barnard) Thurston, 1973: 166-167. Griffiths, 1981: 91, fig. 3H-I.

Material examined

Types. Lectotype and paratypes, SAM A4875, from the Sneeugat Valley near Tulbagh.

Other material. SAM A40230, from a stream on the path to Sneeugat Peak above the farm Bergplaas, foot of the Winterhoek Mountains, near Tulbagh. SAM A40231, from a stream in the Ceres municipal campsite. SAM A40232, from a tributary of the Molenaar's River, Worcester end of Du Toits Kloof Pass.

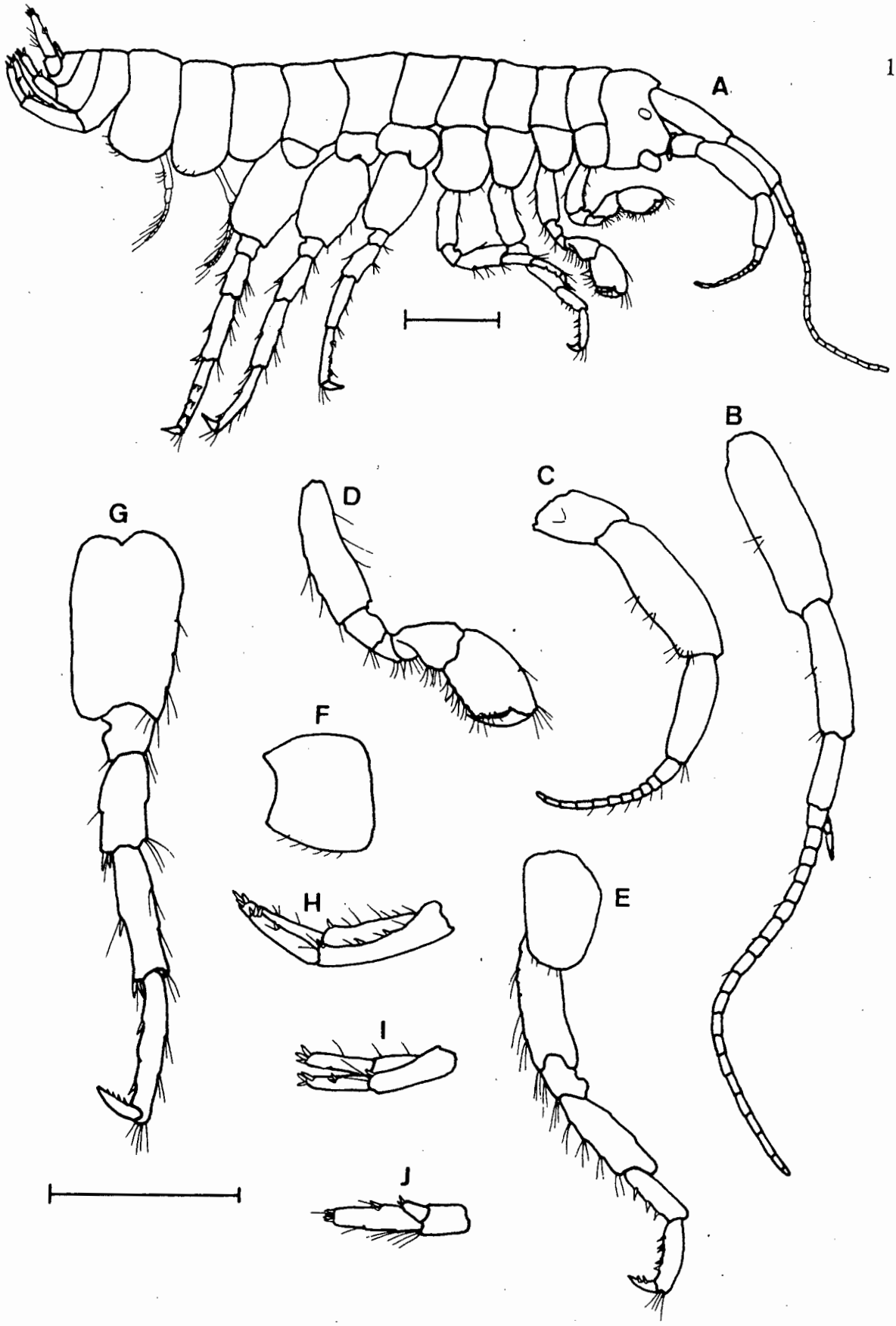

Fig. 8. *Afrocrangonyx tulbaghensis*, male, 6,3 mm. A. Lateral view. B. Antenna 1. C. Antenna 2. D. Gnathopod 2. E. Pereopod 3. F. Coxa 4. G. Pereopod 6. H. Uropod 1. I. Uropod 2. J. Uropod 3. Scale lines represent 1 mm.

Diagnosis

Eyes white. Antenna 1 sparsely setose, flagellum 16-25 articulate, accessory flagellum 3-4 articulate. Antenna 2 shorter than 1, sparsely setose, slender in females, articles 3, 4 and 5 of peduncle swollen in males, article 4 the longest, flagellum 9-12 articulate. Coxa 4, posterior margin with a distinct, but shallow emargination. Gnathopod 2, palm transverse to slightly oblique, with 2-4 spines. Pereopods 3 and 4 unmodified, dactyls usually with two, but sometimes with one spinule, Pereopods 5-7, dactyls usually with 2-4, but sometimes with one spinule. Uropod 1, peduncle with spines and setae, rami subequal, usually with marginal, and always with apical spines, sometimes with a few marginal setae. Uropod 2, peduncle with spines and setae, inner ramus longer than outer, both with marginal and apical spines, inner ramus with at least one seta. Uropod 3, inner ramus about 0.3 length of outer, with some apical setae, outer ramus with a few marginal and apical spines and setae, second segment rudimentary. Telson deeply cleft, each lobe usually with a single spine and a few setae.

Remarks

A. tulbaghensis is most similar to *A. crassicornis*, from which it is most easily distinguished by the possession of usually 2-4 spinules on the dactyls of pereopods 3-7, and by the lack of teeth-like spines on article 5 of pereopod 3.

Distribution

This species has been collected from the Winterhoek Mountains near Tulbagh in the north to Du Toit's Kloof on the Dutoitsberg in the south (Fig. 26).

Aquadulcaris gen nov.

Type species. *Paramelita platypus* Stewart & Griffiths, 1991a: 35-40, figs 7, 8.

Diagnosis

Eyes white. Antenna 1 in males as long as 2, sparsely setose, articles 1 and 2 approximately subequal, article 1 about three times longer than wide, flagellum sparsely setose, 28-41 articulate, accessory flagellum 4-5 articulate. Antenna 2 sparsely setose, both peduncle and flagellum extremely elongate and stout in males, flagellum 16-22 articulate. Gnathopod 2, article 2 medially spinose, palm markedly convex and with a palmar tooth. Pereopods 3 and 4 modified in males, densely setose posteriorly, article 4 greatly expanded laterally, dactyl with 3-5 spinules. Coxa 4, posterior margin only slightly emarginate. Pereopods 5-7 dactyl with 6-10 spinules. Segments 2-7 with 1-4 sausage-shaped sternal gills, coxal gill 7 present. Uropod 3, inner ramus 0.2 length of outer, outer ramus with a small but distinct second segment.

Etymology

From the Latin *aqua dulcis*, meaning fresh water, and *caris*, meaning shrimp.

Aquadulcaris platypus (Stewart & Griffiths, 1991)

Fig. 9A-J

Paramelita platypus Stewart & Griffiths, 1991a: 35-40, figs 7, 8.

Material examined

Types. Holotype, SAM A40020, paratypes, SAM A40021, from a tributary of the Fernkloof River in the Fernkloof Nature Reserve.

Other material. SAM A40022, from a stream near Stanford.

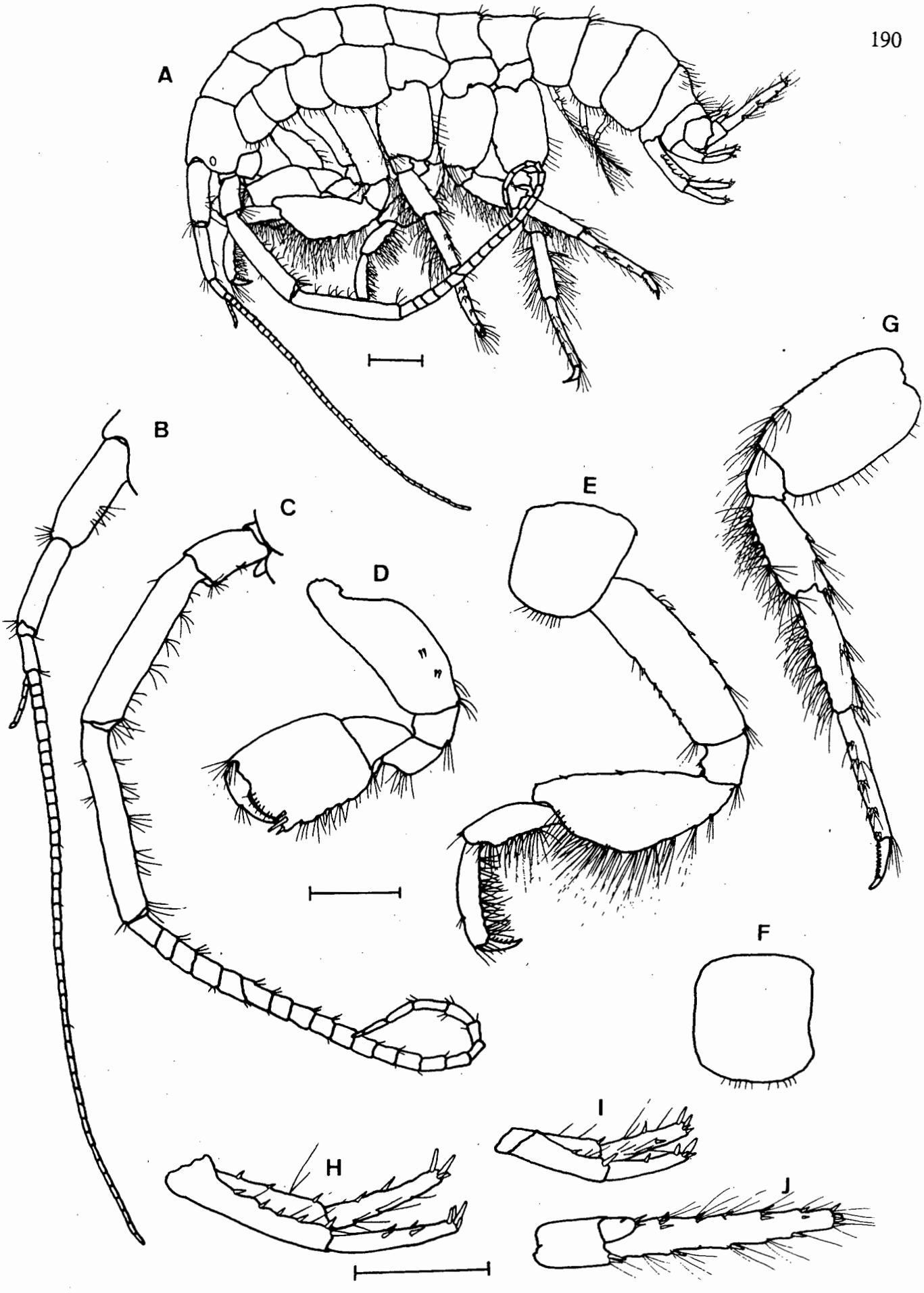

Fig. 9. *Aquadulcaris platypus*, male, 12,8 mm. A. Lateral aspect. B. Antenna 1. C. Antenna 2. D. Gnathopod 2, right side, medial view. E. Pereopod 3. F. Coxa 4. G. Pereopod 6. H. Uropod 1. I. Uropod 2. J. Uropod 3. Scale lines represent 1 mm.

Diagnosis

Eyes white. Antenna 1 sparsely setose, flagellum 28-41 articulate, accessory flagellum 4-5 articulate. Antenna 2 sparsely setose, of equal length to 1 in adult males, both peduncle and flagellum extremely elongate and stout, flagellum with 16-22 articles. Coxa 4, posterior margin very slightly emarginate. Gnathopod 2, article 2 sparsely medially spinose, palm transverse, markedly convex, with a tooth and 2-5 spines. Pereopods 3 and 4 densely setose posteriorly, modified in males, article 4 greatly expanded laterally, dactyl each with 3-5 spinules. Pereopods 5-7 moderately to densely setose, dactyls each with 6-10 spinules. Uropods 1 and 2, peduncle spinose and setose, inner rami with marginal spines and setae, outer rami with marginal spines, lacking setae, each rami with apical spines. Uropod 3, inner ramus 0.2 length of outer, apically sponose, outer ramus with marginal and apical spines, moderately setose, second segment small but distinct. Telson deeply cleft, each lobe with one spine and 6-8 setae.

Remarks

The combination of an extremely elongate antenna 2, a markedly convex palm with a distinct palmar tooth in gnathopod 2, laterally expanded article in pereopods 3 and 4, almost quadrate coxa 4, and a distinct, albeit small second segment on the outer ramus of uropod 3 make this species unmistakable.

Distribution

This species has been collected from two streams draining the slopes of the Kleinriviersberge between Hermanus and Stanford (Fig. 26).

Paramelita Schellenberg, 1926

Type species. *Paramelita ctenodactyla* Schellenberg, 1926: 367-370, fig. 57. (=*Paramelita capensis* (Barnard), 1916).

Rediagnosis

Eyes white or black. Antenna 1 0.7-1.8 length of 2, peduncle sparsely setose, article 1 1.1-1.6 length of 2, 2.6-3.7 longer than wide, flagellum sparsely setose, 18-80 articulate, accessory flagellum 3-8 articulate. Antenna 2, sparsely to densely setose, rarely toothed or lobed, peduncle either shorter or longer than flagellum, often stout in males, article 4 2.1-4.5 longer than wide, flagellum 11-35 articulate. Gnathopod 2, article 2 medially spinose or not, palm slightly to strongly oblique. Pereopod 3 usually unmodified, rarely subchelate. Coxa 4, posterior margin slightly emarginate to strongly excavate. Pereopods 3 and 4, dactyls with 2-8 spinules. Pereopods 5-7, dactyls with 3-14 spinules. Segments 2-7 with 1-4 sausage-shaped sternal gills, coxal gill 7 present. Uropod 3, inner ramus 0.1-0.4 length of outer, second segment on outer ramus present or absent.

Key to Paramelita species

1 Eyes black. 2

Eyes white 3

2 Antenna 2, posterior margins and pereopods 3-7 densely setose posteriorly (Fig. 18C). . . . . *P. nigroculus* var. *persetosus*

Antenna 2, posterior margins and pereopods 3-7 sparsely to moderately setose posteriorly, lacking setal brushes . . . . . *P. nigroculus*

3 Antenna 2, peduncle either toothed, lobed or ridged (Figs 14C, 19C, 22C, 24C). 4

Antenna 2, peduncle lacking teeth, lobes or ridges (Fig. 10C, etc). 7

4 Antenna 2, articles 4 or 5 of peduncle toothed (Figs 19C, 24C) 5

Antenna 2, article 3 posteriorly lobed (Fig. 13C) or article 5 with lateral ridges (Fig. 22C). 6

5 Antenna 2 shorter than 1, article 4 of peduncle strongly laterally swollen, with a posterodistal, terminal tooth and a proximal, medial lobe (Fig. 24A). . . . . .*P. spinicornis*

Antenna 2 extremely elongate, exceeding 1 in length, article 4 of peduncle with a posterodistal, subterminal tooth (Fig. 19A) . . . . . *P. odontophora*

6 Antenna 2, article 3 of peduncle posteriorly lobed, pereopod 3 normal (Fig. 13A) . . . . . .*P. flexa*

Antenna 2, article 5 of peduncle with lateral ridges, pereopod 3 subchelate (Fig. 22A) . . . . . .*P. pinnicornis*

7 Antenna 2 densely setose (Figs 21C, 23C) 8

Antenna 2 sparsely to moderately setose (Fig. 10C, etc). 9

8 Antenna 2, peduncle stout, flagellum shorter than peduncle, with 8-12 articles; pereopods 5-7, article 2 moderately expanded; uropod 1, outer ramus lacking setae; uropod 2, inner ramus usually with some setae (Fig. 23A). . . . . .

. . . . . .*P. seticornis*

Antenna 2, peduncle elongate and slender, flagellum as long as peduncle, with 13-16 articles; pereopods 5-7, article 2 markedly poorly expanded; uropod 1, outer ramus with some setae; uropod 2, inner ramus lacking setae (Fig. 21A) .

. . . . . *P. pillicornis*

9 Coxa 4, posterior margin poorly emarginate (Figs 10F, 14A). 10

Coxa 4, posterior margin moderately to strongly excavate (Figs 11F, 12F, etc)

. 11

10 Gnathopod 2, article 2 medially spinose, palm strongly convex, defining angle forming a small projecting rounded tooth; pereopod 3, and sometimes 4, article 2 strongly spinose medially, article 4 often considerably longer and wider than 5 (Fig. 14A). . . . . .*P. granulicornis*

Gnathopod 2, article 2 sometimes weakly spinose medially, palm moderately convex, lacking tooth at defining angle; pereopods 3 and 4 article 2, lacking medial spines, article 4 moderately longer and wider than 5 (Fig. 10A). . . . . .

. . . . . *P. aurantius*

11 Antenna 2 as long as, or exceeding 1 in length, peduncle markedly stout (Figs 16C, 17C, 25C). 12

Antenna 2, distinctly shorter than 1, peduncle slender to moderately stout (Figs 11C, 12C, etc) 14

12 Uropod 3, outer ramus 3.0 length of peduncle; uropods 1 and 2, inner rami always with a few setae, outer rami lacking setae (Fig. 25A). . . *P. validicornis*

Uropod 3, outer ramus 2.0-2.6 length of peduncle; uropods 1 and 2, inner rami with or without setae, outer rami sometimes with setae (Figs 16H-J, 17G-I). .13

13 Pereopod 3, article 4 unmodified; urosome densely setose dorsally; uropods 1 and 2, inner and outer rami with setae; body colour brown (Fig. 16A). . . . . .*P. magna*

Pereopod 3, article 4 posterodistally protruded to from a 'tooth'; urosome moderately setose dorsally; uropod 1, inner ramus with a few setae, outer ramus without, uropod 2, rami lacking setae; body colour white (Fig. 17A). . . . . .*P. magnicornis*

14 Antenna 1, flagellum with 22-27 articles; antenna 2, flagellum 11-18 articulate (Figs 15B,C, 20B,C) 15

Antenna 1, flagellum with 33-80 articles; antenna 2, flagellum 15-35 articulate (Figs 11B,C, 12B,C) 16

15 Pereopods 3 and 4, dactyls with 2-3 spinules; uropod 2, inner ramus lacking marginal setae; uropod 3, outer ramus moderately to densely setose (Fig. 20A). . . . . *P. parva*

Pereopods 3 and 4, dactyls with four spinules; uropod 2, inner ramus with a few marginal setae; uropod 3, outer ramus, poorly setose (Fig. 15A). . . . . *P. kogelensis*

16 Antenna 2, flagellum with 15-17 articles; pereopods 3 and 4, dactyls with 2-3 spinules; pereopods 5-7, dactyls with 5-7 spinules; coxa 4 distinctly, but moderately excavate posteriorly; uropods 1 and 2, rami lacking setae; uropod 3, outer ramus poorly setose (Fig. 11A). . . . . *P. barnardi*

Antenna 2, flagellum usually with more than 17 articles; pereopods 3 & 4, dactyls with 3-6 spinules; pereopods 5-7, dactyls with 8-13 spinules; uropods 1 & 2, inner rami with marginal setae; uropod 3, outer ramus, strongly setose (Fig. 12A) . . . . . *P. capensis*

Paramelita aurantius (Barnard, 1927)

Fig. 10A-K

Gammarus aurantius Barnard, 1927: 173-174; pl. 10, figs 6.16.

Paramelita aurantius (Barnard) Thurston, 1973: 167. Griffiths, 1981: 82, fig. 2J.

Material examined

Types. Lectotype and paratypes, SAM A3997, from Landrost Kloof, Hottentots Holland Mountains.

Other material. SAM A4005, from valley at foot of Vallei Berg, Hottentot Holland Mountains. SAM A4014, from Moordenaars Kop, Hottentot Hollands Mountains. SAM A4869, from Caledon side of Landdrost Kloof, Hottentot Holland Mountains. SAM A40234, from a tributary of the Du Toits River on Franchhoek Pass, Villiersdorp side. SAM A40235, from a stream flowing through the Nuweberg State Forest on Viljoen's Pass.

Diagnosis

Eyes white. Antenna 1 sparsely setose, flagellum about 20-32 articulate, accessory flagellum 3-4 articulate. Antenna 2 sparsely to moderately setose, shorter than 1, only slightly stouter than 1 in males, flagellum with about 12-13 articles. Coxa 4, posterior margin with a slight emargination. Gnathopod 2, article 2 sometimes weakly spinose medially, palm transverse to slightly oblique, with 2-5 defining spines. Pereopods 3-7 unmodified, dactyls with 2-10 spinules. Uropod 1, peduncle spinose, sometimes with a few setae, rami subequal, with marginal and apical spines, lacking setae. Uropod 2, peduncle spinose, sometimes bearing at least one seta, inner ramus slightly longer than outer ramus, both with marginal and apical spines, lacking setae. Uropod 3, inner ramus about 0.3 length of outer, apically spinose, outer ramus with

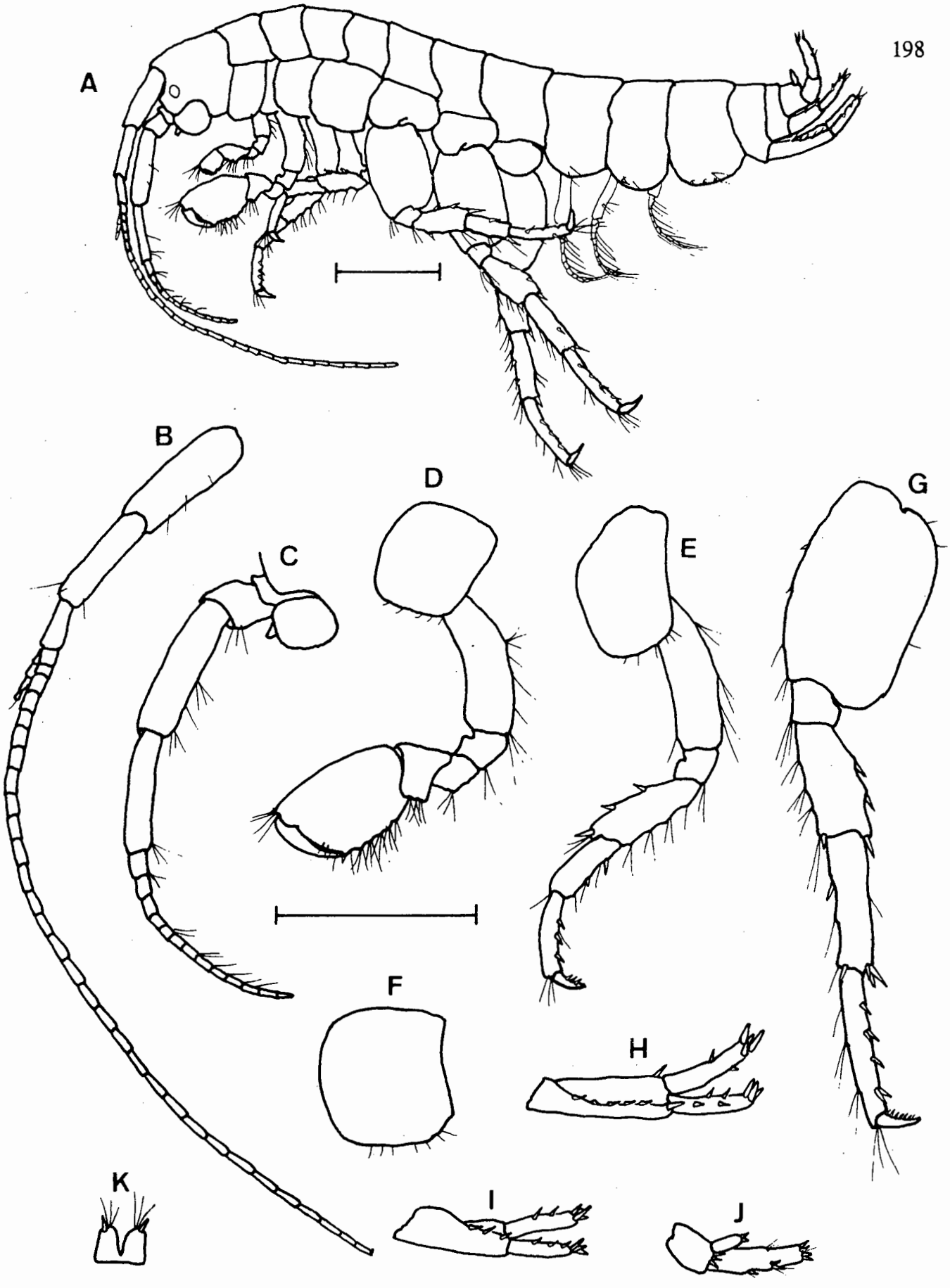

Fig. 10. *Paramelita aurantius*, male, 6,8 mm. A. Lateral aspect. B. Antenna 1. C. Antenna 2. D. Gnathopod 2. E. Pereopod 3. F. Coxa 4. G. Pereopod 6. H. Uropod 1. I. Uropod 2. J. Uropod 3. K. Telson. Scale lines represent 1 mm.

marginal and apical spines, sparsely setose, second segment rudimentary. Telson deeply cleft, each lobe bearing one spine and a few setae.

Remarks

This species is identified by the possession of an unmodified, sparsely to moderately setose, and relatively short antenna 2 in males, an almost quadrate coxa 4, the relative lack of setae on uropods 1 and 2, and its orange colour when alive. It most closely resembles *P. granulicornis*, but can be distinguished from this species by the lack of a tooth on the palm of gnathopod 2, and the absence of spines on the medial surfaces of articles 2 of pereopods 3 and 4.

Distribution

From streams draining the slopes of Hottentots Holland and Franschhoek Mountains (Fig. 26).

Paramelita barnardi Thurston, 1973

Fig. 11A-K

Paramelita barnardi Thurston, 1973: 159-168, figs 1-3. Griffiths, 1981: 85, fig. 2A-C.

Material examined

Types. Allotype, SAM A16808, from Boomslang Cave, Kalk Bay Mountains.

Other material. SAM A40239, from Boomslang Cave, Kalk Bay.

Diagnosis

Eyes white. Antenna 1 sparsely setose, flagellum 33-36 articulate, accessory flagellum with 4-5 articles. Antenna 2 shorter than 1, moderately setose, flagellum with 15-17 articles. Coxa 4 distinctly, but shallowly excavate posteriorly. Gnathopod 2, palm oblique, with 4-5 defining spines. Pereopods 3 and 4 moderately setose,

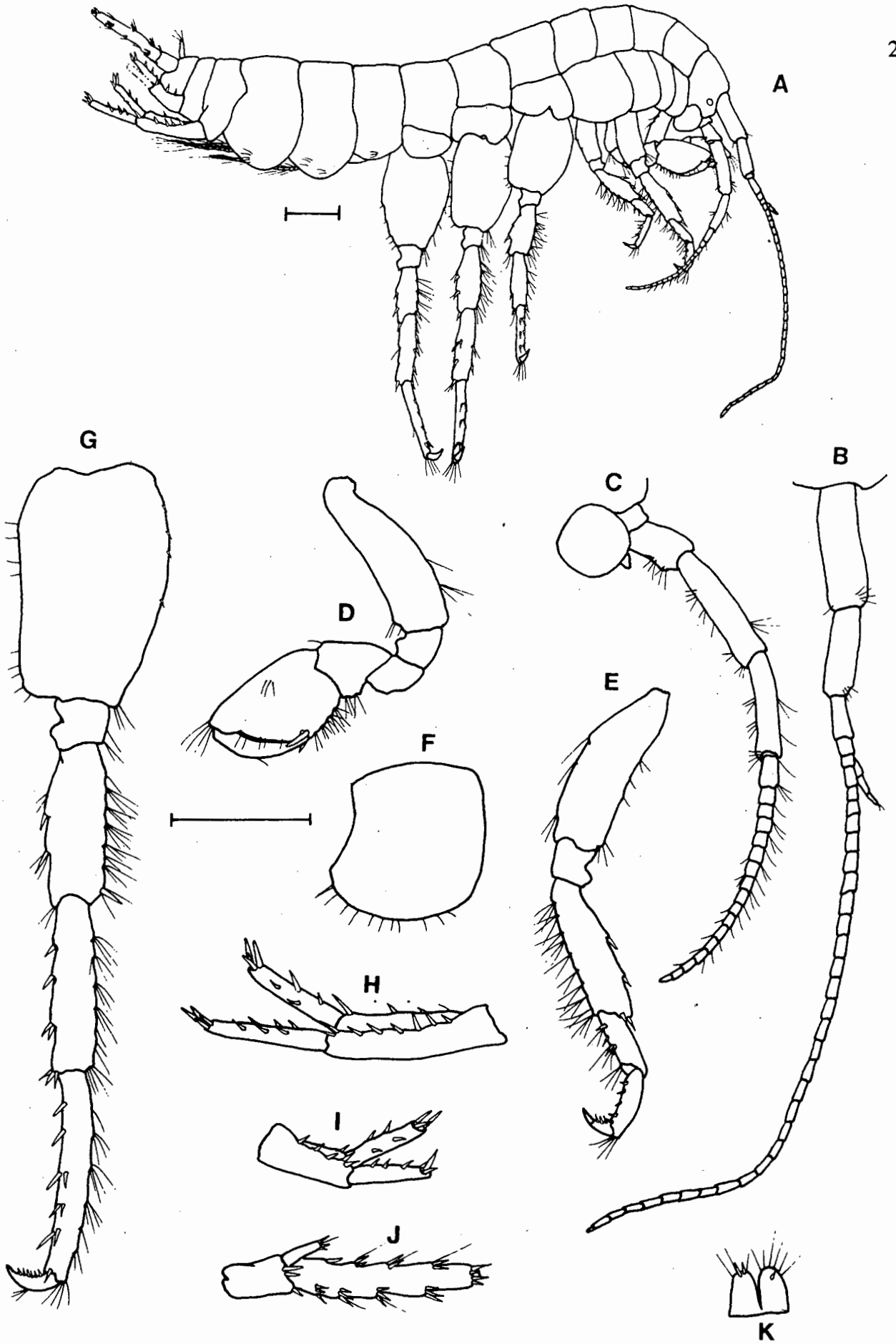

Fig. 11. *Paramelita barnardi*, male, 9,5 mm. A. Lateral aspect. B. Antenna 1. C. Antenna 2. D. Gnathopod 2, medial view. E. Pereopod 3. F. Coxa 4. G. Pereopod 6. H. Uropod 1. I. Uropod 2, left side. J. Uropod 3, right side. K. Telson. Scale lines represent 1 mm.

unmodified, dactyls with 2-3 spinules each. Pereopods 5-7 moderately setose, dactyls with 5-7 spinules each. Uropod 1, peduncle spinose, sometimes with a single seta, rami subequal, with marginal and apical spines, lacking setae. Uropod 2, peduncle spinose, inner ramus slightly longer than outer, both rami with marginal and apical spines, lacking setae. Uropod 3, inner ramus about 0.4 length of outer, apically spinose, outer ramus very poorly setose, with several groups of marginal and apical spines, second segment short, about 4% length of first. Telson deeply cleft, left lobe bearing a single spine and some setae, right lobe with 1-2 spines and some setae.

Remarks

This species is morphological similar to *P. capensis*, *P. kogelensis* and *P. parva*, but can be separated from these species based on its relatively weakly excavate coxa 4, its poorly setose uropod 3, and the number of articles in the flagellum of antenna 2.

Distribution

Known only from Boomslang Cave, Kalk Bay Mountains, Cape Peninsula (Fig. 26).

Paramelita capensis (Barnard, 1916)

Fig. 12A-J

Gammarus capensis Barnard, 1916: 203-205, pl. 27, (figs 20-22) *part.*, non SAM A3083; 1927: 169.

?Paramelita ctenodactyla Schellenberg, 1926: 367, fig. 57.

Material examined

Types. Lectotype and paratypes, SAM A2259, from Table Mountain.

Other material. SAM A195, A2258, A2459, A2552, A2598, A2963, A2967, A2968, A3033, A3866 and A4008, all from various localities on Table Mountain.

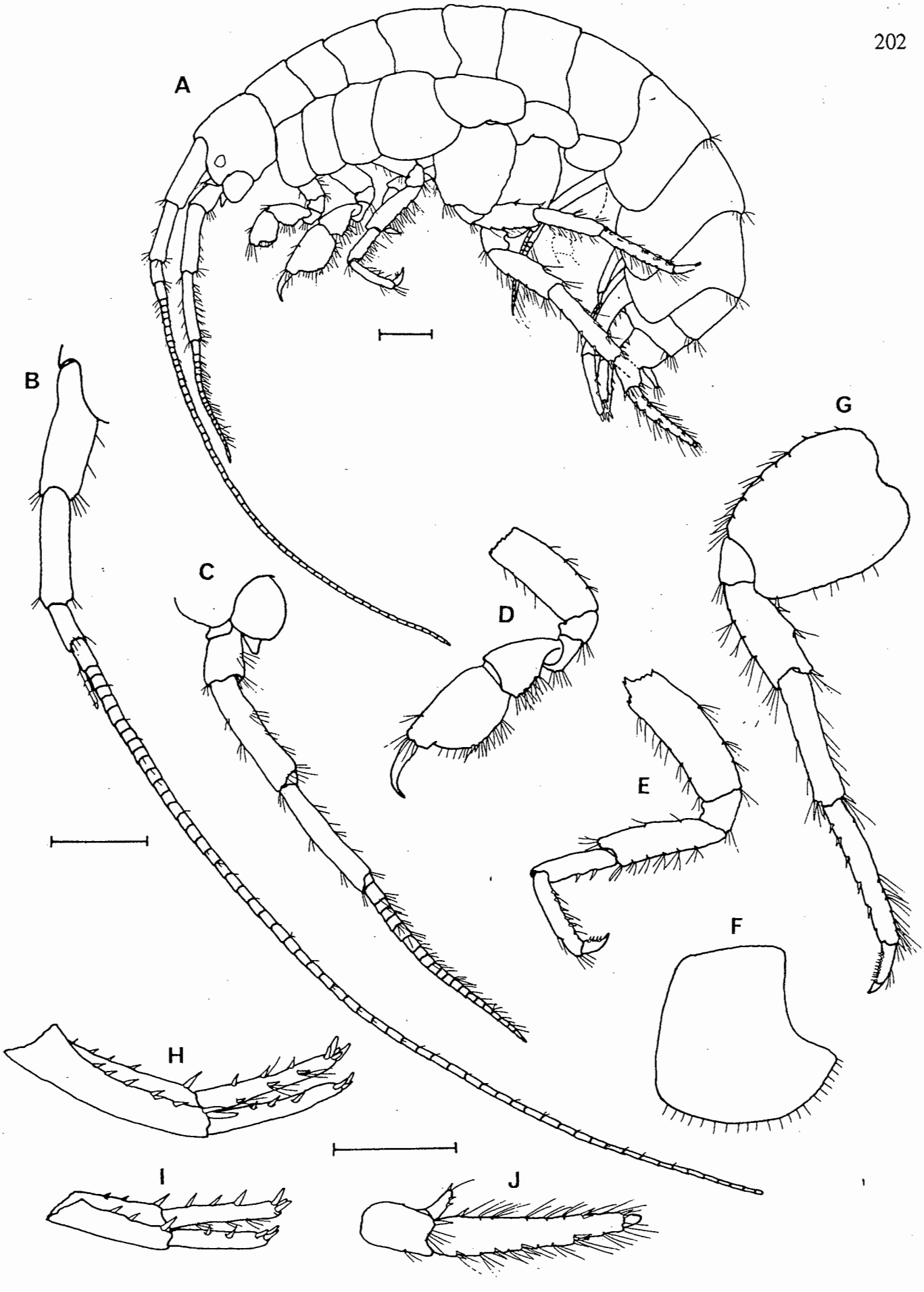

Fig. 12. *Paramelita capensis*, male, 16,3 mm. A. Lateral aspect. B. Antenna 1. C. Antenna 2. D. Gnathopod 2. E. Pereopod 3. F. Coxa 4. G. Pereopod 5. H. Uropod 1. I. Uropod 2. J. Uropod 3. Scale lines represent 1 mm.

SAM A2960, from Muizenberg Mountains. SAM A4565, from Hout Bay. SAM A6604, from the Cedarberg. SAM A7328, from Noordhoek forest. SAM A40259, tributary of the Spansemat River, Cape Peninsula. SAM A40260, The Baths, Citrusdal. SAM A40261, Grotto Ravine, Table Mountain. SAM A40262, Platteklip Gorge, Table Mountain. SAM A40263, Blackburn Ravine, Hout Bay.

Diagnosis

Eyes white. Antenna 1 sparsely setose, flagellum approximately 40-80 articulate, accessory flagellum 5-8 articulate. Antenna 2 usually shorter than 1, moderately setose, peduncle long and slender, stouter than 1, flagellum with about 15-35 articles. Coxa 4 strongly excavate posteriorly. Gnathopod 2, articles 2 either with long spine-like setae, or with stout spines on medial, posterior margin, palm strongly oblique, with 3-5 defining spines. Pereopods 3 and 4 moderately setose, unmodified, article 2 usually either with spine-like setae or strong spines medially, dactyls each with 3-6 spinules. Pereopods 5-7 moderately setose, dactyls with 8-13 spinules. Uropod 1, peduncle spinose, with very few setae, rami subequal, inner ramus with marginal spines and setae, outer ramus with marginal spines, rarely with setae, both ending in apical spines. Uropod 2, peduncle spinose, inner ramus slightly longer than outer, both with marginal and apical spines and marginal setae. Uropod 3, inner ramus 0.2 length of outer, apically spinose, outer ramus with marginal and apical spines, strongly setose, distinct second segment. Telson deeply cleft, each lobe bearing one spine and many apical and subapical setae.

Remarks

This species closely resembles *P. barnardi, P. kogelensis* and *P. parva*, but is distinguished from these species by its large size at maturity, strongly oblique palm of gnathopod 2, deeply excavate coxa 4, and densely setose uropod 3.

Distribution

Apparently widespread, Collected from streams in the Cedarberg area in the north, to the Cape Peninsula in the south (Fig. 26).

Paramelita flexa Griffiths, 1981

Fig. 13A-F

Paramelita flexa Griffiths, 1981: 86-89, fig. 5.

Material examined

Types. Holotype, Albany Museum MISC 52B, from a tributary of the Palmiet River between Elgin and Grabouw. Paratypes, SAM A16776, from the same locality as the holotype.

Diagnosis

Eyes white. Antenna 1 sparsely setose, flagellum about 26 articulate, accessory flagellum 5 articulate. Antenna 2 shorter than 1, in males, article 3 strongly lobed posterodistally, article 4 curved ventrally and article 5 bent at right angles to 4, flagellum 16 articulate. Coxa 4 distinctly excavate posteriorly. Gnathopod 2, palm slightly oblique, with three defining spines. Pereopods 3 and 4 unmodified, dactyls with 3-4 spinules each. Pereopods 5-7, dactyls with 4-9 spinules each. Uropod 1, peduncle spinose, lacking setae, rami subequal, with marginal and apical spines, no setae. Uropod 2, peduncle spinose and setose, inner ramus slightly longer than outer, both with marginal and apical spines, lacking setae. Uropod 3, inner ramus 0.3 length of outer, with some spines on apex, outer ramus with marginal and apical spines and setae, second segment distinct. Telson deeply cleft, each lobe with one spine and some setae.

Remarks

The combination of a protruded posterior margin in article 3 of antenna 2, an excavate coxa 4, multispinose dactyls and the presence of a second segment on the outer ramus of uropod 3 makes this species distinctive. It is highly unlikely that the

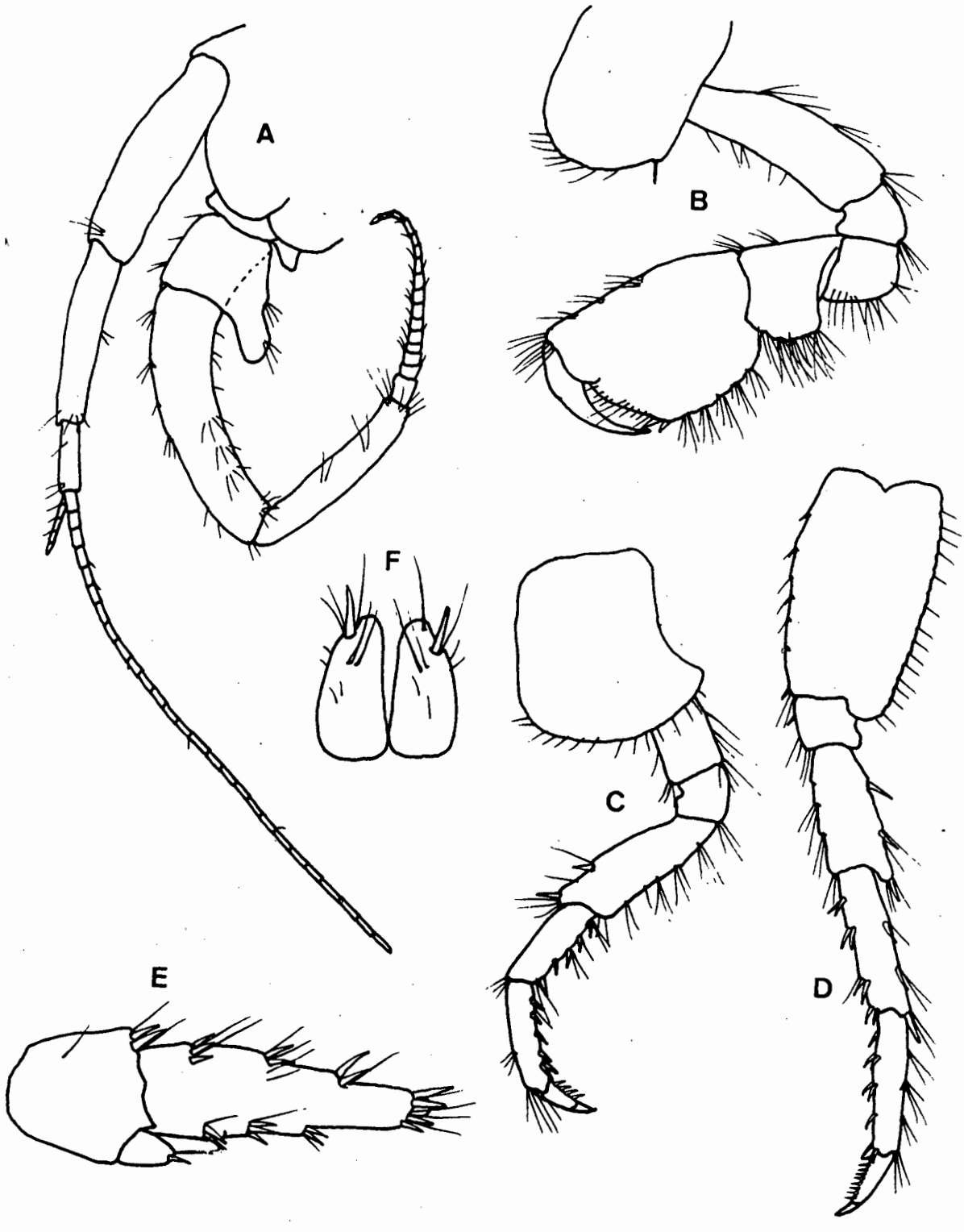

Fig. 13. *Paramelita flexa*, male, 7,0 mm. A. Antenna 1 and 2. B. Gnathopod 2. C. Coxa and Pereopod 4. D. Pereopod 7. E. Uropod 3. F. Telson.

"lobe" on article 3 of antenna 2 is at all homologous to the semi-circular lobe found in *A auricularius*, *A. andronyx* and *A. pheronyx*. As yet, male specimens have not been recollected since 1952, and in 1979, only one ovigerous female and four juveniles were found at the type locality.

Distribution

Known only from the type locality, a tributary of the Palmiet River on the Grabouw-Elgin road, between Hottentots Holland and Groenland Mountains (Fig. 26).

Paramelita granulicornis (Barnard, 1927)

Fig. 14A-I

Gammarus granulicornis Barnard, 1927: 175-177, pl. 10, (figs 10-11, 20).
Paramelita granulicornis (Barnard) Thurston, 1973: 167. Griffiths, 1981: 89, fig. 2H-I.

Material examined

Types. Lectotype and paratypes, SAM A4874 and A5178, from Steenbras River.

Other material. SAM A5182, A5183, A5185, all from the Hottentots Holland Mountains. SAM A40236, from a stream on Houhoek Pass. SAM A40237, from a tributary of the Palmiet River flowing near the Orchard's Farm Stall. SAM A40238, from a tributary of the Palmiet River below Elephant Rock.

Diagnosis

Eyes white. Antenna 1, sparsely setose, flagellum about 27-40 articulate, accessory flagellum 4-5 articulate. Antenna 2 sparsely to moderately setose, shorter than 1, moderately stout, peduncle sometimes elongate in males, flagellum with about 12-16 articles. Coxa 4 quadrate. Gnathopod 2, article 2 posteriorly spinose, palm

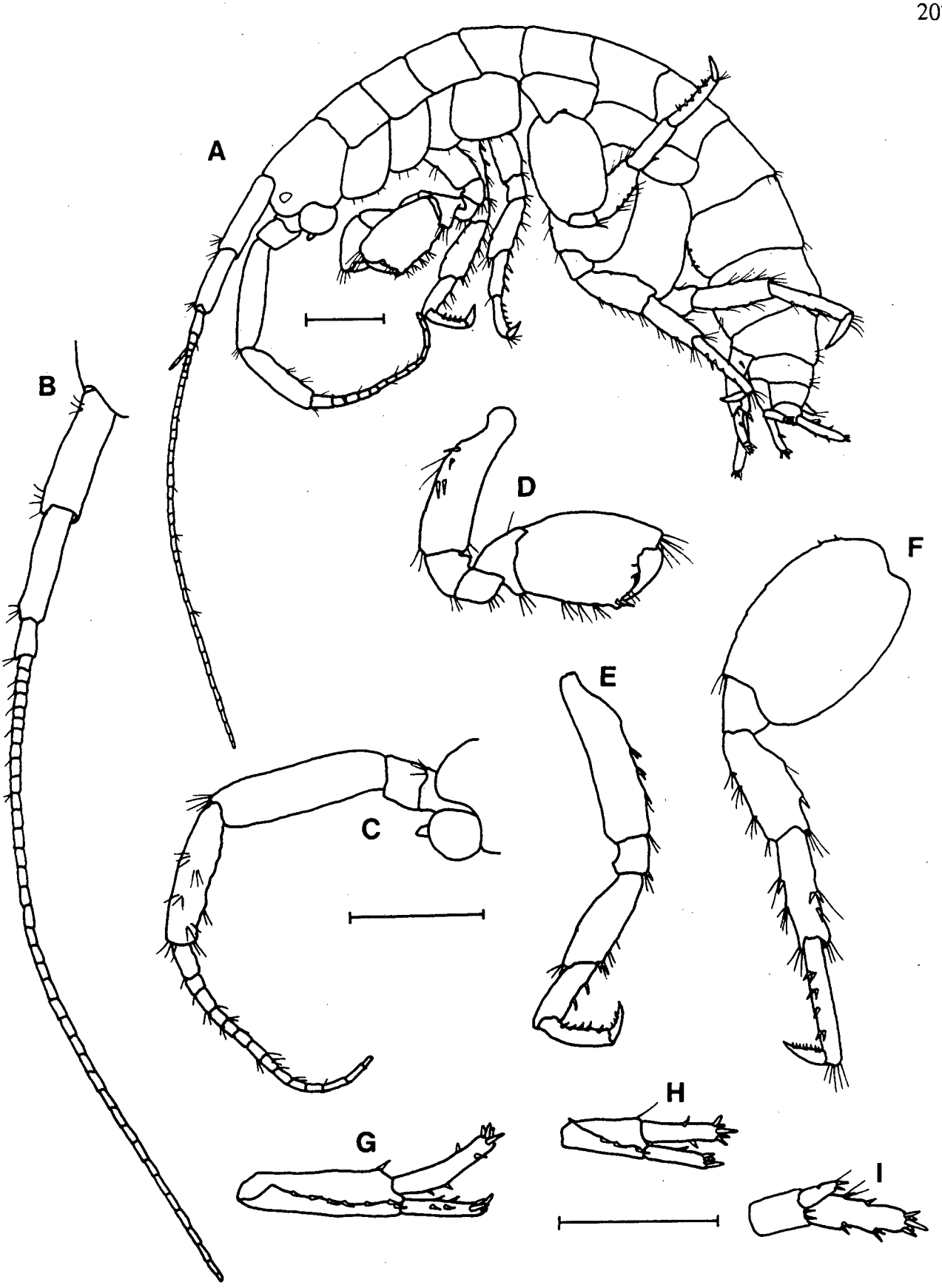

Fig. 14. *Paramelita granulicornis*, male, 10,3 mm. A. Lateral aspect. B. Antenna 1. C. Antenna 2. D. Gnathopod 2, medial view. E. Pereopod 3. F. Pereopod 6. G. Uropod 1. H. Uropod 2. I. Uropod 3. Scale lines represent 1 mm.

transverse, defining angle forming a small protruding rounded tooth, with three spines. Pereopods 3 and 4, articles 2 strongly spinose posteriorly, article 4 often considerably longer and wider than 5, dactyls with 2-3 spinules each. Pereopods 5-7, dactyls with 4-8 spinules. Uropod 1, peduncle spinose, lacking setae, rami subequal, bearing marginal and apical spines, lacking setae. Uropod 2, peduncle with spines and usually at least one seta, inner ramus longer than outer, both with marginal and apical spines, lacking setae. Uropod 3, inner ramus about 0.4 length of outer ramus, with some apical setae and at least one seta, outer ramus with marginal and apical spines and setae, second segment rudimentary. Telson deeply cleft, each lobe bearing one spine and several setae.

Remarks

This species is most like *P. aurantius*, from which it is distinguished by the possession of a strongly convex palm with palmar tooth in gnathopod 2. *P. granulicornis* shares this condition with *Aquadulcaris platypus*, and it is possible that these two species are closely related. Both species have almost quadrate fourth coxal plates, and Barnard (1927) has commented on how article 4 in pereopods 3 and 4 is "strongly expanded distally", and article 5 is "noticeably shorter" than article 4 in *P. granulicornis*. This condition is extremely well developed in *A. platypus*. Any further decisions regarding the position of *P. granulicornis* will be taken once genetic analysis of *Paramelita* is complete.

Distribution

Known from the Hottentots Holland Mountain and adjacent areas (Fig. 26).

Paramelita kogelensis (Barnard, 1927)

Fig. 15A-J

Gammarus kogelensis Barnard, 1927: 172-173, pl. 10, (figs 9, 21).

Paramelita kogelensis (Barnard) Thurston, 1973: 167. Griffiths, 1981: 89, fig 2G.

Material examined

Types. Lectotype and paratypes, SAM A4873, west of Kogelberg.

Other material. SAM A5174, Kogelberg. SAM A5190, on way to Kogelberg from Steenbras. SAM A40243, Viljoen's Pass, Nuweberg State Forest.

Diagnosis

Eyes white. Antenna 1 sparsely setose, peduncle sometimes spinose, flagellum 27-34 articulate, accessory flagellum 3-5 articulate. Antenna 2 shorter than 1, sparsely to densely setose, flagellum 12-16 articulate. Coxa 4, posterior margin, distinctly excavate. Gnathopod 2, article 2 not medially spinose, palm slightly to moderately oblique, with 2-3 defining spines. Pereopods 3 and 4 unmodified, dactyls with 2-4 spinules. Pereopods 5-7, dactyls with 5-8 spinules. Uropod 1, peduncle spinose, lacking setae, rami subequal, with marginal and apical spines, inner ramus rarely with setae. Uropod 2, peduncle spinose, rarely with one seta, inner ramus slightly longer than outer, both with marginal and apical spines, inner ramus rarely with 1-2 setae. Uropod 3, inner ramus 0.2-0.3 length of outer, apically spinose and sometimes with 1-2 setae, outer ramus with marginal and apical spines, sparsely setose, distinct second segment present. Telson deeply cleft, each lobe with one spine and 3-4 setae.

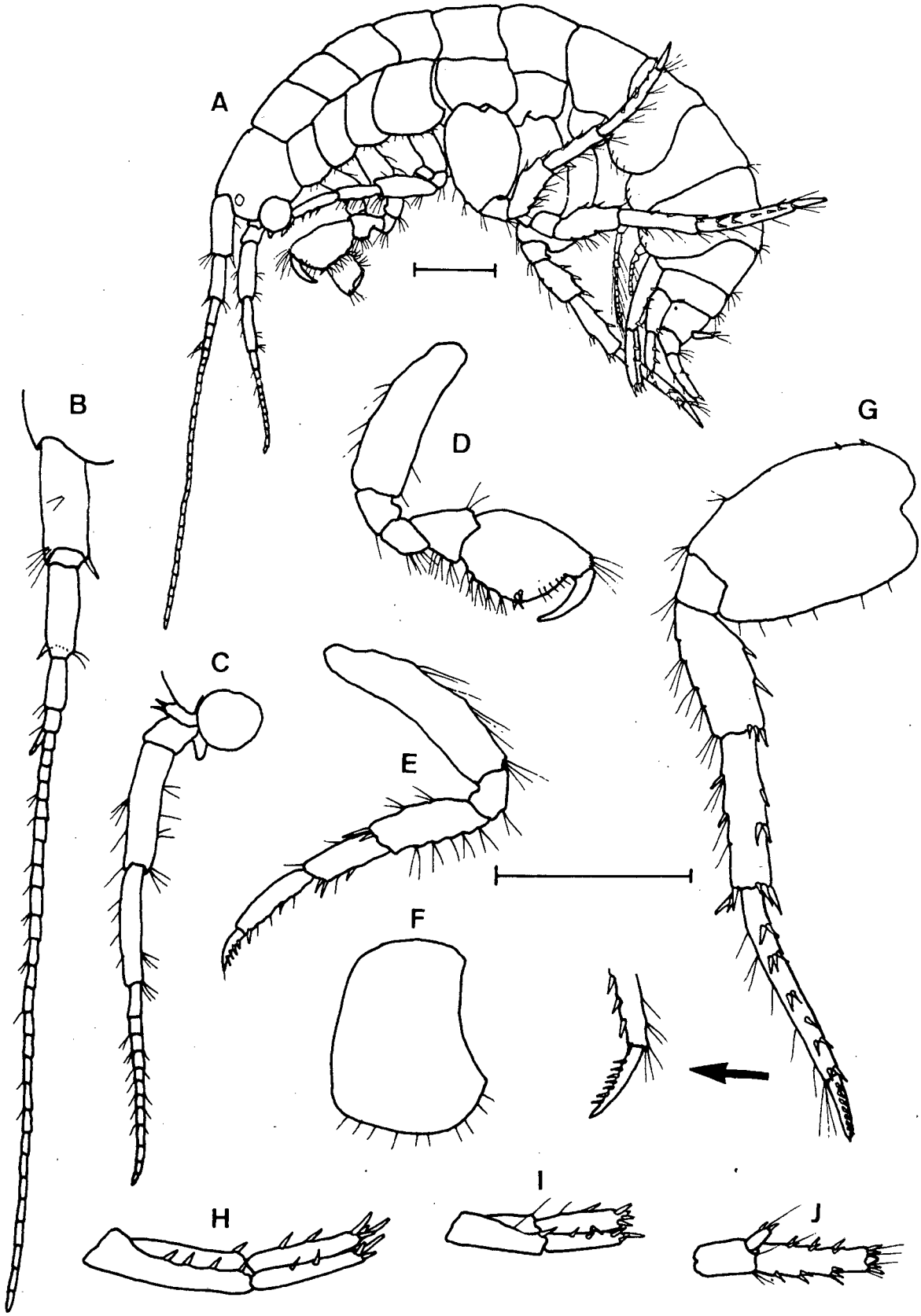

Fig. 15. *Paramelita kogelensis*, male, 8,0 mm. A. Lateral aspect. B. Antenna 1. C. Antenna 2. D. Gnathopod 2, medial view. E. Pereopod 3. F. Coxa 4. G. Pereopod 6. H. Uropod 1. I. Uropod 2. J. Uropod 3. Scale lines represent 1 mm.

Remarks

One of four morphologically similar species, *P. kogelensis* is distinguished from *P. parva, P. barnardi* and *P. capensis* by the number of articles in the flagella of antennae 1 and 2, spination of the dactyls of pereopods 3 and 4, and the setation of the inner rami of uropod 1 and the outer ramus of uropod 3.

Distribution

Collected from localities on the Hottentots Holland and adjacent mountains (Fig. 26).

Paramelita magna Stewart & Griffiths, 1991

Fig. 16A-J

Paramelita magna Stewart & Griffiths, 1991b: 79-83, figs 2, 3.

Material examined

Types. Holotype, SAM A40208, and paratypes, SAM A40209, from a tributary of the Krom River in the Cape of Good Hope Nature Reserve.

Other material. SAM A3083, Kalk Bay (Barnard's (1916: 205) SAM A3084, typographical error). SAM A4563, from Noordhoek. SAM A40210, from the Booiskraal River, and SAM A40211, from the Buffels River, both in the Cape of Good Hope Nature Reserve. SAM A40212, from Nellies Pool, and SAM A40213, from the Silvermine River, both in the Silvermine Nature Reserve.

Diagnosis

Eyes white. Antenna 1 sparsely setose, flagellum 37-42 articulate, accessory flagellum 6-8 articulate. Antenna 2 sparsely to moderately setose, in males, peduncle stout and elongate so that antenna 2 equal to, or exceeding 1 in length, flagellum with 16-19 articles. Coxa 4, posterior margin excavate. Gnathopod 2, medial posterior

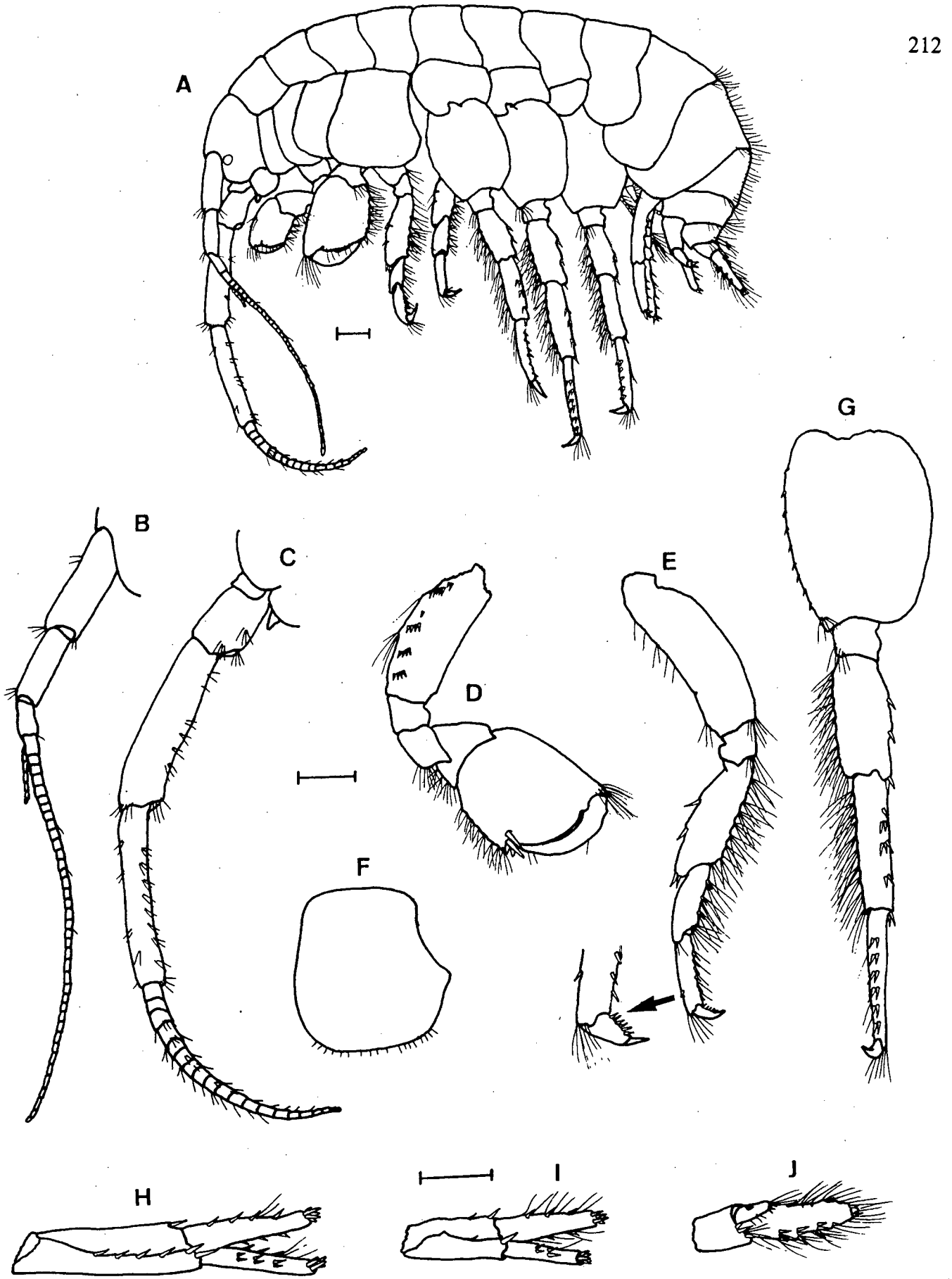

Fig. 16. *Paramelita magna*, male, 22,3 mm. A. Lateral aspect. B. Antenna 1. C. Antenna 2. D. Gnathopod 2, medial view. E. Pereopod 3. F. Coxa 4. G. Pereopod 6. H. Uropod 1. I. Uropod 2. J. Uropod 3. Scale lines represent 1 mm.

margin of article 2 strongly spinose in males, palm slightly oblique, defined by five spines. Pereopods 3 and 4 unmodified, moderately to densely setose posteriorly, dactyls with 4-6 spinules. Pereopods 5-7 strongly setose anteriorly, dactyls with 8-10 spinules. Uropod 1, peduncle spinose, usually lacking setae, rami subequal, with marginal spines and setae and apical spines. Uropod 2, peduncle spinose, sometimes with a few setae, inner ramus longer than outer, both with marginal spines and setae and apical spines. Uropod 3, inner ramus 0.3 length of outer, apex spinose and setose, outer ramus with marginal and apical spines and setae, second segment distinct, about 6% length of first. Telson deeply cleft, each lobe usually with one spine and many setae, right lobe sometimes with two spines.

Remarks

Some of the largest specimens of *Paramelita* collected are members of this species. *P. magna* is easily recognised by its dark brown colour, markedly setose urosome and pereopods, and the possession of stout elongate second antennae in males. It is distinguished from *P. validicornis* by the relative length of the peduncle and outer ramus in uropod 3, and from *P. magnicornis* by the setation of the uropods, body colour, and the lack of modification of pereopod 3.

Distribution

In streams draining mountainous areas in the southern part of the Cape Peninsula (Fig. 26).

Paramelita magnicornis Stewart & Griffiths, 1991

Fig. 17A-J

Paramelita magnicornis Stewart & Griffiths, 1991: 25-30, figs 3, 4.

Material examined

Types. Holotype, SAM A40009, and paratypes, SAM A40010, from a stream draining the Swartkop Mountains near Miller's Point.

Other material. SAM A40011 and SAM A40015, from a stream draining Chapman's Peak. SAM A40012, from a stream in the Kalk Bay Mountains near Clovelly. SAM A40013, from a stream near Miller's Point. SAM A40014 and A40016, from Peck's Valley on Boyes Drive.

Diagnosis

Eyes white. Antenna 1 sparsely setose, flagellum 27-40 articulate, accessory flagellum 4-6 articulate. Antenna 2 sparsely to moderately setose, in males, articles 3, 4 and 5 of peduncle elongate and stout, with articles 4 and 5 distally swollen, antenna 2 exceeding 1 in length, flagellum 13-21 articulate. Coxa 4, posterior margin excavate. Gnathopod 2, article 2 spinose on medial posterior margin in males, palm oblique, defined by 3-4 stout spines. Pereopods 3 and 4 moderately to densely setose posteriorly, in males, articles 4 posterodistally protruded into a triangular tooth, dactyls with 4-6 spinules. Pereopods 5-7, moderately to densely setose, dactyls with 6-9 spinules. Uropod 1, peduncle spinose and setose, rami subequal, both with marginal and apical spines, inner ramus with a few setae. Uropod 2, peduncle spinose and setose, inner ramus slightly longer than outer, both with marginal and apical spines, inner ramus sometimes with a few setae, outer ramus lacking setae. Uropod 3, inner ramus 0.3 length of outer, apex spinose, outer ramus with marginal and apical spines

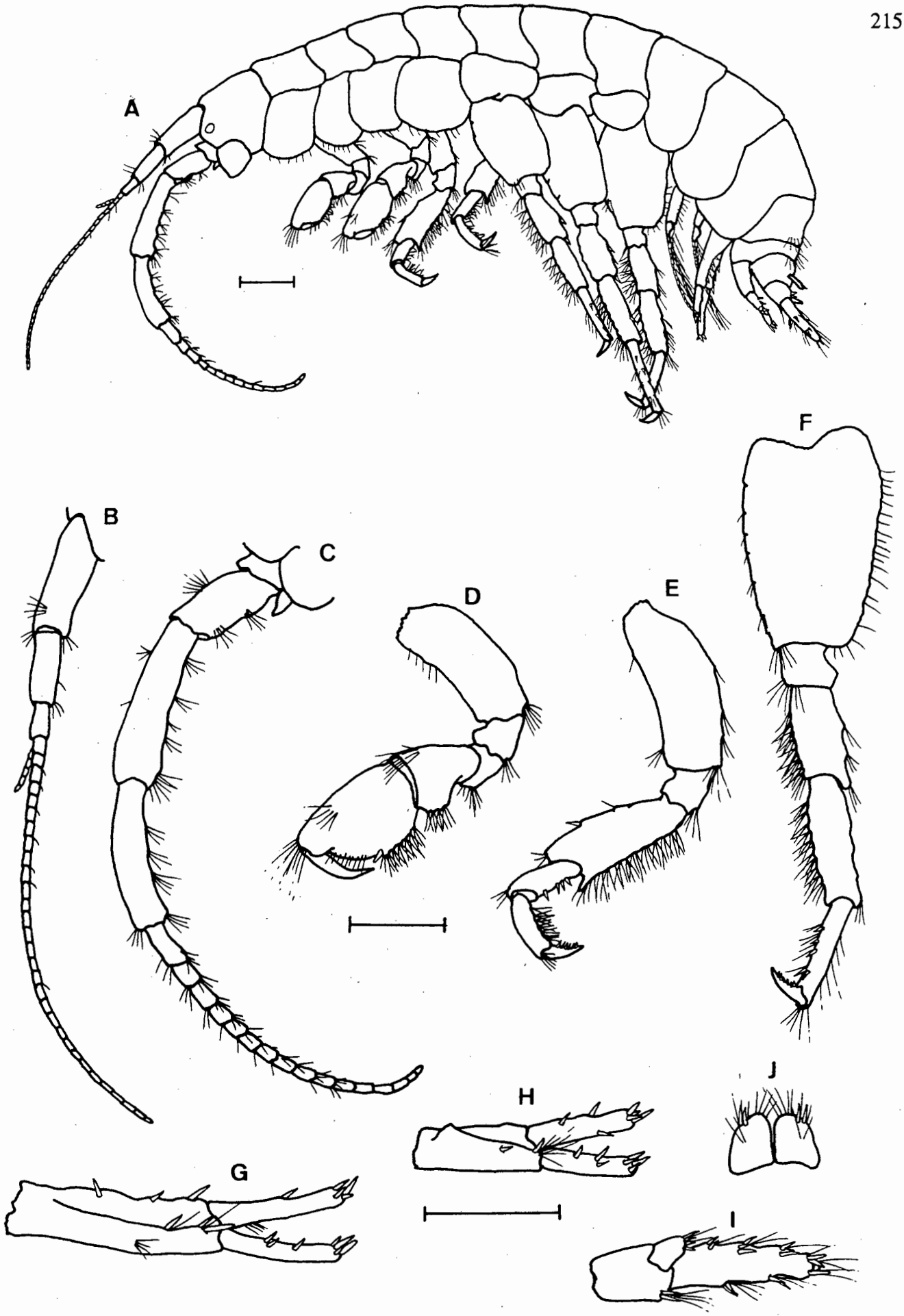

Fig. 17. *Paramelita magnicornis*, male, 15,0 mm. A. Lateral aspect. B. Antenna 1. C. Antenna 2. D. Gnathopod 2. E. Pereopod 3. F. Pereopod 7. G. Uropod 1. H. Uropod 2. I. Uropod 3. J. Telson. Scale lines represent 1 mm.

and setae, second segment distinct but small, about 5% length of first. Telson deeply cleft, each lobe with 1-2 spines and many setae.

Remarks

This species is usually distinguished by the elongation of antenna 2, and the posterodistal projection of article 4 in pereopods 3 and 4. Populations which have this 'tooth' on article 4 absent or poorly developed are distinguished from *P. magna* by setation of the uropods and body colour and size.

Distribution

Confined to the Cape Peninsula, from Constantiaberg in the north to Swartkopberg in the south (Fig. 26).

Paramelita nigroculus (Barnard, 1916)

Fig. 18A-J

Gammarus nigroculus Barnard, 1916: 206-207, pl. 27, (fig. 23); 1927: 168-169.
Paramelita nigroculus (Barnard) Thurston, 1973: 166. Griffiths, 1981: 89-90, fig. 6.

Material examined

Types. SAM A3059, from a stream above Oranjezicht, Table Mountain. Lectotype and paratypes, variety *persetosus*, SAM A4877, from Sneeugat near Tulbagh.

Other material. SAM A1270, Devil's Peak, Table Mountain. SAM A2461 and 4009, Platteklip Gorge, Table Mountain. SAM A2966, 3060-62, Table Mountain. SAM A3038, Kirstenbosch. SAM A4002, north of Landdroskloof, Caledon side. SAM A4016 and 4871, Steenbras River. SAM A4560, Jonkershoek, opposite Diep Gat. SAM A4876, Tulbagh. SAM A4878-84, all from localities near Sneeugat, north of Tulbagh. SAM A4885, Franchhoek Mountains. SAM A4887, Vlakte, Ceres.

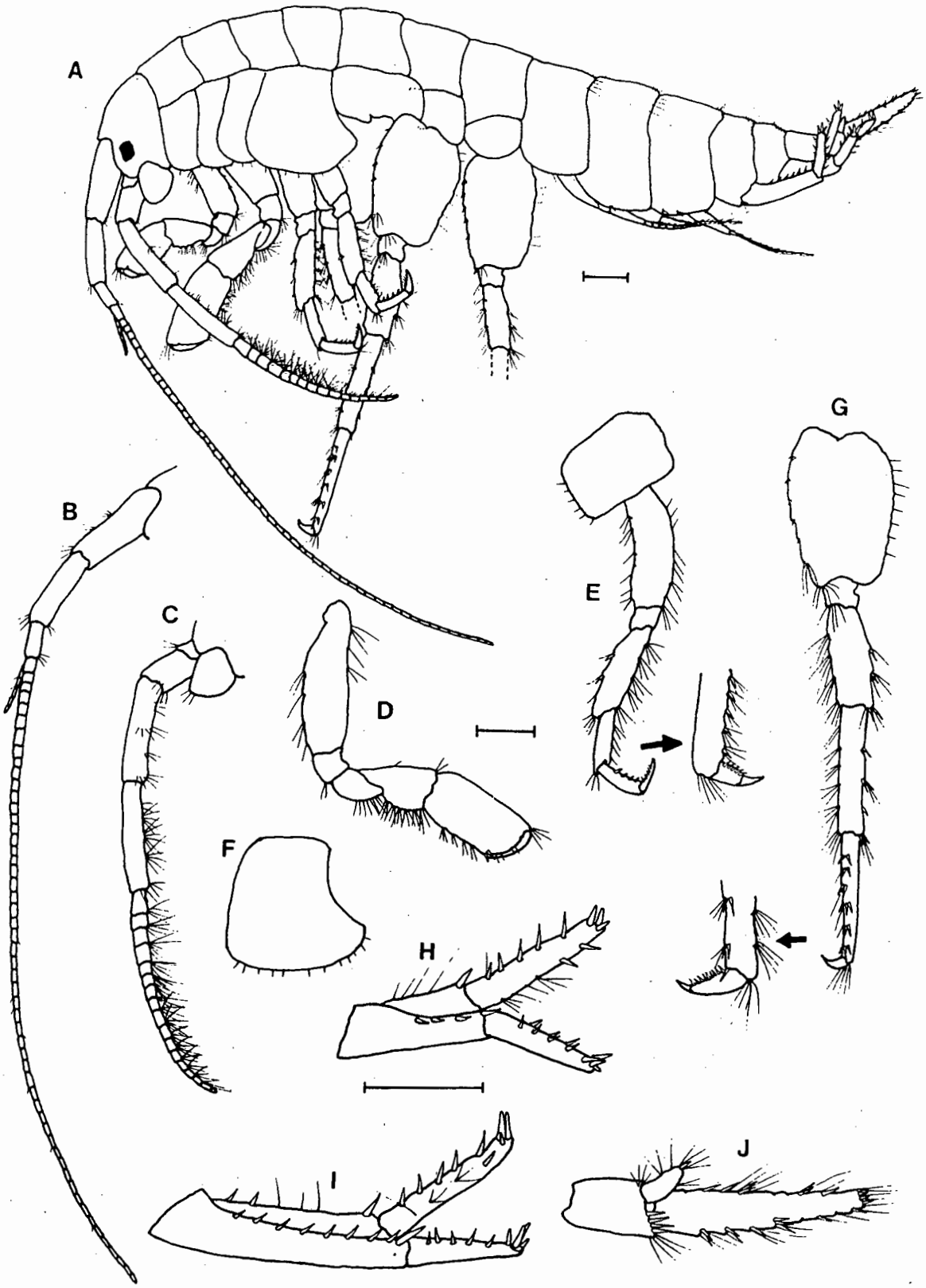

Fig. 18. *Paramelita nigroculus*, male, 18,0 mm. A. Lateral aspect. B. Antenna 1. C. Antenna 2. D. Gnathopod 2, medial view. E. Pereopod 3. F. Coxa 4. G. Pereopod 6. H. Uropod 2. I. Uropod 1. J. Uropod 3. Scale lines represent 1 mm.

SAM A5188, Hottentot Holland Mountains. SAM A6054, 6055 and 8273, Zonderend Mountains. SAM A6296, Montagu. SAM A6602, Middelberg plateau, Cedarberg. SAM A6603 and 6965, Tafelberg, Cedarberg. SAM A6936-38, Swellendam Mountains. SAM A6944, Simonsberg. SAM A6945, Witte River. SAM A7335, Schuiffenberge, east of Citrusdal. SAM A8196, Krom River, Cedarberg. SAM A12308, Porterville. SAM A40264, A40266 and A40271, Du Toit's Kloof. SAM A40265, Goudini Spa. SAM A40267 and A40268, Bain's Kloof Pass. SAM A40269, Steenboks Nature Reserve. SAM A40270, Paarl Rocks. SAM A40272 and A40273, Dwarsrivierhoek, near Stellenbosch. SAM A40274, Wemmershoekdam. SAM A40275, Franchhoek Pass.

Diagnosis

Eyes black. Antenna 1 sparsely setose, flagellum 20-70 articulate, accessory flagellum 4-5 articulate. Antenna 2 shorter than 1, sparsely to densely setose, slender to stout, flagellum 15-22 articulate. Coxa 4, posterior margin strongly excavate. Gnathopod 2, article 2 not medially spinose, articles 5 and 6 markedly elongate or not, palm slightly oblique, with 2-4 defining spines. Pereopods 3 and 4 unmodified, moderately to densely setose, dactyls with 3-7 spinules. Pereopods 5-7 moderately to densely setose, dactyls with 5-10 spinules. Uropod 1, peduncle spinose and setose, rami subequal, both with marginal and apical spines, inner ramus setose or not. Uropod 2, peduncle spinose and setose, both rami with marginal and apical spines, inner ramus setose or not. Uropod 3, inner ramus 0.2-0.3 length of outer, apically spinose, margins and apex setose, outer ramus with marginal and apical spines, sparsely to densely setose, second segment present or not. Telson deeply cleft, each lobe with one spine and many apical and dorsal setae.

Remarks

All populations of *Paramelita* which have black eyes have been considered members of a single widespread species, *P. nigroculus*, despite morphological variation between them. For example, some populations have individuals with elongate, stout

second antennae, while in others, these antennae are relatively slender and short. Barnard (1927) recognised a variety, *persetosus*, based mainly on specimens from the Sneeugat valley north of Tulbagh. These animals have densely setose second antennae, pereopods and uropods. Since setation in *P. nigroculus* can vary considerably between, and within in one population, and is usually related to maturity, with the larger, older individuals more setose than younger specimens, Barnard (1927) was reluctant to consider these populations with highly setose second antennae as a separate species. There is a need for a thorough, morphological and genetic investigation of all black eyed *Paramelita* populations.

Distribution

Widely distributed from the Cedarberg in the north to Swellendam in the east (Fig. 26).

Paramelita odontophora Stewart & Snaddon, 1991

Fig. 19A-J

Paramelita odontophora Stewart & Snaddon, 1991: 155-159, figs 6, 7.

Material examined

Types. SAM A40240, holotype, SAM A40241, paratypes, from a tributary of the Palmiet River near Elgin.

Other material. SAM A40250, tributary of the Palmiet River, near Kleinmont.

Diagnosis

Eyes white. Antenna 1 sparsely setose, flagellum 35-38 articulate, accessory flagellum 4-5 articulate. Antenna 2 sparsely setose, in males, both peduncle and flagellum extremely elongate so that antenna 2 is considerably longer than 1, article 4 with a subterminal, posterodistal tooth, flagellum with 19-22 articles. Coxa 4,

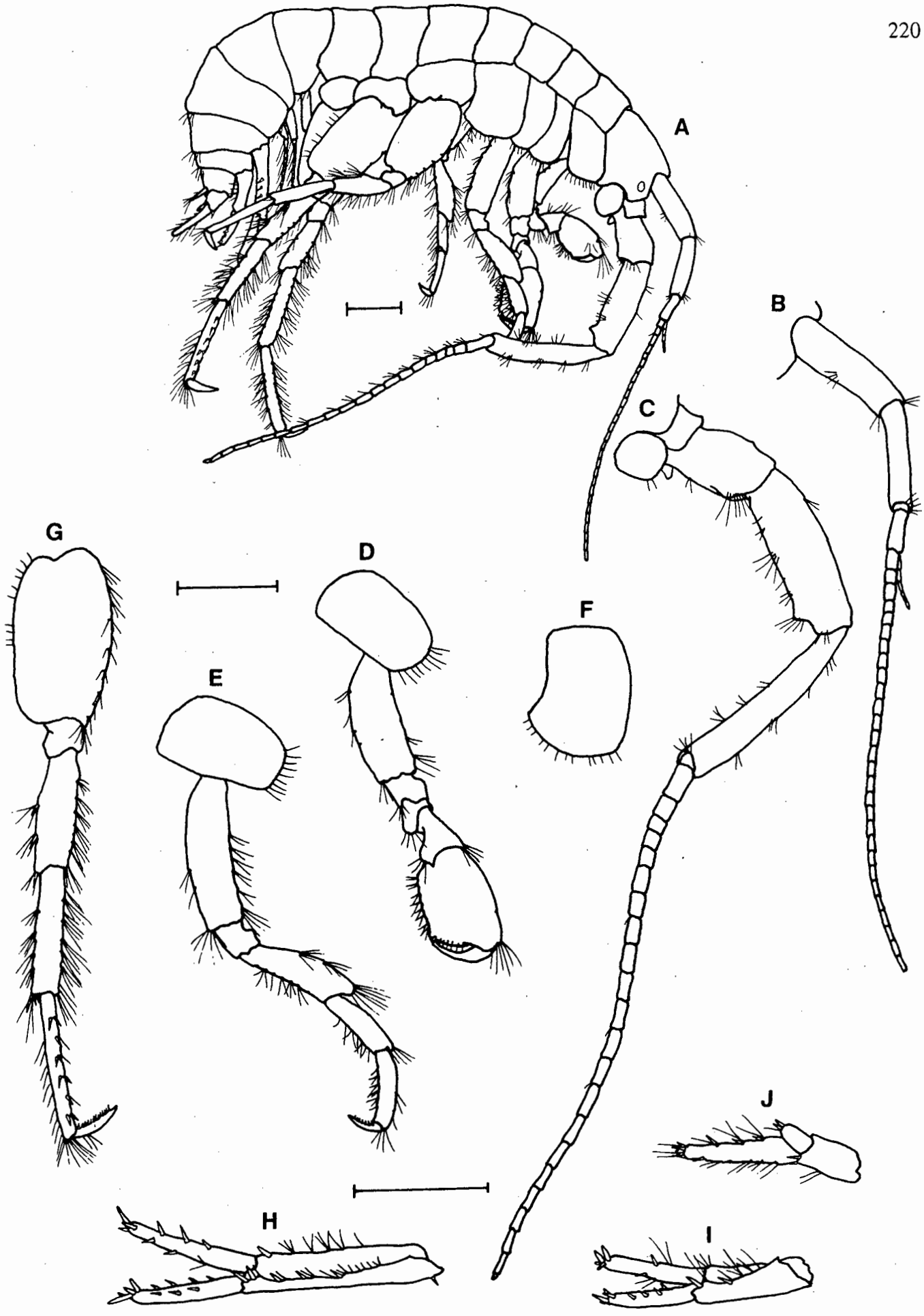

Fig. 19. *Paramelita odontophora*, male, 11,1 mm. A. Lateral aspect. B. Antenna 1. C Antenna 2. D. Gnathopod 2. E. Pereopod 3. F. Coxa 4. G. Pereopod 6. H. Uropod 1 I. Uropod 2. J. Uropod 3. Scale lines represent 1 mm.

posterior margin excavate. Gnathopod 2, article 2 spinose on posterior, medial margin in males, palm slightly oblique, with four defining spines. Pereopods 3 and 4 unmodified, moderately setose, dactyls with 4-7 spinules. Pereopods 5-7 moderately to densely setose, dactyls with 11-13 spinules. Uropod 1, peduncle spinose and setose, rami subequal, both with marginal and apical spines, inner ramus sometimes with a single seta. Uropod 2, peduncle spinose and setose, rami approximately subequal, both with marginal and apical spines, inner ramus with a few marginal setae. Uropod 3, inner ramus 0.3 length of outer, with two apical spines, outer ramus with marginal and apical spines and setae, second segment distinct but small, about 4-5% of first. Telson deeply cleft, each lobe with a single spine and about 6-8 setae.

Remarks

The extremely elongate antenna 2 with a subterminal 'tooth' on article 4 of the peduncle in males makes this species unmistakable.

Distribution

Known from two tributaries of the Palmiet River (Fig. 26).

Paramelita parva Stewart & Griffiths, 1991

Fig. 20A-J

Paramelita parva Stewart & Griffiths, 1991b: 93-97, figs 8, 9.

Material examined

Types. Holotype, SAM A40226, paratypes, SAM A40227, from a tributary of the Storms River, eastern Cape.

Other material. SAM A40228, A40229, A40230 and A40231, all from tributaries of the Storms River, eastern Cape.

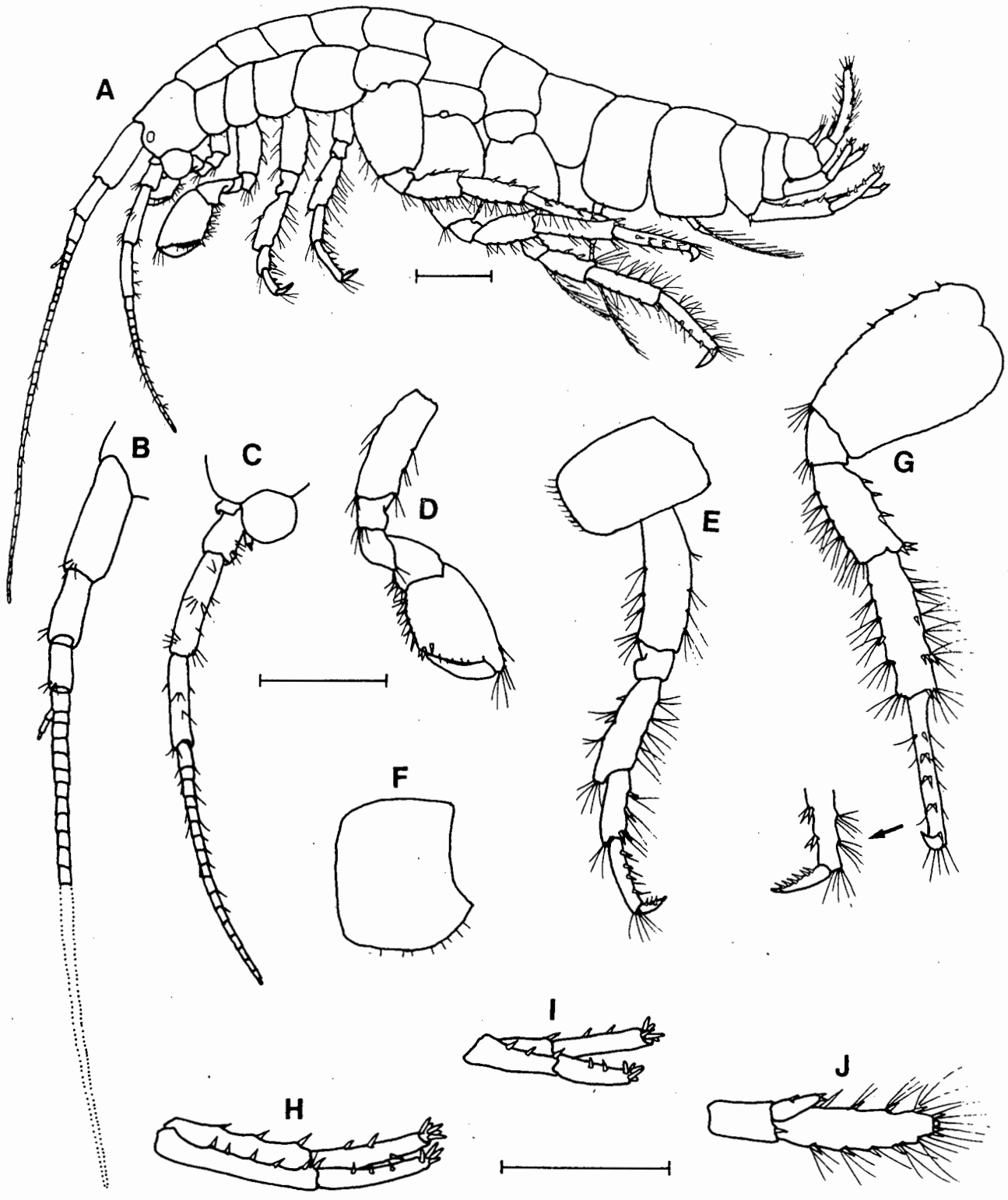

Fig. 20. *Paramelita parva*, male, 8,7 mm. A. Lateral aspect. B. Antenna 1. C. Antenna 2. D. Gnathopod 2, medial view. E. Pereopod 3. F. Coxa 4. G. Pereopod 6. H. Uropod 1. I Uropod 2. J. Uropod 3. Scale lines represent 1 mm.

Diagnosis

Eyes white. Antenna 1 sparsely setose, flagellum 22-26 articulate, accessory flagellum 4-5 articulate. Antenna 2 sparsely to moderately setose, shorter than 1, peduncle not enlarged in males, flagellum with 11-18 articles. Coxa 4, posterior margin excavate. Gnathopod 2, palm moderately oblique, with 3-4 defining spines. Pereopods 3 and 4 moderately setose, unmodified, dactyls each with 2-3 spinules. Pereopods 5-7 moderately setose, dactyls each with 4-7 spinules. Uropods 1 and 2, peduncle spinose, lacking setae, rami with marginal and apical spines, lacking setae. Uropod 3, inner ramus 0.3 length of outer, apically spinose, outer ramus with marginal and apical spines, moderately to densely setose, second segment small but distinct. Telson deeply cleft, each lobe with 1-2 spines and a few 1-4 setae.

Remarks

This species is morphologically similar to *P. kogelensis*, from which it is distinguished by the number of spinules on the dactyls of pereopods 3 and 4, and the setation of the uropods.

Distribution

The most isolated of all the paramelitid species, *P. parva* has been collected from the Storms River catchment, eastern Cape (Fig. 26).

Paramelita pillicornis Stewart & Griffiths, 1991

Fig. 21A-K

Paramelita pillicornis Stewart & Griffiths, 1991b: 84-88, figs 4, 5.

Material examined

Types. Holotype, SAM A40214, paratypes, SAM A40215, from a tributary of Waboomsrivier on Gydo Pass, north of Ceres.

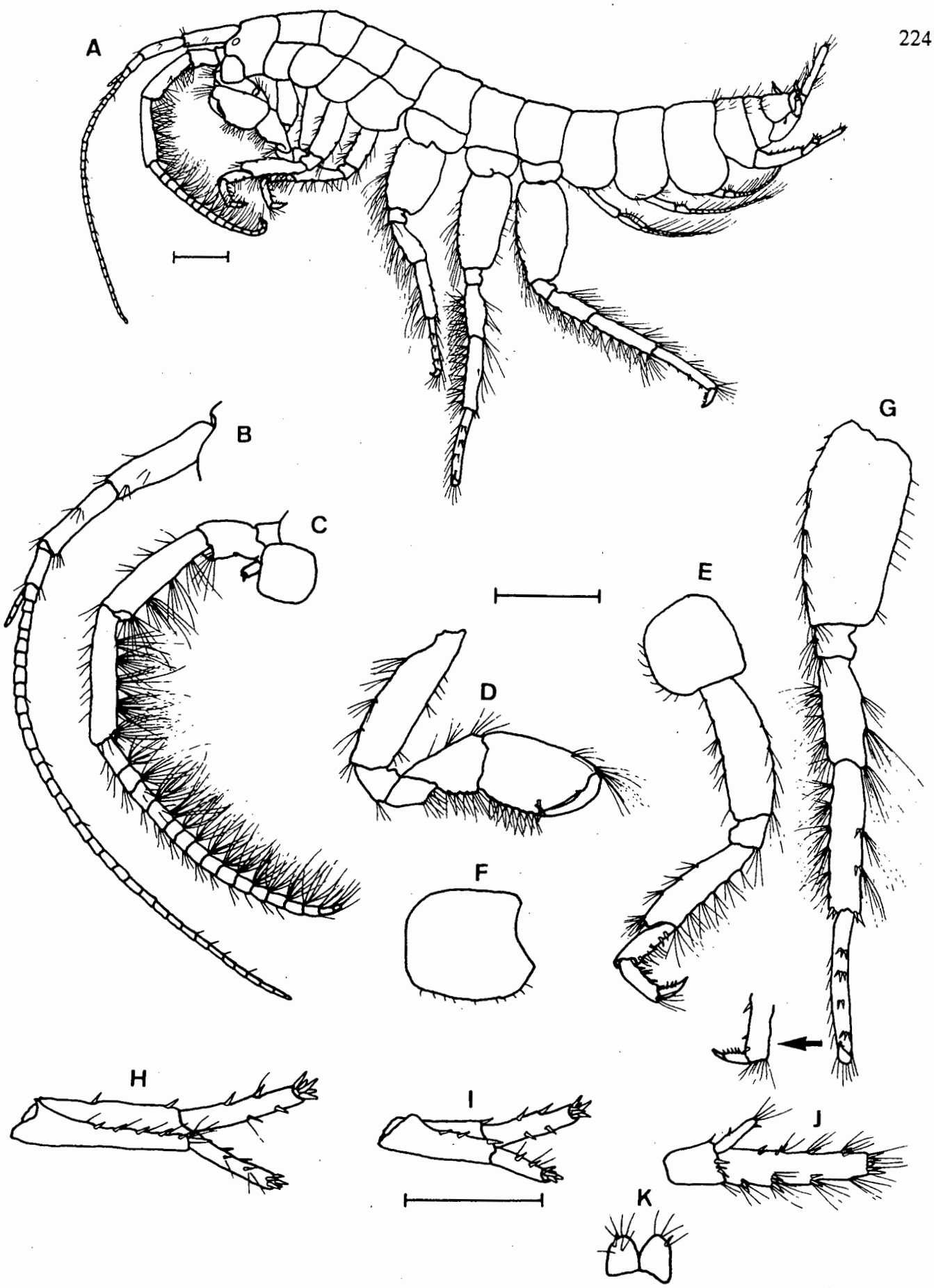

Fig. 21. *Paramelita pillicornis*, male, 10,8 mm. A. Lateral aspect. B. Antenna 1. C. Antenna 2. D. Gnathopod 2, medial view. E. Pereopod 3. F. Coxa 4. G. Pereopod 6. H. Uropod 1. I. Uropod 2. J. Uropod 3. K. Telson. Scale lines represent 1 mm.

Diagnosis

Eyes white. Antenna 1 sparsely setose, flagellum 18-33 articulate, accessory flagellum 3-4 articulate. Antenna 2 shorter than 1, densely setose posteriorly in males, flagellum with 13-16 articles. Coxa 4, posterior margin excavate. Gnathopod 2, palm distinctly oblique, with three defining spines. Pereopods 3-7 moderately to densely setose, article markedly poorly expanded, dactyls each with 3-5 spinules. Uropod 1, peduncle spinose and setose, both rami with marginal spines and sometimes setae, ending in apical spines. Uropod 2, peduncle spinose, lacking setae, inner ramus with marginal spines, outer ramus with marginal spines and sometimes setae, both with apical spines. Uropod 3, inner ramus 0.3-0.4 length of outer, apically spinose and setose, outer ramus with marginal and apical spines, moderately setose, second segment small but distinct. Telson deeply cleft, each lobe with one spine and 3-8 setae.

Remarks

One of two white-eyed *Paramelita* species with a highly setose antenna 2, *P. pillicornis* is distinguished from *P. seticornis* by the relative length of the peduncle and the number of articles in the flagellum of this antenna, the width of article 2 in pereopods 5-7, and the setation of the rami in uropods 1 and 2.

Distribution

Known only from the type locality, Gydo Pass, north of Ceres (Fig. 26).

Paramelita pinnicornis Stewart & Griffiths, 1991

Fig. 22A-J

Paramelita pinnicornis Stewart & Griffiths, 1991a: 20-25, figs 1, 2.

Material examined

Types. Holotype, SAM A40004, paratypes, SAM A40005, from a tributary of the Burgersbos River, Cape Peninsula.

Other material. SAM A10017, from Newlands, Cape Peninsula. SAM A40008, from Kenilworth Race Course, Cape Peninsula. SAM A40006 and A40007, from adjacent streams in the Cape Hangklip area, east coast of False Bay.

Diagnosis

Eyes white. Antenna 1 sparsely setose, flagellum 31-46 articulate, accessory flagellum 5-6 articulate. Antenna 2 sparsely to moderately setose, of equal length to 1, articles 4 and 5 of peduncle in males extremely elongate and outer margin and tip of article 5 extended into an elongate triangular flange, flagellum with 16-23 articles. Coxa 4, posterior margin excavate. Gnathopod 2, article 2 strongly spinose medially, palm oblique, with 3-5 defining spines. Pereopod 3 moderately setose, modified in males, article 4 elongate, article 5 with a posterior lump and a few long, blade-like spines, article 6 curved, attached at right angles to 5, dactyl with 6-7 spinules. Pereopod 4 unmodified, dactyl with 6-7 spinules. Pereopods 5-7, dactyls with 10-14 spinules. Uropods 1 and 2, peduncle spinose and setose, rami with marginal spines and setae and apical spines. Uropod 3, inner ramus 0.1-0.2 length of outer, apically spinose, outer ramus with marginal and apical spines, densely setose, second segment small but distinct, apically spinose. Telson deeply cleft, each lobe with one spine and several setae.

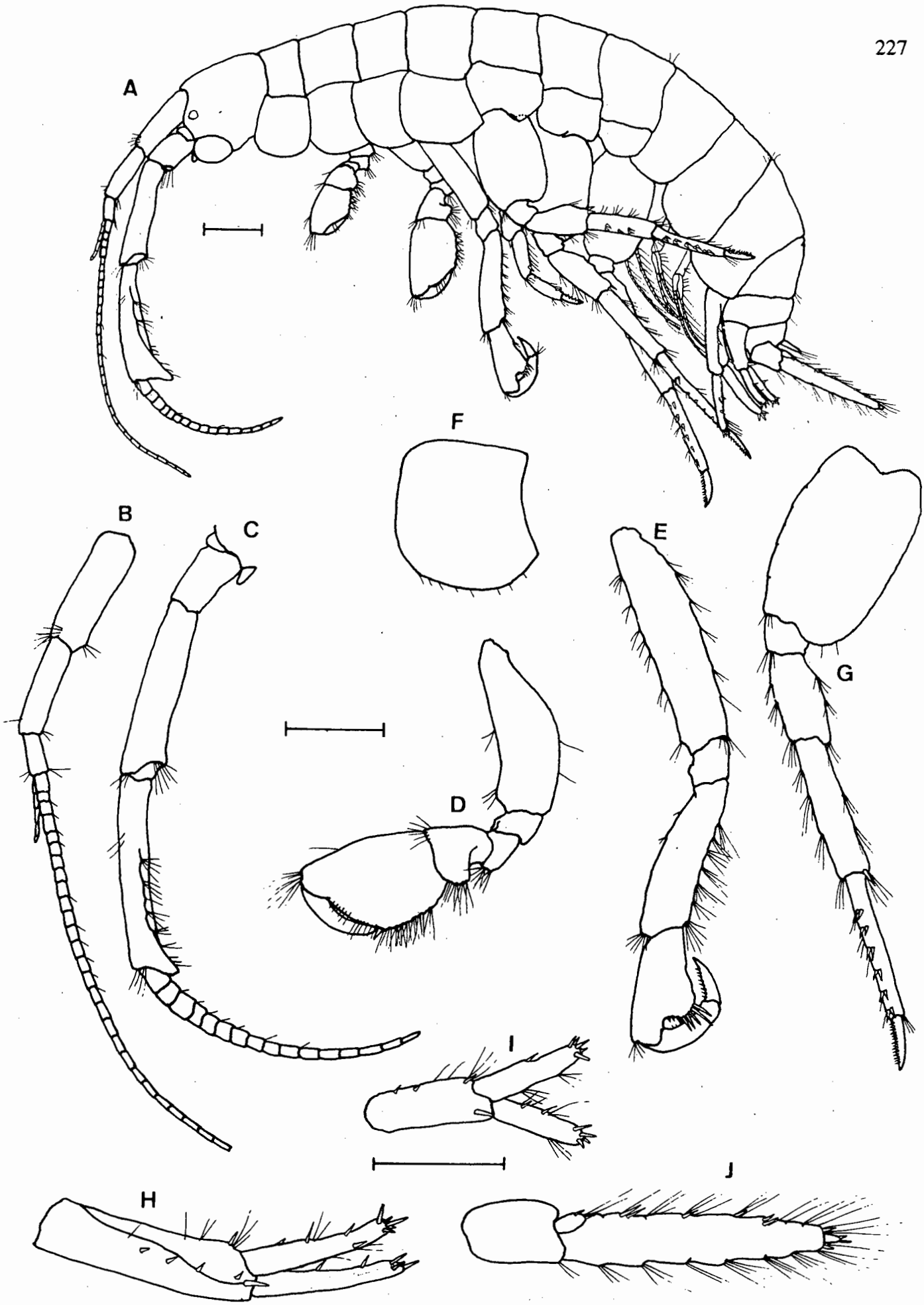

Fig. 22. *Paramelita pinnicornis*, male, 13,5 mm. A. Lateral aspect. B. Antenna 1. C. Antenna 2. D. Gnathopod 2. E. Pereopod 3. F. Coxa 4. G. Pereopod 7. H. Uropod 1. I. Uropod 2. J. Uropod 3. Scale lines represent 1 mm.

Remarks

The unusual form of antenna 2 and pereopod 3 is unique to this species.

Distribution

This species has a rather disjunct distribution, and is known from the northern and eastern parts of the Cape Peninsula and also the Cape Hangklip area, along the east coast of False Bay (Fig. 26).

Paramelita seticornis (Barnard, 1927)

Fig. 23A-K

Gammarus seticornis Barnard, 1927: 171-172, pl. 10, (figs 7, 17).

Paramelita seticornis (Barnard) Thurston, 1973: 166-167. Griffiths, 1981: 90, fig. 2D-F.

Material examined

Types. Lectotype and paratypes, SAM A3994, from Landdrost Kloof, Caledon side, Hottentots Holland Mountains.

Other material. SAM A40228, from Sir Lowry's Pass, Somerset West side, Hottentot Hollands Mountains. SAM A40229, from Malkopvlei, Betty's Bay.

Diagnosis

Eyes white. Antenna 1 sparsely setose, flagellum 20-30 articulate, accessory flagellum with 4-5 articles. Antenna 2 stout and densely setose in males, moderately stout and setose in females, flagellum 8-12 articulate. Coxa 4, posterior margin with a shallow but distinct emargination. Gnathopod 2 palm oblique, with 2-4 palmar spines. Pereopods 3 and 4 unmodified, dactyls with 2-4 spinules. Pereopods 5-7, dactyls with 4-10 spinules. Uropod 1, peduncle with spines and setae, rami subequal, with marginal and apical spines, inner ramus with some setae. Uropod 2, peduncle with spines and setae, inner ramus longer than outer, both with marginal and apical spines, inner ramus

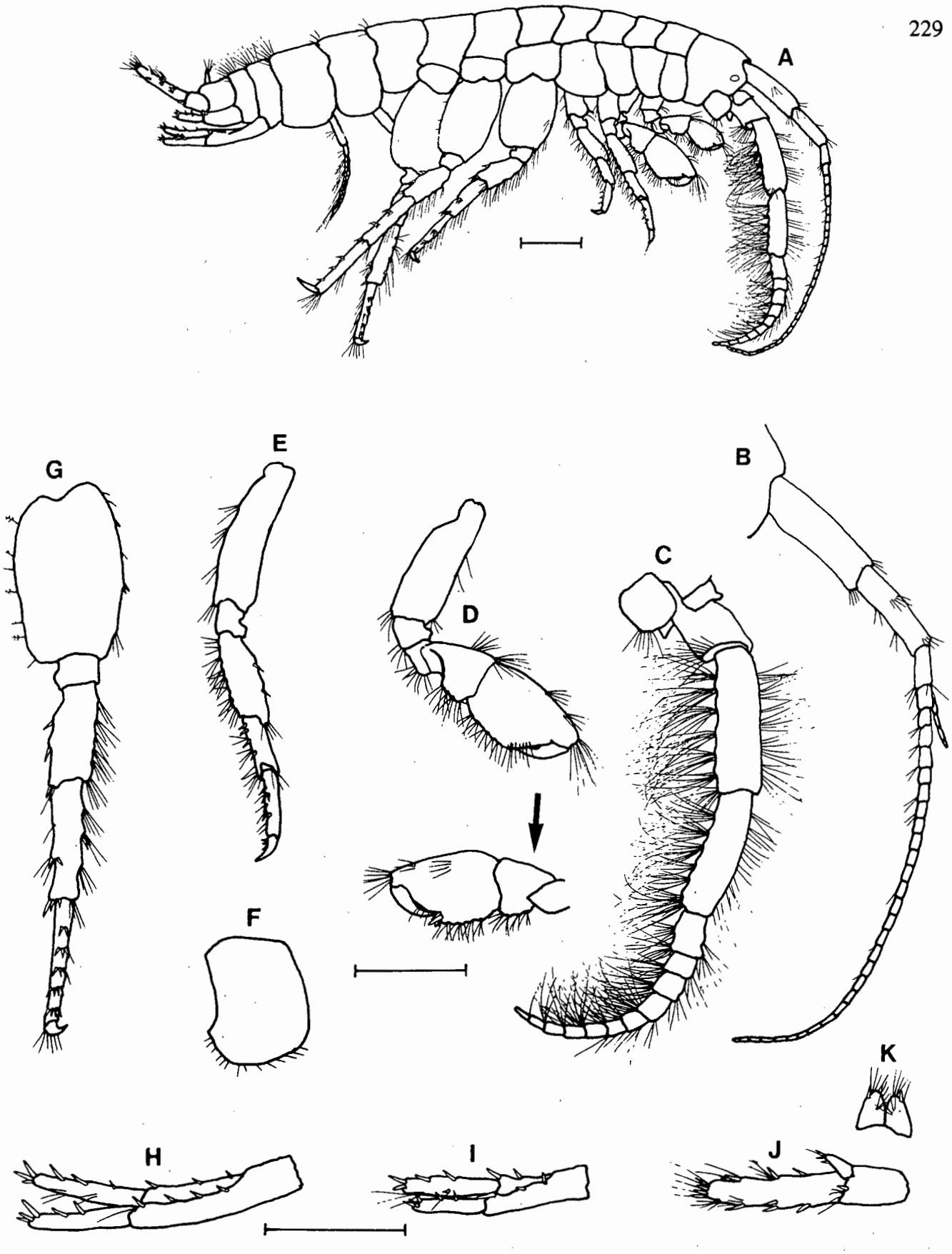

Fig. 23. *Paramelita seticornis*, male, 9,0 mm. A. Lateral aspect. B. Antenna 1. C. Antenna 2. D. Gnathopod 2, lateral and medial views. E. Pereopod 3. F. Coxa 4. G. Pereopod 6. H. Uropod 1. I. Uropod 2. J. Uropod 3. K. Telson. Scale lines represent 1 mm.

with some setae. Uropod 3, inner ramus about 0.3 length of outer ramus, with 2-3 apical spines, outer ramus with marginal and apical spines and setae, second segment rudimentary. Telson deeply cleft, each lobe with a single spine and several setae.

Remarks

This species is distinguished from an allied Hottentots Holland form, *P. kogelensis*, by its densely setose antenna 2, a condition it shares with *P. pillicornis*.

Distribution

Known from Hottentots Holland and adjacent mountain ranges (Fig. 26).

Paramelita spinicornis (Barnard, 1927)

Fig. 24A-I

Material examined

Types. Lectotype and paratypes, SAM A5177, from Hottentot Hollands Mountains.

Other material. SAM A5180, Hottentot Holland Mountains. SAM A5186, Steenbras Valley. SAM A6053, Zonderend Mountains. SAM A6939, Swellendam Mountains. SAM A6940, Swellendam Mountains. SAM A6941, Zuurbraak Peak. SAM A6942, Tradouw Peak. SAM A6943, south of Barrydale. SAM A40253, Betty's Bay. SAM A40254, Disa Kloof, Betty's Bay. SAM A40255, Fernkloof Ravine, Hermanus. SAM A40256, Harold Porter Gardens, Betty's Bay. SAM A40257, Lamloch Stream, Kleinmond. SAM A40258, Between Betty's Bay and Kleinmond.

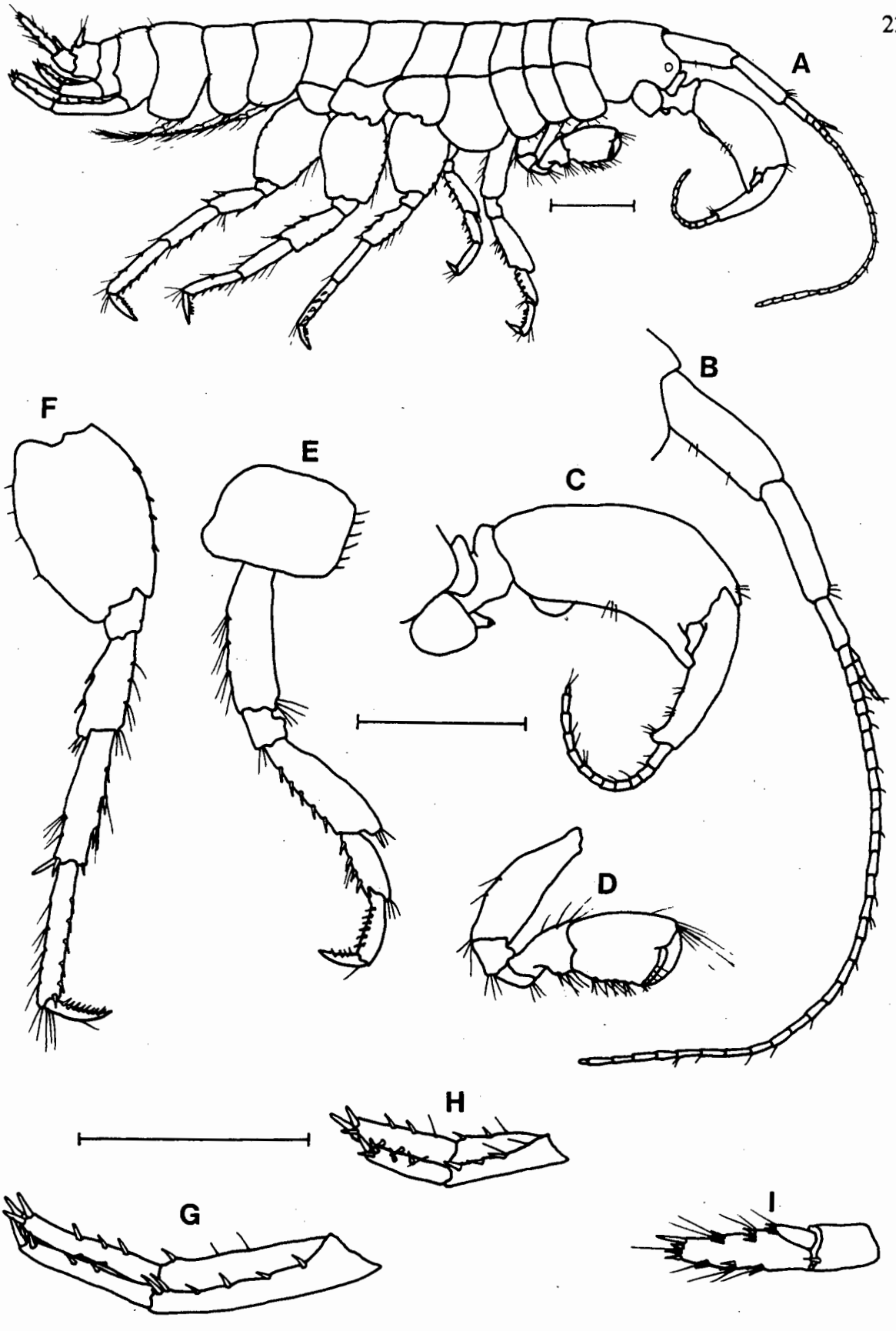

Fig. 24. *Paramelita spinicornis*, male, 7,0 mm. A. Lateral aspect. B. Antenna 1. C. Antenna 2. D. Gnathopod 2. E. Pereopod 3. F. Pereopod 6. G. Uropod 1. H. Uropod 2. I. Uropod 3. Scale lines represent 1 mm.

Diagnosis

Eyes white. Antenna 1 sparsely setose, flagellum 20-25 articulate, accessory flagellum 4 articulate. Antenna 2 shorter than 1, sparsely setose, article 4 of peduncle strongly laterally swollen and with a proximal medial lobe and a posterodistal tooth in males, flagellum 9-11 articulate. Coxa 4, posterior margin excavate. Gnathopod 2, article 2 not medially spinose, palm slightly oblique, with 3-4 defining spines. Pereopods 3 and 4 sparsely setose, unmodified, articles 4, 5 and 6 strongly spinose, dactyls with 3-4 spinules. Pereopods 5-7 sparsely to moderately setose, dactyls with 5-8 spinules. Uropod 1, peduncle spinose, usually with a single seta, rami subequal, both with marginal and apical spines, lacking setae. Uropod 2, peduncle spinose, usually with 1-2 setae, outer ramus longer than inner, both rami with marginal and apical spines, inner ramus sometimes with a single seta. Uropod 3, inner ramus 0.3 length of outer, apically spinose, outer ramus with marginal and apical spines, sparsely setose, small but distinct second segment present. Telson deeply cleft, each lobe with one spine and 3-5 setae.

Remarks

The possession of a terminal, posterodistal tooth on the laterally swollen article 4 of antenna 2 in males, excavate coxa 4, multispinose dactyls in the pereopods, and the presence of a distinct second segment on the outer ramus of uropod 3 make this species unmistakable, despite its superficial resemblance to *Afrocrangonyx dentata* and *Paramelita odontophora*.

Distribution

Collected from Hottentots Holland to Swellendam Mountains (Fig. 26).

Paramelita validicornis Stewart & Griffiths, 1991

Fig. 25A-J

Paramelita validicornis Stewart & Griffiths, 1991b: 88-92, figs 6, 7.

Material examined

Types. Holotype, SAM A40216, paratypes, SAM A40217, from a stream flowing into Kleinriviervlei, near Hermanus.

Other material. SAM A7394, from near Bredasdorp. SAM A40218, from a tributary of the Afdaksrivier. SAM A40219, from the Fernkloof Nature Reserve, Hermanus.

Diagnosis

Eyes white. Antenna 1 sparsely setose, flagellum with 44-48 articles, accessory flagellum 5-6 articulate. Antenna 2 sparsely setose, peduncle elongate and stout in males, flagellum with 19-22 broad, flattened articles. Coxa 4, posterior margin excavate. Gnathopod 2, article 2 medially spinose, palm distinctly oblique, with 4-5 spines. Pereopods 3-7 moderately setose, unmodified, dactyls with 4-9 spinules. Uropods 1 and 2, peduncle spinose, 1 sometimes with 1-2 setae, inner rami with marginal spines and setae, outer rami with marginal spines, lacking setae, all rami with apical spines. Uropod 3, inner ramus 0.2 length of outer, apically spinose and setose, outer ramus with marginal and apical spines, moderately to densely setose, second segment small but distinct. Telson deeply cleft, each lobe with 1-2 spines and 6-10 setae.

Remarks

The most distinguishing features of this species are the stout and elongate antenna 2, particularly in males, and the relatively long outer ramus in uropod 3. The latter condition, along with differences in setation of the rami of uropods 1 and 2, is used to separate *P. validicornis* from *P. magna* and *P. magnicornis*.

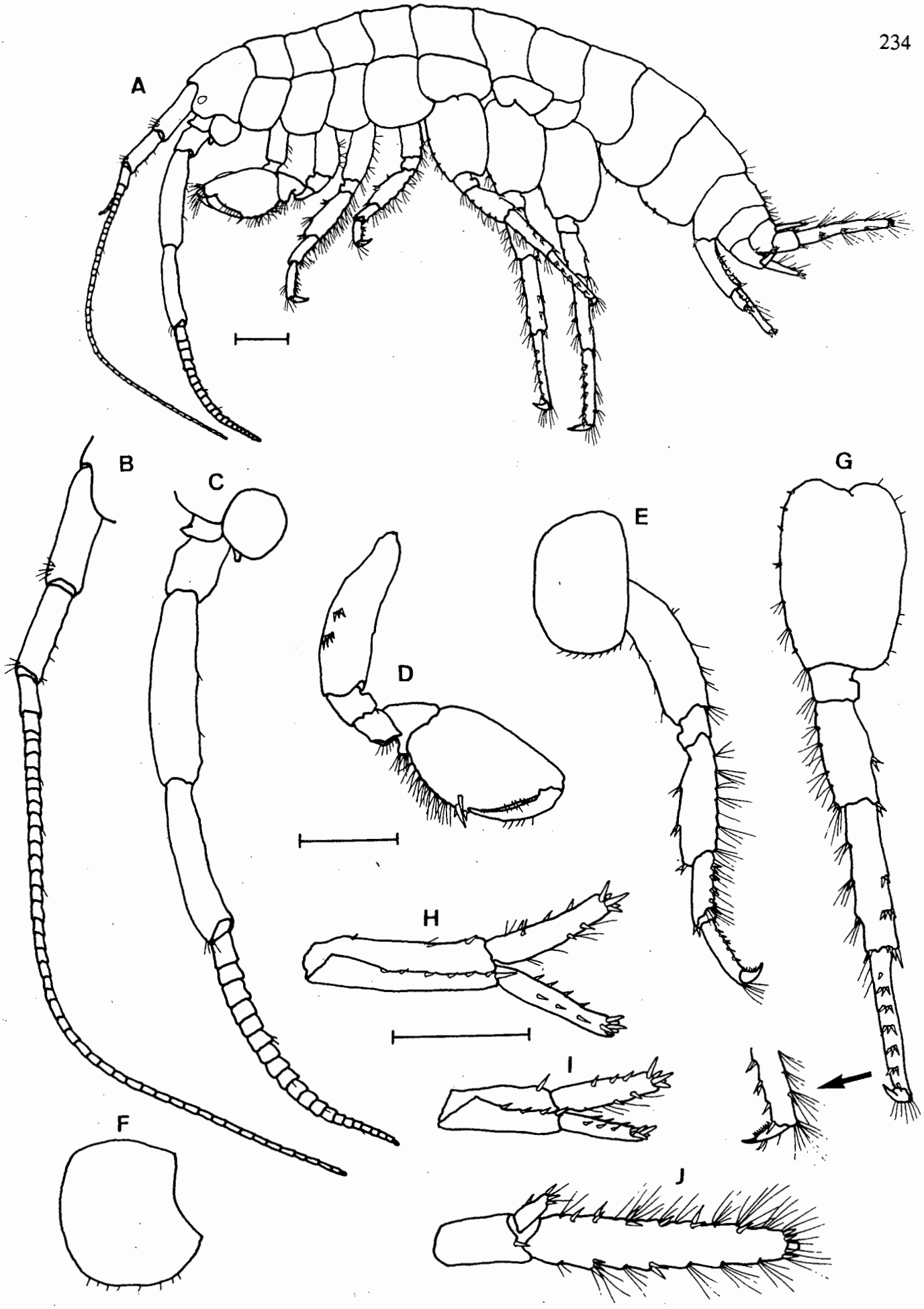

Fig. 25. *Paramelita validicornis*, male, 13,8 mm. A. Lateral aspect. B. Antenna 1. C. Antenna 2. D. Gnathopod 2, medial view. E. Pereopod 3. F. Coxa 4. G. Pereopod 6. H. Uropod 1. I. Uropod 2. J. Uropod 3. Scale lines represent 1 mm.

Distribution

Although this species is known from Bredasdorp in the west to the Kleinriviersberge in the east, it is possibly more widespread (Fig. 26).

Key to all the South African paramelitid species

A key to all the known paramelitid species, irrespective of genus, is also included, the purpose of which is to facilitate identification to species level when initial separation into genera is uncertain.

1 Eyes black. 2

Eyes white, invisible in preserved material 3

2 Antenna 2, posterior margins and pereopods 3-7 densely setose posteriorly (Fig. 18C) . . . . . *P. nigroculus* var. *persetosus*

Antenna 2, posterior margins and pereopods 3-7 moderately setose, lacking setal brushes . . . . . .*P. nigroculus*

3 Pereopod 3 modified, either article 4 weakly to strongly posterodistally protruded (Figs 2E, 7E, 17E), or greatly expanded laterally (Fig. 9E), or article 5 posteriorly lobed (Figs 3E, 22E), or with 2-4 teeth-like spines (Figs 4E, 6E) 4

Pereopod 3 unmodified (Figs 4E, 10E, etc) 10

4 Pereopod 3, article 4 weakly to strongly posterodistally protruded (Figs 2E, 7E, 17E) 5

Pereopod 3, article 4, not posterodistally protruded 7

5 Pereopod 3, article 4, posterodistally protruded into a triangular tooth, dactyl with about six spinules; antenna 2, article 3, not lobed (Fig. 17A) . . . . . .P. *magnicornis*

Pereopod 3, article 4 strongly posterodistally protruded, dactyl with a single spinule; antenna 2, article 3, with a semicircular lobe posteriorly (Figs 2A, 7A). 6

6 Pereopod 3, article 4 short, posterodistally protruded into a long, narrow 'spur'; antenna 2, article 3 strongly swollen and enlarged (Fig. 7A). . . . . *Afrocrangonyx pheronyx*

Pereopod 3, article 4 long, posterodistally protruded into a triangular shaped lobe; antenna 2, article 3 moderately swollen (Fig. 2A) . . . . . .*Afrocrangonyx andronyx*

7 Antenna 2, peduncle with article 3 lobed (Fig. 3C) or article 5 strongly ridged (Fig. 22C). 8

Antenna 2, peduncle swollen or elongate, but without lobes or ridges (Figs 4C, 9C) 9

8 Antenna 2, article 3 bearing a semicircular lobe posteriorly, article 5 not ridged; pereopods 3-7, dactyls with 1-2 spinules (Fig. 3A) . . . . . *Afrocrangonyx auricularius*

Antenna 2, article 3 not lobed, article 5 with outer margin and tip extended into an elongate triangular flange, or ridge; pereopods 3-7, dactyls with 7-14 spinules (Fig. 22A) . . . . . .*P. pinnicornis*

9 Pereopod 3, article 4, greatly expanded laterally, densely setose posteriorly, article 5 lacking teeth-like spines, dactyl with about seven spinules; antenna 2, article 4 extremely elongate (Fig. 9A) . . . . . *Aquadulcaris platypus*

Pereopod 3, article 4 not greatly expanded laterally, moderately setose posteriorly, article 5 with 2-4 teeth-like spines, dactyl with a single spinule; antenna 2, article 4 strongly laterally swollen (Fig. 4A) . . . . . *Afrocrangonyx crassicornis*

10 Antenna 2, peduncular articles 3, 4 or 5 lobed (Fig. 13C) or toothed (Figs 5C, 19C, 24C) 11

Antenna 2, peduncular articles without lobes or teeth. 14

11 Antenna 2, article 3 posterodistally lobed (Fig. 13A). . . . . *P. flexa*

Antenna 2, article 3 not lobed (Figs 5C, 19C, 24C) 12

12 Antenna 2 extremely elongate, exceeding 1 in length, article 4 of peduncle with a posterodistal, subterminal tooth, article 5 not toothed (Fig. 19A). . . . . *P. odontophora*

Antenna 2 shorter than 1, article 4 of peduncle strongly swollen, with a posterodistal, terminal tooth and a posteroproximal lobe (Fig. 24C), or with no teeth (Fig. 5C), article 5 usually with a posterodistal tooth 13

13 Antenna 2, article 4 of peduncle with a posterodistal tooth, article 5 sometimes with a posterodistal tooth; pereopods 3-7, dactyls with 4-10 spinules; coxa 4, posterior margin moderately excavate (Fig. 24A). . . . . *P. spinicornis*

Antenna 2, article 4 of peduncle not toothed, article 5 with a posterodistal tooth; pereopods 3-7, dactyls always with a single spinule; coxa 4, posterior margin slightly emarginate (Fig. 5A) . . . . . *Afrocrangonyx dentata*

14 Antenna 2 posteriorly densely setose (Figs 21C, 23C). 15

Antenna 2 posteriorly sparsely to moderately setose (Fig. 10C, etc) 16

15 Antenna 2, peduncle stout, flagellum shorter than peduncle, with 8-12 articles; pereopods 5-7, article 2 moderately expanded; uropod 1, outer ramus lacking setae, uropod 2, inner ramus usually with some setae (Fig. 23A) . . . . *P. seticornis*

Antenna 2, peduncle elongate and slender, flagellum as long as peduncle, with 13-16 articles; pereopods 5-7, article 2 markedly poorly expanded; uropod 1, outer ramus with some setae, uropod 2, inner ramus lacking setae (Fig. 21A) . . . . *P. pillicornis*

16 Antenna 2, article 4 of peduncle strongly laterally swollen, article 5 often bent at right angles to 4 (Figs 4C, 8C); gnathopod 2, article 2 lacking medial spines (Figs 4D, 8D). 17

Antenna 2, article 4 sometimes enlarged and elongated (Figs 16C, 25C, etc), but not disproportionately laterally swollen, article 5 attached normally to 4; gnathopod 2, article 2 usually with medial spines (Fig. 16D, etc). 18

17 Pereopod 3, article 5, usually with 1-4 stout, teeth-like spines; pereopods 3-7, dactyls, always with one spinule each; coxa 4, posterior margin, very slightly emarginate (Fig. 4A) . . . .*Afrocrangonyx crassicornis*

Pereopod 3, article 5 lacking stout, teeth-like spines; pereopods 3 and 4, dactyls, usually with two, but sometimes with one spinule, pereopods 5-7, dactyls, usually with 2-4, but sometimes with one spinule each; coxa 4, posterior margin, with a distinct but shallow emargination (Fig. 8A) . . . .*Afrocrangonyx tulbaghensis*

18 Antenna 2 as long as, or exceeding 1 in length, peduncle markedly stout (Figs 16C, 17C, 25C). 19

Antenna 2, distinctly shorter than 1, peduncle slender to moderately stout (Figs 11C, 12C, etc) 21

19 Uropod 3, outer ramus 3.0 length of peduncle; uropods 1 and 2, inner rami always with a few setae, outer rami lacking setae (Fig. 25A) . . *P. validicornis*

Uropod 3, outer ramus 2.0-2.6 length of peduncle (Figs 16J, 17J); uropods 1 and 2, inner rami with or without setae, outer rami sometimes with setae 20

20 Urosome densely setose dorsally, uropods 1 and 2, inner and outer rami with setae, body colour brown (Fig. 16A). . . . . .*P. magna*

Urosome moderately setose dorsally, uropod 1, inner ramus with a few setae, outer ramus without, uropod 2, rami lacking setae, body colour white (Fig. 17A). . . . . .*P. magnicornis*

21 Coxa 4 quadrate, or posterior margin with a slight emargination (Figs 10F, 14A) 22

Coxa 4 weakly to strongly excavate (Figs 11F, 12F, etc). 23

22 Gnathopod 2, article 2 medially, palm strongly convex, defining angle forming a small projecting rounded tooth; pereopod 3, and sometimes 4, article 2 strongly spinose posteriorly, article 4 often considerably longer and wider than 5 (Fig. 14A) . . . . . *P. granulicornis*

Gnathopod 2, article 2 sometimes weakly spinose medially, palm moderately convex, lacking tooth at defining angle; pereopods 3 and 4 article 2, lacking

medial spines, article 4 moderately longer and wider than 5 (Fig. 10A). . . . . .
. . . . . .*P. aurantius*

23 Antenna 1, flagellum with 22-27 articles, antenna 2, flagellum 11-18 articulate (Figs 15B,C; 20B,C) 24
Antenna 1, flagellum with 33-80 articles, antenna 2, flagellum 15-35 articulate (Figs 11B,C; 12B,C) 25

24 Pereopods 3 and 4, dactyls with 2-3 spinules; uropod 2, inner ramus lacking marginal setae; uropod 3, outer ramus moderately to densely setose (Fig. 20A)
. . . . . *P. parva*
Pereopods 3 and 4, dactyls with four spinules; uropod 2, inner ramus with a few marginal setae; uropod 3, outer ramus, poorly setose (Fig. 15A) . . . . . .
. . . . . .*P. kogelensis*

25 Antenna 2, flagellum with 15-17 articles; pereopods 3 and 4, dactyls with 2-3 spinules; pereopods 5-7, dactyls with 5-7 spinules; coxa 4 distinctly, but moderately excavate posteriorly; uropods 1 and 2, rami lacking setae; uropod 3, outer ramus poorly setose (Fig. 11A) . . . . . .*P. barnardi*
Antenna 2, flagellum usually with more than 17 articles; pereopods 3 & 4, dactyls with 3-6 spinules; pereopods 5-7, dactyls with 8-13 spinules; uropods 1 & 2, inner rami with marginal setae; uropod 3, outer ramus, strongly setose (Fig. 12A) . . . . . .*P. capensis*

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PAPER 7

PHYLOGENETIC RELATIONSHIPS AMONG SOUTH AFRICAN PARAMELITID AMPHIPODS (CRANGONYCTOIDEA: PARAMELITIDAE) BASED ON MORPHOLOGICAL VARIATION

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ABSTRACT

The phylogenetic relationships among freshwater species of two South African crangonyctoid amphipod genera, *Afrocrangonyx* and *Paramelita* were determined based on morphological variation. Three Australian paramelitid genera were chosen as outgroups for polarising the character states, and the most parsimonious trees constructed by means of the HENNIG86 cladistic analyses software package. A fully resolved cladogram was calculated for *Afrocrangonyx*. Although the cladogram for *Paramelita* was not resolved, some monophyletic groups within the genus were identified. The phylogeny of each genus was related to geographical distribution of the species. Shortcomings in the data were also discussed.

INTRODUCTION

The crangonyctoid family Paramelitidae was first erected by Bousfield (1977) to accommodate "*Gammarus*" (now *Austrogammarus* Barnard & Karaman, 1983), *Uroctena* and *Hurleya* from Australia, *Paramelita* from South Africa and *Falklandella* from the Falkland Islands. Bousfield (1983) later added *Sternophysinx* from South Africa. After amending the diagnosis of the family in order to accommodate their

revision of the Australian paramelitids, Williams & Barnard (1988) added four more Australian genera, *Protocrangonyx*, *Giniphargus*, *Austrocrangonyx*, and *Antipodeus*, and excluded *Falklandella*, *Sternophysinx* and *Paracrangonyx*.

Although a cladistic analysis of the family Paramelitidae has yet to be undertaken, Williams & Barnard (1988) did comment on relationships between the genera in their revision of the Australian crangonyctoid amphipods. Thus, they regarded *Austrogammarus* as the most primitive of Australian paramelitids, *Antipodeus* as being "much closer" to *Austrocrangonyx* than to *Austrogammarus*, *Hurleya* as having "more affinities with paramelitid genera than with neoniphargid and perthiid genera", and *Uroctena* as having "strong affinities" with *Paramelita*.

Paramelita was first erected by Schellenberg (1926) to accommodate the description of amphipods collected during an expedition in 1903 from South Africa. Later, he transferred the 10 "*Gammarus*" species described by Barnard (1916, 1927) from freshwater streams in the south-western Cape, South Africa, to this genus (Schellenberg, 1937). Additional new species have since been described (Thurston, 1973; Griffiths, 1981; Stewart & Griffiths, 1991a,b,c; Stewart & Snaddon, 1991), so that the total of known *Paramelita* species now stands at 24. In a recent revision of the genus, Stewart (in prep.) split the 24 *Paramelita* species into three genera. In this revision, seven species were recognised as belonging to the new genus *Afrocrangonyx*, 16 species remained in *Paramelita*, and the monospecific genus *Aquadulcaris* was erected to accommodate *P. platypus*. Phylogenetic relationships between these species are unknown. The purpose of this paper is to determine the phylogeny of South African paramelitid species based on a cladistic analysis of morphological data. The polarity of the characters used was determined by outgroup comparison (Watrous & Wheeler, 1981), and the computer programme HENNIG86 (written by J.S. Farris) used to generate most parsimonious trees. Monophyletic groups within the genera, unresolved phylogenetic relationships between taxa, and inadequacies in the data are discussed below.

MATERIALS AND METHODS

Material for this study was derived mainly from collections made by the author during 1989 and 1990. Additional material was obtained from earlier collections held by the South African Museum. Depending on the material available, at least five adults of both sexes were examined using a Wild dissecting microscope. Fully mature adults were used as morphological differentiation between the species was most marked in these individuals. Specimens were partially dissected when necessary, and measurements were made by means of an eyepiece micrometer. Body length was measured from behind antenna 1 to the base of the telson. A total of 20 characters each in *Afrocrangonyx* and *Paramelita*, referring to the external morphology of the amphipods, could be successfully polarised. Quantitative characters were gap-coded by plotting histograms of all quantitative characters, and coding the character states according to 'identifiable' gaps (Conlan, 1988; Notenboom, 1988). Homologous characters were recognised by similarity in positions and connection with other body parts. Character states were polarised using outgroup comparison, where character state distributions in other paramelitid genera were determined largely by a survey of the literature (e.g. Williams & Barnard, 1988). In the case of quantitative characters not supplied in the descriptions, these were scored from illustrations (see also Conlan, 1988). Unknown polarities were coded in the data matrix with a question mark. Unique character states found only in one species were considered to be autapomorphies, and were excluded from the numerical analysis. The data matrix was analysed by means of the HENNIG86 package of J.S. Farris. Most parsimonious trees, which minimise the number of changes in character states needed to explain the pattern of character state distribution among the taxa, were derived from the character state matrix by means of the "ie*", "mhennig*" and "bb" commands. Although the "ie*" command is certain to find all trees of minimal length, this command proved to

be prohibitively time-consuming for the analysis of *Paramelita*. The "mhennig*" command was therefore selected as the next-best choice as recommended by J.S. Farris in the documentation accompanying the programme. The "xsteps w" command, used in combination with the tree-calculating commands, provided a successive weighting facility. Farris has suggested that successive weighting provides a means of grouping on more reliable characters without making prior decisions on weighting.

RESULTS AND DISCUSSION

a) Selection of outgroups. - It is commonly held (e.g. Ridley, 1986) that the most suitable outgroup to choose is that of a closely related species or genus. Obvious candidates would therefore be other genera within the Paramelitidae. Unfortunately, the choice of suitable outgroups is complicated by the fact that the composition of Paramelitidae is still under question (e.g. Bousfield, 1983; Williams & Barnard, 1988). Notenboom (1988) encountered a similar situation in his study of the phylogeny of *Pseudoniphargus*, and commented that "an important obstacle in phylogenetic studies of amphipods at lower taxonomic levels is the highly debated classification into families and superfamilies". For the phylogenetic analysis of *Paramelita*, *Austrogammarus* and *Austrocrangonyx* were chosen as outgroups. Like *Paramelita*, these two genera possess sternal gills, a gill on coxa 7, a short second segment on the outer ramus of uropod 3 and a cleft telson. Williams & Barnard (1988) regard *Austrogammarus* as the most plesiomorphic genus of Australian paramelitids. Although these authors have suggested (prior to the formation of *Afrocrangonyx*) that *Uroctena* has strong affinities with *Paramelita*, a *Uroctena* type ancestor for *Paramelita* would have involved losing and regaining coxal gill 7, which is a plesiomorphic crangonyctoid 'marker'. Similarly, *Hurleya*, *Protocrangonyx* or *Giniphargus* type ancestors would also involve the loss and regaining of coxal gill 7, and an *Antipodeus* type ancestor, the loss and regaining of

sternal gills. Thus, it seems more probable that *Austrogammarus* and *Austrocrangonyx* are closest to the hypothetical ancestor of *Paramelita*.

It could be argued that *Afrocrangonyx* and *Uroctena* shared a common ancestor prior to the loss of coxal gill 7 in the latter genus. The two genera have similarities in the form of their sternal gills, gnathopods, oostegites, telson, epimeral plates, urosome setosity, head sinus, maxillae, spinose dactyls of the pereopods, and the "pediformity" of antenna 2 in males (Williams & Barnard, 1988). Thus, *Uroctena* was used as the outgroup for the phylogenetic analysis of *Afrocrangonyx*.

b) Selection and polarity of characters. - The characters which differentiated species of *Afrocrangonyx* and *Paramelita* and which were used in the numerical analysis are listed in Tables I and II, and the distributions of character states over the species are given in Tables III and IV. Characters were either 'qualitative', such as presence or absence or differences in shape, 'quantitative' and 'discontinuous', such as counts of spinules, or 'quantitative' and 'continuous', such as the relative lengths of limbs. As in Notenboom's (1988) and Conlan's (1988) cladistic studies on amphipods, ratios and counts were included in the present analysis, despite reservations by some authors (e.g. Pimental & Riggins, 1987) about quantitative data. These characters avoided the use of subjective character state definitions, and were needed because ofthe shortage of usable characters. Care was taken to identify clear gaps when coding these data.

The structure of antenna 2 in adult males showed interesting differences between the paramelitid species (fig. 1). In all seven species of *Afrocrangonyx* and one *Paramelita* species (*P. spinicornis*), the "pediformity" alluded to by Williams & Barnard (1988) in *Uroctena*, was clearly evident, with articles 3, and particularly 4, strongly swollen in adult males. Based on outgroup analysis, this condition was considered plesiomorphic in *Afrocrangonyx*, but an autapomorphy in *P. spinicornis*. The presence of teeth, lobes and ridges on antenna 2 in four *Afrocrangonyx* and four *Paramelita* species was apomorphic for these genera, as was the elongation of the

TABLE I

Descriptions of the 20 characters used for cladistic analysis of *Afrocrangonyx*. Characters apply to adult males, and have been polarised using *Uroctena* as an outgroup.

No.	Character	States
	Antenna 1	
0	setation	0: sparse 1: moderate to dense
	Antenna 2	
1	no. of articles in flagellum	0: <12 1: 12-20
2	peduncle/flagellum length	0: 1.2-1.6 1: 0.8 2: 1.8-2.1
3	article 3, lobe	0: absent 1: present
4	article 4/5 length	0: 1.1-1.7 1: 2.1
5	article 5 length/width	0: 2.2-3.5 1: 4.2-4.9
	Gnathopod 1	
6	article 2, medial spines	0: absent 1: present
	Gnathopod 2	
7	article 2, medial spines	0: absent 1: present
	Pereopod 3	
8	article 2, medial spines	0: absent 1: present
9	article 4 width	0: normal 1: widening distally
10	article 4, projection	0: absent 1: present
11	article 5, teeth-like spines	0: absent 1: present
12	article 6, shape	0: normal 1:arched
13	article 7, spinules	0: 2-6 1: 1
	Pereopod 4	
14	article 7, spinules	0: 2-6 1: 1
	Pereopods 5-7	
15	article 7, spinules	0: 2-6 1: 1
	Uropod 1	
16	outer ramus, setation	0: absent 1: present

	Uropod 2	
17	inner ramus, setation	0: absent 1: present
18	outer ramus, setation	0: absent 1: present
	Uropod 3	
19	outer ramus, article 2	0: 35-45% of article 1 1: absent or rudimentary

TABLE II

Descriptions of the 20 characters used for cladistic analysis of *Paramelita*. Characters apply to adult males, and have been polarised using *Austrocrangonyx* and *Austrocrangonyx* as outgroups.

No.	Character	States
	Antenna 1	
0	antenna 1/2 length	0: > 1.2 1: 0.7-1.0
1	article 1 length/width	0: 1.8-2.3 1: 2.6-3.7
	Antenna 2	
2	no. of articles in flagellum	0: 12-30+ 1: < 12
3	article 3 length/width	0: 0.7-0.9 1: 1.1-1.5 2: 1.9
4	article 4, size	0: normal 1: elongate & stout
5	article 5, size	0: normal 1: elongate & stout
	Gnathopod 1	
6	article 2, medial spines	0: absent 1: present
	Gnathopod 2	
7	angle of palm	0: transverse to slightly oblique 1: moderately to strongly oblique
8	article 2, medial spines	0: absent 1: present
	Pereopod 3	
9	article 2, medial spines	0: absent 1: present
10	article 7, spinules	0: 1 1: 2-6 2: 7-8
	Pereopod 4	
11	coxa 4, posterior margin	0: distinctly excavate 1: slightly emarginate
12	article 7, spinules	0: 1 1: 2-6
	Pereopods 5-7	
13	article 7, spinules	0: 1 1: 2-10 2: > 10
	Uropod 1	
14	outer ramus, setation	0: absent 1: present

	Uropod 2	
15	inner ramus, setation	0: absent 1: present
16	outer ramus, setation	0: absent 1: present
	Uropod 3	
17	inner/outer ramus length	0: 0.6-0.7 1: 0.1-0.4
18	outer ramus, article 2	0: 7-20% of article 1 1: < 7% 2: rudimentary
	Telson	
19	spination	0: 0-1 spines per lobe 1: > 2 per lobe

TABLE III

Distribution of character states in *Afrocrangonyx*. (See Table I for description of characters and character states.)

Taxa	Characters																			
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Uroctena (outgroup)	0	0	0	0	0	0	0	?	0	0	0	0	0	0	0	0	0	0	0	0
A. andronyx	0	1	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
A. auricularius	0	0	0	1	0	1	0	0	0	1	0	1	1	1	1	1	0	0	0	1
A. crassicornis	1	0	2	0	1	0	0	0	0	0	0	1	0	1	1	1	0	0	0	1
A. dentata	1	0	2	0	1	0	0	0	0	0	0	0	0	1	1	1	0	0	0	1
A. marunguis	1	1	2	0	0	0	0	0	0	0	0	1	0	1	1	1	0	1	0	1
A. pheronyx	0	0	0	1	0	0	1	1	1	1	1	0	0	1	1	1	0	1	0	1
A tulbaghensis	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1

TABLE IV

Distribution of character states in *Paramelita*. (See Table II for descriptions of characters and character states.)

Taxa	Characters																			
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Austrogammarus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Austrocrangonyx	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
P. aurantius	0	1	0	0	0	0	0	0	1	0	1	1	1	1	0	0	0	1	2	0
P. barnardi	0	1	0	1	0	0	0	0	0	0	1	0	1	1	0	0	0	1	1	1
P. capensis	0	1	0	?	0	0	1	1	1	1	1	0	1	2	1	1	1	1	1	0
P. flexa	0	1	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	1	1	0
P. granulicornis	0	1	0	0	0	0	0	0	1	1	1	1	1	1	0	0	0	1	2	0
P. kogelensis	0	1	0	0	0	0	0	0	0	0	1	0	1	1	0	1	0	1	1	0
P. magna	1	1	0	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1	1
P. magnicornis	1	1	0	2	1	1	1	1	1	0	1	0	1	1	0	1	0	1	1	1
P. nigroculus	0	1	0	1	0	0	0	0	0	0	1	0	1	1	0	1	0	1	1	0
P. odontophora	1	1	0	1	1	1	0	0	1	0	2	0	1	2	0	1	0	1	1	0
P. parva	0	1	0	1	0	0	0	1	0	0	1	0	1	1	0	0	0	1	1	1
P. pillicornis	0	1	0	1	0	0	0	1	0	0	1	0	1	1	1	0	1	1	1	0
P. pinnicornis	1	1	0	1	1	1	0	1	1	0	2	0	1	2	0	1	1	1	1	?
P. seticornis	0	1	1	0	1	0	0	1	0	0	1	0	1	1	0	1	0	1	2	0
P. spinicornis	0	1	1	0	0	0	0	0	0	0	1	0	1	1	0	1	0	1	1	0
P. validicornis	0	1	0	1	1	1	0	1	1	0	1	0	1	1	0	1	0	1	1	1

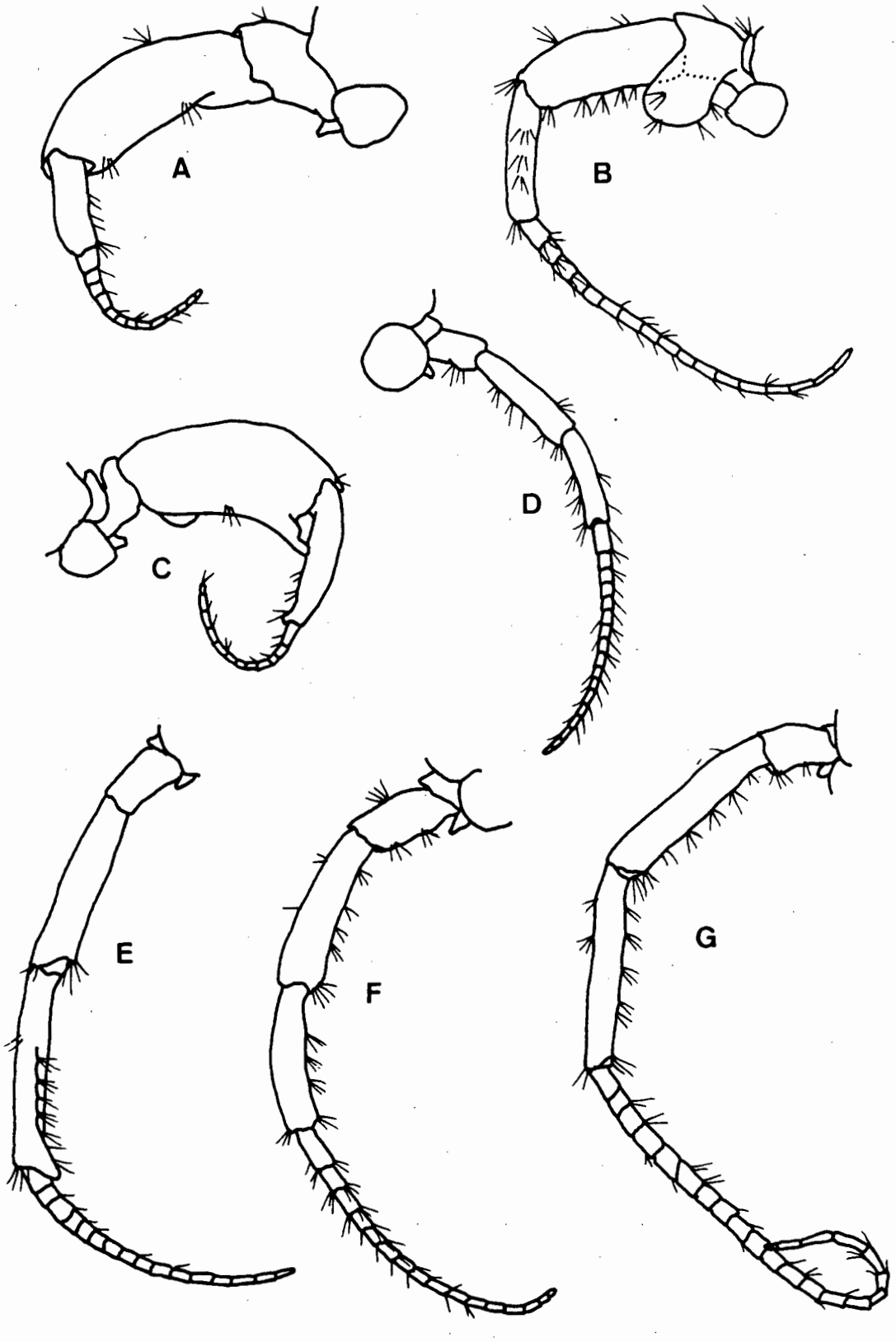

Fig. 1. Antenna 2, lateral view. A. *Afrocrangonyx crassicornis*. B. *A. andronyx*. C. *Paramelita spinicornis* . D. *P. barnardi*. E. *P. pinnicornis*. F. *P. magnicornis*. G. *Aquadulcaris platypus*.

peduncle of this antenna in five *Paramelita* species and the single species of *Aquadulcaris*. The flagellum of this antenna has relatively few articles (less than 12) in adult males of *Uroctena*; a condition shared by two *Afrocrangonyx* and one *Paramelita* species. This condition was considered plesiomorphic in the former genus, but apomorphic in the latter.

Gnathopod 2 differed amongst *Afrocrangonyx* and *Paramelita* species in terms of spination of article 2 and the nature of the palm in article 6 (fig. 2). Like *Austrogammarus* and *Austrocrangonyx*, all seven species of *Afrocrangonyx*, seven species of *Paramelita*, and the single *Aquadulcaris* species had transverse to slightly oblique palms, whilst nine *Paramelita* species had moderately to strongly oblique palms. Unlike *Afrocrangonyx*, *Uroctena* species generally have their palms moderately to strongly oblique, suggesting that the poorly oblique palms in *Afrocrangonyx* are apomorphic. The presence of spines on article 2 of gnathopod 2 was common amongst South African paramelitids, with *Aquadulcaris*, two *Afrocrangonyx* and eight *Paramelita* species sharing this condition. A survey of the literature (e.g. Williams & Barnard, 1988) revealed that three of the four species of *Uroctena* possess similar spines, but that none of the six *Austrogammarus* or two *Austrocrangonyx* species have spines of this type. Their presence was thus considered as apomorphic in *Paramelita*.

None of the 12 species examined in the three Australian genera possessed a claw-like pereopod 3 of the type found in adult males of four *Afrocrangonyx* and one *Paramelita* species (fig. 3). Closer examination of this condition revealed that the 'claw' is achieved in several different ways through various modifications of either articles 4 or 5, and 6. This condition has obviously arisen in some of these species as a result of convergent evolution, so that the possession of a claw-like pereopod 3 is not necessarily evidence of close affinity between them.

Coxa 4 varies from being quadrate to having its posterior margin strongly excavate in *Paramelita* species (fig. 4). In *Austrogammarus* and *Austrocrangonyx* this coxal plate is excavate, but like *Afrocrangonyx*, it is poorly emarginate posteriorly in

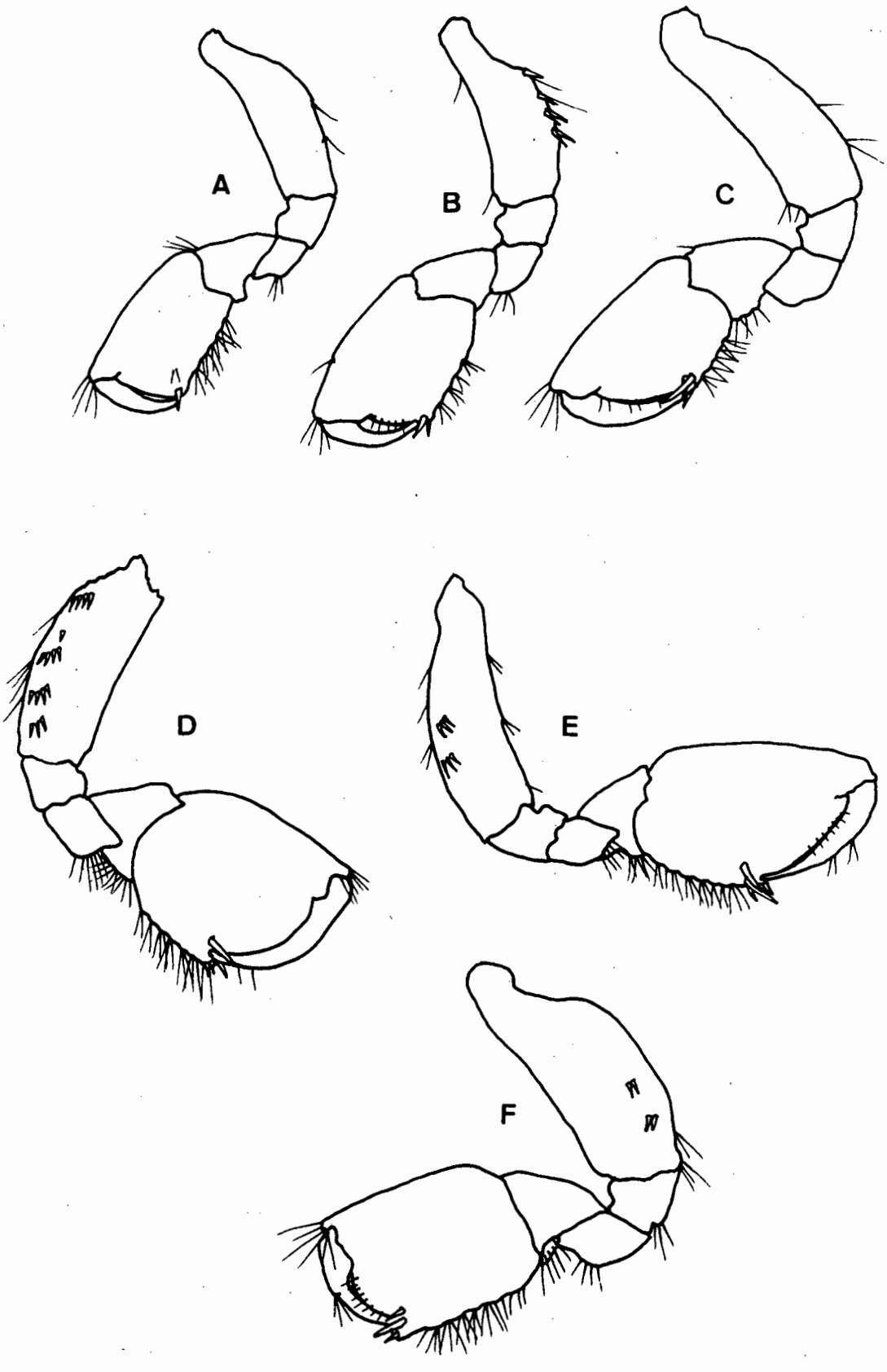

Fig. 2. Gnathopod 2, medial view. A. *Afrocrangonyx auricularius*. B. *A. pheronyx*. C. *Paramelita barnardi*. D. *P. magna*. E. *P. validicornis*. F. *Aquadulcaris platypus*.

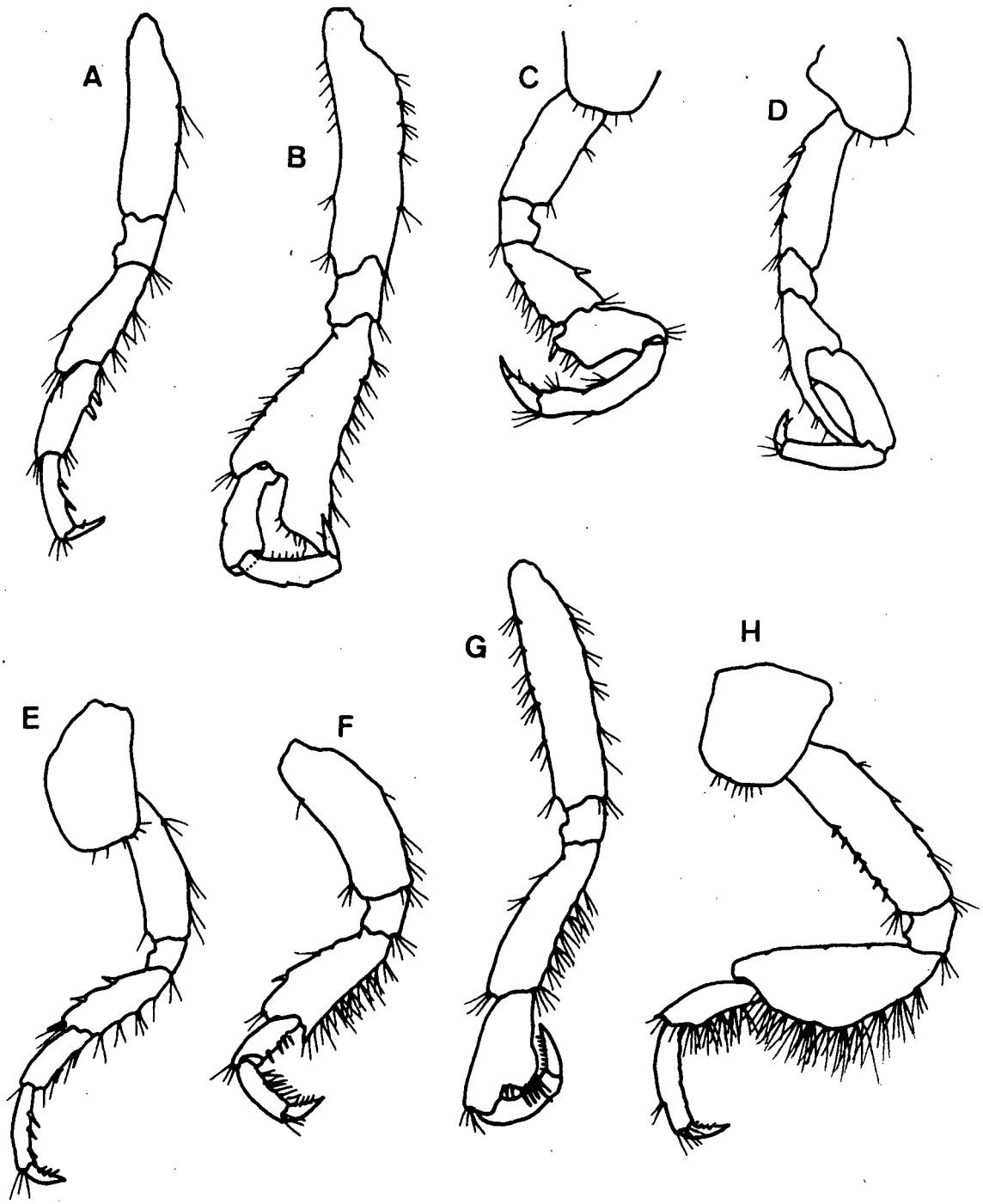

Fig. 3. Pereopod 3, lateral view. A. *Afrocrangonyx crassicornis*. B. *A. andronyx*. C. *A. auricularius*. D. *A. pheronyx*. E. *Paramelita aurantius*. F. *P. magnicornis*. G. *P. pinnicornis*. H. *Aquadulcaris platypus*.

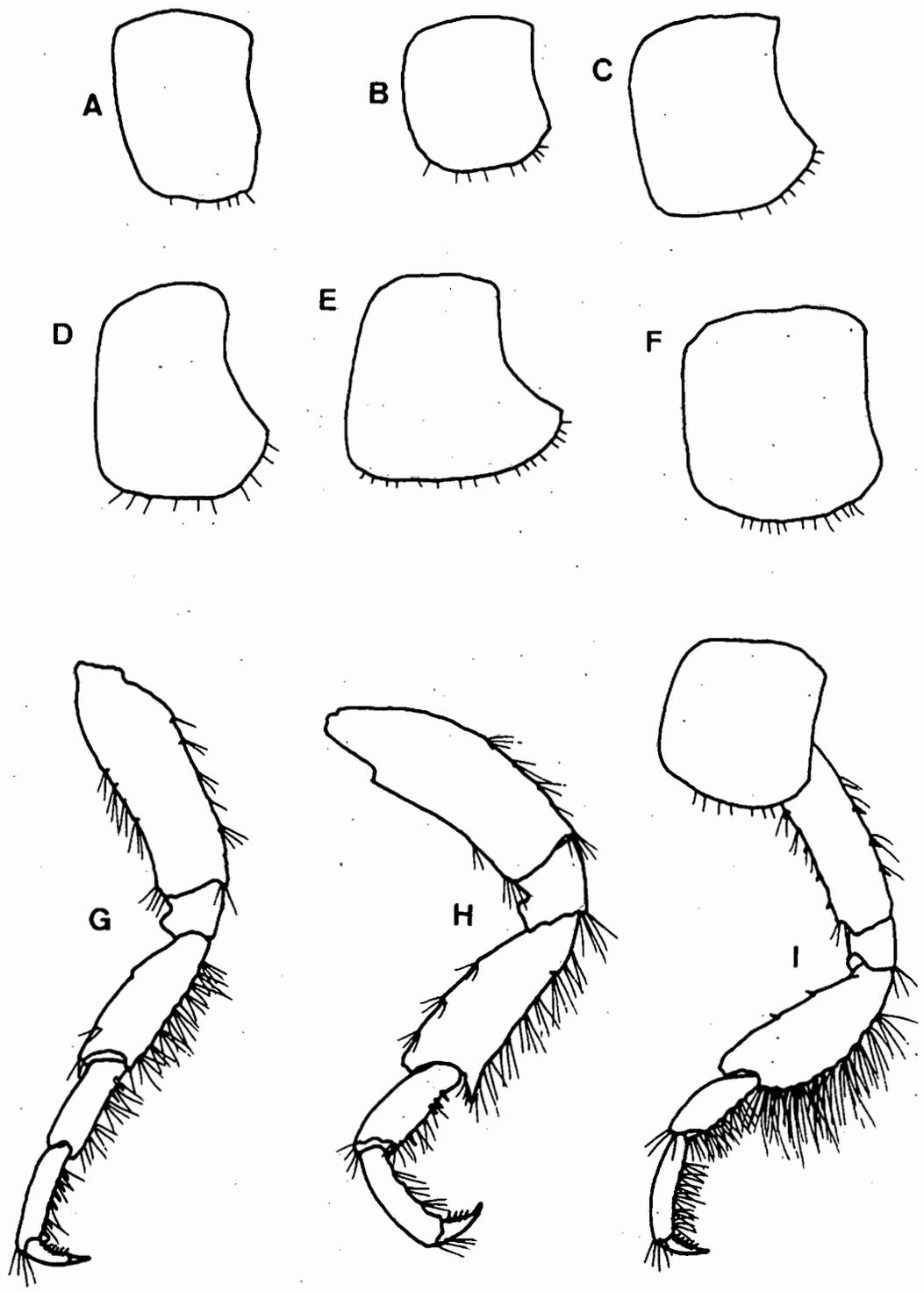

Fig. 4. Coxa 4 left side. A. *Afrocrangonyx crassicornis*. B. *Paramelita aurantius*. C. *P. parva*. D. *P. kogelensis*. E. *P. nigroculus*. F. *Aquadulcaris platypus*. Pereopod 4, lateral view. G. *P. magna*. H. *P. magnicornis*. I. *Aquadulcaris platypus*.

segment on this ramus in *Uroctena*. Three *Paramelita* species had this article absent, while the remaining 13 *Paramelita* species and *Aquaducaris* had a small, but distinct article 2 on the outer ramus of uropod 3.

On the whole, relatively few characters (20 each for *Afrocrangonyx* and *Paramelita*) could be polarised, and were therefore usable. Many characters, such as eye colour, setation of the antennae and limbs, and the relative lengths of the peduncle and flagellum in the antennae, had variable character states within the outgroups. Notenboom (1988) also complained of a shortage of usable characters in his study of the amphipod genus *Pseudoniphargus*, and pointed out that this was a general problem when working at a low taxonomic level.

c) Phylogeny of *Afrocrangonyx*. - In the initial analysis, use of the "ie*" and "bb" commands resulted in two trees with consistency indices of 0.70. Successive weighting was then employed by the repeated use of the sequence "ie*; bb; xs w;" in the HENNIG86 programme, which yielded a fully resolved cladogram for *Afrocrangonyx* with a consistency index of 0.96 (fig. 5). All of the *Afrocrangonyx* species were separated from the outgroup *Uroctena*, by the apomorphic condition of the outer ramus of uropod 3. In *Uroctena*, this ramus has a relatively long second article, but in *Afrocrangonyx*, it is either absent or rudimentary. *A. tulbaghensis*, with its typically pediform antenna 2 and multispinose dactyls appears to be most like the hypothetical ancestor presumed to have given risen to the *Afrocrangonyx* species. The remaining *Afrocrangonyx* species form a monophyletic group defined by the possession of only a single spinule on the dactyls of pereopods 3-7 (characters 13, 14 and 15).

Within this group, two monophyletic subgroups were evident. The first of these, comprising *A. pheronyx*, *A. andronyx* and *A. auricularius* were defined by two synapomorphies - the possession of a posterior lobe on article 3 of antenna 2, and a characteristic distal widening of article 4 of pereopod 3. Two of these species are known from adjacent streams, but *A. andronyx* is known only from an isolated

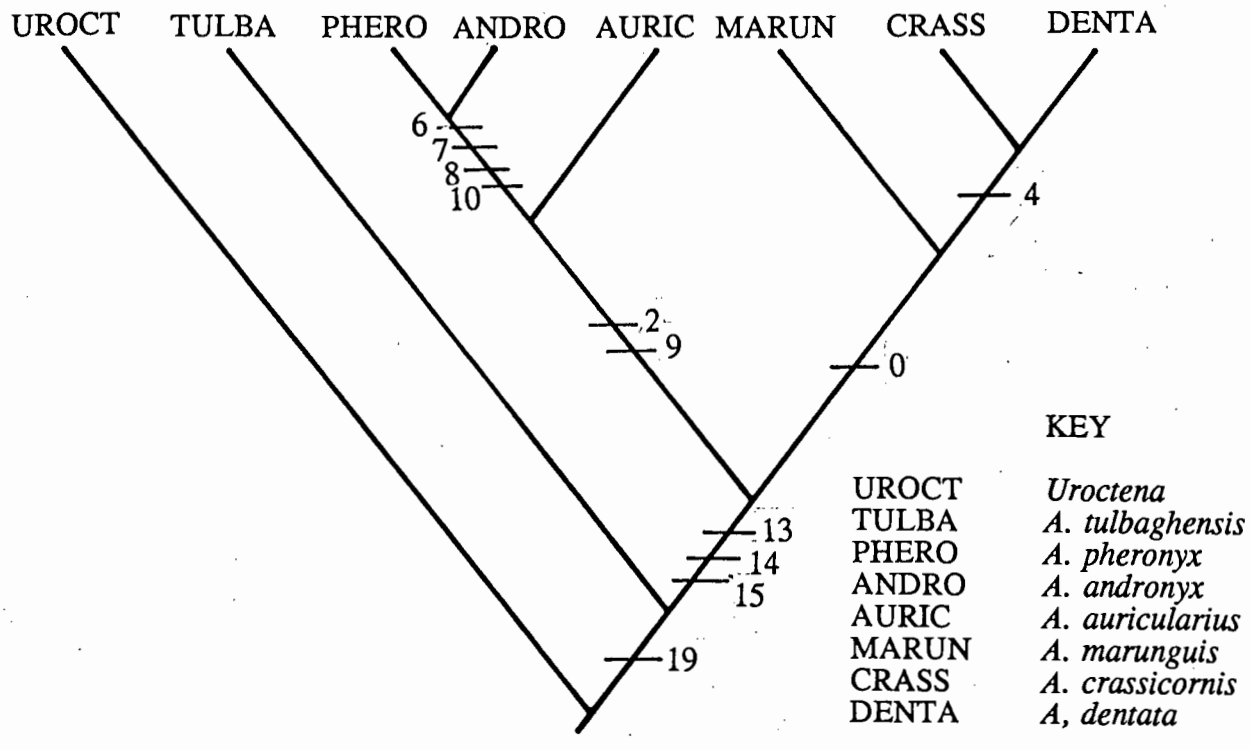

Fig. 6. Cladogram of *Afrocrangonyx*, with *Uroctena* as outgroup.

mountain massif over 75 km north of the *A. auricularius* and *A. pheronyx* localities. This clade, thus, has a rather disjunct distribution.

The monophyletic group consisting of *A. crassicornis*, *A. dentata* and *A. marunguis* was defined by a single apomorphy - the possession of a moderately to densely setose flagellum on antenna 1. These species have been found in streams on the Cape Peninsula which are no more than 15 km apart. The geographical distribution of these species, therefore, supports the proposed existence of this clade.

Although the assumption has been made that all existing *Afrocrangonyx* species have been included in the cladistic analysis, it is possible that a more thorough search of streams in the mountainous areas of the south western Cape could turn up more as yet undescribed forms. Four of the seven species in the genus have only recently been discovered (Stewart & Griffiths, 1991c), and many locations remain to be sampled.

d) Phylogeny of *Paramelita*. - In the initial analysis of the 20 unweighted characters using "mh*; bb;", one most parsimonious tree, with a consistency index of 0.60 was constructed. After repeated use of the sequence "mh*; bb; xs w;", which employs successive weighting, the tree remained the same, but the consistency index increased to 0.81. This tree is presented in fig. 6.

The unresolved nature of the tree can be attributed to a combination of the relative shortage of 'good' apomorphies, and the possibility that the genus, as presently composed, is not monophyletic. As many as seven of the 20 characters analysed fitted the tree badly, and were consequently given weightings of 0 to 2 (range 0-10) during the successive weighting procedure. These 'weak' synapomorphies are more likely to be shared due to parallel evolution than common descent. A good example of this is the possession of medial spines on article 2 of pereopod 3, a condition shared by *P. granulicornis* and *P. capensis*, and given a weighting of zero in the analysis. Myers (1988) was equally concerned by the affect of parallel evolution on the development of an synapomorphic scheme, and concluded from his study of amphipods in the family Aoridae, that parallel evolution was a far more common phenomenon than previously

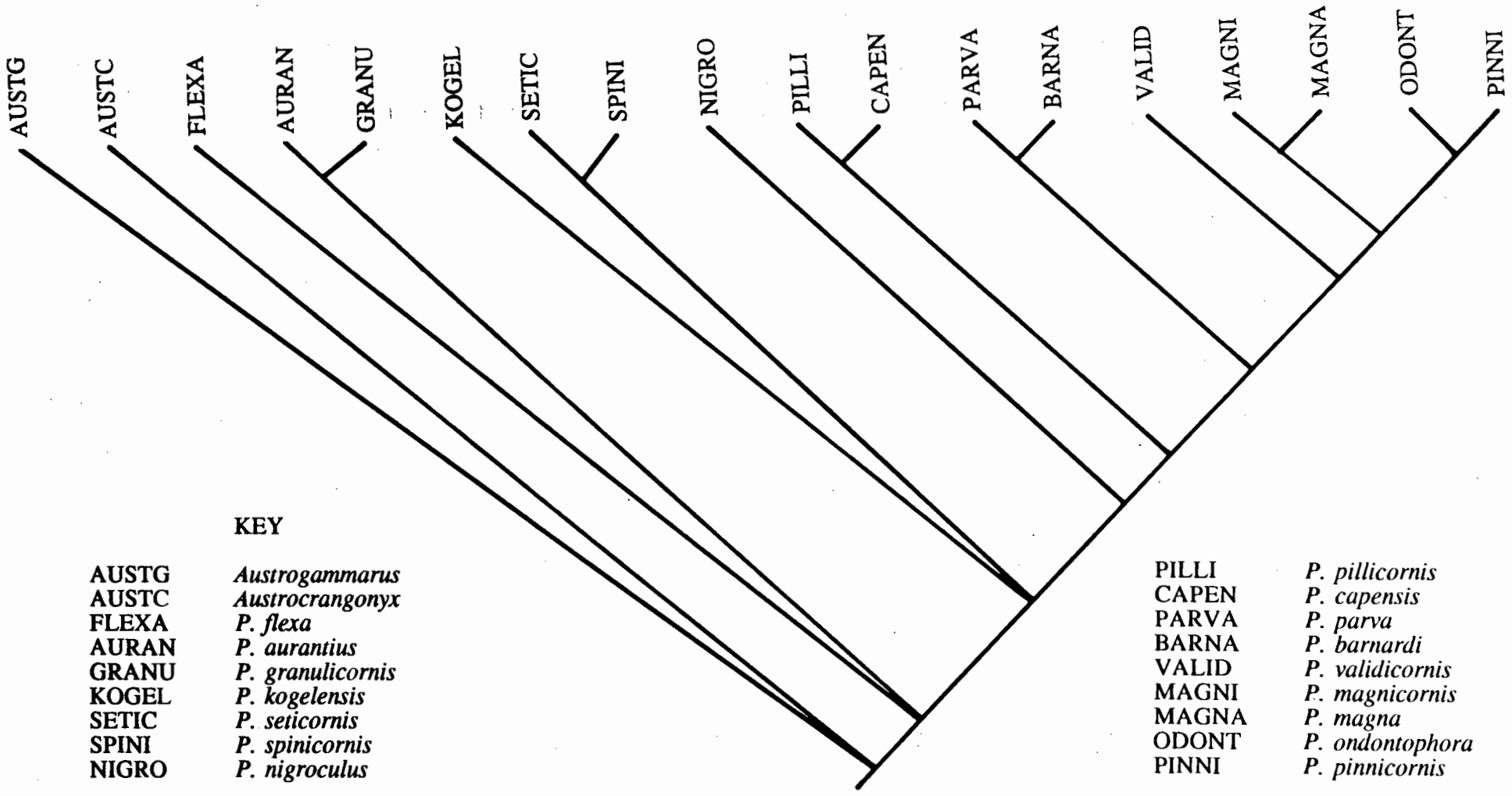

Fig. 7. Cladogram of *Paramelita*, with *Austrogammarus* and *Austrocrangonyx* as

believed. He proposed that in amphipods in general, complex character states are rare and variation morphoclinal, thus making it difficult to detect cases of parallel evolution in these animals (Myers, 1988). In addition, Notenboom (1988) has pointed out that at low taxonomic levels, species share similar gene pools, thus increasing the chances of the occurence of parallelisms.

The fact that the monophyletic group of *P. aurantius* and *P. granulicornis* is geographically related provides additional evidence for the validity of this clade. Griffiths (1981) has already suggested in the past that these 'Hottentot Holland Mountain' species, along with *P. kogelensis* and *P. seticornis*, form a " closely related group". *P. aurantius* and *P. granulicornis* share many apomorphic conditions, such as an almost quadrate coxa 4, medial spines on article 2 of gnathopod 2, and the absence of a second article on the outer ramus of uropod 3. *P. seticornis* and *P. spinicornis* are also probably closely related, as suggested by the cladogram. Again, both species occur in the same geographical area (Hottentot Holland Mountains), often in adjacent streams. Although *P. flexa* and *P. kogelensis* were unable to be grouped, these species also occur on the Hottentot Holland Mountains. When Barnard (1927) first described *P. kogelensis*, he considered it to be "closely allied" to *P. seticornis*. It is highly probable that the present analysis does not include all the extant species of this group, so that a fully resolved cladogram is not possible at this stage. Many streams in the area remain unvisited, but it is hoped that this will be rectified in the near future.

The clade consisting of *P. magna*, *P. magnicornis*, *P. odontophora* and *P. pinnicornis* is characterised mainly by the possession of elongate and stout second antennae. *P. magna* and *P. magnicornis* occur, often in sympatry, in streams in the southern part of the Cape Peninsula. *P. odontophora* and *P. pinnicornis* are also relatively close geographically to each other, with the most easterly known population of *P. pinnicornis* only about 15 km away from the nearest population of *P. odontophora*. *P. pinnicornis* is also known from two localities on the Cape Peninsula. The most unlikely monophyletic group is that of *P. parva* and *P. barnardi*, as these

species occur over 400 km apart, with the latter species known only from a single cave on the Cape Peninsula.

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PAPER 8

Life history and reproductive biology of the mountain stream amphipod *Paramelita nigroculus* (Barnard)

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Abstract

The life history and reproductive biology of the freshwater crangonyctoid amphipod *Paramelita nigroculus* (Barnard) were investigated. Monthly densities of this amphipod were generally high, varying from 264-12 227 individuals m^{-2}. The mean density of 7972 individuals m^{-2} in summer was significantly higher than the value of 1 071 calculated for winter. Sizes of animals ranged from 1-11 mm, with females reaching a larger size than males. As a result of continuous breeding throughout the year, size frequency distributions were similar for all months, and cohorts difficult to discern. Preliminary observations indicate that the life history parameters of *P. nigroculus* are not necessarily characteristic of all *Paramelita* species.

Introduction

An investigation of macroinvertebrate community structure in Window Stream, Table Mountain ($33^{o}58'$ S, $18^{o}25'$ E) revealed that this community was dominated by the crangonyctoid amphipod *Paramelita nigroculus* (Barnard). The black-eyed *P. nigroculus* is the most widespread of the *Paramelita* species, all of which are restricted to the inland waters of the south-western Cape Province, South Africa (Griffiths, 1981). Little is known of the biology of these freshwater amphipods, with Barnard's (1927) study being the only account to date. In this paper, Barnard (1927) commented

on the systematics, distribution, histology of the eye, and timing of breeding of some of the *Paramelita* species. Thus, between 1984 and 1986, the population of *P. nigroculus* in Window Stream was intensively studied in order to gain some insight into the ecology of these amphipods. Buchanan *et al.* (1988) reported on the thermal acclimation and tolerance to lethal high temperatures in this species, while the present paper examines its life cycle and reproductive biology. Results on the energy budget and feeding preferences of *P. nigroculus*, and on the role that this amphipod plays in leaf litter breakdown are to be presented elsewhere.

Study area

Window Stream is a first order headwater stream draining the eastern slopes of Table Mountain (33°58' S, 18°25' E). Riparian trees provide abscissed leaves which form the major food source of *P. nigroculus*. The leaves are dropped throughout the year, with peak leaf fall occurring in summer. The streambed is uneven, and consists of a mixture of large boulders, stones and gravel. Water depth varies from a few centimeters to about 20 cm in winter, stream width is about 4.0 to 5.0 m, temperatures range from 10°C to 18°C, and pH varies from 3.6 to 4.7.

Materials and methods

Between four and six randomly distributed benthic samples (either 0.06 or 0.10 m^2) were collected monthly from June 1984 to May 1985 along a 10 m stretch at Window Stream. Miller (1982) found that six samples were sufficient to get representative population estimates for *Gammarus pseudolimnaeus* Bousfield in a small headwater stream in Wisconsin, USA. Samples were obtained by means of a modified Surber Sampler placed on the stream bottom. Amphipods in the water column and in the benthos to a depth of 5 cm were sampled. The material collected was preserved in

70% alcohol until further processing in the laboratory. All *P. nigroculus* specimens retained by a 125 um sieve were extracted from the benthic samples, and where possible, the size and sex of each individual was determined. Measurements (to the nearest millimetre) for body length were taken from the base of the first antennae to the base of the telson (Hynes, 1954). Animals were grouped into 1 mm size classes.

Males were recognised by the possession of paired papillae projecting from the ventral surface of the last thoracic segment, while females were characterised by the presence of developing or mature oostegites (brood plates). Since it was often difficult to determine sex in individuals less than 4 mm, this length was set as the division between juveniles and immature individuals. Accordingly, individuals were placed in one of the following categories: juveniles, males, immature females, nonbreeding mature females and breeding mature females (either ovigerous or carrying young). Female individuals were considered to be mature when their oostegites were fully developed and fringed by bristles (Hynes, 1954; Hynes & Harper, 1972). Animals which had neither projecting genital papillae nor oostegites were included in the immature female category. Welton (1979) was able to distinguish between immature and mature males of *Gammarus pulex* (L.), where he considered those specimens smaller than individuals observed in precopula to be immature. Miller (1982) separated breeding pairs of *G. pseudolimnaeus* Bousfield, and counted the number of segments in the flagellum of the left first antenna in males, and concluded that males with 20 or more such segments were mature. Since no animals were observed in precopula in the present study, such divisions could not be adopted. No animals possessing both male and female characteristics simultaneously were found. The number of eggs in intact brood pouches of ovigerous females was counted. Data has been expressed as mean + standard deviation unless specified otherwize, and Student's t-test was used to test for significant differences where appropriate.

Results and discussion

Population density

The densities of *P. nigroculus* recorded monthly (Fig. 1) were very variable, a factor probably due to the small number of samples taken (between three and six each month). Figures ranged from 264 (June 1984) to 12 227 individuals m^{-2} (November 1984). A mean density of 7 972.3 + 6 995.9 individuals m^{-2} for the summer months (December, January and February) was significantly higher than the figure of 1 071.2 + 1 239.5 calculated for the winter months (June, July and August; t-test, $p < 0.05$).

Two possible explanations can be offered for the apparent 'seasonal' trend in the population density. The high densities in summer are due largely to a high number of juveniles during this period (Fig. 2). These individuals are either absent in winter, or are present, but due to stronger flows during the wet winter months, occur deeper in the hyporheas, and are not captured by the sampling method used. Low winter and high late summer densities have also been noted for *Gammarus pulex* in an English (Welton, 1979) and a Danish (Mortensen, 1982) stream.

The densities recorded here are similar to those of Chambers (1977), who found densities of *G. tigrinus* (Sexton) in a lake in the Netherlands to be between 481 and 16 034 individuals m^{-2}, and considered these values to be "very high". Goedmakers (1981) reported population densities of 500-10 000 gammarids m^{-2} in a French chalk stream, and Welton (1979) also found densities to be high and extremely variable in a population of *G. pulex* in a stream in England. In the first year of his study, values ranged from 887 to 10 137 individuals m^{-2}, while in the second year, densities ranged from 820 to 6 329 individuals m^{-2}. Mortensen (1982) reported densities of 500-5 500 m^{-2} for the same species in a Danish stream. Many authors have reported much lower densities for amphipods. For example, Marchant & Hynes (1981) reported values ranging from 9 to 1 620 individuals m^{-2} for *G. pseudolimnaeus* in a Canadian river,

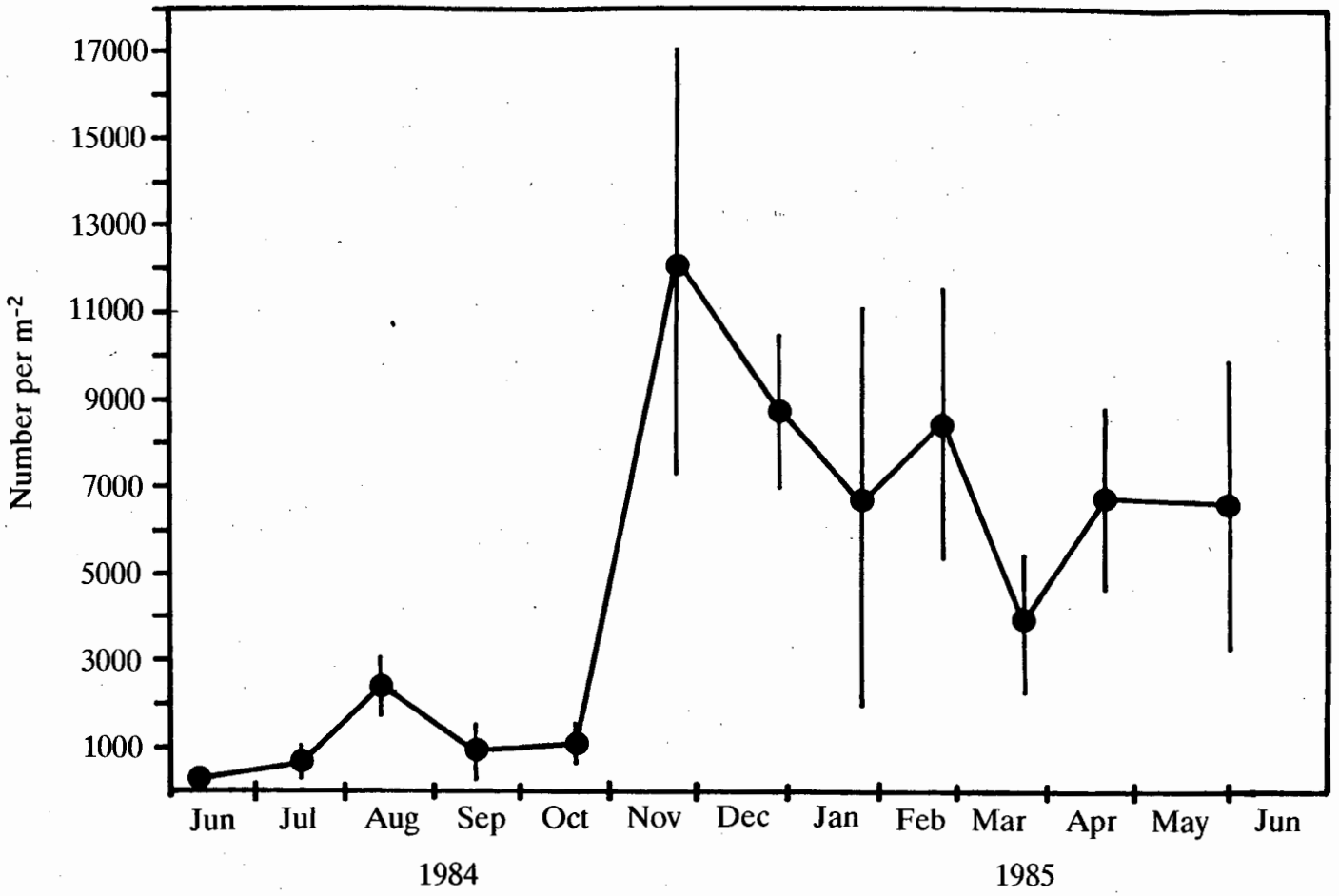

Fig. 1. Densities with time of *Paramelita nigroculus* in Window Stream. Vertical lines represent one standard error above or below the mean.

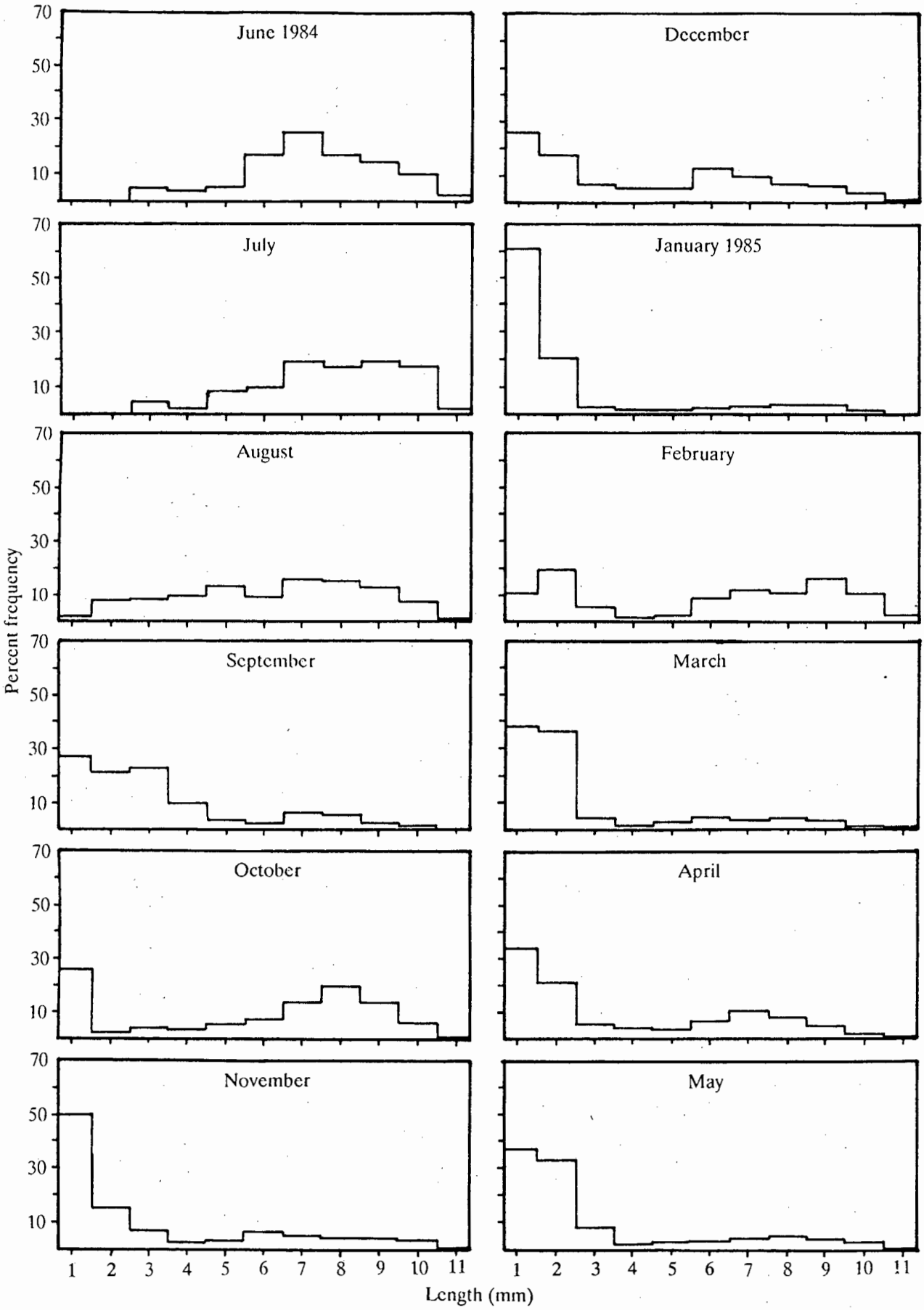

Fig. 2. Size frequency distribution (in terms of numbers) of *Paramelita nigroculus* at Window Stream.

Mathias (1971) found a mean density of 300 *Crangonyx richmondensis occidentalis* individuals m^{-2} in a Canadian lake, and King *et al.* (in prep.) only found 2-8 individuals m^{-2} of *P. nigroculus* in Langrivier, a second order stream draining a fynbos catchment.

Population structure

The sizes of *P. nigroculus* individuals (Fig. 2) retained in the samples ranged from 1.0 to 11.0 mm in length, with only one individual out of the total number of 11 239 examined exceeding 12.0 mm! As suggested by Barnard (1927), reproduction of *P. nigroculus* was continuous throughout the year, and juveniles (1.0 to 3.9 mm) were present all year round. The percent frequency of juveniles was relatively low (4.9 to 18.1 % of the total population) during winter (June, July and August), but increased during the rest of the sampling period, ranging from 31.7 to 83.8%. Welton (1979) found that in a population of *G. pulex* which was breeding throughout the year, the percentage of juveniles was always the highest of all the age classes, and varied from 39 to 76%. The size frequency distribution of immature and adult individuals only (> 4 mm) is also represented in Fig. 3. Here, the frequency of males and females have been plotted separately. With the exception of June and September, the size frequency distribution of the immature and mature individuals remained similar throughout the year. The mean size of females was between 9.0 and 9.9 mm, while males between 6.0 and 8.9 mm were most frequent. Adult males, therefore, were clearly smaller than mature females, with very few males having exceeded 10.0 mm in length (four 0f 1 902 examined).

The fact that females are clearly larger than males in *P. nigroculus* appears to be unusual amongst the gammarids. For example, in *G. pulex* (Welton, 1979) *G. pseudolimnaeus* (Marchant & Hynes, 1981) and *G. tigrinus* (Chambers, 1977), males reach a larger maximum size. However, in three other crangonyctoid species,

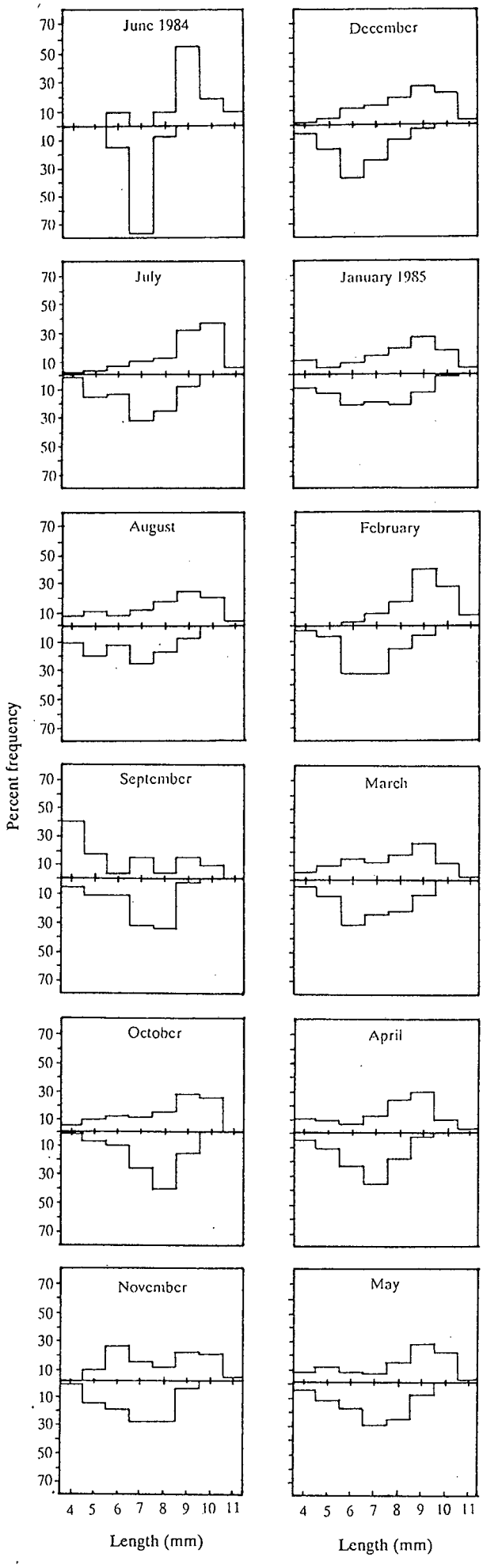

Fig. 3. Size frequency distribution (in terms of numbers) of females (above the line) and males (below the line) of *Paramelita nigroculus* in the 4-11 mm size range.

Crangonyx forbesi (Hubricht & Mackin), *C. gracilis* Smith, and *C. richmondensis occidentalis*, males are smaller than females (Hynes, 1955; Mathias, 1971; Crawford & Tarter, 1979). This is probably not the case in the other *Paramelita* species. Although the population structures of these species have not been studied in any detail, the largest individuals caught from populations of these species are always male (unpubl. data).

Sizes of freshwater gammarids are very variable, and are unfortunately measured in different ways. In other studies where body length has been measured in the same way as in the present study, maximum sizes of 13.0 to 16.0 mm have been recorded (Chambers, 1977; Crawford & Tarter, 1979; Kostalos, 1979; Welton, 1979; Marchant & Hynes, 1981; Miller, 1982).

Reproductive biology

With the exception of June 1984, mature females (ovigerous, bearing young, or having bristled oostegites) were present all year round (Fig. 4). The June sample, however, was small, and only included 11 females in total. When the proportion of mature females relative to the total number of females was considered, values of three (September) to 27% (October) were obtained (Fig. 5). Seasonal trends were not obvious, although it appeared that there might have been a higher frequency of mature females in summer than in winter. However, a mean density of mature females of 223.6 individuals m^{-2} in summer was not significantly higher than a mean density of 55.7 individuals m^{-2} for winter (t-test, $p > 0.05$). Of these mature females, anything from 4.4 (November) to 100.0% (September) were either ovigerous or carrying young. It was difficult to ascertain how many of the females with bristled oostegites might have already reproduced.

Oostegite development is first visible in individuals greater than 7.0 mm. Oostegite 'buds' are rarely present in individuals smaller than this, for example, of the 409 specimens examined measuring between 4.0 and 6.9 mm, only nine showed any

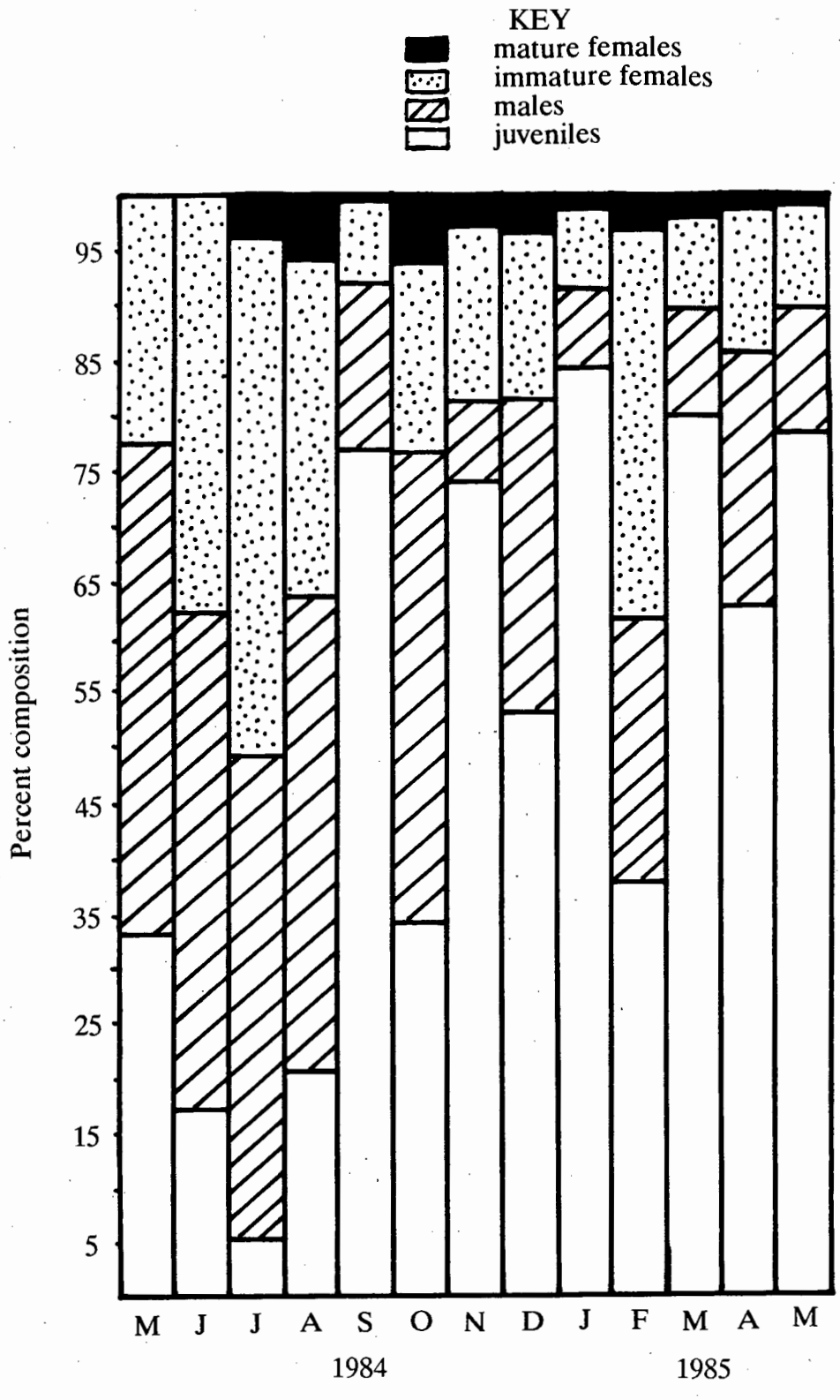

Fig. 4. Percent composition of the *Paramelita nigroculus* population.

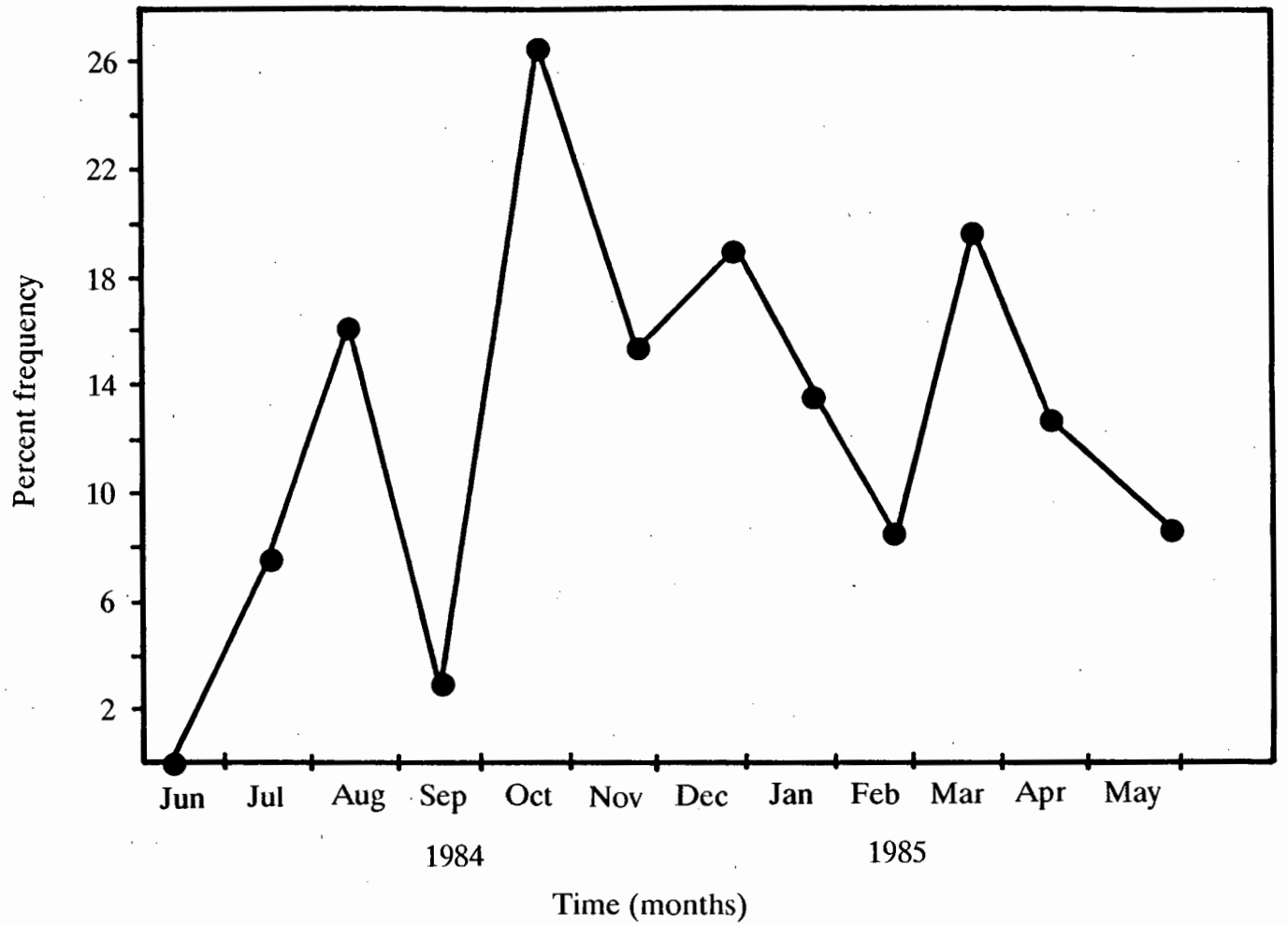

Fig. 5. Percentage of females of *Paramelita nigroculus* that were mature.

signs of oostegite development. Oostegites develop bristles when individuals are 9.0 mm in length. Fifty-one of the 53 ovigerous females encountered during the study were between 9.0 and 10.9 mm long. Intact brood pouches in these animals contained an average of 41.1 + 9.7 eggs (range: 31-60). Since 51 of the 53 ovigerous females encountered all fell into two size classes, a regression analysis of egg number against body length was not suitable in the present study.

The ratio of males to females was very variable, and showed no seasonal trend. A mean ratio of 1.2 + 0.5 was calculated. Welton (1979) also found this ratio to be very variable in *G. pulex*.

In contrast to *P. nigroculus*, both the crangonyctoids *C. forbesi* and *C. richmondensis occidentalis* have been reported to be seasonal breeders (Mathias, 1971; Crawford & Tarter, 1979). Marked seasonal trends in the breeding patterns of gammarid amphipods are common, and have been recorded in *G. pseudolimnaeus* (Hynes & Harper, 1972; Waters & Hokenstrom, 1980; Miller, 1982), *G. pulex* (Iversen & Jessen, 1977; Gee, 1988), *G. lacustris* (Hynes & Harper, 1972) and *G. tigrinus* (Chambers, 1977). Although *G. minus* Say breeds throughout the year, Kostalos (1979) recorded a distinct peak in breeding activity during winter for this species. Welton (1979) found ovigerous females all year round in *G. pulex* in England, but noted that the numbers of these females rose in summer, the time of maximum breeding activity. Marked seasonal breeding patterns have also been recorded in five other marine *Gammarus* species by Kolding & Fenchel (1979).

Williams & Wan (1972; cited in Smith & Williams, 1984) have hypothesised that a lack of a precisely timed autumnal leaf fall in Australian streams would be reflected by a lack of seasonality in the life cycles of Australian stream invertebrates. Although Smith & Williams (1984) decided to test this hypothesis in their study of the life cycles of two Australian amphipod species, they were unable to come to any firm conclusions. They suggested that the lack of seasonality in the life cycle of *Pseudomoera gabrieli* was as a response to the absence of seasonal variations in

temperature and flow rates, and that seasonality in *Austrochiltonia australis* was probably due to marked seasonal changes in flow regime. Similar problems are encountered when testing this hypothesis in Window Stream. Allochthonous input occurs throughout the year in this stream, peaking in summer. Flow rate, on the other hand, is highly seasonal, with high flow in winter and low flow in summer. The life cycle of *P. nigroculus*, therefore, is likely to be affected by both the hydrological regime and by food availablity.

The number of eggs carried by *P. nigroculus* females is in the range reported for other gammarid species. Hynes & Harper (1972) recorded means of 16.8-49.8 eggs pouch^{-1} in *G. pseudolimnaeus*, while Miller (1982) counted a mean of 38.7 eggs pouch^{-1} and Marchant & Hynes (1981) means of 26.2 to 39.3 in the same species. Mean values for other species are 11.0-21.8 for *G. lacustris limnaeus* Smith (Hynes & Harper, 1972), and 39.5-63.0 for *G. tigrinus* (Sexton) females in the 9+ mm size class (Chambers, 1977).

Standing stocks

The monthly standing stock of *P. nigroculus* was calculated by using a regression of dry weight on length and the density estimates of each size class (Fig. 6). The latter estimates were obtained by multiplying the percent frequency of each size class by the total densities on each sampling occasion. Dry weights were obtained from the regression equation

$$\ln y = 2.65 \ln x - 11.2,$$

where y represented the weight in grams and x the length in millimetres. Standing stocks were variable and appeared to be higher in summer than in winter. However, owing to the few samples taken, the mean value of 14.81 g dry weight m^{-2} for the summer months (December to February) was not significantly higher than the value of 3.15 g dry weight m^{-2} calculated for winter (June to August; t-test, $p > 0.05$). The

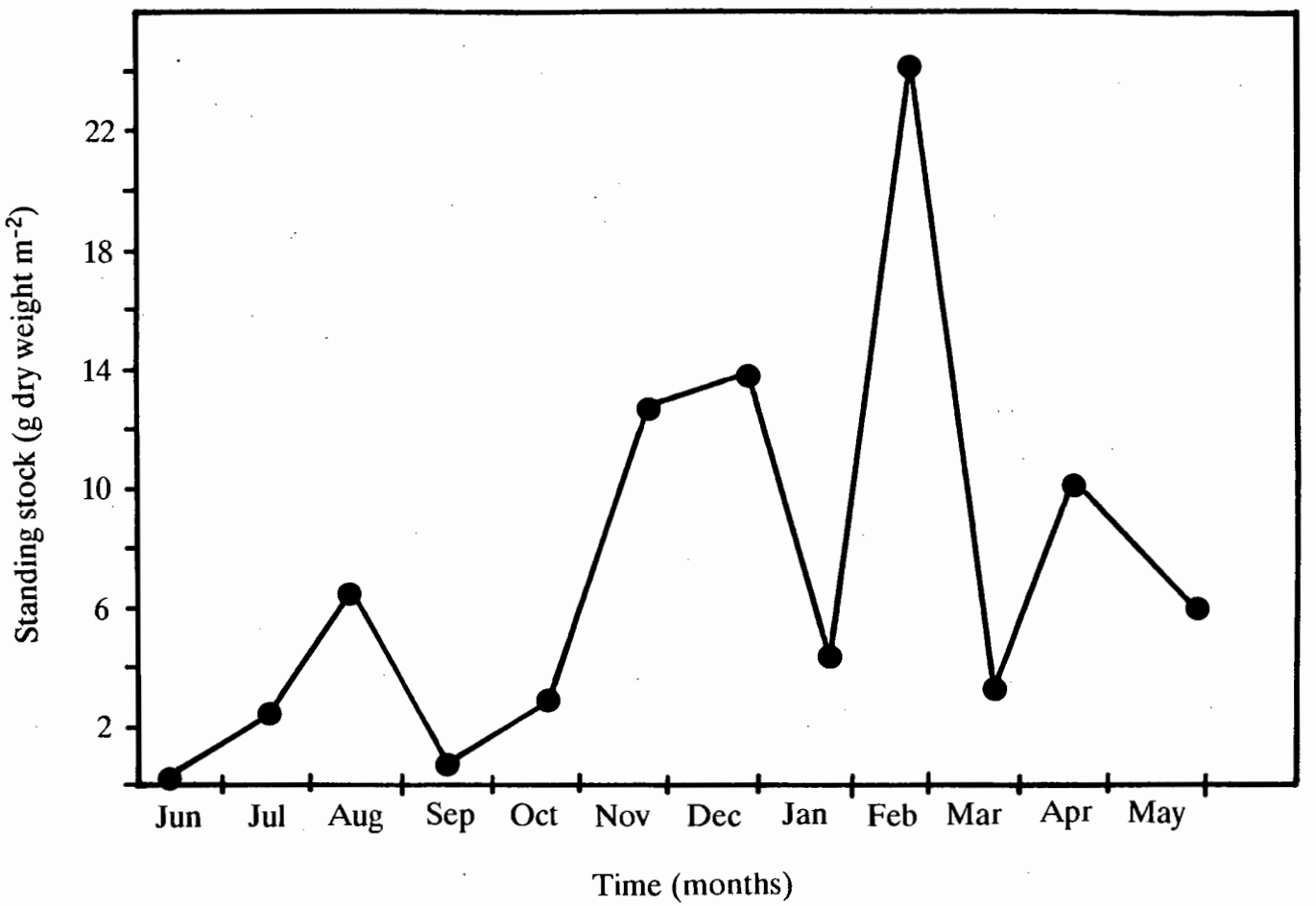

Fig. 6. Standing stocks of *Paramelita nigroculus* in Window Stream.

highest value recorded was 24.32 g dry weight m^{-2} (February), while the lowest value of 0.37 g dry weight m^{-2} was obtained for June. Animals in the size classes 6 to 10 mm formed the major part of the biomass for all months (Fig. 7). Juveniles (1.0-3.9 mm) were never responsible for more than 13% of the total standing stock. During winter, they formed between 0.5-5.7% of the standing stock, and for the rest of the study period, between 1.1 and 13.0%.

Welton (1979) recorded biomass values of 1.5 to 11.0 g dry weight m^{-2} for a population of *G. pulex*. In his study, the major proportion of the biomass consisted of animals 9.3 to 13.2 mm in length (his largest individuals were 15.3-16.2 mm in length). Mortensen's (1982) biomass values for the same species in a Danish stream, namely 0.2-3.4 g dry weight m^{-2}, are considerably lower than those obtained by Welton (1979) and those in the present study.

Acknowledgements

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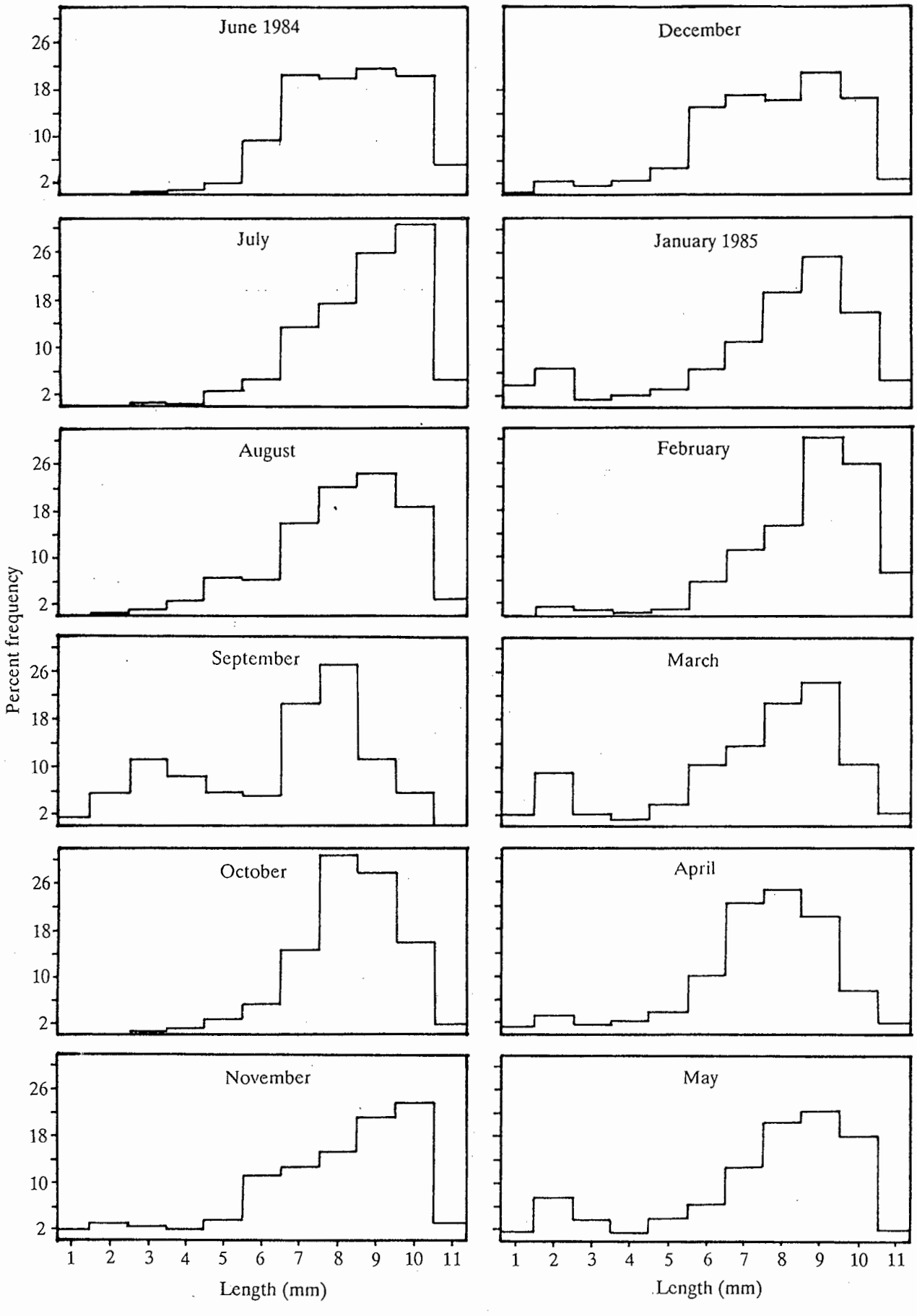

Fig. 7. Size frequency distribution (in terms of biomass) of *Paramelita nigroculus* at Window Stream.

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PAPER 9

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THERMAL ACCLIMATION AND TOLERANCE TO LETHAL HIGH TEMPERATURE IN THE MOUNTAIN STREAM AMPHIPOD *PARAMELITA NIGROCULUS* (BARNARD)

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(*Received* 8 *June* 1987)

Abstract—1. Rates of upward and reverse acclimation were studied in *Paramelita nigroculus* (Barnard) from a mountain stream using a modification of the Critical Thermal Maximum (CTM) method. The upper lethal temperatures were measured using the LT_{50} method.

2. Acclimation rates were found to be typical of most crustaceans, with a gain of resistance to high temperature, following transfer from 8.5 to 20°C, being completed in 1–2 days. Loss of heat resistance took slightly longer (3 days).

3. The species is strongly sexually dimorphic, males being significantly smaller than females. Neither sex, body length nor feeding status was found to affect tolerance levels.

4. The LT_{50} (min) for animals acclimated to 13.5°C ranged from approximately 300 min at 27°C to 4 min at 31°C.

5. LT_{50} values for 20°C-acclimated individuals were significantly higher than those acclimated at 13.5°C at corresponding test temperatures.

INTRODUCTION

Thermal acclimation is a mechanism whereby organisms are able to exploit and survive extremes of environmental conditions (Brattstrom, 1970). This typically involves a compensatory change by the organism in response to temperature changes (Edney, 1964). Adjustments of thermal tolerance have been demonstrated in a wide variety of organisms (Dourdoroff, 1942; Brattstrom, 1970; Bradley, 1978; Claussen and Walters, 1982), including amphipods (Krog, 1955; Sprague, 1963; Savage, 1982; Hamasshima and Morino, 1984), but nothing is known about thermal acclimation of freshwater amphipods of South Africa.

Paramelita nigroculus (Barnard), an important member of the "shredder" (Cummins, 1974) community, is endemic to certain freshwater streams of the south-western Cape Province (Fig. 1) and is the most widespread of the 12 *Paramelita* species which occur in the region (Griffiths, 1981). The climate of the southwestern Cape is mediterranean, with a winter rainfall (July–September) and a dry summer (December–March). During summer, many small streams cease to flow and often form a series of isolated pools. Such pools act as refugia, often supporting large numbers of the amphipod (Stewart, unpublished data), and ambient temperatures at this time may reach 35°C or more, with pool temperature rising accordingly. Within the context of a broader study of the autecology of *P. nigroculus*, it was considered of interest to investigate the physiological adaptations of these animals which may enable them to survive extreme daily temperature fluctuations.

In this study the rates of thermal acclimation and the tolerances to high temperatures of *P. nigroculus* were investigated. Experiments were carried out using the Critical Thermal Maximum (CTM) method (Hutchinson, 1961; Claussen, 1980) to measure rates of gain or loss of heat resistance, and the LT_{50} method (Fry, 1957) to measure upper lethal temperatures. Although the CTM method has been reported to yield diverse results (Fry, 1957), it has nevertheless been successfully applied to a wide variety of organisms (Brattstrom, 1970; Gatz, 1971; Johnson, 1972; Otto and Gerking, 1973; Claussen, 1977, 1980; Claussen and Walters, 1982; Floyd, 1985).

MATERIALS AND METHODS

Collection and holding

Paramelita nigroculus specimens for CTM experiments were collected from Skeleton Gorge on Table Mountain (33°58'S, 18°25'E) in April 1986. Animals were housed in groups of 12 in 300 ml plastic jars containing river water obtained from the collecting site. Jars and all other apparatus with which the amphipods came into contact, were leached in river water for at least 24 hr prior to holding. Water lost through evaporation was replaced with fresh stream water at 2–3-day intervals.

After an initial 5-day holding period at 15°C, which approximated stream temperature, one third of the individuals were transferred to a constant-temperature bath maintained at an acclimation temperature of 8.5 ± 0.1°C, and the remainder at 20 ± 0.1°C. One group at 20°C and the group at 8.5°C comprised two feeding CTM experiments and were provided with abcissed leaves from riparian trees. Animals used in non-feeding CTM experiments were placed in jars containing a substratum of small stones. Acclimation proceeded for 12 days during which time the jars were well aerated using compressed air passed through airstones. Animals for the LT_{50} experiments were sorted and housed

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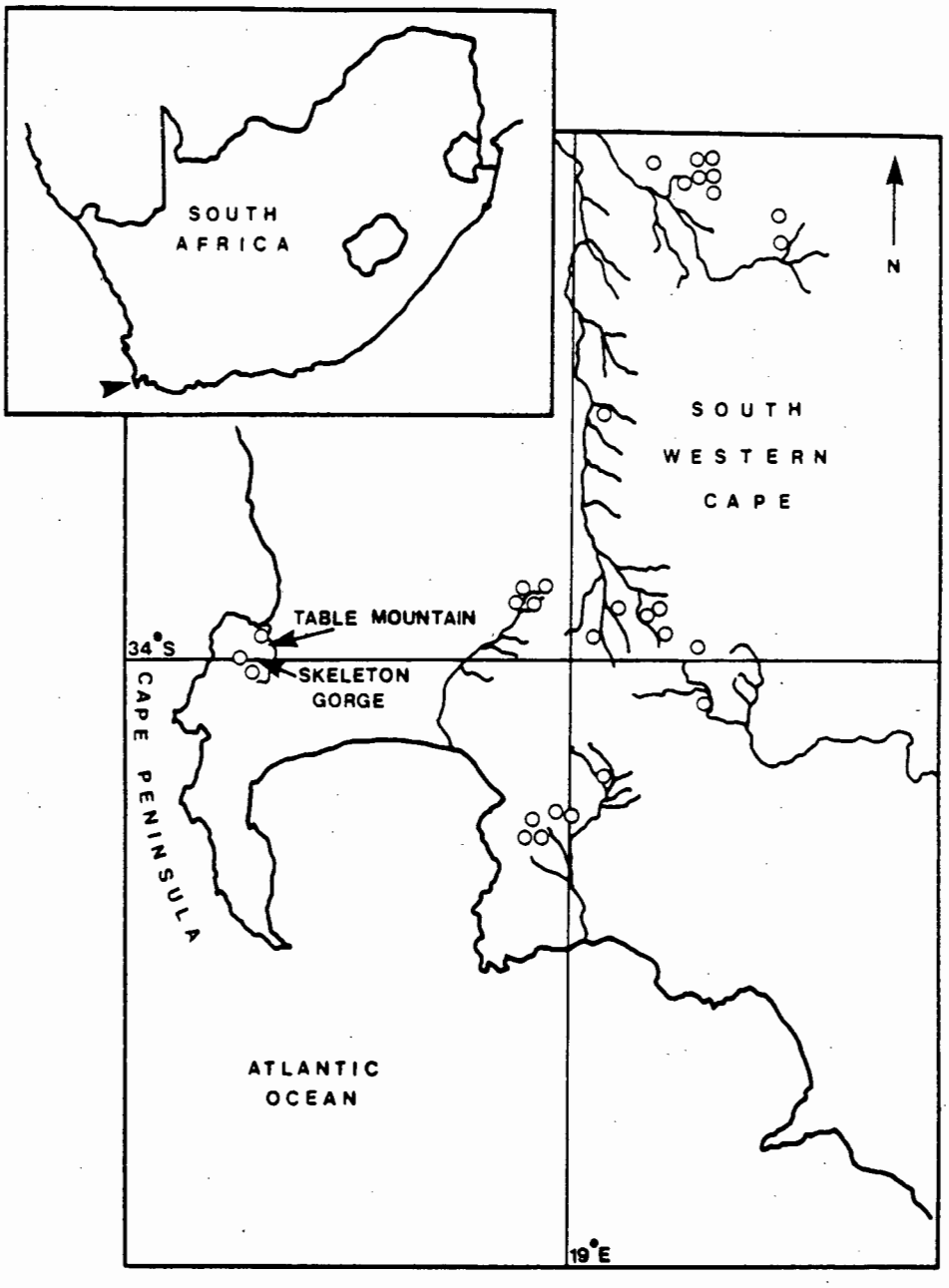

Fig. 1. Map of the southwestern Cape Province showing the distribution of *Paramelita nigroculus* (Barnard) (depicted by the open circles) and the collection site, Skeleton Gorge, on Table Mountain. (Modified from Griffiths, 1981).

in the same way. Acclimation proceeded at 13.5 ± 0.75°C and at 20 ± 0.1°C for 1 month.

CTM test procedure

One jar each of feeding and non-feeding cold-acclimated animals (8.5°C; 12 days) represented day zero for the 8.5–20°C transfer test. The remaining jars were transferred to a water bath maintained at 20 ± 0.1°C and were CTM tested at various intervals. A similar procedure was employed for groups of animals maintained at 20°C (20–8.5°C transfer tests). CTM testing was carried out between 0800 and 1300 hr with the exception of the 6 hr CTM tests, which were conducted between 1400 and 1900 hr on day 0.

Animals for CTM tests were placed in 10 ml of stream water in test-tubes suspended in a beaker containing 800 ml of water at the acclimation temperature (either 8.5 or 20°C). The water in the beaker was then heated at a constant rate of 1.4°C/min using a 100 W aquarium heater, and was continuously aerated to maintain a uniform temperature throughout (Otto and Gerking, 1973). Water temperature in the test-tubes was monitored using a mercury thermometer.

At a certain critical temperature, near to that of the lethal temperature, a wide variety of animals are observed to perform a characteristic action that serves to mark the end-point of the CTM experiments; for example, loss of equilibrium in *Dugesia* (Claussen and Walters, 1982). Such an end-point criterion was absent in *P. nigroculus* and total cessation of movement (CM), was instead used as the end-point. Animals were not found to recover after a period of 5 min at 20°C. Once dead, animals were sexed and body-length from the base of the antennae to the tip of the telson was measured to the nearest 0.1 mm.

LT_{50} *test procedure*

The method used was that of Fry (1957). Jars of 12 animals, fully acclimated to 13.5 ± 0.75°C, were introduced into water baths maintained at a series of test temperatures ranging from 27 to 31 ± 0.1°C. In order to reduce thermal shock, water temperature in each jar was allowed to rise over a 30 min period to that of the test temperature, after which, timing commenced. Each jar was checked at progressive time increments, more frequently during the initial stages, and the dead animals removed. The same

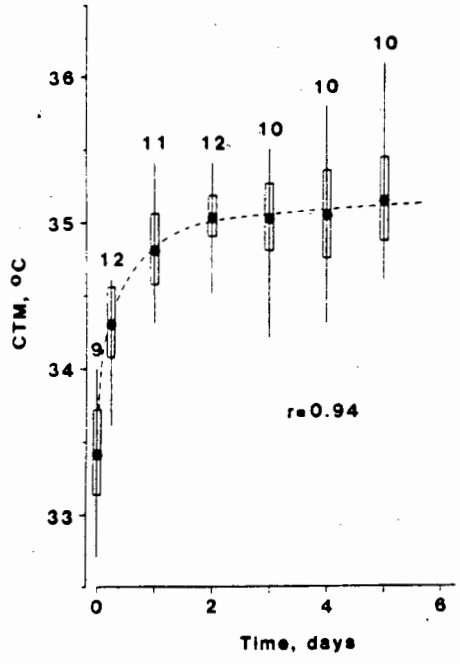

Fig. 2. Thermal time-course pattern for feeding individuals following transfer from 8.5 to 20°C. For each point, the vertical line represents the range, the closed circle the mean CTM value, and the rectangle, the 95% confidence limits about the mean. The number above each symbol indicates the sample size (N). The correlation coefficient (r; $P < 0.005$) is shown below and to the right of the derived hyperbolic curve of best-fit (Claussen, 1977, 1980) as depicted by the broken line.

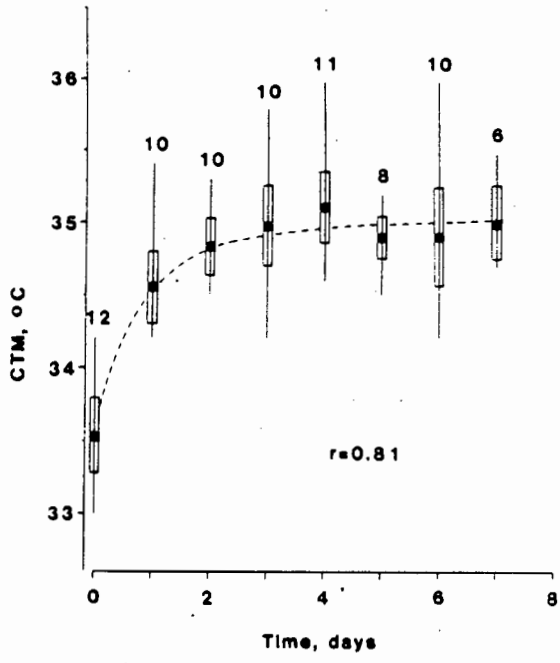

Fig. 3. The thermal time course for forward acclimation of non-feeding individuals, following transfer from 8.5 to 20°C. For each point, the vertical line represents the range, the closed circle the mean CTM value, and the rectangle, the 95% confidence limits about the mean. The sample size (N) is indicated above each symbol. The correlation coefficient (r; $P < 0.01$) is shown below and to the right of the derived hyperbolic line of best-fit as depicted by the broken line.

procedure was followed for 20°C-acclimated animals, although due to high mortality during holding, there were only sufficient individuals remaining to allow testing at only 2 test temperatures; namely, 29 and 31°C. Three replicates were carried out at each test temperature and the results were statistically analysed using stepwise Student's t-tests and ANOVA.

RESULTS

CTM experiments

In all three experimental treatments the response to a change in acclimation temperature led to a change in tolerance to high temperature by less than 2°C. The upward acclimation time course patterns of both the feeding and non-feeding groups (Figs 2 and 3) appeared to be of Type I (Claussen, 1977), with no apparent overshoot when compared with the derived hyperbolic curves (see Claussen, 1977, 1980 for methodology). Acclimation was initially rapid for both groups, the $\frac{1}{2}$AT (half acclimation time) of the feeding group, 0.28 days, being shorter than that of the non-feeding group (0.50 days). This difference, however, is not statistically significant ($P > 0.2$; Modified Student's t-test). Differences in CTM values between feeding and non-feeding groups at any one time, from day 0 to day 5 inclusive, also were not significant ($P > 0.05$; Student's t-test). The results of these experiments are presented in Table 1. Upward acclimation in the feeding group (Fig. 2) does not appear to be complete after 5 days exposure

Table 1. Statistical analyses of the thermal acclimation time courses for *Paramelita nigroculus* based on a one-way ANOVA (Sokal and Rohlf, 1969) and stepwise Student's t-tests computed using Bonferroni's probability corrections (Dixon, 1985). Alphabetic values for each test (i.e. within each column) which do not share a common character, differ significantly ($P < 0.05$). Similarly, numerical values (in parentheses) for each time (i.e. within each row) which do not share a common number also differ significantly ($P < 0.05$)

Acclimation time	Feeding 20–8.5°C		Feeding 8.5–20°C		Non-feeding 8.5–20°C	
Day 0	A	(1)	A	(1)	A	(1)
6 hr	A	(0)	B	(1)	—	—
Day 1	B	(0)	B,C	(1)	B	(0.1)
Day 2	B,C,D	(0)	C	(1)	B	(1)
Day 3	C,D	(0)	C	(1)	B	(1)
Day 4	C,D	(0)	C	(1)	B	(1)
Day 5	C	(0)	C	(1)	B	(1)
Day 6	C,D	(0)	—	—	B	(1)
Day 7	C	—	—	—	—	—
Day 9	D	—	—	—	—	—

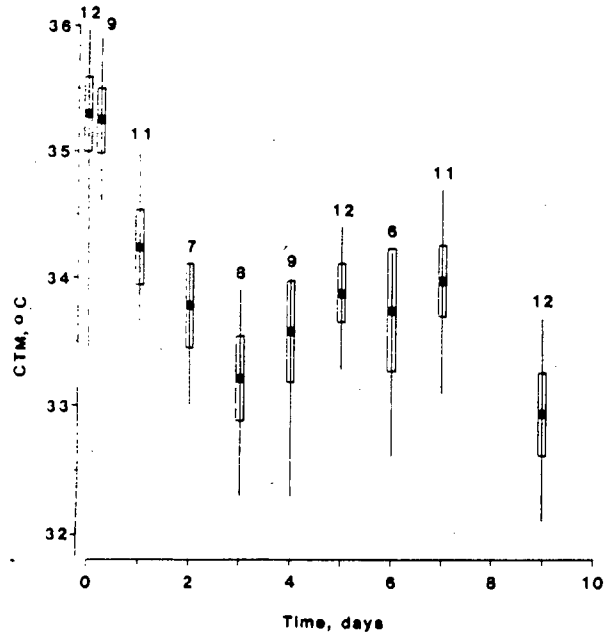

Fig. 4. Loss of heat resistance of *Paramelita nigroculus* individuals following transfer from 20 to 8.5°C. For each point, the vertical line represents the range, the closed circle the mean CTM value, and the rectangle, the 95% confidence limits about the mean. The sample size (N) is shown above each data point. The derived hyperbolic curve of best-fit is omitted due to poor correlation between the variables ($r = 0.30$; $P > 0.05$).

to 20°C. This is suggested by the slightly higher observed CTM value of 35.3 at day 0 for 20°C-acclimated (20–8.5°C) animals, a value which is also in close agreement with the expected CTM value of 35.2. The expected CTM is obtained by adding a ΔCTM of 1.8 to the day 0 CTM of 33.4 (12 days acclimated to 8.5°C) for cold-acclimated individuals (see Claussen, 1977, 1980 for methodology). The fact that acclimation does not appear to be complete after this 5-day period is in conflict with the results of a series of t-tests which failed to show any significant differences between the observed CTM of 35.3 and CTM data from, and including, day 1 onwards ($P > 0.05$; Student's t-test). A similar situation prevailed for the non-feeding group (Fig. 3); acclimation, according to the expected CTM of 35.1 and the ΔCTM of 1.6 was not complete after 7 days at 20°C, but t-tests showed no significant difference in the CTMs, and hence complete acclimation, after 2 days ($P > 0.05$).

The data for reverse acclimation (20–8.5°C transfer test; Fig. 4) approximate poorly to the derived hyperbolic curve ($r = 0.30$). For this reason, the derived ΔCTM of 1.8 (Claussen, 1980) is not considered to be a reliable estimate of the true value. Individuals appear to be fully acclimated by day 2 where the CTM value of 33.8°C no longer differs significantly ($P > 0.05$; Student's t-test) from the day 0 CTM (see above) of cold-acclimated individuals (8.5–20°C transfer test: feeding). Reverse acclimation appears to follow a Type IIIa response pattern (Claussen, 1977) with a graphical estimate of $\frac{1}{2}$AT (Fig. 4) approximating to 0.8 days. Within each of the three time course patterns (Figs 2–4), the differences among most of the CTM values for the various acclimation times were not significant ($P > 0.05$; Table 1).

Males were found to be significantly smaller ($P < 0.001$; Student's t-test) than females, the average length of all the males used in this study being 7.7 ± 1.4 mm and females, 9.8 ± 1.7 mm. The sex of individuals was not found to affect the tolerance to high temperature either in fully or partially acclimated animals ($P > 0.10$; Student's t-test), neither was size in any way related to resistance to high temperature ($r = 0.08$; $P \gg 0.05$).

High mortalities encountered for animals held at 20°C were attributed to increased pollution of the water due to accelerated plant decay caused by the relatively high temperature. The problem was not found in jars without food.

LT_{50} *experiments*

After 1 month of holding, at least 80% of the individuals acclimated to 20°C had died, whereas at 13.5°C very few individuals died. Results of LT_{50} experiments are presented graphically in Fig. 5. Lines of best fit were calculated using Model I linear regression (Sokal and Rohlf, 1969).

The LT_{50}s (in min) obtained from these regression lines were found to be exponentially correlated ($r = -0.99$; $P < 0.005$) to the test temperature to which the individuals were exposed (Fig. 6; Table 2). Extrapolation of the best-fit line to the 0.1 min level yielded an absolute upper lethal temperature of

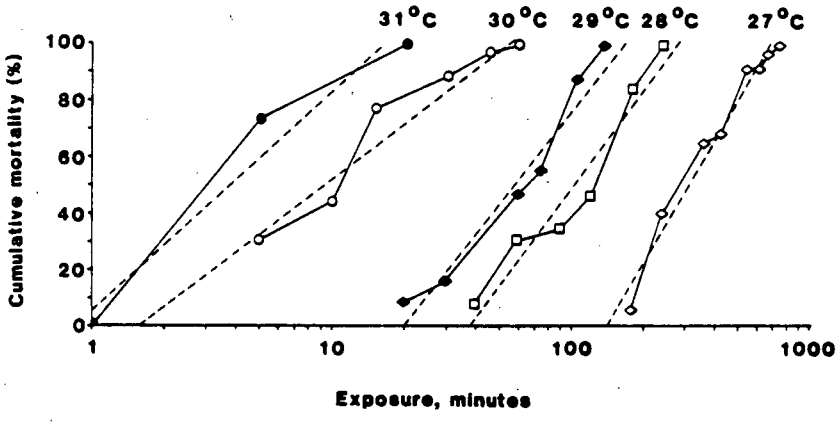

Fig. 5. Cumulative percentage mortalities of *Paramelita nigroculus* (acclimated for 30 days at 13.5°C) expressed as a function of the log of exposure time to the relevant test-temperatures. Test temperatures are shown above each time–percent graph.

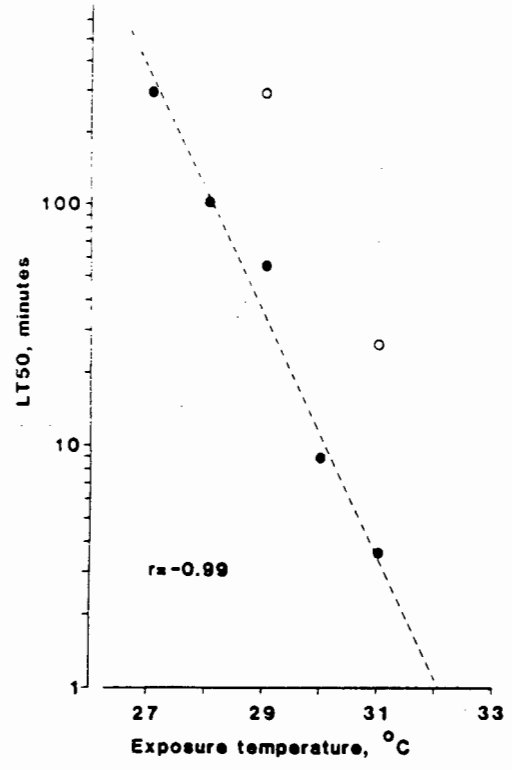

Fig. 6. LT_{50}, in min, expressed as a function of the test-temperature (°C) to which fully acclimated animals were exposed. Open circles represent 20°C-acclimated individuals and the closed circles 13.5°C-acclimated individuals. The best-fit line (broken) was calculated using Model I regression statistics (Sokal and Rohlf, 1969) for the 13.5°C-acclimated animals. The correlation coefficient (r) is given below and to the right of this line.

34.1°C for 13.5°C-acclimated individuals. This value lies between the CTM values obtained for 20 and 8.5°C-acclimated feeding individuals. Claussen (1980) demonstrated a good approximation to linearity between CTM and acclimation temperature over a range of 15–25°C for the crayfish *Oronectus rusticus*. Assuming that *P. nigroculus* displays a similar relationship over the range 8.5–20°C, we can calculate, by simple proportion, an expected CTM of 34.1 for an acclimation temperature of 13.5°C. This expected value exactly corresponds with the observed LT_{50} value reported here.

DISCUSSION

The observed heat tolerance of 33.4–35.3°C found in *P. nigroculus* falls well within the range of typical CTM values recorded for other Crustacea (Claussen, 1980). The rate at which animals were heated during CTM testing in this study (1.4°C/min) was faster than that found in most other studies (Johnson, 1972; Otto and Gerking, 1973; Claussen and Walters, 1982). Johnson (1972) proposed that the ideal rate of heating should equal the rate at which the core temperature of the organism responds to changes in water temperature. A rate that is too slow will allow partial acclimation during experimentation but a rate that is too rapid will erroneously yield unrealistically high CTM values (Lowe and Vance, 1955; Zweifel, 1957). Johnson (1972) subsequently reported, using a rate of 0.3°C/min in his study on anurans, the same rate as that used later by Otto and Gerking (1973) for the desert pupfish, *Cyprinodon*. In view of the small body size of *P. nigroculus* in comparison with these animals, the rate of heating employed in this study is considered to be acceptable.

The rates of upward acclimation compare favourably with those of other workers. The freshwater amphipod *Asellus intermedius* Forbes completed acclimation from 20 to 30°C in 3 days and *Hyalella azteca* Saussure increased its heat resistance over a period of 2 days (Sprague, 1963). Spoor (1955) reported a much faster rate of increase in the crayfish, which changed its heat resistance over 1 day when transferred from 4 to 24°C.

Sprague (1963) observed a decrease in heat resistance of 5% for the freshwater amphipods *Gammarus fasciatus* Say and *H. azteca* as a result of prolonged holding in the laboratory. This may account for the apparently lower than expected mean heat tolerance attained by cold-acclimated *P. nigroculus* in the 8.5–20°C CTM transfer tests. *Paramelita nigroculus* had at this stage been held for approximately 18 days. Under these circumstances the $\frac{1}{2}$AT values may be slightly more rapid than one might first be led to believe, and acclimation would indeed be complete in the 1–2-day period as reflected in the results of the t-tests (Table 1).

Reverse acclimation was slower than that of forward acclimation, as is generally the case (Dourdoroff, 1942). The crayfish, *O. rusticus*, exhibited a $\frac{1}{2}$AT of 2.3 days for reverse acclimation as opposed to 0.5 days for forward acclimation (Claussen, 1980). The stonefly, *Paragnetina media* Walker lowered its heat resistance only slightly over 39 days (Heiman and Knight, 1972), while the tropical salt-marsh fish, *Cyprinodon dearborni*, followed a similar trend (Chung, 1981). Loss of heat resistance in *P. nigroculus* was initially slow, but increased more rapidly before levelling off at a lowered level of heat resistance (Type IIIa sigmoid curve; Claussen, 1977). This is important from an ecological perspective, as the individual may be expected to acclimate more closely to higher daytime temperatures rather than to lower night-time temperatures. Thus one might expect to find low $\frac{1}{2}$ATs and large ΔCTMs in organisms subjected to large temperature fluctuations such as in tidal pools (Claussen, 1977; Macisaac *et al.*, 1985).

As a general rule, organisms are not fed during the holding and acclimation periods (McLeese, 1956; Sprague, 1963; Brattstrom, 1970; Claussen, 1980), although Vernberg and Tashian (1959), Hamashima

Table 2. The time to 50% mortality (LT_{50}) of fully acclimated (1 month) *Paramelita nigroculus* individuals exposed to a range of test-temperatures. All correlation coefficients (r) are significant (95% confidence level)

Acclimation temperature (°C)	Test temperature (°C)	LT_{50} (min)	r
13.5	27	306.9	0.985
13.5	28	100.6	0.970
13.5	29	56.3	0.980
13.5	30	9.0	0.964
13.5	31	3.7	0.975
20.0	29	308.3	0.975
20.0	31	27.2	0.980

and Morino (1984) and Macisaac *et al.* (1985) supplied their experimental animals with food. Starvation has been shown not to affect the heat tolerance of lobsters (McLeese, 1956) or the larvae of the giant toad, *Bufo marinus* (Floyd, 1985). However, when starved, animals with a high metabolic rate and little food reserve may exhibit a more rapid loss of vitality and Sprague (1963) has reported starvation to cause death in amphipods after 2 weeks as opposed to 6 weeks for satiated individuals. In our study, the presence of food was found to have no significant effect on the heat tolerance of *P. nigroculus* (Student's t-test; $P > 0.05$). On the other hand, an excess of food in the water has been suggested to act as a pollutant (McLeese, 1956) also lowering the vitality of the laboratory organism, which may explain the high mortality of certain experimental groups in our study. Although Wilder (1940), could not demonstrate such an effect for *H. azteca* it nevertheless remains a plausible hypothesis.

The lack of any significant influence of sex on resistance to high temperatures, either during acclimation or in fully acclimated individuals, is in accord with previous observations on other crustaceans (Spoor, 1955; Vernberg and Tashian, 1959; Sprague, 1963; Edney, 1964; Claussen, 1980). Sprague (1963) has, however, reported one such isolated incident. Similarly, the absence of any significant intraspecific size effect is in agreement with other experiments performed on fish (Dourdoroff, 1942), lobsters (McLeese, 1956), terrestrial isopods (Edney, 1964), terrestrial amphipods (Lazo-Wasem, 1984) and aquatic amphipods (Macisaac *et al.*, 1985). Sprague (1963) gives evidence to the contrary in other freshwater amphipod genera and Fraenkel (1968) reported that larger individuals of Littorinidae have an enhanced tolerance to high temperature as opposed to small individuals. Sutcliffe *et al.* (1981) observed that at higher temperatures, the survival of *G. pulex* juveniles was greatly decreased when compared with the adults. Age would therefore seem to play a significant role in affecting heat tolerance and to eliminate this effect in future experimentation, animals of uniform age should be used.

The effects of seasonality and photoperiodism were not considered in this study. Temperature tolerance is known to change with the season (Krog, 1955; Sprague, 1963; Heiman and Knight, 1972). Our experiments were restricted to autumn and the effects of seasonality on the heat tolerance of *P. nigroculus* remain unresearched. Photoperiod has been reported to have a significant diel effect on lethal temperatures (Hutchinson, 1961; Heath, 1963; Johnson, 1972; Floyd, 1985) and for this reason most researchers choose to maintain a constant photoperiod (Edney, 1964; Brattstrom, 1970; Claussen, 1977, 1980; Claussen and Walters, 1982) during acclimation; as far as possible we adopted this approach.

The major difficulty encountered during the LT_{50} experiments was the exact definition of when to commence the exposure period. As the temperature of the water in the jars neared that of the water bath, the rate at which it did so become progressively slower. This meant that animals may have spent a significant period at a temperature just below that of the water bath and this could greatly have affected the duration of the subsequent survival period once the test-temperature was finally attained. This would be most obvious at the higher test temperatures (30–31°C), where survival was already extremely short. Obviously, a more clearly defined starting point to the exposure period, as well as the reduction of thermal shock to which individuals would otherwise be exposed, is desirable. The importance of reducing thermal stock has been clearly demonstrated by Fraenkel (1968): increasing the tempering period led to a 1–2° increase in thermal tolerance over a 1 hr period in four species of Littorinidae.

Although the CTM and LT_{50} methods of experimentation appear to be highly comparable, yielding very similar values for 13.5°C-acclimated individuals, this is purely as a result of death being used as the CTM end-point criterion in this study. Under normal circumstances, the CTM value is typically lower than that of the LT_{50} value (Claussen and Walters, 1982).

The temperature of 13.5°C possibly underestimates the average summer temperature to which *P. nigroculus* acclimatizes. Over an annual cycle (1984) in Skeleton Gorge, Table Mountain, we have recorded a water temperature range of between 10 and 18°C. The stream has a well-established riparian canopy and even in dry periods, when pools form, temperatures have not been observed to exceed the temperature tolerance ranges of *P. nigroculus* reported here. Of great interest, however, is the distribution (Fig. 1) of the species in a number of open canopy headwaters, which may well exhibit much higher water temperatures during summer. Further investigations of specimens from open canopy habitats might elucidate some of the apparent distributional anomalies of this species.

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PAPER 10

has had a profound effect on invertebrate life cycles (e.g. Petersen & Cummins, 1974). In contrast to this, evergreen riparian trees in the Southern Hemisphere have peak litter fall during spring/summer (Winterbourn, 1976; King, 1982; King *et al.*, 1987a, b), and King *et al.* (1987a) have suggested that this difference might have implications for the timing of life cycles of Southern Hemisphere stream invertebrates.

Literature on litter input and accumulation in Southern Hemisphere streams is scarce. Blackburn & Petr (1979) examined litter fall from a sclerophyllous forest into a mountain stream over four months in Victoria, Australia, while Towns (1985) investigated litter input over five months into a second-order intermittent stream in *Eucalyptus* forest in South Australia. Further, Winterbourn (1976) monitored beech litter fall into a New Zealand stream over one year. The only southern African studies are those of King (1982) and King *et al.* (1987a, b). King (1982) examined leaf fall at two sites on the Eerste River, an undisturbed mountain stream site and a second lower site disturbed by agriculture and deciduous exotic trees. In the same catchment, King *et al.* (1987a, b) investigated the organic matter dynamics of Langrivier, a second – order perennial tributary of the Eerste River.

The Eerste River lies in the Fynbos Biome. Confined to the southern tip of Africa, and recognised as one of the six main floristic kingdoms in the world, the biome is characterised by three main elements; the heath-like Ericaceae, the sedge-like Restionaceae and members of the Proteaceae family. Trees, which are often of Afromontane origin (White, 1978), are rare, and generally confined to riverine habitats such as along the Eerste River. Dominant species include *Ilex mitis* (L.) Radlk, *Cunonia capensis* (L.) and *Kiggelaria africana* L.

Some fynbos streams support large shredder populations. For example, Window Stream on Table Mountain, is dominated by the shredding amphipod, *Paramelita nigroculus* (Barnard). Others, such as Langrivier, lack large shredder populations (King *et al.*, 1987a). Since leaf litter entering Window Stream appeared to form the major food source of *P. nigroculus*, a study was initiated to investigate whether or not leaf litter dynamics could explain the differences in macroinvertebrate community structure between the two streams.

Study area

Window Stream is a first-order headwater stream draining the fynbos-covered eastern slopes of Table Mountain (33° 58′ 30″ S, 18° 25′ 15″ E) (Fig. 1). Like Langrivier, it is heavily shaded by Afromontane endemics, the most notable being *I. mitis*, *C. capensis*, *K. africana*, *Halleria lucida* L. and *Rapanea melanophloeos* (L.) Mez. The uneven stream bottom comprises a mixture of large boulders, stones and gravel. Water depth is variable, ranging from a few centimetres in summer (December-February) to about 20 cm in winter

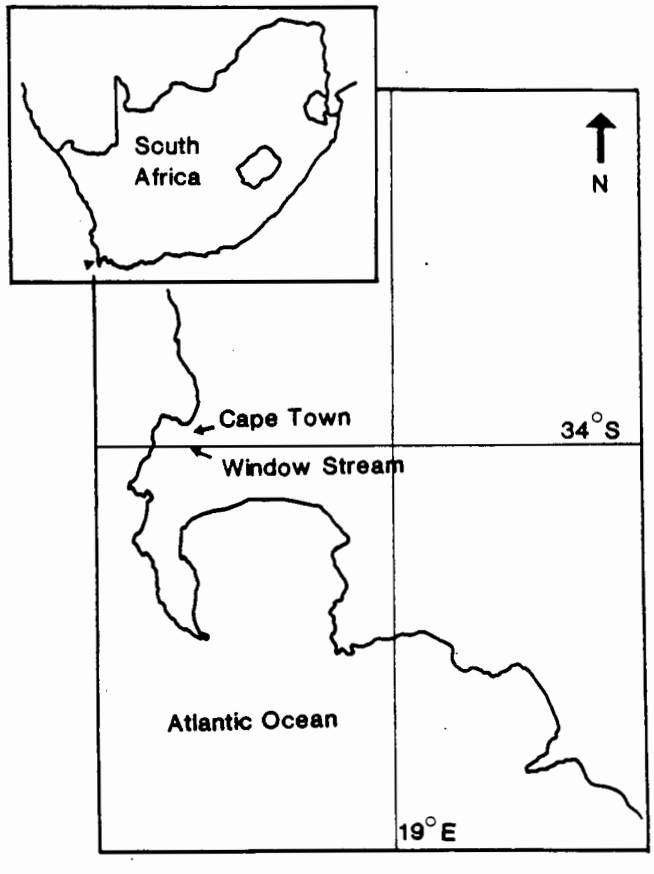

Fig. 1. Map of the southwestern Cape Province showing the locality of the study site, Window Stream, on Table Mountain.

(June-August), while stream width varies from 1.5 to 2.4 m. The temperature range encountered during the study was 10 to 18 °C. The brown colour and acidic nature (pH 3.6–4.7) of the water is characteristic of many fynbos streams.

Methods

Allochthonous input was measured by suspending five litter traps randomly in riparian trees along a 50 m stretch of Window Stream. The traps were reinforced, tightly woven, plastic mesh bags (1.2 × 0.8 m) held open by wire rings and which had drainage holes at their bases (King *et al.*, 1987a). The mean mouth area was 0.26 m^2, and the total area sampled by the five traps was 1.3 m^2. Since these traps were too small to capture large woody debris, the woody component collected comprised small branches, twigs and bark (see also Conners & Naiman, 1984). Lateral transport of allochthonous material into the stream was not measured (lateral transport accounted for only 8% of total input into Langrivier; King *et al.*, 1987a). Litter was collected monthly between May 1984 and April 1985, and after pooling the contents of the five bags, leaves, twigs, bark, flowers and seeds were separated and identified. Samples were oven-dried at 60 °C for 48 h and then weighed. The values obtained were not corrected for leaching losses which must have occurred prior to collection primarily in the winter months.

Between four and six samples of benthic coarse particulate organic matter (benthic CPOM; particles > 1 mm) were collected randomly from the stream bed using square-sided benthic samplers (either of 0.06 or 0.12 m^2; King *et al.*, 1987a). Each sample was preserved separately in 70% alcohol and stored for later analysis in the laboratory when the detritus was oven-dried at 60 °C for 48 h and weighed. Results are expressed as g dry mass m^{-2} of stream bed and, means ± one standard deviation are quoted. Analysis of Variance (ANOVA) was used to test for significant differences where appropriate (Zar, 1974).

Results

Monthly total litter fall ranged from 11 (September) to 79 g m^{-2} (March) (Fig. 2). A total of 426 g m^{-2} a^{-1}, which corresponds to 1.2 g m^{-2} d^{-1} fell during the year (Table 1).

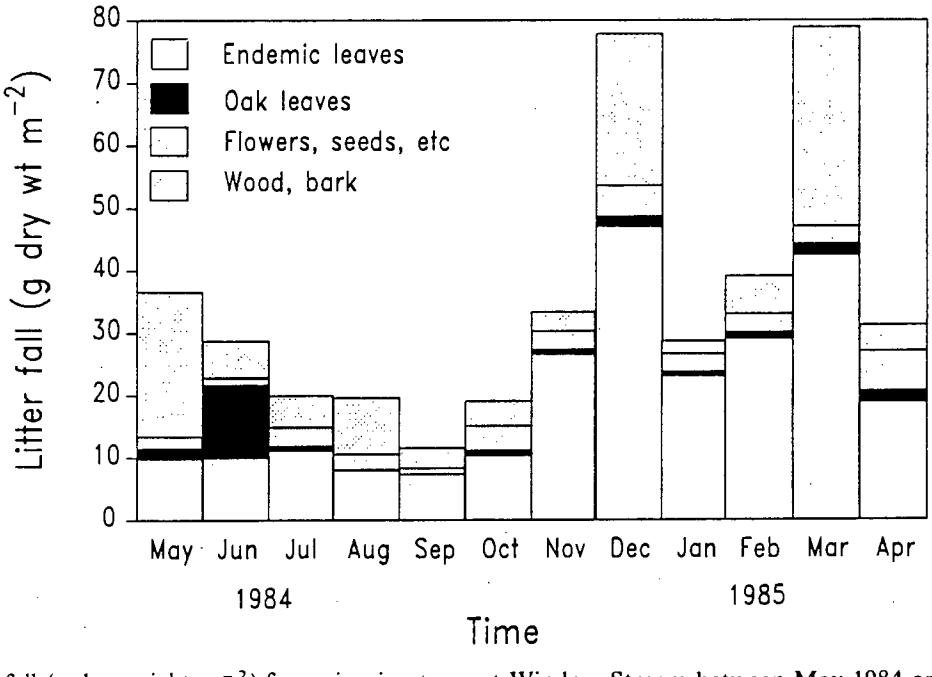

Fig. 2. Litter fall (g dry weight m^{-2}) from riparian trees at Window Stream between May 1984 and April 1985.

Table 1. Annual fall of litter from riparian trees at Window Stream.

Component	Litter fall $g\ m^{-2}\ a^{-1}$	%
Endemic leaves	244	57
Oak leaves	23	5
Flowers, fruits & seeds	37	9
Twigs, branches & bark	122	29
Total	426	

Total litter fall (all components combined) was not significantly higher in summer than in winter (Table 2; ANOVA, $P > 0.05$). However, fall of leaves from native trees was significantly higher in summer (ANOVA, $P < 0.05$), while it was similar in spring and in autumn (ANOVA, $P > 0.05$). During the study, between 7 (September) and 47 $g\ m^{-2}\ mth^{-1}$ (December) of leaves fell, with a mean of $20.3 \pm 13.5\ g\ m^{-2}\ mth^{-1}$ (Table 2), and a total of $244\ g\ m^{-2}\ a^{-1}$ (Table 1). This leaf component dominated total litter fall, contributing between 32 and 82% each month.

The study site included a few individuals of the introduced oak *Quercus robur* L. Monthly fall of leaf litter of oak was generally low (0 (September) to $2.0\ g\ m^{-2}$ (May)), with the exception of June, when $12\ g\ m^{-2}$ fell. This autumn/early winter peak leaf fall is consistent with periods of peak fall from deciduous trees of the Northern Hemisphere.

The flower, fruit and seed components contributed between 1 (June and September) and $6\ g\ m^{-2}\ mth^{-1}$ (April) (Fig. 2), a mean of $3.0 \pm 1.5\ g\ m^{-2}\ mth^{-1}$ (Table 2), or a total of $37\ g\ m^{-2}\ a^{-1}$ (Table 1). The fall of woody elements was very variable, ranging between 2 (January) and $32\ g\ m^{-2}\ mth^{-1}$ (March), and totalled $122\ g\ m^{-2}$ for the year (Table 1), with a mean over the year of $10.1 \pm 10.2\ g\ m^{-2}\ mth^{-1}$ (Table 2). The contributions to total litter fall of oak were between 0 and 39%, flowers, seeds and fruits between 3 and 21%, and wood and bark between 7 and 65%.

Several Afromontane and other endemic forest species were responsible for the leaf fall from native trees (Fig. 3). For example, leaves belonging to 13 species entered the traps in November, while at least five species were identified in May and June. The dominant species included *C. capensis* (19–78% of monthly leaf fall), *I. mitis* (5–20%), *Secamone alpini* Schultes (3–28%), *Rhoicissus tomentosa* (Lam.) Wild & Drummond (2–18%) and *H. lucida* (0–11%). Although the monthly contribution of *K. africana* was usually low (0–9%), this species was responsible for 15% of the leaf litter in August, 17% in September and 28% in October. Similarly, *R. melanophloeos* showed peak litter fall (20%) in September.

Peak litter fall of *C. capensis* ($24\ g\ m^{-2}\ mth^{-1}$), *H. lucida* ($4\ g\ m^{-2}\ mth^{-1}$)

Table 2. Mean seasonal litter fall at Window Stream, between May 1984 and April 1985. Standard deviation and sample sizes (n) are shown.

Litter	Winter $n = 3$	Spring $n = 3$	Summer $n = 3$	Autumn $n = 3$	Annual $n = 12$
Endemic leaves	9.8 ± 1.6	14.9 ± 10.3	32.9 ± 12.4	23.6 ± 16.8	20.3 ± 13.5
Oak leaves	4.3 ± 6.8	0.4 ± 0.3	1.1 ± 0.5	1.8 ± 0.1	1.9 ± 3.3
Flowers, seeds, fruits	2.2 ± 1.0	2.7 ± 1.5	3.7 ± 1.2	3.7 ± 2.3	3.0 ± 1.5
Wood, bark	6.7 ± 2.1	3.3 ± 0.6	10.7 ± 11.7	19.9 ± 14.3	10.1 ± 10.2
Total	23.3 ± 6.7	21.3 ± 11.1	48.0 ± 24.8	48.7 ± 26.1	35.3 ± 21.2

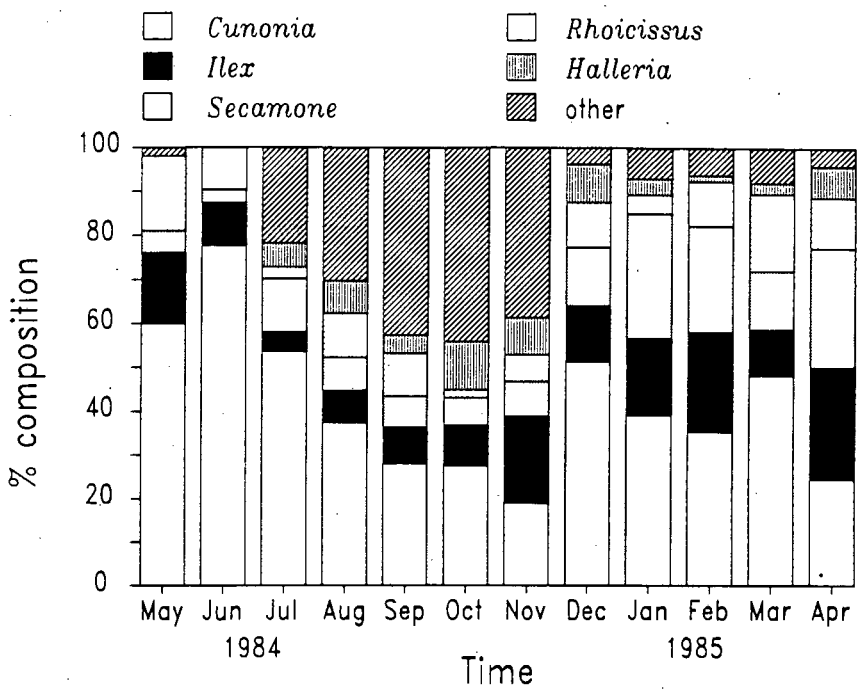

Fig. 3. Percentage composition of endemic leaf litter fall at Window Stream between May 1984 and April 1985.

and *R. tomentosa* (5 g m^{-2} mth^{-1}), occurred in December, although 20 g m^{-2} mth^{-1} of *Cunonia* and 8 g m^{-2} mth^{-1} of *Rhoicissus* fell later in March. A steady fall (4–6 g m^{-2} mth^{-1}) of *I. mitis* fell from November through April and a similar increase in litter fall was also observed for *S. alpini* (5–7 g m^{-2} mth^{-1}) from December through April.

Standing stocks were determined for soft benthic CPOM (leaves, flowers, fruit and seeds), hard benthic CPOM (twigs, bark and branches), and total benthic CPOM on the stream bed between May 1984 and April 1985 (Fig. 4). Monthly means were calculated from between four to six randomly chosen samples. The standard deviations, often high due to the variability of the data, are not shown. Total standing stocks of CPOM ranged from 14 (January) to 69 g m^{-2} mth^{-1} (August), of which 5 (January) to 45 g m^{-2} mth^{-1} (May) was soft litter, and 9

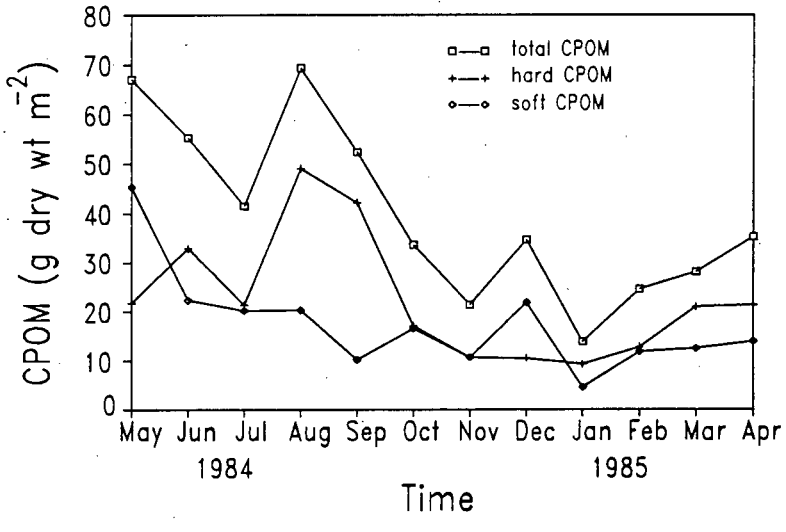

Fig. 4. Standing stocks of CPOM on the streambed in Window Stream between May 1984 and April 1985.

(January) to 49 g m^{-2} mth^{-1} (August) was hard litter. Mean seasonal standing stocks of total CPOM, soft CPOM and hard CPOM (Table 3) showed no significant seasonal differences (ANOVA, $P > 0.05$), although the mean standing stocks appeared to be higher in winter than in summer. This was in contrast to the strongly seasonal input of litter (Table 2; Fig. 2). Accordingly, annual means have been calculated (Table 3), and show that the mean total CPOM standing stock of 41 g m^{-2} mth^{-1} consisted of approximately 18 g m^{-2} mth^{-1} soft litter and 23 g m^{-2} mth^{-1} hard litter. Hard litter accounted for 30 to 81% of the total CPOM, with an average of 57%, while leaves were responsible for 8 to 76%, and an average of 34% of the total CPOM. Flowers, fruits and seeds, and oak leaves, were less important.

The amounts of *Cunonia*, *Ilex*, *Rhoicissus*, *Kiggelaria* and *Halleria* in the standing stocks of leaves on the stream bed reflected the contribution of these species to the allochthonous input to the stream (ANOVA, $P > 0.05$) (Fig. 5). However, there were significantly greater amounts of *Diospyros whyteana* (Hiern) F. White leaves on the stream bed compared to the incoming litter (ANOVA, $P < 0.05$), while there were far fewer *Secamone* leaves on the stream bed than would

Table 3. Mean seasonal standing stock of CBOM (g m^{-2}) in Window Stream, between May 1984 and April 1985. Standard deviation and sample sizes (n) are shown.

	Winter Jun–Aug $n = 16$	Spring Sept–Nov $n = 13$	Summer Dec–Feb $n = 14$	Autumn Mar–May $n = 15$	Annual $n = 58$
Soft CBOM	21.1 ± 25.2	12.3 ± 9.1	12.8 ± 15.6	24.0 ± 23.2	17.9 ± 20.0
Hard CBOM	34.3 ± 44.9	24.8 ± 27.9	10.7 ± 11.1	21.4 ± 15.4	23.2 ± 29.4
Total CBOM	55.4 ± 50.5	37.1 ± 29.8	24.4 ± 20.4	44.0 ± 33.1	40.9 ± 36.8

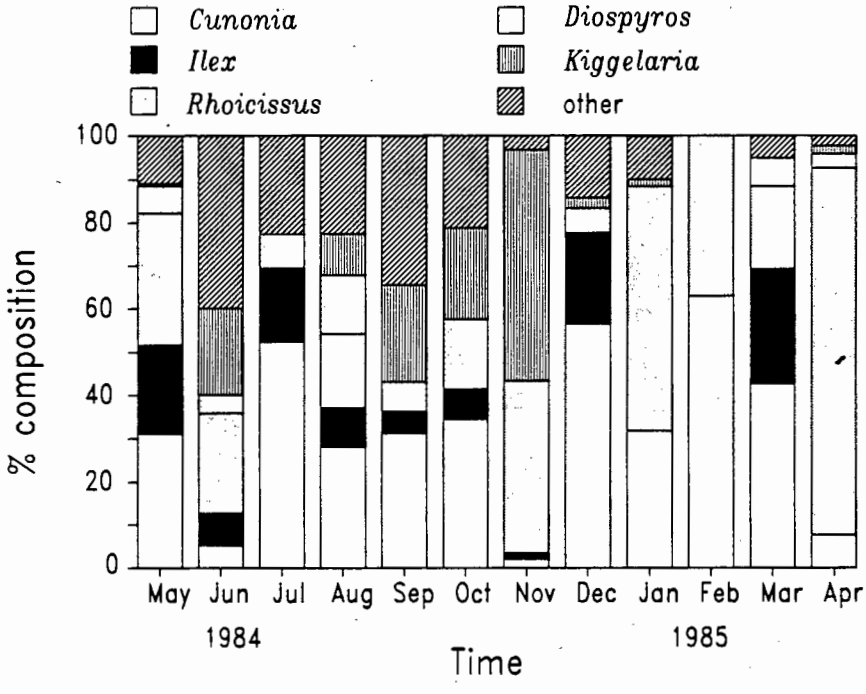

Fig. 5. Percentage composition of the standing stocks of endemic leaves on the streambed in Window Stream between May 1984 and April 1985.

have been expected from the contribution of this species to allochthonous input (ANOVA, $P < 0.05$).

Discussion

Litter fall

This study has confirmed earlier findings (King, 1982; King *et al.*, 1987a), that litter from riparian trees in the Fynbos Biome falls throughout the year, with peak inputs occurring in summer. Winterbourn (1976) found that beech litter entering a New Zealand mountain stream also fell all year, but that maximum leaf fall occurred in late spring, summer and autumn. Since Blackburn & Petr (1979) and Towns (1985) sampled over only a few months, they were unable to comment on the seasonality of litter fall into their Australian mountain streams. The 426 g m^{-2} a^{-1} which entered the traps at Window Stream was similar to the 434–500 g m^{-2} a^{-1} measured by King *et al.* (1987a), but higher than the 268 g m^{-2} a^{-1} obtained by King (1982) for a different fynbos site (Table 4). All of these values are well within the range obtained by other authors (Table 4) for mountain streams in Southern Hemisphere countries.

The very marked autumnal peak in litter fall, mainly of deciduous leaves, in Northern Hemisphere streams is well documented (e.g. Gosz *et al.*, 1972; Fisher & Likens, 1973; Conners & Naiman, 1984). Conners & Naiman (1984) collected between 64 and 78% of the total annual inputs to a Canadian headwater stream during maximum leaf fall (September–November; 22 g m^{-2} d^{-1}). At Window Stream, only 34% of the total annual input occurred at the time of *maximum* litter fall (3 g m^{-2} d^{-1}). Thus, although total annual litter fall for the Canadian stream (534 g AFDW m^{-2} a^{-1}) was not much more than that for Window Stream (426 g m^{-2} a^{-1}), and in the following year was less (307 g AFDW m^{-2} a^{-1}), the intensity of *peak* litter fall into the Canadian stream was far higher.

In some cases autumn leaf fall is not quite so pronounced as that found by Conners & Naiman (1984). For example, Fisher & Likens (1973) reported that the autumn leaf fall of 4 g m^{-2} d^{-1} represented 54% of total litter input into Bear Brook, New Hampshire, USA. Nearly half of total litter fall into Roaring Brook (New England, USA) occurred in a 12-day period (McDowell & Fisher, 1976) when 4 g m^{-2} d^{-1} fell. Field measurements by McIntire & Colby (1978) in an Oregon stream revealed a maximum of 3 g m^{-2} d^{-1} in November. Nevertheless, most of these figures still exceed the value obtained for Window Stream. Clearly, autumnal litter fall in Northern Hemisphere systems is of a far greater magnitude than the summer peak falls into Southern Hemisphere stream systems.

As in other studies (e.g. Fisher & Likens, 1973; Winterbourn, 1976; King, 1982; Conners & Naiman, 1984; Towns, 1985; King *et al.*, 1987a), leaf litter at Window Stream formed an important component of allochthonous input. Leaf litter has contributed over 60% of total litter fall (Table 4) in all but one study (Blackburn & Petr, 1979) in Southern Hemisphere streams. Blackburn & Petr (1979) attributed the high proportion of wood and bark in their study to the fact that they sampled only in winter, a time of strong winds, and of peak fall of bark and branches.

Benthic particulate organic matter

Benthic CPOM accumulation in Langrivier (King *et al.*, 1987a) and Window Stream differed. In Langrivier, the standing stocks of benthic CPOM on the stream bed reflected the pattern of litter fall, in Window Stream it did not (Table 5). Although several other studies have also shown that standing stocks of CPOM match the pattern of leaf input (e.g. Winterbourn, 1976; Malmqvist *et al.*, 1978; Mulholland, 1981; Bärlocher, 1983), this is not always the case. Wakefield *et al.* (1980) observed negligible seasonal changes in CPOM standing stocks in a Southern Ontario second-order stream, while in Idaho, Minshall *et al.* (1982) recorded the highest standing stocks of

Table 4. A review of the literature on riparian litter input into streams in the Southern Hemisphere. The percentage contribution of leaves, woody debris, fruit, seed and flowers to the total litter fall is shown. Total litter is quoted (1) as g m^{-2} a^{-1}, and (2) g m^{-2} d^{-1}. Figures for lateral transport are presented as g m^{-1} a^{-1}.

Study area	Dominant species	Total litter 1	2	Lateral transp.	% leaves	% woody	% fruit seed, flowers	References
S. Island New Zealand	*Nothofagus solandri*	458	1.5	110	62	24	14	Winterbourn (1976)
Victoria Australia	*Eucalyptus regnans* *Atherosperma moschatum* *Nothofagus cunninghamii*	604	1.7	–	25	66	9	Blackburn & Petr (1979)
Adelaide Australia	*Eucalyptus obliqua* *Eucalyptus leucoxylon*	183[a]	0.5[a]	–	68		32[b]	Towns (1985)
S-west Cape South Africa	*Brabejum stellatifolium* *Metrosideros angustifolia* *Brachylaena neriifolia* *Hartogia schinoides*	268	0.7	–	97[c]	3[d]		King (1982)
S-west Cape South Africa	*Ilex mitis* *Cunonia capensis* *Brabejum stellatifolium* *Metrosideros angustifolia*	434 – 500	1.2 – 1.4	32	64 – 81	19 – 29	< 1 – 7	King *et al.* (1987a)
S-west Cape South Africa	*Ilex mitis* *Cunonia capensis* *Secamone alpini* *Rhoicissus tomentosa*	426	1.2	–	62	29	9	present study

[a] These values have been calculated from Fig. 8 in Towns (1985).
[b] This figure includes twigs and branches.
[c] Comprises leaves fruits and flowers.
[d] Comprises wood and bark.

Table 5. A comparison of CBOM standing stocks in various first-to third-order streams.

Study area	Mean Seasonal Standing Stocks ($g\ m^{-2}\ mth^{-1}$)				Mean Annual Standing Stocks ($g\ m^{-2}\ mth^{-1}$)	References
	Spring	Summer	Autumn	Winter		
Bear Brook, New Hampshire, USA	–	1190	–	–	–	Fisher & Likens (1973)[a]
Stampen Stream, Sweden	4–92	5–14	38–224	31–85	33–96	Malmqvist *et al.* (1978)
Devils Club Creek, Oregon, USA	1082	1364	397	3142	1496	Naiman & Sedell (1979)[b]
Mack Creek, Oregon, USA	379	1061	37	128	402	Naiman & Sedell (1979)[b]
Hunsberger Creek, Ontario, Canada	–	–	–	–	205–237	Wakefield *et al.* (1980)[c]
Broadstone Stream, England	27	22	38	33	30	Hildrew *et al.* (1980)[d]
Smith Creek, Michigan, USA	183	16	129	141	117	Cummins *et al.* (1981)[e]
Upper 43rd Site, Michigan, USA	80	99	53	88	80	Cummins *et al.* (1981)[e]
Kellogg Forest, Michigan, USA	76	239	152	65	133	Cummins *et al.* (1981)[e]
McKenzie River, Oregon, USA	–	56–138	–	–	–	Murphy *et al.* (1981)
Camp Creek, Idaho, USA	182	56	30	34	76	Minshall *et al.* (1982)
Lützel, Switzerland	9	4	43	33	30	Bärlocher (1983)
Non-acid stream, Sweden	0	–	26–58	–	–	Otto & Svensson (1983)
Acid stream, Sweden	29	–	47–88	–	–	Otto & Svensson (1983)
LaTrobe River, Victoria, Australia	–	–	–	–	11–36	Marchant *et al.* (1985)[f]
Langrivier, South Africa	20–52	62–86	30–121	6–19	35–66	King *et al.* (1987a)[g]
Window Stream, South Africa	37	24	44	55	41	present study

[a] Reported as 'total detritus'.
[b] AFDW, CBOM > 1 mm, < 10 cm.
[c] AFDW, FBOM and CBOM, 133 μm mesh used.
[d] FBOM and CBOM, 330 μm mesh used.
[e] AFDW.
[f] FBOM and CBOM, 150 μm mesh used.
[g] FBOM and CBOM.

CPOM in a second-order stream in spring, and not in autumn. In view of the significant autumn leaf fall, Naiman & Sedell (1979) were surprised at the lack of seasonal trends in total amount of benthic organic matter in streams in Oregon, USA.

Although mean CPOM standing stocks appeared to be higher in winter ($55\ g\ m^{-2}$) than

in summer (24 g m^{-2}) at Window Stream, seasonal differences were not significant (ANOVA, $P > 0.05$). However, this was probably due to the high variability of the data. The apparent 'reversed' pattern in Window Stream is surprising, and in the case of the winter samples, unexpected. Not only was litter input lower in winter than in summer, but increased discharge and subsequent winter spates should flush organic material from the stream bed resulting in relatively 'debris-free' systems (King *et al.*, 1987a). One would thus expect lower standing stocks in winter than in summer. However, it is possible that biological interactions are responsible for depressing summer standing stocks of CPOM in Window Stream, these values being considerably lower than the summer values for Langrivier (Table 5). In summer, Window Stream supports very high densities of amphipods (mean density, 7972 individuals m^{-2}), and these animals have a significant effect on CPOM breakdown (unpubl. data). It is probable, therefore, that the relatively low CPOM standing stocks in summer were due to the feeding activities of these shredders. Langrivier does not support such shredder populations (King *et al.*, 1987a). The considerably lower amphipod densities (mean, 1071 individuals m^{-2}) in winter in Window Stream coincide with higher standing stocks of CPOM.

Another explanation, although less likely, for the higher winter CPOM standing stocks in Window Stream is seasonal variation in stream width. The mean width in summer (1.5 m) was considerably increased in winter (2.4 m) due to discharge increases. A considerable accumulation of detritus on the dry stream bed occurred in summer (pers. obs.), and it was only when water levels rose, that this material entered the stream. High winter values of CPOM therefore, might reflect the incorporation of this material. The retention time of this CPOM in the stream is unknown, although winter spates would probably result in its rapid removal. In a study in a New England stream, McDowell & Fisher (1976) reported that approximately 17% of litter input was delayed until the stream expanded and incorporated litter in previously dry areas.

It is questionable if Langrivier (and Window Stream) has lower standing stocks '... than that reported for most other studies...' (King *et al.*, 1987a). Although some studies have reported higher standing stocks (e.g. Fisher & Likens, 1973; Naiman & Sedell, 1979), the figures for Langrivier and Window Stream are well within other reported ranges (Table 5). Naiman & Sedell (1979) incorporated 'bole' wood (> 10 cm diameter) into their standing stock estimates, whereas most other authors have not done this. If the standing stocks of CPOM, without bole wood are considered in their streams, then their values are similar to those of Fisher & Likens (1973), and in the case of autumn and winter in Mack Creek, similar to Langrivier (King *et al.*, 1987a), Window Stream and other studies (Table 5).

King *et al.* (1987a) reported that the proportions of *Ilex* and *Cunonia* leaves in Langrivier were always less than in leaf fall, and suggested that this might be due to physical abrasion and animal feeding. However, despite high amphipod densities, the amounts of *Ilex* and *Cunonia* in Window Stream reflected their contribution to allochthonous input. This is surprising since, when artificially constructed leaf packs of *Ilex* and *Cunonia* were placed in Window Stream, leaves vanished within one month! *Diospyros* leaves are pubescent and are, therefore, unlikely to be attractive to shredders. This may account for their rather high standing stocks in Window Stream relative to their input. The converse is possibly true for *Secamone* leaves.

Total benthic POM standing stocks were not estimated in this study. Minshall *et al.* (1982) and King *et al.* (1987a) have warned that by excluding the measurement of fine particles, total POM standing stocks can be grossly underestimated. Course benthic organic matter was responsible for only 10.7 to 44.6% of total POM measured by Minshall *et al.* (1982) in a second-order stream. CPOM and FPOM combined constituted 46–64% of total POM in Langrivier (King *et al.*, 1987a).

If high shredder densities reflect efficient organic retention, and absence of shredders poor retention in streams (Winterbourn *et al.*, 1981),

then CPOM standing stocks would be poor indicators of the retentive abilities of streams. Window Stream and Langrivier have similar standing stocks, yet Window Stream supports high shredder densities while Langrivier does not. These streams have similar physical and chemical regimes, with the exception of discharge, which is an order of magnitude higher in Langrivier. Therefore, it is possibly discharge and *not* retention which explains the differences in shredder densities within our systems, and possibly also in the New Zealand systems. Obviously, the whole question of the effect of organic matter retention by streams on invertebrate community structure requires review. Also noteworthy is the fact that the autumnal leaf fall peak recorded for many Northern Hemisphere streams is of far greater magnitude and is of shorter duration than the peak summer leaf fall recorded for Window Stream. The implications of this difference between our Southern Hemisphere system and those of the Northern Hemisphere for the synchronisation of invertebrate life cycles to energy inputs also warrants study.

Acknowledgements

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PAPER 11

Leaf litter retention and its implications for shredder distribution in two headwater streams

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With 3 figures and 2 tables in the text

Abstract

Leaves of two riparian species (*Cunonia capensis* L. and *Brabejum stellatifolium* L.) were released in two south-western Cape streams (Window Stream and Langrivier) to determine the retentive characteristics of these systems. The distance travelled by each leaf, as well as the hydrological and substratum feature of retention and the presence of sticks and wood was noted after three hours and daily thereafter for five days. All of the leaves were retained within the study reach (50m) in Window Stream, while only 83 - 68% were retained in Langrivier. The distance from the release point necessary to retain 90% of the leaves was 19m in Window Stream and 91m in Langrivier. Riffles and backwaters were the most important hydrological features of retention and cobbles the most efficient substratum retention feature. The highest numbers and biomass of shredders were associated with those features which had the greatest trapping efficiencies. It was concluded that discharge probably plays a major role in determining the retentive capabilities of a stream.

Introduction

Many studies have shown that leaf fall from riparian trees is the primary energy source in headwaters, and it has been predicted (River Continuum Concept) that these shaded streams which receive large amounts of allochthonous input will be dominated by organisms adapted to shred leaf litter (Sedell et al., 1978; Vannote et al., 1980; Naiman & Sedell, 1981; Cummins et al., 1983; Minshall et al., 1983). On the other hand, several authors (Winterbourn et al., 1981; Rounick & Winterbourn, 1983; Cummins et al., 1984) have stressed that retention, rather than the amount of leaf litter entering a stream, plays a more important role in structuring stream communities, although Cummins et al. (1989) have suggested that input and retention are directly correlated. Most studies, with the exception of Young et al. (1978) and Speaker et al. (1984) have examined retention by quantifying the standing stock of benthic organic matter (BOM).

Retention of released material, rather than the measurement of BOM standing stocks, has been investigated by Young et al. (1978), Speaker et al. (1984) and Cummins et al. (1989), providing data which are useful in indicating inter-stream differences. In these experiments, marked leaves were released into streams and the distance travelled by each leaf was recorded after a time interval.

Window Stream (Stewart & Davies, 1990) and Langrivier (King et al., 1987), both headwaters draining areas of sclerophyllous mediterranean-type shrubland (fynbos), have similar magnitudes of allochthonous input and BOM on the stream bed, yet they differ considerably in their invertebrate community structure. Window Stream is dominated by amphipod shredders while in Langrivier there appears to be little shredder activity. Given the great similarities between the two streams in terms of their allochthonous inputs, riparian characteristics, temperature, pH, flow, nutrient regimes and substratum types, there appear to be only two factors which could explain the recorded differences in shredder distribution: discharge and retention capacity.

Discharge is generally an order of magnitude greater in Langrivier than in Window Stream (King et al., 1987; Stewart & Davies, 1990). Winterbourn et al. (1981) suggested that high shredder densities reflect efficient organic retention, while absence of shredders indicates poor retention. The retentive capacities of Window Stream and Langrivier are unknown. Thus, using similar methods to those of Young et al. (1978) and Speaker et al. (1984), a study was initiated in order to test whether or not the retentive abilities of these two streams differed. Our prediction was that Window Stream with its very high shredder numbers would be far more retentive than Langrivier. The influence of different streambed features on retention was also investigated.

Study sites

Detailed descriptions of the study sites, namely Window Stream (first-order; 33o 58' S, 18o 25' E) and Langrivier (second-order; 34o 0' S, 18o 55' E), have been dealt with by Stewart & Davies (1989) and King et al. (1987) respectively. The riparian zones of both streams are dominated by small evergreen trees, with the red elm, *Cunonia capensis*, being the dominant species in Window Stream and the wild almond, *Brabejum stellatifolium*, being dominant in Langrivier. The substrata of the two streams are extremely heterogeneous, comprising mostly boulders and cobbles. Window Stream is smaller (mean width = 1.8m) than Langrivier (mean width = 3.6m), and although flow rates were identical during the study period (0.37 m s-1), discharge was far greater in Langrivier (mean = 0.49 m3 s-1) than in Window Stream (mean = 0.05 m3 s-1). Annual temperature ranges are similar (10-18^{o}C :Window Stream; 8-19^{o}C :Langrivier) and both streams are acidic (pH = 3.6-4.7: Window Stream; pH = 4.3-6.4: Langrivier).

Methods

The short-term retentive properties of Window Stream and Langrivier were investigated by a closed-system release and recapture method (see Young et al., 1978; Speaker et al., 1984) using two species of marked leaves. A 10mm stretched-mesh net was secured across each stream channel 50m below the release point. Abscissed leaves were marked by spray-painting half of each leaf with orange (*B. stellatifolium*) and pink (*C. capensis*) non water-based paint. Only half of each leaf was painted as a compromise between the requirements for visual impact in order to follow released leaves and retaining the wetting characteristics and flexibility of the leaves.

Five hundred marked leaves of each species were released by distributing them evenly across the channel in areas of moderate flow in both streams. The distance from the release point, the hydrological and substratum features of retention, and the association with sticks and/or wood were recorded for each leaf three hours after release and every 24 hours subsequently for a further four days. Speaker et al. (1984) recorded little change in retention after three hours in a preliminary investigation in their studies. Hydrological features included riffles, pools, chutes and backwaters, while substratum features comprised sand, gravel, cobble and boulders (Speaker et al., 1984). Wood was defined as material $>$ 10mm in diameter.

The relative trapping efficiencies of each hydrological and substratum feature, as well as those of sticks and wood, were calculated by dividing the percent leaves retained by each feature by the proportion that the feature contributed to the 50m reach of stream studied. Speaker et al. (1984) calculated their trapping efficiencies by dividing the percent leaves retained by each feature by the total area occupied by that feature in the reach. Chi-squared analysis performed on absolute numbers was used to test for significant differences in the retentive abilities of the hydrological and substratum features.

Depth profiles and wetted perimeter were measured every 5m downstream, starting at the release point (0m) and ending at 45m. Depth was measured every 10cm and the flow rate recorded with an OTT C2 portable current meter every 50cm across the channel at each 5m interval. Discharge was calculated by multiplying increments of the cross-sectional area of the channel by the flow measured at two-thirds of the depth at fixed points across the channel. The ratio of wetted perimeter to cross-sectional area of flow was used to determine an index of channel irregularity (ICI; Speaker et al., 1984).

Stream biota were sampled using a 64*um* mesh box sampler covering an area of 0.625m2. Samples were taken from each retention feature within the streams, and were preserved in 5% formalin. Samples were sorted and organisms identified, counted and assigned to functional feeding groups using Merritt & Cummins (1984).

Results

Although mean flow rates in the two streams were identical during the study period (Table 1), discharge was far greater in Langrivier (mean = 0.49 m3 s-1) than in Window Stream (mean = 0.05 m3 s-1). In addition, the indices of channel irregularity were less for Langrivier (mean = 7.07) than for Window Stream (mean = 33.01).

As was the case in Speaker et al. (1984), the negative exponential model best explained the retention of leaves in our streams (Fig. 1). Linear regression of semi-logged data gave significant r-values for the distribution of leaves in Window Stream, but insignificant values for Langrivier. This suggests that leaves were randomly distributed in the Langrivier system, but were concentrated near the release point in Window Stream. Over the five days of monitoring, the percentage retention in the study reach of both leaf species was always 100% in Window Stream, but decreased

TABLE 1. Flow rates (Flow) m s^{-1}, discharge (Dis.) m^3 s^{-1} and indices of channel irregularity (ICI) at 5m intervals in Window Stream and Langrivier.

	WINDOW STREAM			LANGRIVIER		
Distance downstream	Flow m s^{-1}	Dis. m^3 s^{-1}	ICI	Flow m s^{-1}	Dis. m^3 s^{-1}	ICI
0	0.9	0.12	4.26	0.63	0.99	5.73
5	0.08	0.02	15.91	0.28	0.28	5.64
10	0.52	0.04	42.35	0.44	0.31	10.59
15	0.15	0.02	28.38	0.19	0.47	4.29
20	0.41	0.08	25.39	0.46	0.49	5.06
25	0.25	0.03	22.32	0.49	0.57	9.65
30	0.06	0.01	40.24	0.39	0.88	4.81
35	1.15	0.09	20.66	0.21	0.28	7.35
40	0.52	0.08	38.10	0.31	0.17	13.16
45	0.46	0.02	100.45	0.31	0.47	4.41
Mean	0.37	0.05	33.01	0.37	0.49	7.07

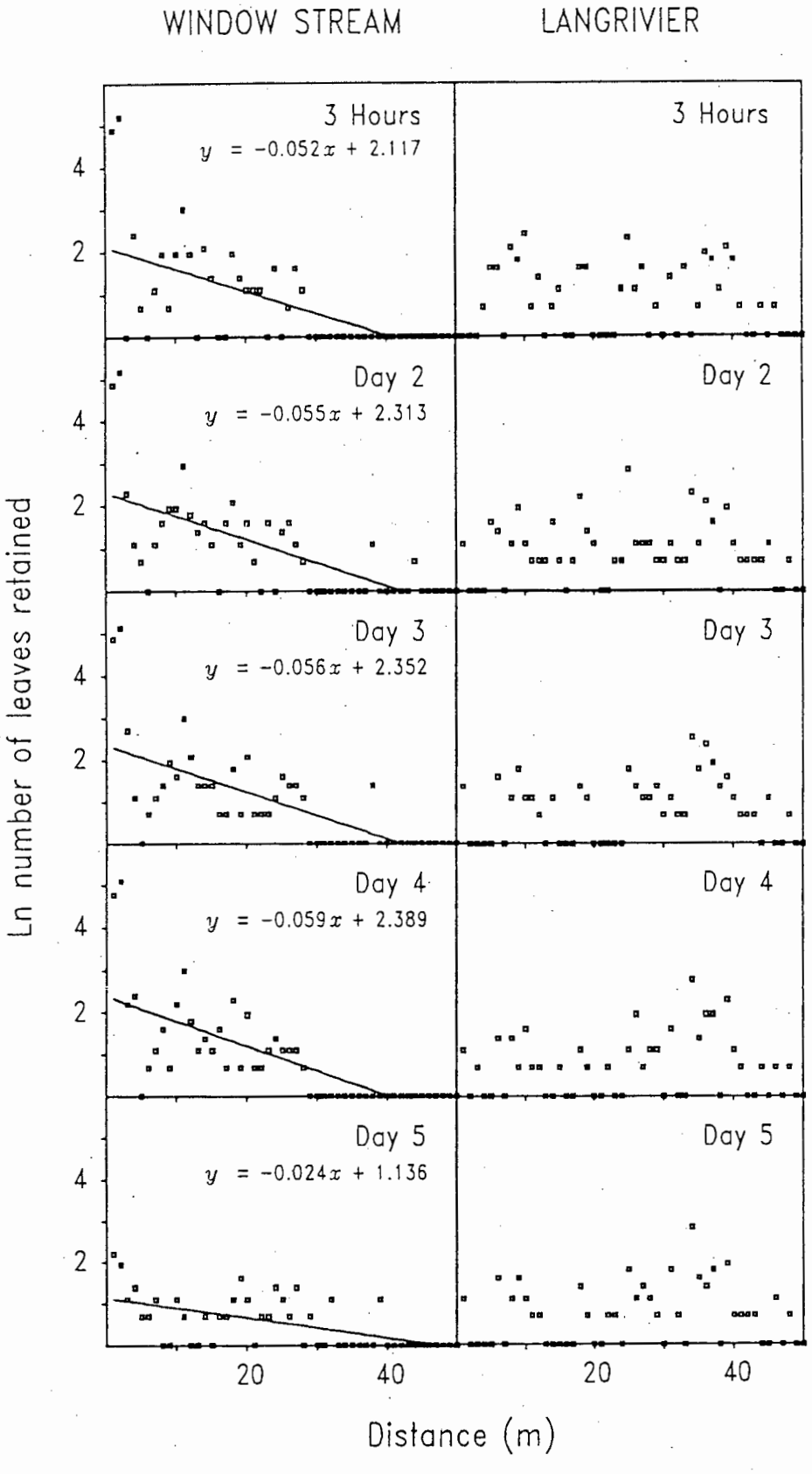

Fig. 1. Log to the base e of number of released leaves retained per metre squared in Window Stream and Langrivier.

from 83% after 3 hours to 71% after 5 days for *C. capensis*, and from 80% after 3 hours to 68% after 5 days for *B. stellatifolium* in Langrivier.

In both streams, and for both leaf species, riffles and backwaters showed significantly greater trapping efficiencies than did pools and chutes (Chi-squared, $p<0.05$). Of the substratum types, gravel had the highest trapping efficiency in Langrivier (10.30: *B. stellatifolium*; 10.00: *C. capensis*) but was relatively unimportant in Window Stream (0.37: *B. stellatifolium*; 0.00: *C. capensis*), In this system, cobbles (29.49: *B. stellatifolium*; 28.84: *C. capensis*) appeared to play a more important role than in Langrivier (3.22: *B. stellatifolium*; 6.00: *C. capensis*). Although cobbles trapped fewer leaves than boulders in Langrivier, they retained a higher number of leaves relative to their occurence on the streambed, and therefore had a higher trapping efficiency than boulders (Table 2), which were relatively unimportant in terms of their trapping efficiencies in both streams for both leaf species. Sticks and wood,which were equally efficient, showed some of the greatest trapping efficiencies (Table 2). Although differences between streams were minimal, species differences were clearly observed, with trapping efficiencies being greater for *C. capensis* than for *B. stellatifolium*.

The distribution of shredding organisms in both streams matched the trapping efficiencies of the hydrological and substratum features of the systems (Fig. 2), with the highest number and biomass of shredders being associated with backwaters and riffles and the lowest with pools and chutes. Between 30 and 60% of the total invertebrate biomass associated with riffles and backwaters were shredders (Fig. 2). Large amphipods were the principle shredders in Window Stream, while smaller plecopteran nymphs dominated the shredder component in Langrivier. Shredder biomass was thus always greater in Window stream than in Langrivier (Fig. 3).

TABLE 2. Relative trapping efficiencies of two leaf species (*Brabejum stellatifolium* and *Cunonia capensis*) by different hydrological and substratum features, as well as sticks and wood in Window Stream and Langrivier.

	WINDOW STREAM		LANGRIVIER	
Feature	*Brabejum*	*Cunonia*	*Brabejum*	*Cunonia*
Hydrological				
Riffle	9.75	10.87	3.88	6.85
Pool	1.04	1.04	0.52	0.26
Chute	0.91	0.64	0.40	0.25
Backwater	17.07	11.60	5.67	7.00
Substratum				
Sand	0.00	0.00	0.00	0.00
Gravel	0.37	0.00	10.30	10.00
Cobble	29.49	28.84	3.22	6.00
Boulder	2.13	1.91	1.87	2.49
Sticks	14.74	25.26	12.24	29.80
Wood	9.09	25.45	11.85	21.48

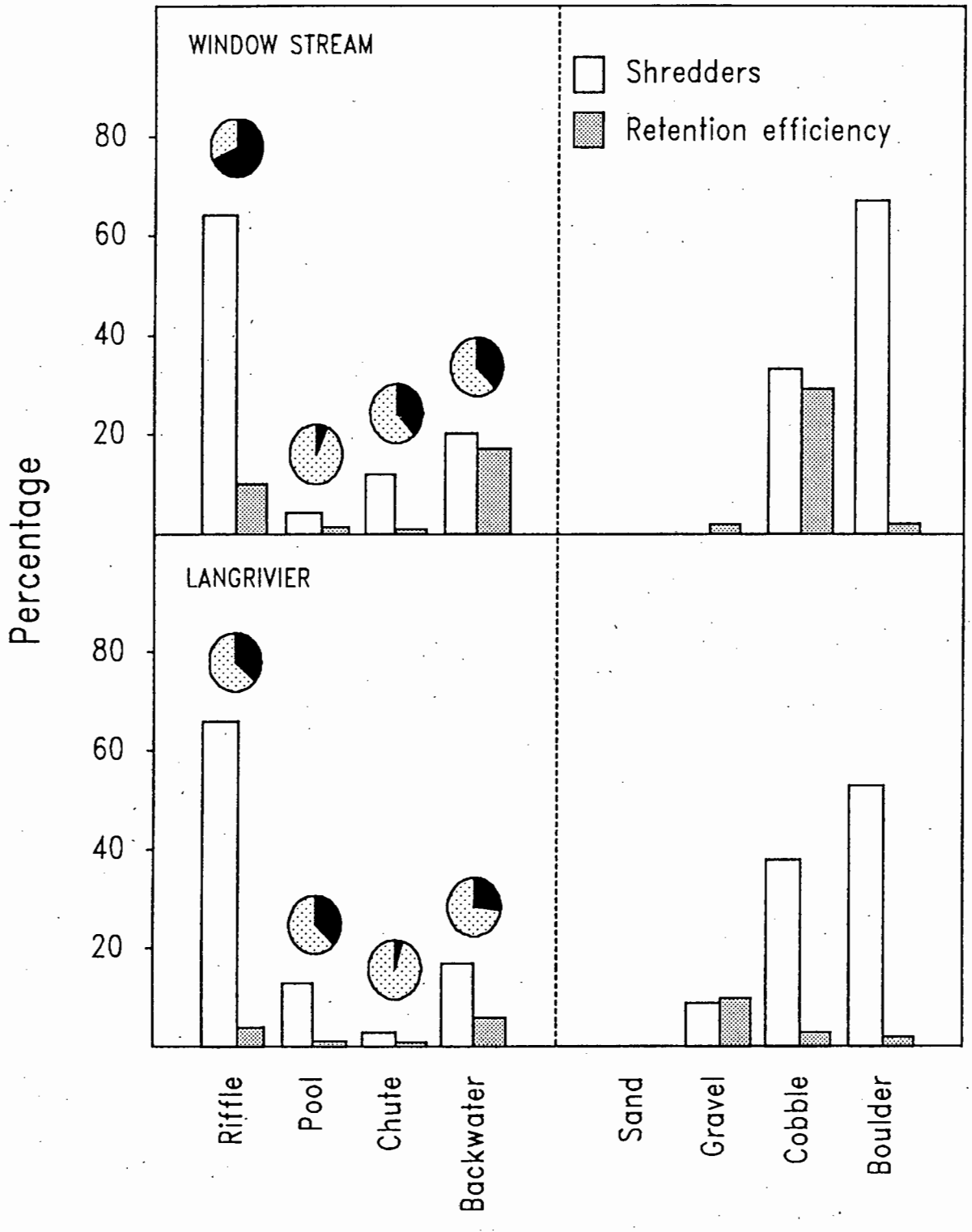

Fig. 2. The percentage of the shredder fauna associated with each hydrological and substratum feature of both streams, and the corresponding retention efficiencies. Pie diagrams indicate the proportion of the shredder component (dark shading) in the stream biota.

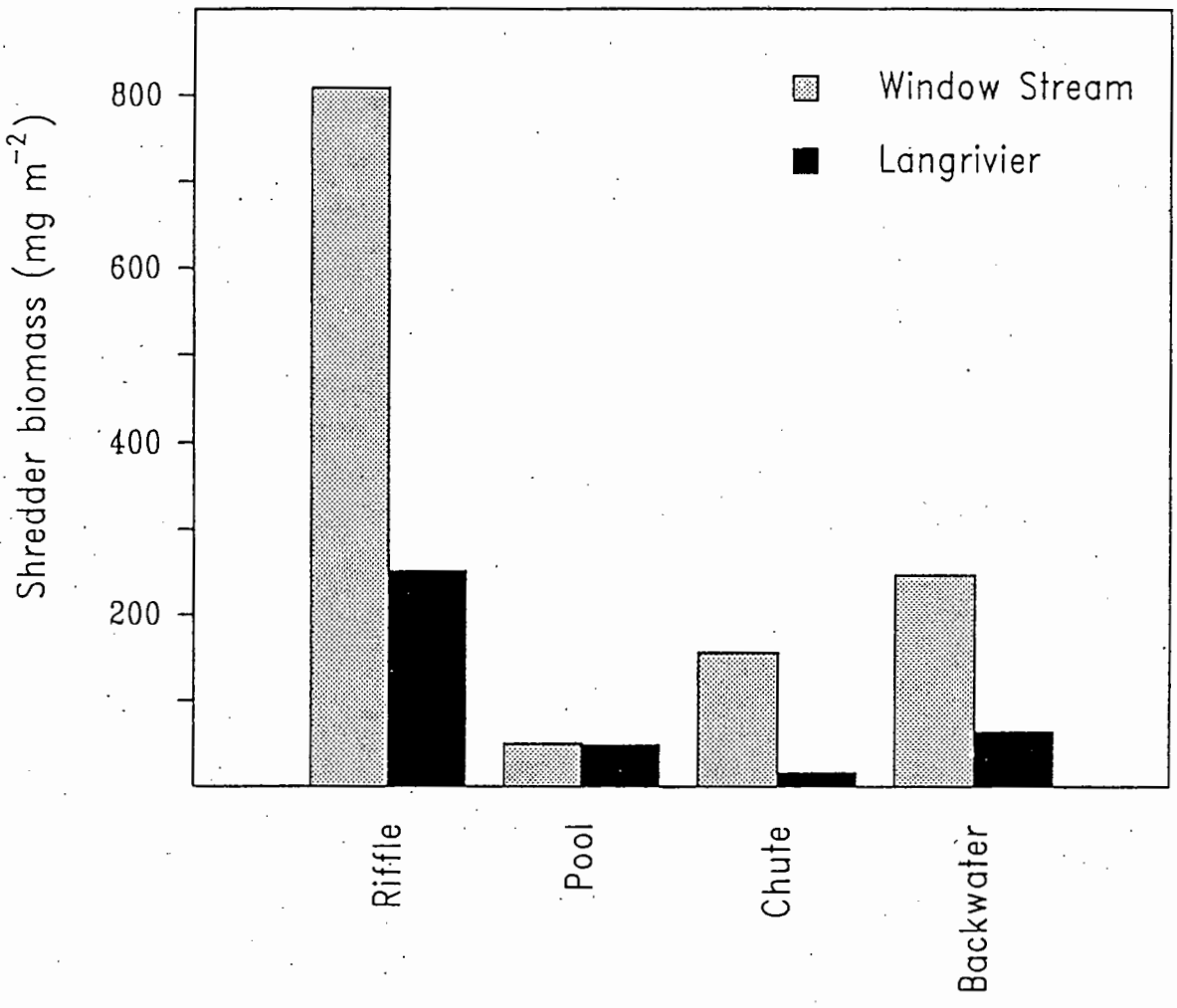

Fig. 3. The biomass of shredding organisms per metre squared in Window Stream and Langrivier.

Discussion

With the exception of discharge, the two study streams were similar in terms of their physical and chemical properties. In addition, leaf input was similar in Window Stream (426 g m3 y-1: Stewart & Davies, 1990) and Langrivier (434-500 g m3 y-1: King et al. 1987). Despite this, retention of leaves varied markedly between the two systems (Fig. 1). All leaves released in Window Stream were retained within the study reach, while retention of leaves in Langrivier varied from 83 - 68%. There was a non-random distribution of leaves in Window Stream, with leaves being retained near the entry point. Leaves were randomly distributed in Langrivier. These data suggest that Window Stream is a more retentive system than Langrivier. On the basis of apparently lower standing stocks of CBOM in Langrivier than reported for most other studies, King et al. (1987a, b) concluded that Langrivier was a poorly retentive system. However, a study in Window Stream (Stewart & Davies, 1990) revealed that CBOM standing stocks in this stream (mean of 41 g m-2 mth-1) were similar to those in Langrivier (35-66 g m-2 mth-1: King et al., 1987a). This illustrates that, contrary to the views of Cummins et al. (1989), the use of CBOM standing stock as a measure of retention is inadequate in our systems.

Although rates of flow in the two streams were identical (Table 1), discharge was an order of magnitude greater in Langrivier than in Window Stream (Table 1). Several workers (Winterbourn, 1976; De La Cruz & Post, 1977; Malmqvist et al., 1978; Sedell et al., 1978; Young et al., 1978) have found an inverse relationship between discharge and retention. Our results seem to concur with this observation, for Window stream with low discharge had higher 'retention' of leaves than Langrivier which had a higher discharge. The index of channel irregularity used by Speaker et al. (1984) gave a greater mean value (33.01: Table 1) for Window Stream than for Langrivier (7.07), suggesting that the channel morphology of Window Stream is more irregular than that of Langrivier. This index, however, is related to discharge and is

difficult to measure in large, deep or fast-flowing streams. Because of these factors, discharge is a preferable measure for comparative purposes.

For Window Stream it was calculated that 90% of released *B. stellatifolium* leaves were retained within 19m of the release point. In Langrivier 81m were required to retain 90% of released leaves. These figures fall within the range of 15m to 210m for study reaches without debris dams reported by Speaker et al (1984) who released 3000 leaves in each of 20 streams. Cummins et al (1989) reported a figure of 250m for 90% removal of released yellow ginkgo leaves. Window Stream was thus as retentive as the most retentive stream that Speaker et al. (1984) investigated, while Langrivier appeared to be moderately retentive.

For both streams, riffles and backwaters were more retentive than pools and chutes (Table 2). This is in accordance with the findings of Speaker et al. (1984) who attributed this pattern to the operation of different depositional mechanisms in pools and riffles. According to these authors, deposition, and therefore retention, in riffles is caused by the presence of obstacles in the water column, whereas retention in pools occurs as a function of low current velocity. Both studies have shown that retention by obstacles is more efficient than retention by low current velocities.

The high trapping efficiency of gravel in Langrivier (Table 2) is attributable to two small patches of backwater where a large number of leaves were retained, and thus it does not appear to be any special characteristic of the gravel itself that is important, but rather that the gravel is associated with depositional areas. A similar line of argument may be used to explain the high retention efficiency of cobble in Window Stream (29.49: *B. stellatifolium*; 28.84: *C. capensis*), although the extreme heterogeneity of this substratum type is also likely to have a large effect. Personal observations revealed that leaves entering cobble areas became trapped firmly between the stones, whereas leaves retained by boulders merely rested against them and were therefore easily dislodged.

The importance of sticks and wood in retention has been emphasised by other workers (Bilby & Likens, 1980; Speaker et al., 1984). Although the study streams have very few debris dams (King et al., 1987b; pers. obs.), sticks and wood are not uncommon and appear to be important in trapping leaves. The greater trapping efficiencies exhibited for *C. capensis* may be attributable to the fact that the leaves of this species are far more flexible than those of *B. stellatifolium* and may thus become entwined around sticks easier than the more rigid leaves of the latter. Flexibility of leaves has previously been found to be important in retention (Young et al., 1978).

The distribution of shredders in the two streams suggested that they utilise habitats which retain the most leaves. This relationship has also been noted by other workers (e.g. Cummins & Klug, 1979; Minshall et al., 1982; Cummins et al., 1989). From this follows the prediction that larger shredder populations would exist in high-retention streams than in low-retention streams (e.g. Winterbourn et al., 1981; Winterbourn, 1982; Rounick & Winterbourn, 1983). The findings of our study are consistent with these predictions.

Leaf release experiments showed that Window Stream was a retentive system while Langrivier was less retentive. This is most likely to be attributable to differences in the discharge of the two streams. Since input into Window stream and Langrivier is similar, it is more likely that the differences in the retentive capabilities of the two streams most influence the distribution of invertebrates. This has been suggested by Winterbourn et al. (1981), Winterbourn (1982) and Rounick & Winterbourn (1983). Relative trapping efficiencies of different hydrological features were apparently related to channel heterogeneity and to the depositional mechanisms operating in each feature.

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Zusammenfassung

Blätter zweier Spezien (*Brabejum stellatifolium* und *Cunonia capensis*) der Uferboschung wurden in zwei Southwestern Cape Strömen (Window Stream und Langrivier) entlassen, um die festhaltende Charakteristika dieses Systems zu bestimmen. Die Distanz die von jedem Blatt zurückgelegt wurde, sowie die hydrologischen und grundlegenden Einschaften der Rückhaltung und das Vorhandensein von Stöcken und Holz wurde nach drei Stunden und danach täglich über fünf Tage registriert. Alle Blätter des Window Stream wurden innerhalb der meßstrecke (50m) zurückgehalten, während es nur 83-68% in Langrivier waren. Die Entfernung vom Startpunkt die nötig war um 90% der Blätter zurückzuhalten, betrug beim Window Stream 19m und Langrivier 91m. 'Riffles' und 'Backwaters' waren die wichtigsten hydrologischen Eigenschaften und Kopfpflaster die wirkungsvollste grundlegende Eigenschaft der Rückhaltung. Die größte Anzahl und Biomasse der Shredder war mit dem wirkungsvollsten Eigenschaften der Rückhaltung verbunden. Es wurde daraus geschlossen, daS der Wasserdurchsatz wahrscheinlich eine Hauptrolle bei der Bestimmung der rückbehaltenden Fähigkeiten eines Stromes spielt.

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PAPER 12

The effect of discharge on leaf retention in two headwater streams

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With 3 figures and 2 tables in the text.

Abstract

Leaf litter retention in two headwater streams was investigated over a range of discharges in order to test the prediction that discharge is a determinant of the retentiveness of the two systems. A closed-system release and recapture method was used to quantify leaf retention. The relative trapping efficiencies of retentive features in both streams were recorded over a range of discharges. Leaf retention decreased markedly with an increase in discharge, as did the streambed complexity, calculated using an index of channel irregularity. Backwaters were found to be the most retentive features in both streams, and their efficiencies increased with increasing discharge. Wood and leafpacks were also significant retainers of leaves. Riffles decreased in trapping efficiency with increased discharge. It was concluded that to quantify the overall retentiveness of a system, seasonal fluctuations in retention and channel irregularity, resulting from seasonal variation in discharge, must be measured.

Introduction

The input of nutrients into headwaters consists largely of allochthonous matter from riparian vegetation, usually in the form of coarse particulate organic matter (CPOM) (e.g. FISHER & LIKENS, 1973; CUMMINS et al., 1989), consequently shredders, feeding primarily on this CPOM, are expected to dominate the biota of the headwaters (CUMMINS et al., 1989). For the shredder community to utilise the litter falling into the stream, this litter must be retained (CUMMINS et al., 1989). MINSHALL et al. (1983) found that headwater reaches were "more retentive" than streams of higher order. Evidence of this was the high ratio of stored to transported organic matter in headwater reaches, when compared with lower reaches. It is not surprising, therefore, that many studies have correlated shredder abundance with the retentiveness of the systems investigated (CUMMINS, 1974; SEDELL et al., 1978; BARMUTA & LAKE, 1982; MINSHALL et al., 1983; STEWART & DAVIES, 1990).

ROUNICK & WINTERBOURN (1983) contrasted two physically different New Zealand streams in an attempt to explain differences in shredder abundance. The "more retentive" system had a greater abundance, while the "less retentive" system had fewer shredders. These authors measured "retention" by quantifying the amount of CPOM on the streambed. PROCHAZKA et al. (1990) made a similar comparison between two south-western Cape streams, but measured retention by the release and recapture of marked leaves. They found that Window Stream, which had a high abundance of shredders also had a higher retention capacity than Langrivier, which had a lower shredder abundance (PROCHAZKA et al., 1990). These authors further predicted that discharge could be expected to play a role in determining retention. The aim of the present study, therefore, was to investigate how discharge affected the retention of CPOM at the two sites used by PROCHAZKA et al. (1990).

The influence of discharge on retention could be expected to be complex. Higher discharges could be expected to decrease the trapping efficiencies of obstructions in the streambed, while also influencing the presence of depositional sites,

where much of the CPOM is retained (BILBY, 1981). Thus, pools may be incorporated into the faster flowing channel, resulting in stored organic matter being transported downstream. Retention features such as debris dams have been found to be of importance in many studies (BILBY & LIKENS, 1980; BILBY, 1981). Small debris dams have been observed in south-western Cape streams (KING et al., 1987a; PROCHAZKA et al., 1990), and are often shifted during periods of high discharge.

Following the work of PROCHAZKA et al. (1990), we hypothesised that the lower CPOM retention, and consequently lower shredder abundances in Langrivier, were due to higher discharges in this system when compared with Window Stream. To investigate the relationship between discharge and retention in these two systems, a closed-system leaf release and recapture method was used at three different discharges at each stream. A similar method has been used in other studies as a technique to quantify the retentiveness of a stream (e.g. YOUNG et al., 1978; SPEAKER et al., 1984; CUMMINS et al., 1989; PROCHAZKA et al., 1990). The trapping efficiencies of the hydrological and substratum features were measured at the varying discharges. In addition, channel irregularity, which reflects the presence of obstructions in the channel, was recorded and its relationship with discharge was investigated.

Study sites

The two study streams drain catchments within the fynbos biome of the south-western tip of southern Africa (KING et al., 1987a). Window Stream (first order) drains the eastern slopes of Table Mountain (33°58'S, 18°25'E); 60 kilometres to the east, Langrivier (second order) drains a south-westerly facing subcatchment in the Jonkershoek Valley (34°0'S, 18°55'E).

The riparian vegetation of both streams consists primarily of Afromontane tree species (KRUGER, 1979). The riparian zone at Window Stream is dominated by *Cunonia capensis* (L.) (red elm), *Ilex mitis* (L.) Radlk. (wild holly) and *Brabejum*

stellatifolium (L.) (wild almond). *Cunonia capensis* and *B. stellatifolium* dominate the riparian vegetation at Langrivier. Total annual litter-fall for both sites falls within the range 400-500 g m^{-2}, with leaves and other soft litter contributing the greatest percentage (KING et al., 1987a; STEWART & DAVIES, 1990). Peak leaf fall occurs in summer.

Boulders and cobble dominate the physically heterogeneous beds of both streams. The mean width was less for Window Stream (1.8 m) than for Langrivier (3.6 m). Mean discharge was greater at Langrivier (0.49 m^3 s^{-1}) than at Window Stream (0.05 m^3 s^{-1}) when recorded by PROCHAZKA et al. (1990) in August 1989 in late winter. Rainfall reaches a maximum in winter in the south-western Cape, and spates are a common phenomenon during this season. This results in a wide variation in width, depth and discharge through the early winter.

Methods

Retention

Retention was measured using a closed-system leaf release and recapture method (e.g. YOUNG et al., 1978). A 50 m-stretch of river was chosen at each site. The site at Window Stream was 60 m below a weir, while that at Langrivier was immediately above a gauging weir. A closed system was established by stretching a 10 mm mesh net across the stream and securing it along the stream bottom using large boulders. Five hundred oven-dried leaves of the species *B. stellatifolium* were marked using non-water based luminous spraypaint applied to half the leaf. This marking was essential for recapture in the brown waters of the two systems. The leaves were released 50 m above the net, and were evenly distributed across an area of medium flow. After three hours, each leaf was collected and the distance it had travelled, and the retentive feature of the stream that had captured it, were recorded. The retentive features fell into two categories, hydrological which included riffles, backwaters, chutes and areas of slow and fast flow, and substratum, which included boulders, cobble, gravel, wood,

leafpacks and foam. The relative trapping efficiency of each feature was calculated as the ratio of the percentage of leaves trapped by the feature to the percentage area each feature contributed to the study reach. Chi-squared analysis performed on absolute numbers was used to test for significant differences in the retentive abilities of the hydrological and substratum features. All of these methods were repeated during each of the three visits at each stream between March and July 1990.

Physical Parameters

Detailed stream profiles were recorded every 5 m along the study reach at each visit. The depth (cm) was measured across the width of the stream, every 5 cm at lowest discharge, every 10 cm at intermediate discharge, and every 20 cm at highest discharge. This last decrease in acuity was due to the difficulty in measuring depth in high flow conditions. The width and wetted perimeter was recorded at each 5 m interval, and flow rate was recorded at several points across the channel using an OTTC2 portable current meter.

According to the position of flow measurements, each profile was divided into cells and discharge calculated by multiplying the flow rate by the cross-sectional area of each cell. Mean discharge was calculated for each visit. An index of channel irregularity (ICI) was calculated as the ratio of wetted perimeter to the cross-sectional area of flow (SPEAKER et al., 1984).

Results

The mean discharge for the two streams over the three visits was 0.218 $m^3 s^{-1}$ for Window Stream, and 0.247 $m^3 s^{-1}$ for Langrivier. These values differed from those of previous studies (Table 1). A marked difference was evident between the two systems when the highest discharges recorded during the present study were compared - at Langrivier this was 0.645 $m^3 s^{-1}$, while at Window Stream it was only 0.335 $m^3 s^{-1}$.

Table 1: Discharge data for Langrivier and Window Stream, from this and previous studies.

LANGRIVIER	WINDOW STREAM	SOURCE	DATE RECORDED
Discharge (m^3s^{-1})			
0.49 (mean)	0.05 (mean)	PROCHAZKA et al. (1990)	winter 1989 (August)
0.03 - 0.22		KING et al. (1987a)	summer - winter (1987)
0.03 - 0.4		KING et al. (1987b)	summer - winter (1987)
0.019	0.059	This study	autumn - winter (1990)
0.077	0.260		
0.645	0.335		
means: 0.247	0.218		

It was evident from a plot of the percentage of leaves retained against discharge (Fig. 1a) that retention decreased dramatically with increased discharge in both streams. Similarly, the indices of channel irregularity decreased with increased discharge in both systems (Fig. 1b). At all discharges channel irregularity was greater for Window Stream (mean of 14.35) than for Langrivier (11.77).

For purposes of representing leaf retention versus distance travelled from the release point (Fig. 2), the study reach was divided into 5 m sections and the leaves were cumulated for each section. The retention of leaves versus distance at low discharge in Langrivier was best described by a negative exponential model (Fig. 2a). At low discharge in Window Stream , the retention of leaves showed a logarithmic relationship (Fig. 2d). Most of the leaves released in both streams were thus retained close to the release point.

With increased discharge the relationship became linear for Langrivier (Fig. 2b), while it was exponential for Window Stream (Fig. 2e). This was because at Langrivier more leaves were carried further down the reach. On the other hand, leaves at Window Stream were still retained close to the release point, but more were carried further downstream than at a lower discharge. The 'intermediate' discharge (for this study) for Langrivier was considerably lower than that for Window Stream. At highest discharges in both systems, the leaves appeared to be distributed randomly through the reach, with no apparent significant relationship with distance (Figs 2c & 2f).

In both systems, backwaters were relatively rare and occupied small percentages of the stream areas (Table 2), but they trapped many leaves, often in association with wood and other leafpacks. Thus, the relative trapping efficiencies of backwaters were highest at all three discharges for Langrivier (Fig. 3a-c), while at Window Stream they formed a significant retention feature at both intermediate (0.26 m^3 s^{-1}) and high (0.335 m^3 s^{-1}) discharges (Fig. 3e, f) (Chi-square, $p < 0.05$).

Riffles occupied the highest proportion of the reaches in both systems with the exception of low discharge in Langrivier (Table 2). Despite their dominance they trapped relatively few leaves (Fig. 3). Riffles are usually made up of cobble stretches,

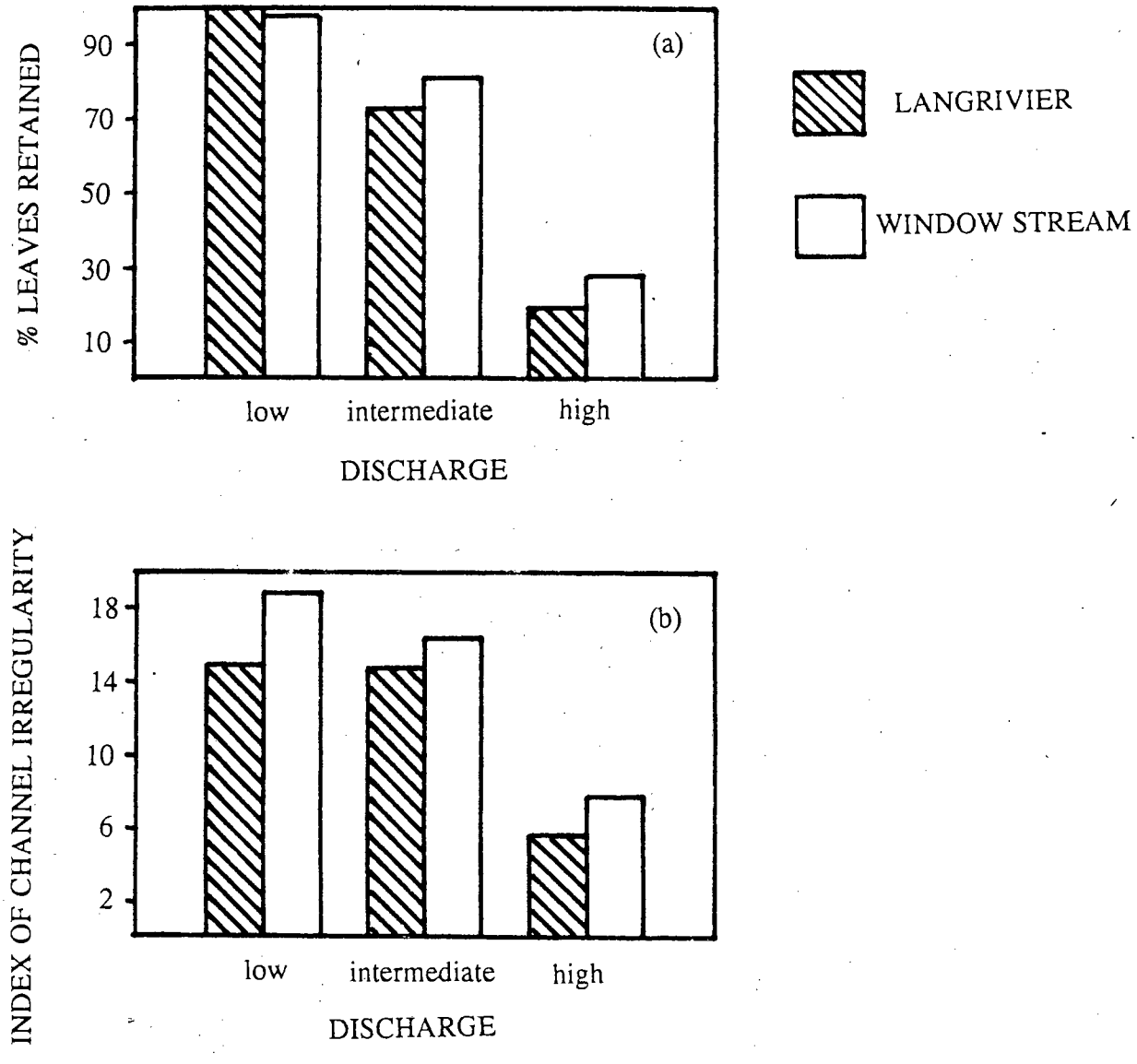

Fig.1. A plot of (a) the percentage of leaves retained, and (b) the index of channel irregularity against discharge in Langrivier and Window Stream.

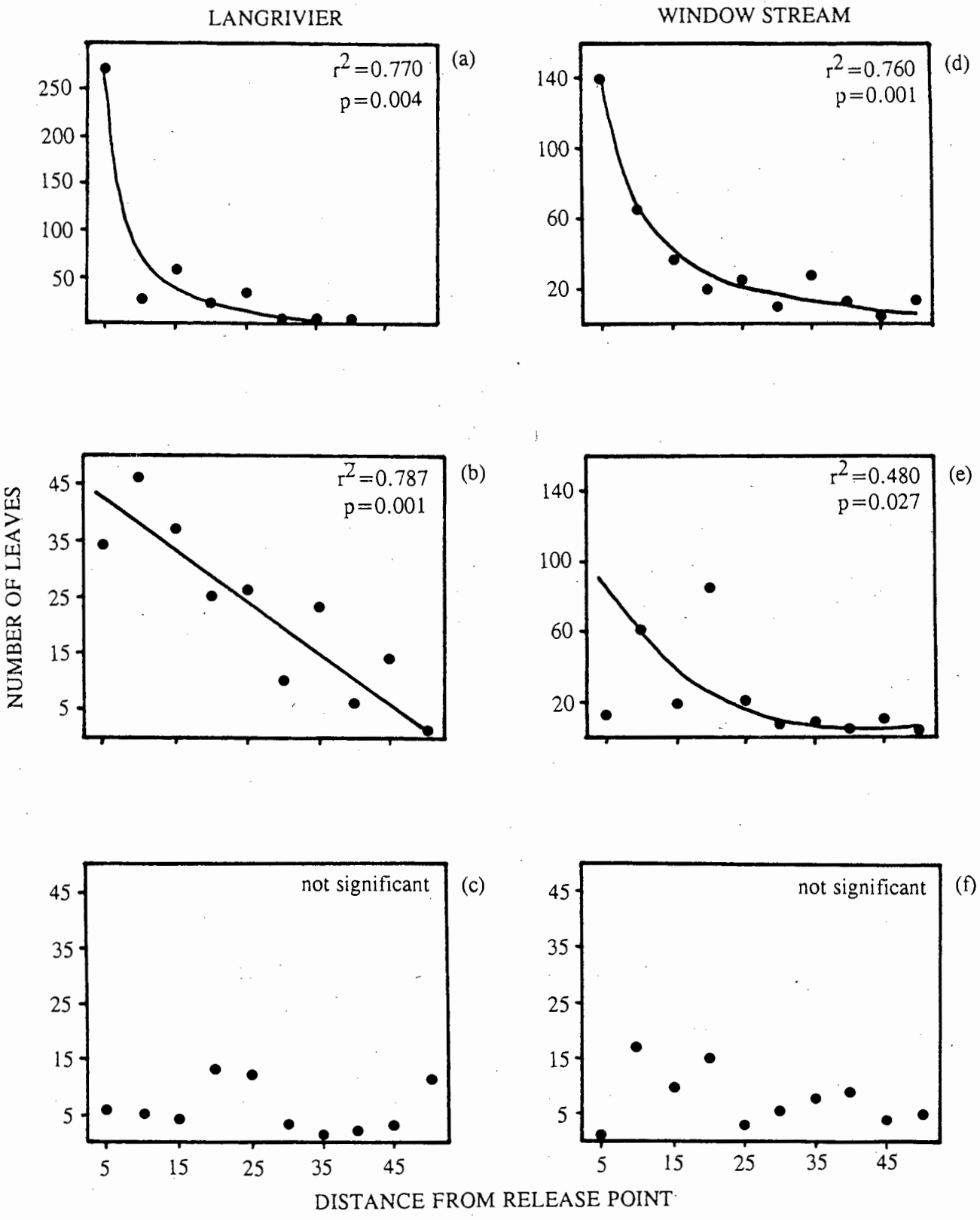

Fig.2. The number of marked leaves retained against the distance travelled from the release point at discharges of (a) 0.019 $m^3 s^{-1}$, (b) 0.077 $m^3 s^{-1}$ and (c) 0.645 $m^3 s^{-1}$ in Langrivier, and (d) 0.059 $m^3 s^{-1}$, (e) 0.260 $m^3 s^{-1}$ and (f) 0.335 $m^3 s^{-1}$ in Window Stream.

Table 2. The percentage of the study reaches occupied by hydrological and substratum features at both sites.

Site	Discharge	Hydrological Features				
		Riffles	Backwaters	Fast flow	Slow flow	Chutes
WINDOW STREAM	low	80	5	10	4	1
	intermediate	60	7	28	3	2
	high	70	5	15	5	5
LANG-RIVIER	low	25	15	40	15	5
	intermediate	70	5	15	5	5
	high	75	2	10	3	10

		Substratum Features					
		Boulders	Cobble	Gravel	Wood	Leafpacks	Foam
WINDOW STREAM	low	50	45	4	1	0	0
	intermediate	35	61	3	1	0	0
	high	20	60	10	5	2	3
LANG-RIVIER	low	33	47	12	8	0	
	intermediate	40	45	10	5	0	
	high	50	40	5	5	0	

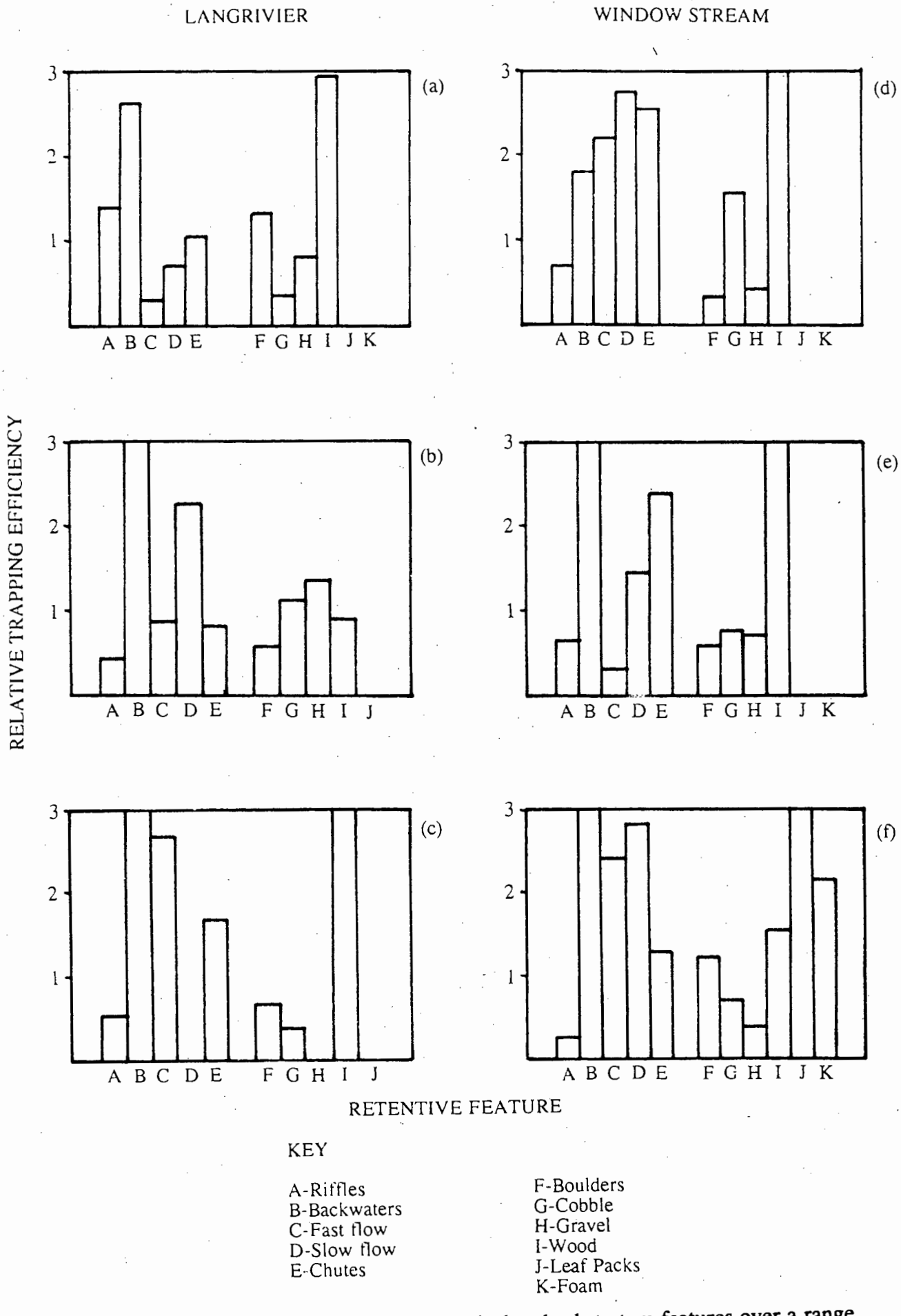

Fig.3. The retention efficiencies of hydrological and substratum features over a range of discharges in Langrivier and Window Stream. Discharge values are given in Fig.1.

and leaves were observed to be trapped singly underneath cobbles. This is in contrast to the bunches of leaves trapped in backwaters. Chutes contributed significantly to retention (Chi-square, $p<0.05$) (Fig. 3). Leaves were channeled towards chutes and either caught between the boulders creating the chute, or were forced under, or behind the cascade of water. Chutes were also responsible for loss of marked leaves, due to the difficulty of extricating them.

Stretches of slow flow in the channel were always more efficient retention features than stretches of medium to fast flow (Fig. 3) with the exception of Langrivier at high discharge, where areas of slow flow did not retain any leaves (Fig. 3c). At low discharge at Window Stream, stretches of slow flow were the most efficient retentive features (Fig. 3d).

Increased discharge appeared to result in increasing the retention efficiency of backwaters in both systems (Fig. 3). Although the proportion that riffles contributed to the stream reach increased with greater discharge, particularly at Langrivier, riffles decreased in retention efficiencies at higher discharges. Stretches of flow (medium to fast and slow) did not show any apparent relationships with discharge. Only at Langrivier did stretches of medium to fast flow increase in trapping efficiency with increased discharge. Chutes were fairly consistent in their retention efficiencies with varying discharge.

Boulders were relatively efficient at retaining leaves, especially boulders forming chutes. Many leaves were stranded on boulders protruding above the water. Cobble stretches usually dominated the substratum at both sites (Table 2) but their relative trapping efficiencies were never significantly high (Fig. 3). As previously mentioned, cobble was generally associated with riffles where leaves were trapped singly. Gravel was usually associated with backwaters and contributed little to the study reaches (Table 2), but relative trapping efficiencies were high (Fig. 3). Leaves sidelined into backwaters were often stranded at the edges of the stream. Wood (sticks, logs etc.) and leafpacks contributed significantly to retention at both sites (Chi-square,

$p<0.05$). Their occurrence was scarce (Table 2), but their retentive properties were efficient (Fig. 3).

An interesting observation was the large number of leaves trapped by foam that results after turbulence caused during spates (Table 2). Foam was caught along the edges of the stream, and bunches of leaves were retained in this thick substance. Its relative retention efficiency was calculated for Window Stream at high discharge only, where its proportional area was recorded. Here its efficiency was only surpassed by that of leafpacks.

The effect of increased discharge on the substratum retention efficiencies did not yield any distinct relationships. They did not increase or decrease consistently with discharge.

Discussion

The evidence provided by this study showed that there was a distinct relationship between retention and discharge, and that the retentiveness of the two systems behaved similarly over a range of discharges. In a previous study using the same two streams, PROCHAZKA et al. (1990) suspected that the retentive nature of a stream is probably largely determined by discharge. Their data suggested that Window Stream was a more retentive system than Langrivier, and in their study, the most significant difference between the two systems was the lower discharge of Window Stream.

The link between retention and discharge has been discussed by other workers. SEDELL et al. (1978) investigated the variation in stream power, calculated using discharge, and its effect on retention, and YOUNG et al. (1978) investigated the influence of current velocity on the distance travelled by leaves released into a stream. KING et al. (1987b) stated that the occurrence and size of leafpacks in Langrivier were

greatly influenced by stream discharge. The present study substantiates these observations.

The percentage of leaves retained once released into a stream decreased markedly when discharge increased (Fig. 1a). At low discharge, the leaves were retained in large numbers around the release point (Figs 2a & 2d). Such a result was also recorded by YOUNG et al. (1978), SPEAKER et al. (1984) and PROCHAZKA et al. (1990). At higher discharges more leaves were retained further from their release point (Figs 2b & 2e). At highest discharge, any predictable relationships between retention and distance were not apparent (Figs 2c & 2f). These observations were similar at both streams used in this study.

In the study of PROCHAZKA et al. (1990), mean discharge values for Langrivier (Table 2) were higher than that for Window Stream, and this was consistent with their findings concerning the retentiveness of both systems. In our study the mean discharge over the study period at Window Stream and at Langrivier was similar. On an annual basis, however, Langrivier probably has a greater discharge, which means that the system spends more time at a lower retention capacity than does Window Stream. Such a situation would account for the greater retentiveness of Window Stream. Discharge data over an annual cycle are lacking for Window Stream to be able to confirm this. Window Stream is, however, dominated by shredders, and WINTERBOURN et al. (1981) have suggested that such a dominance is characteristic of highly retentive systems. In contrast, Langrivier is probably not as suitable for colonisation by large numbers of shredders because of its low retentiveness over the long term.

The morphology of the streambed and its complexity have often been implicated as determinants of retention (e.g. SEDELL et al., 1978; MINSHALL et al., 1983; ROUNICK & WINTERBOURN, 1983; SPEAKER et al., 1984). In our study, streambed complexity was measured using the index of channel irregularity (ICI) (SPEAKER et al., 1984). The effect of discharge on the ICI (Fig. 1b) was similar to that on the percentage of leaves retained (Fig. 1a), with channel irregularity decreasing markedly with increasing

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PAPER 13

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The influence of different litter bag designs on the breakdown of leaf material in a small mountain stream

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Abstract

Leaf breakdown of two riparian tree species, *Cunonia capensis* L. and *Ilex mitis* (L.) Radlk. was investigated *in vitro* at Window Stream, Table Mountain, using three different designs of litter bag. Breakdown of *Cunonia* and *Ilex* in coarse-mesh (5 mm) litter bags was very rapid (respectively 14.79 and 13.93% loss d^{-1}), and was significantly greater than the loss of leaf material of 1% d^{-1} for both species from fine-mesh bags (180 μm). Differences recorded between fine-mesh and composite-mesh bags (180 μm mesh with 5 mm mesh top) represented macro-invertebrate ingestion, and at t = 28 d, amounted to 67.57% material loss in *Cunonia* and 62.58% in *Ilex*. The losses due to microbial activity and leaching, 31.28% in *Cunonia* and 29.17% in *Ilex* were not significantly different. Weight loss of *Cunonia* in coarse-mesh bags (14.79% loss d^{-1}) and in composite-mesh bags (13.93% loss d^{-1}) did not differ, but this was not the case for *Ilex*, where a significantly higher rate of loss in coarse-mesh bags (13.93% loss d^{-1}) than in composite-mesh bags (7.69% loss d^{-1}) was observed. This difference was used to quantify fragmentation losses. It was concluded that future leaf breakdown experiments in mountain streams must take cognisance of differential fragmentation losses before inferences can be made as to both invertebrate feeding preferences and biological decomposition of leaves.

Introduction

In many small mountain streams, riparian leaf litter (allochthonous input) is the major source of energy. A number of authors have measured the decomposition, or breakdown of leaf litter, using either 'leaf packs' (Reice, 1974, 1977, 1980; Petersen & Cummins, 1974; Herbst, 1980; Herbst & Reice, 1982; Short & Ward, 1980; Short *et al.*, 1980), 'litter bags' (Winterbourn, 1978; Blackburn & Petr, 1979; McCammon, 1980; Pidgeon & Cairns, 1981; Dudgeon, 1982; Hanlon, 1982; Rounick & Winterbourn, 1983; Benfield & Webster, 1985), or both methods (Anderson & Sedell, 1979; Benfield *et al.*, 1979; Cummins *et al.*, 1980; Webster & Waide, 1982; Mutch *et al.*, 1983). Although leaf packs are generally thought to be more representative of the natural leaf accumulations on stream beds (Petersen & Cummins, 1974; Cummins *et al.*, 1980), measurement of weight loss from leaf packs does not solely represent 'decomposition' (Hanlon, 1982) of the leaf material because of additional losses due to physical fragmentation (Petersen & Cummins, 1974; Hanlon, 1982; Byren & Davies, 1986). Hanlon (1982) has defined decomposition as 'the degradation of plant materials into their constituent elements by microbial activity and digestion by animals'. We have adapted this definition to include losses due to leaching, for it is impractical to separate this physical component from the biological com-

ponents of the process. In the present study, leaf 'breakdown' has been taken to represent weight losses due to physical fragmentation (caused by animal feeding and abiotic factors), animal ingestion, microbial activity and leaching.

The use of large-mesh bags (frequently 1–13 mm) which allow invertebrate feeding also leads to fragmentation losses, and therefore, weight loss in these bags is again a measure of leaf 'breakdown' and not of 'decomposition' (Hanlon, 1982). On the other hand, small-mesh bags (80–500 μm) which eliminate fragmentation losses do not facilitate macro-invertebrate feeding. Therefore, many authors have measured leaf breakdown rates in streams by using coarse- and fine-mesh litter bags (Winterbourn, 1978; Blackburn & Petr, 1979; McCammon, 1980; Hanlon, 1982; Rounick & Winterbourn, 1983), and have attributed the greater loss of leaf material in coarse-mesh bags to invertebrate feeding activity. However, such studies have not attempted to differentiate between weight loss due to ingestion by invertebrates and that due to fragmentation caused both by the shredding activity of invertebrates and by abiotic abrasion. The need to differentiate between these components has been highlighted by Mutch *et al.* (1983).

In the experiment reported here we have attempted to quantify these components by using three 'litter bag' designs; fine-mesh bags (180 μm – macro-invertebrate exclusion), coarse-mesh bags (5 mm – macro-invertebrate inclusion) and 'composite-mesh' bags with both fine- and coarse-mesh (invertebrate inclusion). In all three, microbial activity and leaching could take place. In addition, coarse-mesh bags allowed measurement of losses of leaf material by both macro-invertebrate feeding activities and abiotic and biological fragmentation, while the 'composite-mesh' bags allowed macro-invertebrate access, while retaining fragments. Our predictions were that losses from both coarse- and 'composite-mesh' bags would be higher than those from fine-mesh bags and that losses from coarse-mesh bags would in turn be higher than those recorded from 'composite-mesh' bags; the differences between these last two allowing quantification of fragmentation *per sê*.

Materials and methods

Freshly fallen, abcissed leaves of *Cunonia capensis* L. and *Ilex mitis* (L.) Radlk. were collected from riparian trees at Window Stream, Table Mountain (33°58′S, 18°25′E) in October (spring) 1984. These species form the dominant component of leaf litter inputs to this first order, temperate (10–18 °C), acid (pH from 3.6 to 4.7) stream. Leaves were oven-dried at 60 °C to constant weight, and 3.0 g (dry weight) samples of each species were placed into 10 × 20 cm nylon-mesh bags, 18 of which were fine-mesh (180 μm netting; here after called 'fine bags'), 18 coarse-mesh (5 mm netting; 'coarse bags'), and 18 bags comprised both mesh types ('composite bags'). Composite bags were constructed such that the main body of the bag consisted of fine mesh (80% of the area) with a coarse-mesh top to ensure macro-invertebrate access to the bag. Litter bags were randomly distributed over the stream bottom in *ca* 10 cm of water and were held in place using steel 'tent pegs'. The composite bags were carefully placed so that the fine-mesh portion of each bag always faced downstream, ensuring that leaf fragments were retained by the bag. Between three and six fine and coarse bags for each leaf species were removed after 7, 14, 21, and 28 days, while three of the composite bags were removed after 2, 5, 8, 14, 21 and 29 days. Loss of leaf material during collection was prevented by placing individual plastic bags under the litter bags as they were lifted from the stream bottom. Bags were returned to the laboratory where they were stored at −20 °C until they could be processed.

All leaf litter remaining was removed from the litter bags, rinsed, placed in aluminium-foil containers and oven dried at 60 °C to constant weight. Weight loss of leaf material was best described by the equation

$$W_t = W_o e^{-kt},$$

where W_t represented weight at time t, W_o the initial weight at t = Od, and k, the decay coefficient. The exponential curves obtained were converted to straight lines ($\log_e$) and an analysis

of covariance was used to test for differences between the slopes of these lines (Zar, 1974). Coefficients of determination (R_2) were calculated by means of the least squares method.

Results and discussion

As predicted, breakdown of both *Cunonia* and *Ilex* in the coarse bags was very rapid (decay coefficients respectively, 0.16 and 0.15), and was significantly greater than leaf material loss from the fine bags (0.01 for both species: ANOCOVA, $P < 0.05$; Fig. 1). Breakdown in composite bags of both leaf species was also significantly greater than that from fine bags (ANOCOVA, $P < 0.05$). It is interesting to note that weight loss of *Cunonia* in coarse bags ($k = 0.16$) and in composite bags ($k = 0.15$) did not differ (ANOCOVA, $P < 0.05$), but this was not the case for *Ilex*, where a significantly higher rate of loss from coarse bags ($k = 0.15$) than from composite bags ($k = 0.08$) was observed (ANOCOVA, $P < 0.05$). In general, losses of *Cunonia* in the presence of macroinvertebrates were greater than those of *Ilex* (ANOCOVA, $P < 0.05$). Table 1 gives weight loss expressed as loss d^{-1} for each treatment. These are calculated from the equation:

$$(1 - e^{-k}) \times 100 = \%\text{loss } d^{-1}.$$

The difference between fine and coarse bags could be attributed to two factors; firstly, macroinvertebrate ingestion in the coarse bags, and secondly, the loss of leaf fragments through the larger mesh of these coarse bags. Window Stream supported large densities (between 264 and 12227 individuals m^{-2}) of a shredding amphipod, *Paramelita nigroculus* (Barnard), and this animal was present in the coarse and composite bags.

Table 1. Leaf material weight loss expressed as % loss d^{-1}.

	Cunonia	*Ilex*
Fine bags	1.00	1.00
Coarse bags	14.79	13.93
Composite bags	13.93	7.69

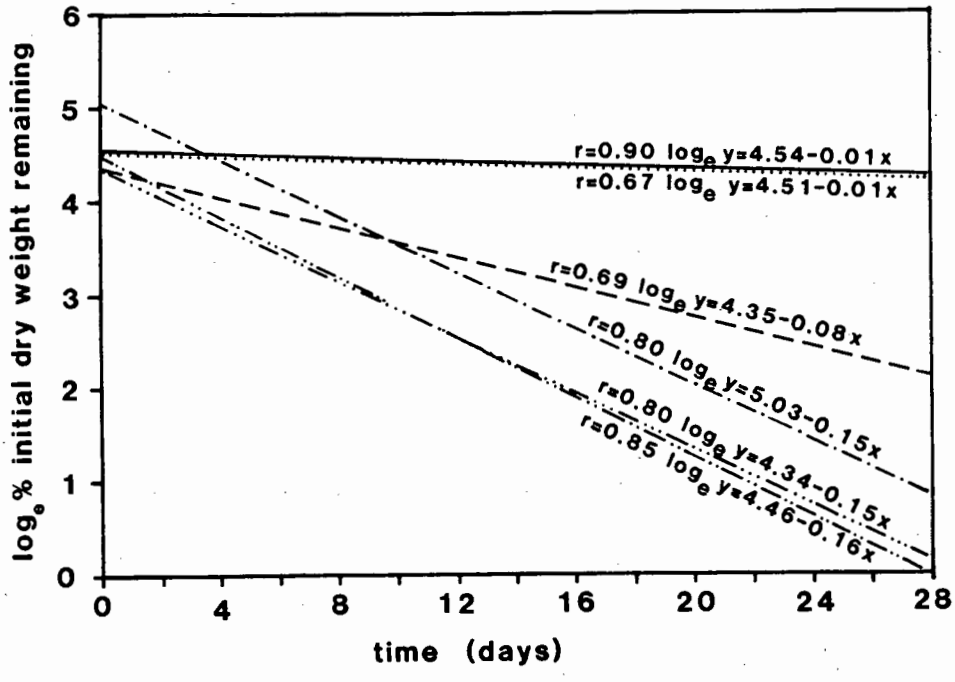

Fig. 1. Breakdown rates of *Cunonia capensis* L. and *Ilex mitis* (L.) Radlk. leaf material expressed as $\log_e$ percentage initial dry weight remaining against time in days.
Ilex: fine mesh litter bags, (————), coarse mesh –·–·–), and composite mesh (– — — -).
Cunonia: fine mesh (·····), coarse mesh (–··–··–), and composite mesh (–···–···–).

Other authors working in streams with high shredder densities have reported similar results for different mesh types. For example, Rounick & Winterbourn (1983) in an experiment they conducted in Middle Bush Stream, New Zealand, found that after 22 weeks, coarse mesh (3 mm) bags had lost all of their *Nothophagus solandri* leaves, while fine mesh (200 μm) bags still had approximately 69% of their leaf material remaining.

Invertebrate ingestion alone must have been responsible for the significant differences recorded between the fine and composite bags. In the case of *Cunonia*, animal ingestion amounted to 67.57% material loss at t = 28 d, while for *Ilex* it was 62.58%. The losses due to microbial activity and leaching (fine bags), 31.28% in *Cunonia* and 29.17% in *Ilex* were not significantly different. Judging from the very rapid loss of *Cunonia* leaves from composite bags, as compared to those of *Ilex*, we can infer that the shredders in Window Stream preferred the former species to the latter.

The differences between weight loss of *Ilex* from coarse and composite bags can be used to quantify fragmentation losses. For example, at t = 28 d, 2.29% of the original *Ilex* leaf material in the coarse bags was remaining, while 8.25% remained in the composite bags which had prevented the loss of small leaf fragments. Therefore, after 28 days, 5.96% of material lost could be attributed to fragmentation.

Nowhere in the literature can we locate reports which quantify fragmentation losses of leaf material from litter bag experiments in mountain streams. Although Hanlon (1982) did not attempt to quantify fragmentation in his experiment, he did suggest that the significantly higher rate of material loss from coarse than from fine bags might have been due to fragmentation rather than to animal processing. Much *et al.* (1983) have also commented that they could not rule out the possibility that a loss of unprocessed material could have accounted for the greater weight loss from bags in riffles, compared with those in pools in their Rocky Mountain stream in Alberta, Canada. This was despite a higher biomass of shredders in their riffles as compared to the pools. Although our results have indicated that fragmentation losses are relatively low in our stream, this does not negate the concerns voiced by these earlier authors, since a wide range of factors; leaf morphology for one, current velocity for another, must contribute to differential fragmentation losses. This is illustrated by our results in so far as our two experimental leaf species exhibit very different fragmentation loss rates. The results of King *et al.* (1987) which demonstrated that, in laboratory experiments, *Ilex* disintegrates easily compared to *Cunonia* supports this conclusion. Therefore, any future experiments which investigate the breakdown rates of leaves in streams must take care to estimate fragmentation losses; each leaf species has different fragmentation 'qualities'. By the same token, inferences as to animal feeding preferences for different leaf species drawn from *in vitro* experiments cannot be attempted with confidence unless litter bag design is improved.

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PAPER 14

The effect of invertebrates on leaf decomposition rates in two small woodland streams in southern Africa

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With 4 figures and 1 table in the text

Abstract

Decay rates were investigated for leaves of three species of trees (*Cunonia capensis* L., *Ilex mitis* L. Radlk and *Rhoicissus tomentosa* (Lam.) Wild and Drummond) retained in fine (180μm) and coarse mesh (5mm) bags (measuring 10 x 20cm) in two headwater streams.

At the site with high shredder densities, decomposition of all three leaf species was significantly faster (decay coefficient, k between 0.04 and 0.14) in coarse than in fine mesh bags (k between 0.003 and 0.01). Weight losses of *Cunonia* and *Ilex* were similar, but significantly faster than that of *Rhoicissus*. The decomposition of *Cunonia* in coarse mesh bags (k = 0.14) was the highest ever recorded for a riparian leaf species. For all leaf species, carbon to nitrogen ratios decreased significantly with time. Ratios for *Ilex* and *Rhoicissus* were similar (25.2-32.4), but lower than those for *Cunonia* (39.6-45.4). Colonisation by the amphipod *Paramelita nigroculus* Barnard was rapid, with 239-740 individuals bag^{-1} being counted after two days. Although there was no significant trend in numbers per bag with time, there was an increase with time in the numbers of amphipods per gram of leaf material.

Breakdown rates at the site with low shredder density were slower. *Cunonia* and *Ilex* disappeared faster from coarse (k=0.02) than from fine mesh (k=0.01) bags, but the slower breakdown rates of *Rhoicissus* (k between 0.003 and 0.01) were not significantly affected by bag type. A mean of 43 *Paramelita capensis* Barnard bag^{-1} was recorded after two days. There were no significant trends with time or leaf species in numbers per bag or numbers per gram dry weight leaf material. Invertebrate feeding can significantly accelerate breakdown rates of detritus in streams. The breakdown rates of particular leaf species can vary considerably according to variations in invertebrate densities, thus making it difficult to fit them into a "slow to fast" continuum for decay rates of riparian leaf species..

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The importance of allochthonous material as the primary energy source in small heterotrophic woodland streams has been stressed by many authors (FISHER & LIKENS, 1973; PETERSEN & CUMMINS, 1974; BIRD & KAUSHIK, 1981). In the Northern Hemisphere, a substantial part of this input is often in the form of autumn shed deciduous leaves (KAUSHIK & HYNES, 1971; FISHER & LIKENS, 1973; ANDERSON & SEDELL, 1979). In contrast, riparian vegetation of Southern Hemisphere river systems is often evergreen; leaves fall throughout the year, with peaks occuring in spring and summer (WINTERBOURN, 1976; KING et al., 1987; BUNN, 1988a, STEWART & DAVIES, 1990).

The fate of these abscissed leaves in stream ecosystems has received much attention in the last two decades. Most leaves are trapped by rocks, debris dams or other obstructions shortly after entering the stream (e.g. SPEAKER et al., 1984), and undergo decay (e.g. PETERSEN & CUMMINS, 1974; ANDERSON & SEDELL, 1979; WEBSTER & BENFIELD, 1986). Earlier studies which attempted to assess the relative importance of microbial and invertebrate feeding activity on *in situ* decomposition rates of riparian leaf litter (e.g. MATHEWS & KOWALCZEWSKI, 1969; KAUSHIK & HYNES, 1971) led to the conclusion that animals were unimportant in leaf decomposition. This conclusion was confirmed by some later studies. For example, McCAMMON (1980) showed that weight loss from beech leaves and twigs in a New Zealand stream was predominantly due to leaching and microbial activity, and in some other studies (e.g. MACKAY & KERSEY, 1985; BUNN, 1988a), differences in breakdown rates in bags of two mesh sizes were negligible. SHORT & WARD (1980) failed to show an increase in leaf breakdown in the presence of greater invertebrate numbers in their study. In addition, MULHOLLAND et al. (1987) ascribed differences in leaf decomposition in their streams to differences in microbial activity rather than to differences in the densities of macroinvertebrates. In contrast, a growing literature

highlights the importance of invertebrates in leaf breakdown (e.g. IVERSEN, 1975; PETERSEN & CUMMINS, 1974; HART & HOWMILLER, 1975; WINTERBOURN, 1978; BENFIELD et al., 1979; SHORT et al., 1980; PIDGEON & CAIRNS, 1981; HERBST & REICE, 1982; ROUNICK & WINTERBOURN, 1983, MACKAY & KERSEY, 1985; BENFIELD & WEBSTER, 1985).

The relative importance of microbial and invertebrate feeding on leaf breakdown is usually quantified by the use of "litter bags" of differential mesh sizes (e.g. ROUNICK & WINTERBOURN, 1983; BUNN, 1988a). Despite some reservations (see WEBSTER & BENFIELD, 1986; BUNN, 1988b), many authors (e.g. PETERSEN & CUMMINS, 1974; SHORT & WARD, 1980; BARNES et al., 1986; WEBSTER & BENFIELD, 1986; McARTHUR & BARNES, 1988) have used an exponential decay model to describe leaf loss from these bags, and PETERSEN & CUMMINS (1974) and WEBSTER & BENFIELD (1986) have used the decay, or "processing" coefficient (k) as an index of breakdown rate when comparing many species. Based on their results, PETERSEN & CUMMINS (1974) described the decay rate of leaf species as either "slow" ($k=0.005$), "medium" (k between 0.005 and 0.010) or "fast" (k between 0.010 and 0.015), and invited others to test the generality of this "slow to fast" continuum. Some authors (e.g. BUNN, 1988a) have fitted their results into this general scheme, whereas others (eg. BENFIELD et al., 1979) have questioned the validity of "species-specific" decay coefficients, since these values have been obtained under a variety of experimental conditions and from different ecosystems. For example, processing coefficients for particular leaf species are affected by temperature (REICE, 1974; IVERSEN, 1975; HERBST & REICE, 1982; BARNES et al., 1986), season (McARTHUR et al., 1988; BUNN, 1988a; GARDEN & DAVIES, 1988), pH (KIMMEL et al., 1985; MACKAY & KERSEY, 1985; ALLARD & MOREAU, 1986; MULHOLLAND et al., 1987) pack size (BENFIELD et al., 1979; REICE, 1974), the addition of sediment (BUNN, 1988b) and nutrient concentration (WEBSTER & BENFIELD, 1986).

Riparian leaf breakdown in southern Africa has received scant attention. The only published studies are those of KING (1982), KING et al. (1987), and STEWART & DAVIES (1989). KING (1982) examined the decomposition of two indigenous and two exotic leaf species confined in "fine" and "coarse" bags in Window Stream, a first-order headwater draining a fynbos catchment. KING et al. (1987) investigated breakdown of three indigenous leaf species, including *Cunonia capensis* L. and *Ilex mitis* (L.) Radlk in bags of two mesh sizes, but reported only on the fauna colonizing the bags, and not on the decomposition of the leaves. Their study was conducted in Langrivier, a second-order fynbos stream. The fynbos biome, which is confined to the southern tip of Africa, is recognised as one of the six main floristic kingdoms in the world, and is characterised by three main elements: the heath-like Ericaceae, the sedge-like Restionaceae, and a variety of members of the Proteaceae. Trees, which are often of Afromontane origin, are rare, and generally confined to riverine habitats. STEWART & DAVIES (1989) were concerned with litter bag design in their examination of the breakdown of leaves of *Cunonia* and *Ilex*.

Against this background, this paper examines the breakdown of three species of leaf at the time (summer) when maximum leaf fall occurred naturally. Two sites were selected: Window Stream, a first-order stream dominated by high densities of the shredding amphipod *Paramelita nigroculus* Barnard, and Noordhoek Stream, a headwater which is frequented by lower densities of another shredding amphipod, *Paramelita capensis* Barnard. Both streams are flanked by Afromontane trees, and drain fynbos catchments. The streams are typically acidic (pH 4-6) and have temperatures in the range 10-20^{o}C. The main purpose of the study was to determine whether leaf breakdown was more rapid in the stream with the higher shredder density. The paper also critically addresses the generality of the "slow to fast" continuum concept of PETERSEN & CUMMINS (1974), the effect of leaf type on breakdown rates, the C:N ratios of detritus in streams, and the colonization of litter bags by stream invertebrates.

Freshly abscissed leaves of the "rooiels" *Cunonia capensis*, the African holly *Ilex mitis*, and the common forest grape *Rhoicissus tomentosa* were collected alongside Window Stream. These were oven-dried for 48h at 60°C, and stored in sealed polythene bags until use. Nylon mesh bags measuring 10 x 20cm and of two mesh sizes were each filled with 3.0g dry weight of leaf material. "Fine" mesh bags (180μm) excluded practically all invertebrates, but allowed loss of leaf material due to leaching and microbial activity, whereas "composite" bags (STEWART & DAVIES, 1989) were constructed such that the main body of the bag consisted of fine mesh (180μm) with a coarse mesh (5mm) top to allow invertebrates access to the bag. Weight loss from these "composite" bags, which retained leaf fragments, therefore represented a measure of leaching, microbial activity and invertebrate ingestion. Oxygen did not appear to be restricted in these bags, and rapid colonization by invertebrates followed their introduction to the stream. The use of bags as opposed to leaf packs allowed the exclusion of selected components of the fauna, and therefore an evaluation of the relative importance of biotic factors in leaf decomposition. Although some studies (e.g. CUMMINS et al., 1980) have shown that leaf packs best simulate natural leaf accumulations, particularly in riffles, MUTCH et al. (1983) found the reverse to be true. Eighteen of each type of bag, with each type of leaf (i.e. 108 bags in all) were placed randomly on the stream bed in November 1985 in approximately 10cm of water. The bags were anchored by steel tent pegs.

Three fine and three composite bags containing each type of leaf were removed after 2, 5, 8, 14, 21 and 29 days from Window Stream and after 2, 4, 8, 15, 23 and 33 days from Noordhoek Stream. Bags were returned to the laboratory where the remaining leaf material was separated from the invertebrate fauna by rinsing, placed into aluminium foil containers and oven-dried at 60°C for 48 hours and weighed. Subsamples of the dry leaf material from Window Stream were ground in a mill, and

between 3.0 and 5.0mg of this material was analysed for carbon and nitrogen using a CHN Analyser. The invertebrates were identified and counted using a dissecting microscope, dried at 60^{o}C for 48 hours and weighed to a level of accuracy of 0.0001g.

Weight loss of leaf material was estimated using the exponential decay model,

$$W_t = W_o e^{-kt},$$

where W_t represented weight at time t, W_o the initial weight at t = 0 d, t the time in days, and k, the decay coefficient. This model best described the data in the present study, and the calculation of k values allowed comparisons with figures obtained in the literature. The exponential curves obtained were converted to straight lines ($\log_e$) and, where appropriate, two sample t-tests or F-tests were used for 'planned' comparisons between slopes, and Student-Newman-Keuls multiple range test was used for 'unplanned' comparisons (ZAR, 1974). Analyses of variances (ANOVA) and Tukey's Multiple Range Tests were used to test for significant differences in C:N ratios and colonisation by invertebrates with leaf species and time.

Results

Leaf breakdown rates

At Window Stream, the site of high shredder density, breakdown of leaf material of all three species was significantly faster in coarse than in fine bags (Fig. 1A; $p < 0.05$, t-test). The breakdown rate of *Cunonia* in coarse bags, although similar to that of *Ilex* ($p > 0.05$), was significantly faster than that of *Rhoicissus* ($p < 0.05$, t-test). No differences were found between the k values of *Ilex* and *Rhoicissus* in coarse bags ($p > 0.05$, t-test). Weight loss of *Cunonia* and *Ilex* in fine bags was similar ($p > 0.05$), but significantly faster than that of *Rhoicissus* ($p < 0.05$, t-test).

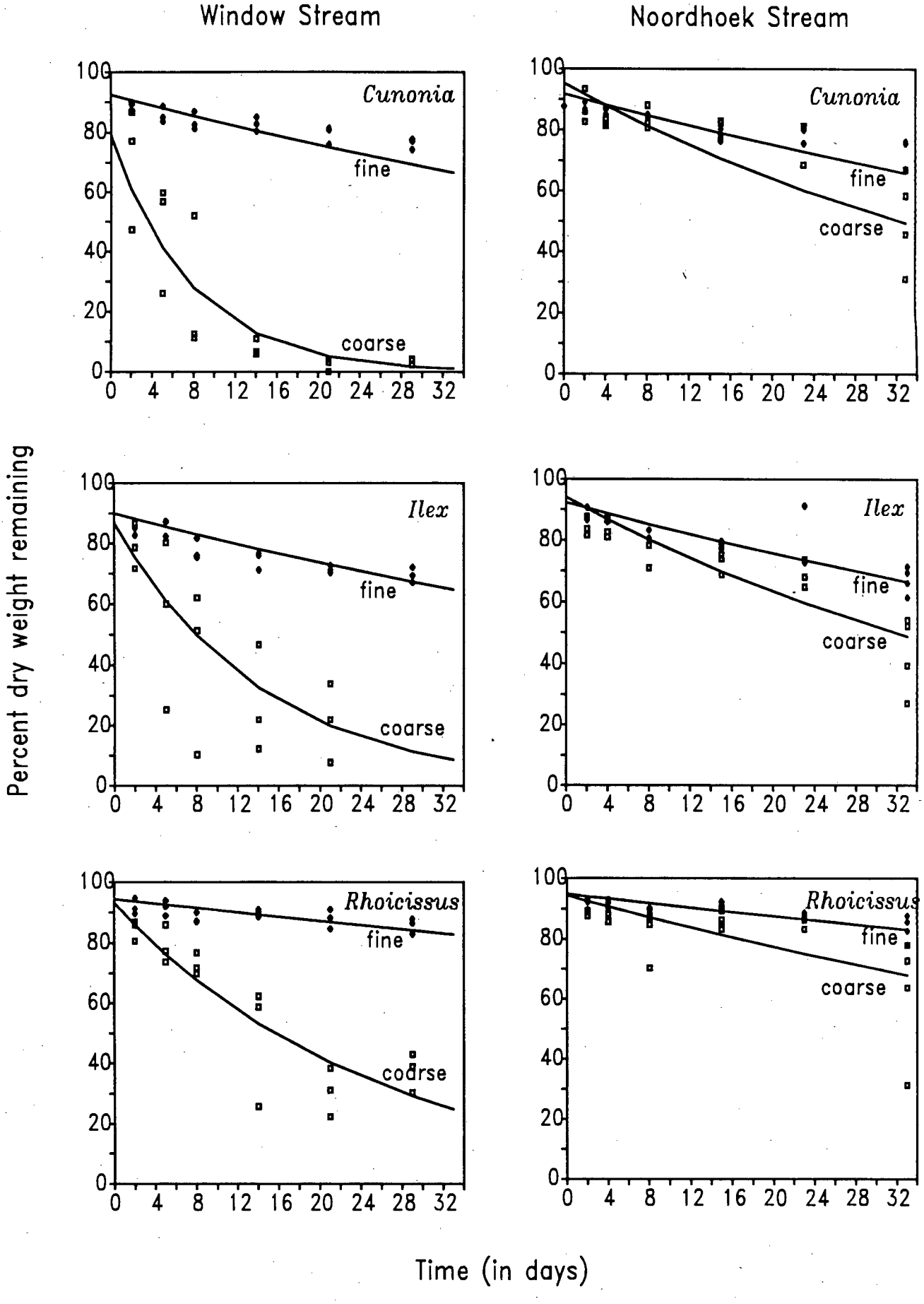

Fig. 1. Percent dry weight remaining versus time for *Cunonia*, *Ilex* and *Rhoicissus* in fine and coarse mesh bags in Window and Noordhoek streams.

At Noordhoek Stream, the site of low shredder density, breakdown of *Cunonia* and *Ilex* in coarse bags, although not as fast as that in Window Stream, was also significantly faster than in fine bags ($p < 0.05$), but the breakdown rates of *Rhoicissus* were the same in coarse and fine bags (Fig. 1B $p > 0.05$, t-test). Breakdown of the three leaf species in fine bags was not the same ($p < 0.05$, F-test); although the loss of *Cunonia* and *Ilex* was similar ($p > 0.05$, t-test), these species disappeared at a significantly faster rate than *Rhoicissus* from fine bags ($p < 0.05$, t-test).

Decay coefficients (k) at both sites for all three species in large mesh bags (k between 0.02 and 0.14) were often considerably higher than those proposed by Petersen & Cummins (1974) for their "fast" category (Table 1). This was particularly true for breakdown in Window Stream with its high shredder densities. Breakdown of *Cunonia* and *Ilex* in fine mesh bags ($k=0.01$) fell into the "medium-fast" category, whereas *Rhoicissus* ($k=0.003$) fitted into the "slow" category (Petersen & Cummins, 1974). The time taken for 50% of leaf material to disappear ($t_{50\%}$) was calculated (Table 1), and revealed ranges of 2.3 to 58.5 d for *Cunonia*, 5.5 to 59.8 d for *Ilex*, and 14.9 to 209.3 d for *Rhoicissus* in fine and coarse bags at the two sites.

C:N ratios

For all three leaf species in Window Stream, C:N ratios (Fig. 2) were significantly higher at the beginning of leaf processing ($t=2$ and $t=5$ d) than at the end of the experiment ($t=21$ and $t=29$ d) ($p<0.05$, Tukey's test). Ratios averaged over the whole time period were similar for *Ilex* and *Rhoicissus* (25.2-32.4) ($p>0.05$), but these were significantly lower than those obtained for *Cunonia* (39.6-45.4) ($p<0.05$, Tukey's test). The type of bag used did not influence C:N ratios in any of the leaf species.

discharge for both systems. ROUNICK & WINTERBOURN (1983) compared two streams of different streambed complexity. They concluded that the presence of retention features, such as boulders and debris dams, significantly increased stream retention. Discharge thus has an important influence on the retentiveness of the system by altering the numbers and proportions of these retention features on the streambed.

There was no marked change in trapping efficiency of substratum features with discharge (Fig. 3), although at Window Stream, cobble showed a decrease in retentiveness at higher discharges (Figs. 3d-f). However, the data did show a change in trapping efficiencies of certain hydrological features - a decrease with discharge for riffles and an increase in trapping efficiency with discharge for backwaters. Riffles are created by cobble disrupting the even flow of water. Their trapping efficiency was probably decreased when greater discharge carried the leaves over the cobble without being retained. Backwaters increased in efficiency with discharge. KING et al. (1987a) mentioned the rarity of backwaters in Langrivier and concluded that they did not play a major role in the retention of benthic organic matter (BOM). This study indicates otherwise, and agrees with the results of PROCHAZKA et al. (1990) and SPEAKER et al. (1984) who found backwaters to be the most retentive of all features. Their increase in efficiency at higher discharges as recorded in this study, emphasises their importance in these conditions. At higher discharges, a greater area of the streambed was submerged and the irregularity of the streams' banks became incorporated into the stream channel. New obstructions such as roots and boulders created many small backwaters into which leaves were sidelined.

It can be concluded that the trapping efficiencies of different retentive features are altered in different ways under conditions of increased discharge. In some studies, retentive features are divided into erosional (riffles, medium to fast flow, slow flow, chutes) and depositional (backwaters, pools) zones, and then compared in terms of retentiveness. However, these categories are not constant under varying discharges. For example, at high flow, pools may become erosional and riffles depositional (MINSHALL et al., 1983; KING et al., 1987a; pers. obs.). This was not observed in

Langrivier or Window Stream, but this example emphasises the need for the careful observation of the behaviour of retentive features over a range of discharges.

In conclusion, discharge plays a major role in determining the retentiveness of a system. At low discharge both streams studied had relatively high indices of channel irregularity, and high percentages of marked leaves were retained, particularly near the point of release. High discharge, such as that occuring during winter spates, resulted in the random distribution of leaves, and very low percentage retention within the study reaches. Every winter these powerful spates appear to scour both systems of their coarse detritus (KING et al., 1987a & b), acting as 'destabilising' mechanisms. In summer when discharge is very low, the study streams are 'stabilised', and leaf fall reaches a peak and retention is extremely high. Thus, discharge is predictably high in winter and low in summer. WINTERBOURN et al. (1981) suggested that the lack of shredders in New Zealand streams was due to the "unpredictable" nature of their streams, caused by the occurrence of unseasonal rainfall. However, rainfall in the south-western Cape occurs on a seasonal basis, and is thus predictable.

It is clear that the retentiveness of a system can only meaningfully be described when the variation in retention, as a result of seasonal fluctuations in discharge, is recorded. The seasonal range of retention must have important consequences for the headwater stream communities, especially shredder communities. It is a parameter that requires further study.

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Table 1. Time taken (in days) till 50% of leaf material has been processed ($t_{50\%}$) and associated k values.

Leaf species	Mesh type	Locality			
		Window Stream		Noordhoek Stream	
		$t_{50\%}$	k value	$t_{50\%}$	k value
Cunonia	coarse	2.3 d	-0.14	32.9 d	-0.02
Cunonia	fine	58.5 d	-0.01	57.8 d	-0.01
Ilex	coarse	5.5 d	-0.08	31.4 d	-0.02
Ilex	fine	55.8 d	-0.01	59.8 d	-0.01
Rhoicissus	coarse	14.9 d	-0.04	63.8 d	-0.01
Rhoicissus	fine	206.0 d	-0.003	209.3 d	-0.003

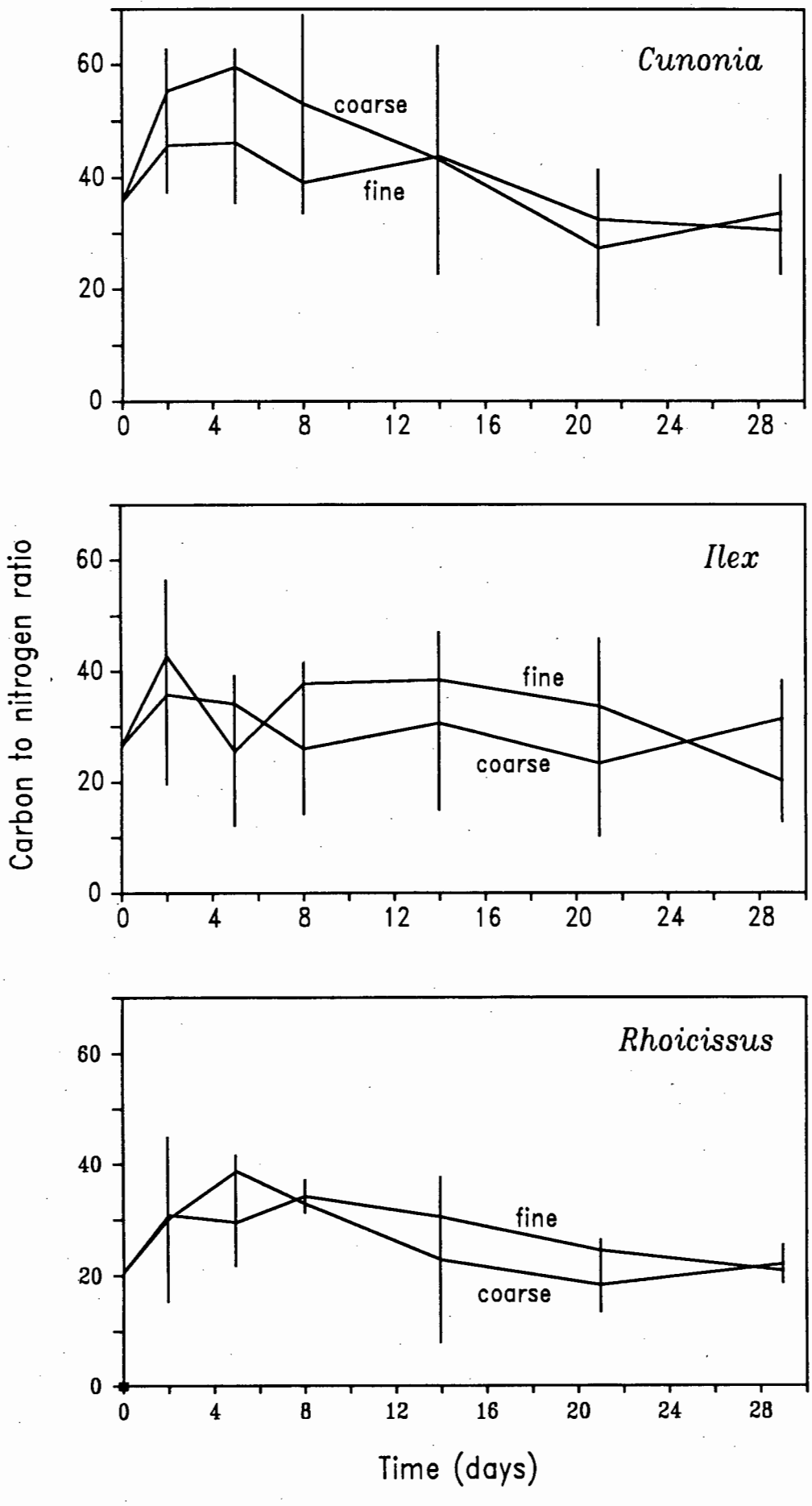

Fig. 2. C:N ratios of decomposing *Cunonia*, *Ilex* and *Rhoicissus* in fine and coarse mesh bags in Window Stream versus time.

The shredding amphipod *P. nigroculus*, by far the largest invertebrate found, dominated the invertebrate fauna associated with leaf decomposition at Window Stream, and represented between 88 and 99% of the total numbers of invertebrates found in the bags. Colonisation was rapid, and within two days, between 239 and 740 individuals of *P. nigroculus* were recorded in the bags (Fig. 3A). Although it appeared that numbers peaked in bags containing *Cunonia* (1024 individuals bag^{-1}) and Ilex (605 individuals bag^{-1}) after only five days, the high variability of the data meant that no statistically significant temporal differences were found. Similarly, the peak value of 1289 individuals bag^{-1} on *Rhoicissus* after 14 days was not significantly different from values recorded at other times ($p > 0.05$. ANOVA). Nevertheless, significantly greater numbers of amphipods were found in the bags containing *Rhoicissus* than in the bags with the other two species ($p < 0.05$, Tukey's test). Although *P. capensis* initially dominated the fauna in all coarse bags at Noordhoek Stream, forming approximately 90% of the total numbers, this figure dropped to about 30% by 23 days due to increasing numbers of much smaller chironomid larvae. The densities of amphipods found in decomposition bags in Noordhoek Stream were considerably lower than those in Window Stream (Fig 3B). There were no significant differences in the numbers of *P. capensis* per bag with leaf species or time ($p > 0.05$, ANOVA). During the study, between 12 and 102 individuals were recorded, with a mean of approximately 43 individuals bag^{-1} for all three leaf species after two days.

At Window Stream, the numbers of *P. nigroculus* per gram dry weight leaf material were not significantly different between the three leaf species (Fig. 4A; $p > 0.05$, ANOVA). For the three leaf species combined, the mean value of 1480 individuals g^{-1} after 21 days was significantly higher than the value of 236 individuals g^{-1} recorded after two days ($p < 0.05$, Tukey's test). Numbers recorded for the other time intervals did not differ ($p > 0.05$, Tukey's test). Again, the high variability of the data meant that numbers

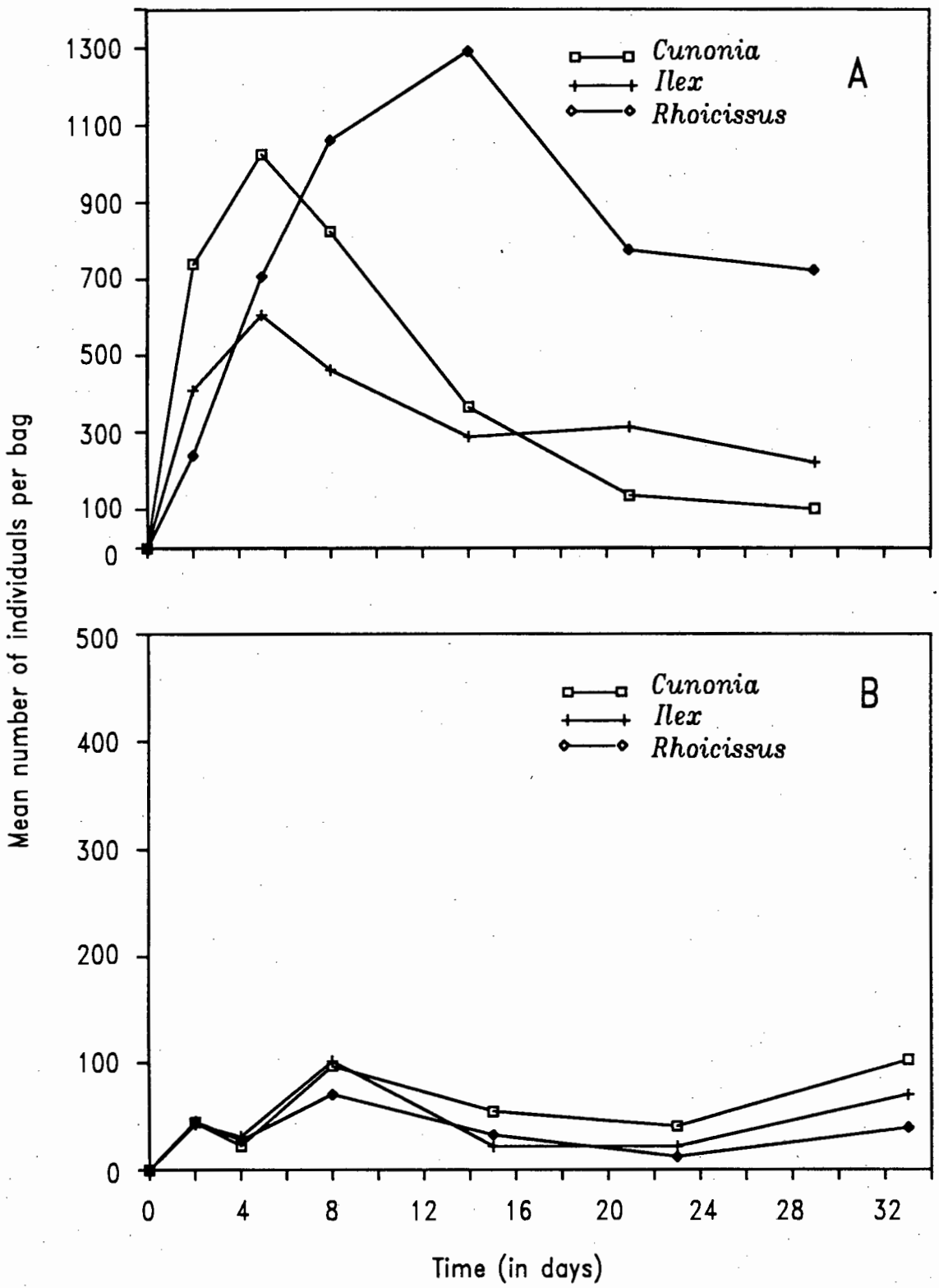

Fig. 3. Mean number of amphipods per bag in (A) Window Stream and (B) Noordhoek Stream.

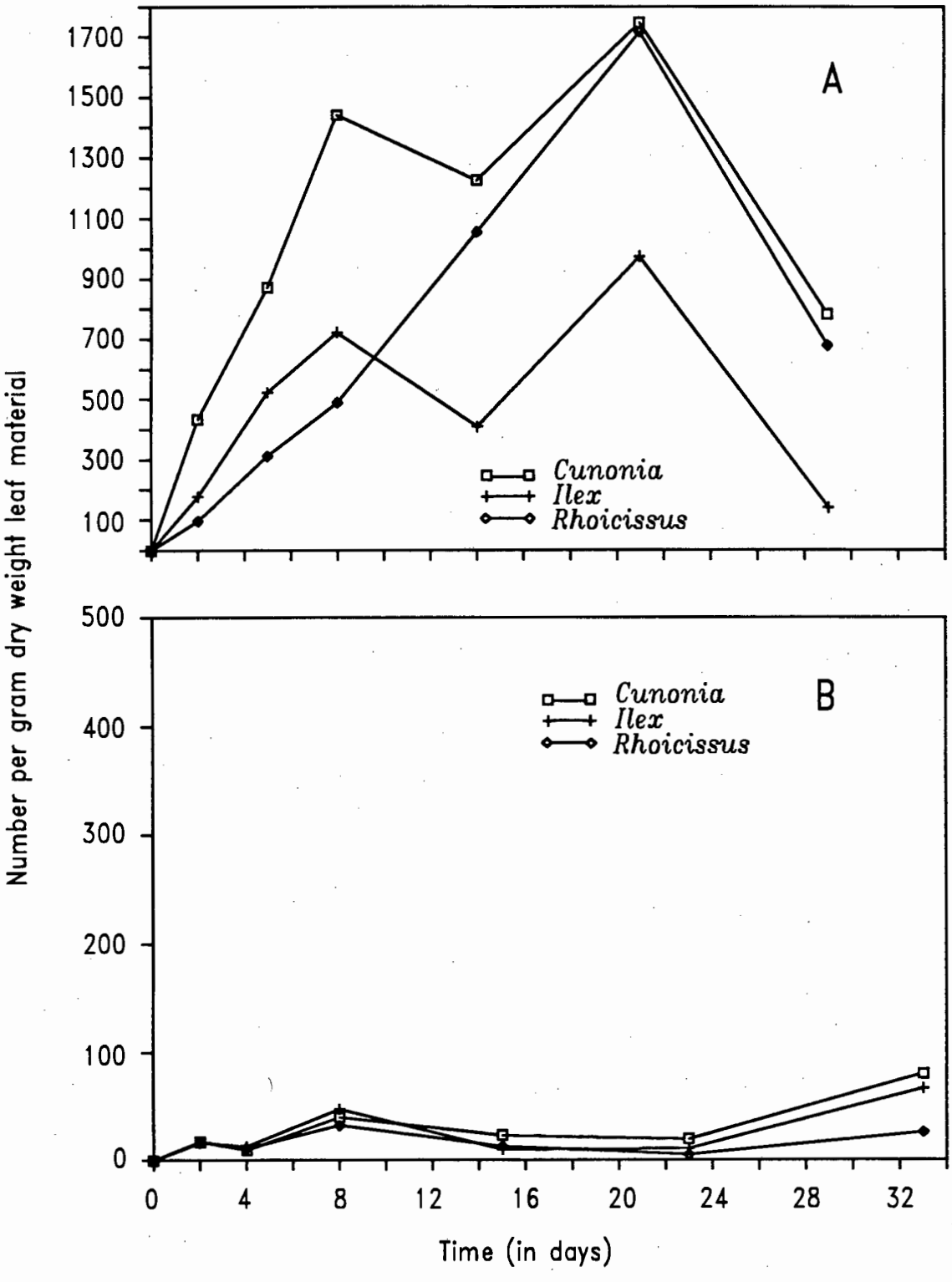

Fig. 4. Mean number of amphipods per gram dry weight leaf material in (A) Window Stream and (B) Noordhoek Stream.

per gram dry weight leaf material at Noordhoek Stream were similar with leaf species and at all time intervals (Fig. 4B; $p>0.05$, ANOVA). The range encountered was between 5 and 79 individuals g^{-1}.

Discussion

The role of invertebrates in leaf breakdown

The results show that shredders can undoubtedly play an important role in leaf breakdown. This effect was most dramatic when shredders occurred in high numbers, but was also clearly demonstrated at the site of lower shredder densities. Several other studies have demonstrated significantly faster processing rates of leaf material exposed to invertebrates. For example, in a similar study carried out in New Zealand, ROUNICK & WINTERBOURN (1983) demonstrated that weight losses of mountain beech in coarse mesh bags incubated in a 'retentive' stream with a large population of shredders were far more rapid than in a stream where large shredders were absent. In addition, breakdown rates in fine mesh bags were similar in both streams - a situation identical to that found in the present study. In the New Zealand stream, weight loss was attributed largely to a single species of large caddisfly; in Window Stream, the amphipod *P. nigroculus* was the dominant shredder in the litter bags.

When BARNES et al. (1986) excluded shredders (mainly amphipods) from leaf packs placed in a cold desert stream in the USA, a significant drop in decay coefficient from $k=0.04$ to $k=0.02$ was recorded. BENFIELD & WEBSTER (1985) attributed faster breakdown rates of leaf packs at their second-order sites in an Appalachian mountain stream, and SHORT et al. (1980) at their third order Colorado stream, to higher numbers of shredders, and MINSHALL et al. (1982) came to a similar conclusion when they found a significant correlation between disappearance of deciduous leaves and numbers of benthic invertebrates in a stream in Idaho, USA. Despite the "better

quality" of poplar leaves placed in spring than in autumn into a headwater in Canada, lower numbers of invertebrates in spring resulted in reduced leaf decay rates from k=0.02 in autumn to k=0.006 in spring (GARDENS & DAVIES, 1988). The fact that, in another study, weight loss from fine bags was considerably slower than loss from coarse bags was taken as evidence of the importance of invertebrate feeding by PIDGEON & CAIRNS (1981).

In the present study, with the exception of very few small chironomid larvae, no macroinvertebrates penetrated the fine (180¤m) mesh bags (see also PIDGEON & CAIRNS, 1981; BUNN, 1988a). In another study of *Cunonia* and *Ilex* breakdown, partly reported on by KING et al., (1987), the composition of the fauna (mainly plecopterans and larval chironomids) was similar in coarse (3-4mm) and fine (250¤m) mesh bags. These authors reported peak numbers after "6-8 weeks" of 93 and 113 individuals g^{-1} dry weight leaf material for coarse and fine bags respectively. KING et al. (1987) found few large shredders, and concluded that the smaller plecopteran nymphs (which had not been excluded by the fine mesh) were the principal shredders in their system. Not surprisingly, therefore, decomposition of *Cunonia* in coarse (k=0.013) and fine (k=0.012) bags was similar (DAY et al., in prep.), as was the case for *Ilex* (k=0.02 in both bag types). Although control packs (k=0.03) and "shredder exclusion cages" (k=0.02) had significantly different processing rates in a Rocky Mountain stream (BARNES et al., 1986), they did not differ in the number of shredders per gram dry weight of leaf material. BARNES et al. (1986) thus concluded that the difference in processing rates was probably due to the fact that only smaller individuals of the principal shredder could enter the "exclusion cages", and warned that not only numbers of shredders, but also size, was an important factor to consider when relating shredder abundance to decomposition rates. Before concluding that invertebrate feeding is unimportant when faced with similar decomposition rates in fine and coarse bags it is, therefore, imperative to confirm that fine mesh did deny shredder access.

The breakdown rate of *Cunonia* ($t_{50\%}$=2.3d, k = 0.14) kept in coarse mesh bags in Window Stream is the highest ever recorded for a riparian leaf species (see WEBSTER & BENFIELD, 1986). Of the six species HART & HOWMILLER (1975) examined, alder leaves lost weight most rapidly, disappearing completely in 34 days. This coincided with a decay coefficient (calculated from the graphs presented) of k=0.05. Alder also disappeared fast (k=0.05, $t_{50\%}$=15 d) in a Colorado stream (SHORT & WARD, 1980). PIDGEON & CAIRNS (1981) calculated a mere seven days for $t_{50\%}$ for Salix babylonica; this represented a decay coefficient of k=0.09. All studies attributed the rapid breakdown to invertebrate feeding. O'KEEFE & LAKE (in press) found that the amphipod *Paramoera* contributed to the "fast" decomposition of *Eucalyptus* leaves, and recorded a k value of 0.05, corresponding to a $t_{50\%}$ of 14 days for these leaves at one of their sites. Previous claims for the "most rapid breakdown rates recorded" include that of DUDGEON (1982) who placed *Liquidambar formosana* in a Hong Kong headwater, and noted a $t_{50\%}$ of 4.5 weeks, and HERBST & REICE (1982) who found that *Eucalyptus* leaves placed in an Israeli stream exhibited a half life of 16 days, coupled with a k value of 0.04. All of the figures quoted here are for riparian leaf species. Decomposition of macrophytes is often much faster - for example, HERBST & REICE (1982) reported a k value of 0.12 for *Phragmites* in an Israeli river.

The present data do not support the idea that leaf litter can be "reliably classified" as fast, medium or slow, or that leaves exhibit 'species-specific' decay coefficients: a fourteen fold increase was recorded for *Cunonia* in the presence of shredders (k between 0.01 and 0.14), while *Ilex* (k between 0.01 and 0.08) disappeared eight times faster when fed upon by invertebrates. In addition, decay coefficients recorded for *Rhoicissus* (k between 0.003 and 0.04) span the entire 'fast to slow' range suggested by PETERSEN & CUMMINS (1974).

Although the exponential decay model was appropriate for the present study, some authors have found that this model was a poor representation of leaf breakdown in their studies. For example, BLACKBURN & PETR (1979), McCAMMON (1980), PIDGEON & CAIRNS (1981) and BUNN (1988b) described decomposition consisting of two stages of rapid weight loss separated by a stage of relatively constant weight. The second period of rapid weight loss was attributed by PIDGEON & CAIRNS (1981) to the loss of large leaf fragments as the leaf began to break up. In the present study, the use of fine and coarse mesh combined, which ensured macroinvertebrate access, yet retained fragments, eliminated this possibility (STEWART & DAVIES, 1989).

Invertebrate colonisation

In Window Stream, the invertebrate community was dominated almost entirely by shredding amphipods, which usually formed more than 90% of the total numbers in decomposition bags. Shredders also dominated total numbers in Noordhoek Stream, especially in the earlier stages of decomposition. Shredders are not always the dominant component of the fauna associated with rapidly decomposing litter. For example, PIDGEON & CAIRNS (1981) and DUDGEON (1982) categorised most of the invertebrates in their studies as "fine particle feeders", and SHORT & WARD (1980) found few shredders on fast decomposing alder leaves.

CUMMINS et al. (1989) have stated that shredders use plant material as food only after microbial colonisation, and that this 'conditioning time' ranges from weeks to months depending on plant species and stream temperatures. However, in their study of *Salix babylonica* decomposition, PIDGEON & CAIRNS (1981) found little evidence for an initial conditioning period prior to invertebrate colonisation, DUDGEON (1982) commented that there was "little time lag" between immersion and macroinvertebrate colonisation in his study, and SHORT & WARD (1980) found high densities of invertebrates on leaf packs of *Alnus tenuifolia* after seven days in their study.

McARTHUR & BARNES (1988), who commented that box elder packs in a Utah alpine stream, decomposed 'fast' (k=0.02) relative to other species, reported immediate colonisation, counting approximately 50 organisms per leaf pack after two days. They suggested that the shredders were either obtaining energy directly from the 'non-conditioned' leaves, or that shredder feeding may promote rapid microbial colonisation, and subsequently, enrichment, of the leaf material. This was probably also the case in the present study, where, particularly in Window Stream, shredder colonisation of the leaf litter was rapid, reaching levels of 239-740 individuals bag^{-1} after only two days. This coincided with rapid leaf breakdown. This lack of time for 'adequate' microbial colonisation implies that the amphipods must be deriving at least part of their nutrition from the leaves *per se*.

It has been predicted that the maximum ratio of shredder biomass to leaf biomass should occur when approximately 50% of the leaf material has been processed (CUMMINS et al., 1989). It is doubtful whether this generalisation is valid for all leaf species. In Window Stream, the mean number of amphipods per gram dry weight leaf material peaked after 21 days, when only 3% of *Cunonia*, 21% of *Ilex* and 30% of *Rhoicissus* remained. COLLIER & WINTERBOURN (1986) have produced a table of peak invertebrate densities on decomposing willow leaves based on four studies - this table revealed peak densities of 41-115 invertebrates g^{-1} at the point when 35 to 45% of willow leaf material remained. In addition, peak numbers occurred on box elder leaves when only 10% of leaf material remained in one study (SEDELL et al., 1975), and when 60% of leaf packs remained in another study (McARTHUR & BARNES, 1988), on *Fraxinus* when 30% remained (PETERSEN & CUMMINS, 1974), on *Liquidambar formosana* when 10% and on *Aleurites montana* when 17% remained (DUDGEON, 1982).

Since shredder feeding was obviously responsible for accelerating decomposition rates in coarse mesh bags, one could expect higher numbers of invertebrates on *Cunonia* and *Ilex* than on *Rhoicissus*. Many authors (e.g. HART & HOWMILLER, 1975;

BLACKBURN & PETR, 1979; PIDGEON & CAIRNS, 1981; DUDGEON, 1982) have reported higher invertebrate numbers on leaf species which decomposed most rapidly. In Window Stream, despite the significantly faster rates at which *Cunonia* and *Ilex* are processed, the densities of amphipods were not significantly different for all three leaf species. When BENFIELD & WEBSTER (1985) found similar densities on leaves which had different breakdown rates, they concluded that either the shredders were using the leaf material as a substrate only, or that feeding occurred at a faster rate on "higher quality" leaves. This could also be the case in the present study.

C:N ratios

Leaves with lower C:N ratios, and therefore probably of greater nutritional value (NAIMAN & SEDELL, 1979), could be expected to decompose more rapidly than leaves with higher C:N ratios. Surprisingly, *Cunonia* with its higher (40-45) C:N ratios decomposed far more rapidly in both fine and coarse bags than *Rhoicissus* with its lower (25-29) C:N ratios , suggesting that *Cunonia* supported greater microbial activity than *Rhoicissus*. NAIMAN & SEDELL (1979) have warned, however, that C:N ratios can be difficult to interpret as leaves can contain refractory nitrogen compounds which are included in the C:N ratios but are unavailable to invertebrates. Whether, or not, *Rhoicissus* contains unpalatable structural components, or chemical inhibitors is unknown.

Carbon to nitrogen ratios calculated for *Cunonia* (46-100) in a previous study (DAY, et al., unpubl. data) were substantially higher, whilst values for *Ilex* (27-48) were similar to values reported here. Ratios for these two species fall into the range obtained by other authors for riparian leaf species. For example, GARDEN & DAVIES (1988) calculated C:N ratios of 30 to 50 for abscissed poplar leaves, and reported a decreasing trend for 50-60 days, followed by an increase in C:N ratios. Carbon to nitrogen ratios of maple in acidic streams in the USA declined from over 65 to about 45 after 105 days

(MULHOLLAND et al., 1987). ALLARD & MOREAU's (1986) C:N ratios for 'sweet gale', *Myrica gale* (9-13) and 'rough alder', *Alnus rugosa*, (9-12) are substantially lower than those in the present and other studies.

Conclusion

In conclusion, it is evident that where shredders occur in high numbers, they can have a marked affect on the rate of leaf breakdown. When they are allowed access to leaves experimentally, they can accelerate calculated decomposition rates tremendously, questioning the validity of the "fast to slow" continuum of leaf species based on literature reviews suggested by other authors. Leaves do differ in terms of their leaching losses, nutrient content and microbial colonisation, and are differentially colonised by invertebrates. These biotic factors, in turn, are influenced by a host of physical conditions, for example, temperature and discharge. Cummins *et al.* (1989) were confident that by 'correcting' for temperature variation by converting the time scale to degree days, the values obtained would be "transferable between streams in different watersheds". The present study shows, however, that invertebrate feeding can also be an overriding factor in leaf decomposition.

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