



BIO4000W Project 2

Resolution of the *Marthasterias* Taxonomic “Disar-star”



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Abstract

Marthasterias glacialis is a sea-star found in the cool-temperate waters of the north-eastern Atlantic as well as along the south-western tip of Africa. The South African *Marthasterias* population is comprised of two distinct morphotypes, a smooth, spineless *rarispinga* form and a spiny *africana* form. These distinct morphotypes have been variably described as separate species, subspecies or forma by various authors over the last century. To test whether these two morphotypes are separate species, or part of a single distinct South African clade, 78 *Marthasterias* individuals were collected from the Cape Peninsula of South Africa. Morphological comparisons were carried out between individuals of the two forms and the results showed no significant clustering of samples. This indicates that there is no morphological separation of the forms into distinct species. The *africana* and *rarispinga* forms were also shown to be genetically indistinguishable, using both a mitochondrial COI sequence and a nuclear ITS1 gene. The COI and ITS sequences of the South African specimens were also compared to that from European specimens, and the p-value distances of 4% and 3% respectively show a significant distinction between the two clades. The South African *Marthasterias* is thus genetically distinct from the European *M. glacialis*, and as such, *Marthasterias africana* sp. nov. will be formally described as a new species elsewhere. *M. glacialis* has a spine armament pattern of a series of three regular rows of spines down the length of each arm, whilst *Marthasterias africana* sp. nov. is either covered in many irregularly-spaced spines, or has an extraordinarily bare surface of only two spine rows per arm. *M. africana* sp. nov. may also have an actinal spine simulating the presence of a third inferomarginal spine. This work resolves a century of taxonomic dispute, separation and amalgamation of the two forms and establishes that will for part of a single, uniquely South African, *Marthasterias* species that is distinct from the European *M. glacialis*.

Plagiarism Declaration

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Introduction

In 1758, Linnaeus established a standardised method for taxonomic classification in his *Systema Naturae*. Despite this creation of this single, rigid classification system, problems arose in the classification of species based purely on morphological characteristics. Often, new species were described from a single specimen, and superficial morphological differences were enough to declare a novel species. The introduction of genetic analysis into the realm of taxonomy has highlighted problems in the exclusive use of subjective morphological features to group specimens. It has been discovered that many morphologically distinct species are actually the same, while some species with a morphological similarity are very different on a genetic level. This has been especially prevalent for the less charismatic orders, where time and financial constraints have resulted in the modern acceptance of classifications derived over a century ago.

Mathasterias glacialis (Linnaeus, 1758) is an asteroid part of the order Forcipulatida (Clark, 1923a) and is widely distributed throughout the littoral habitats of the north-east Atlantic, from northern Norway to the Mediterranean Sea (Harmelin et al., 1980; Nichols and Barker 1984; Savy 1987). The species was also described in the Cape of Good Hope, South Africa by Müller and Troschel (1842) as a “spinier” version *africana*, and a smoother version *rarispinia* (Perrier, 1875) relative to the original European specimen.

The taxonomic history of the species is complex, with the South African forms being amalgamated and separated both from each other and the European species multiple times over the last century. Originally classified as *Asterias glacialis* forma *africana* by Linnaeus (1758), Jullien (1878) placed the sea-star into the subgenus *Marthasterias* due to the presence of a single adambulacral spine. These adambulacral plates are monacanthid, the spines somewhat flattened, not tapering and blunt at the tip (Clark and Courtman-Stock, 1976). This was confirmed by Fisher (1906), who compared specimens (n = 3) and noted that the South African variety agreed very closely with the specimens taken from European waters. The diagnostic characteristics of the genus *Marthasterias* were elaborated on by Verrill (1914) to include five angular rays with three dorsal radial rows of large, mostly conical spines (in addition to the superomarginal rows of spines), inferomarginal plates with two rows of spines and monacanthid adambulacral plates.

Two morphotypes are present in South African waters – *M. africana* (as described by Müller and Troschel, 1842) and *M. rarispina*, already described by Perrier (1875) as *Asterias rarispina* and placed in the genus *Marthasterias* by Verrill (1914). *M. africana* was first described from a single specimen by Müller and Troschel (1842) who acknowledge that in describing the species, a live specimen had never actually been figured or fully described. Perrier (1875) first described *A. rarispina* as having an extraordinarily bare dorsal surface, with only 10-12 spines. He noted that the spines were less stout and sharper than those of *M. glacialis* (Perrier, 1875), and that many superomarginal plates had no spines at all, especially the basal half of each arm.

Clark (1923a) provides the first key to identify all three *Marthasterias* species, as they were then known. All have monacanthid adambulacral spines, and both *M. glacialis* and *M. africana* have large slender pedicellariae of the adambulacral furrows, the length 3-4 times the thickness (Clark, 1923a). *M. rarispina* is described as having large, stout adambulacral furrow pedicellariae, the length being twice the thickness (Clark, 1923a).

Mortensen (1933) described the two South African morphotypes as varieties of *M. glacialis*. The *M. rarispina* form did not seem to grow as big as the European *M. glacialis*, instead only attaining half the average size. *M. africana* was noted as having irregularly arranged spines, while the *rarispina* form only had three series of main dorsal spines. However, despite these morphological differences, Mortensen (1933) also noted the presence of intermediates, suggesting that the two morphotypes cannot rank higher than 'variety - *M. glacialis* var. *africana* and *M. glacialis* var. *rarispina*.

Fisher (1940) changes the classification again to *M. glacialis* forma *rarispina* and *M. glacialis* forma *africana*. This is due to the fact that there are small but consistent differences between the size of the crossed pedicellariae between the European and South African specimens. However, these morphological traits did not provide strong enough evidence to completely separate the South African forms separate from the European. However, Fisher (1940) only compared 11 specimens, hardly a large enough sample size to account for any intermediate morphotypes present. Despite this, Fisher (1940) went on to hypothesise that eventually the South African species will be classified as *M. glacialis africana* (as from Perrier, 1875) with forma *rarispina*.

Clark (1974) provides a summarising description of *M. glacialis* forma *africana* from one specimen taken from False Bay. Ten proximal actinal plates were noted, each with a spine which simulates the presence of a third inferomarginal spine series, a trait unusual in asteroid sea-stars. Pedicellariae wreaths occur on the outer inferomarginal spines, wrapping well around and apparently fused to some of the inner spines as well. This trait is also present in the European *M. glacialis*. The carinal lateral spines have up to four spines per plate. The largest straight pedicellariae have broad rounded tips, sometimes with digits.

The most recent and thorough taxonomic description of South African *M. glacialis* is that of Clark and Courtman-Stock (1976). Here, the official titles remain *M. glacialis* forma *africana* and forma *rarispina* (Figure 1), with an apparent geographical separation of the forms – *rarispina* appears to be found offshore and eastwards of False Bay, while *africana* is found in shallow depths of Table Bay to the western Cape Peninsula, with outliers in False Bay. There also appears to be some colour variations, with the *africana* specimens being predominantly “orange with light and dark mottlings” and the *rarispina* forms being “mottled lilac and blue, and rarely brown”. Noted are the apparent ‘zig-zagging’ of the distal carinal plates in the *rarispina* form, with numerous slightly spaced blunt spines of moderate length all over. Some plates have more than one spine (*africana*), and few spines on the dorsolateral plates characterises forma *rarispina*. The pedicellariae are straight for both forms, tapering and rounded terminally.



Figure 1: Photograph composite of the two identified morphological forms of the South African *Marthasterias* group: forma *rarispina* (Fisher, 1940) with three distinct rows of spines on the cranial, superomarginal and inferomarginal plates and forma *africana* (Clark, 1974) with spines found on the cranial, superomarginal and inferomarginal plates as well as randomly distributed in-between

Most sea-star taxonomy has been conducted exclusively on morphological characteristics. However, O' Loughlin and Waters (2004) argue that the majority of defining morphological characteristics are based on historical preference and ease of access, and that these characteristics may have no phylogenetic value. Hart et al. (1997) produced one of the first molecular phylogenies for sea-stars, exposing the wealth of information DNA sequence analysis provides on phylogenetic relatedness. Thus, the importance of historical

morphological analysis is being eclipsed by the more objective line of evidence genetics provides in the classification of taxa.

It is clear that the two South African forms of *M. glacialis* can be separated from each other, as well as potentially from the European species, through morphological work. However, historical work is scarce and sample sizes used were small. Sampling may have been biased against any intermediate forms. Morphological work alone is thus not sufficient to disentangle this enigma, and genetic work is required to lend further line of evidence to the study.

This research seeks to resolve the classification of the *M. glacialis* group in South Africa, using both morphological and phylogenetic-based evidence to determine the most appropriate classification of the South African specimens. Work by Pérez-Portela (pers. com.) has shown that the South African *Marthasterias* group is genetically distinct from the European *M. glacialis*, essentially turning a century of morphological analysis on its head. However, the form from which the genetic evidence was taken is not known. There are thus multiple possible outcomes to this research: neither of the two morphs are the European *M. glacialis*; one of the morphs (most likely the *M. africana* morph due to its morphological similarity) is actually *M. glacialis*, but the *rarispinga* morph is a separate species; there may be one main species and two subspecies; or the *africana* and *rarispinga* forma are single variable South African species that is genetically separate to the European *M. glacialis*. The main question that will be asked in this work is whether the South African *Marthasterias* forms are two separate species in their own right, or one single South African species distinct from the European species.

Methods

Sampling

Sampling was conducted in April 2013 around the Cape Peninsula of south-western South Africa (Figure 2). Sea-stars were collected through SCUBA at 9-11 m depth at four localities between Simon's Town (34°11'S, 18°26' E) and the Twelve Apostles site (33°58' S, 18°21' E). At each site, divers collected the first individuals they came across, regardless of morphological form. Some sites had few *Marthasterias* individuals, and thus a smaller sample was collected from these sites. A total of 78 sea-stars were collected for morphological analysis - 30 were collected from Simon's Town, 20 from Smitswinkel, 19 from Oudekraal and nine from the Twelve Apostles site. For genetic analysis, a subsample ($n = 12$) of *africana*, *rarisipina* and intermediate-looking specimens were taken from the Simon's Town's sample. Both morphotypes were present at Simon's Town, and time constraints meant only a few individuals could be analysed genetically. Tube feet were removed from each of these 12 animals with forceps and immediately fixed in absolute ethanol and preserved at -20°C until processing.

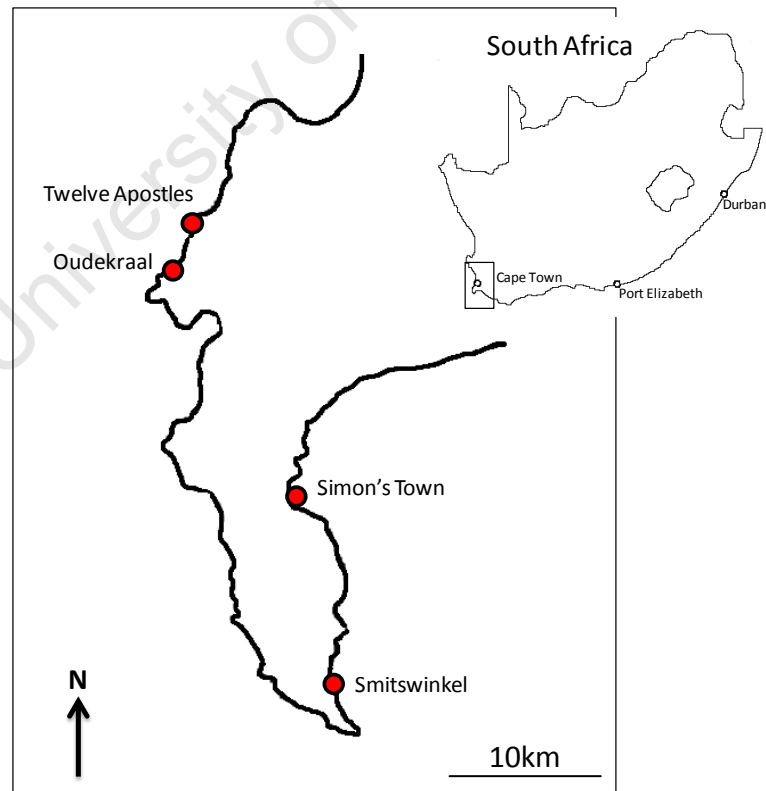


Figure 2: The Cape Peninsula, showing its geographic position on the South African coastline. Sampling was conducted at four localities around the peninsula as indicated by the red dots

Genetic Analysis

Using the same methodology as Pérez-Portela et al. (2010), total DNA was extracted from the collected tube feet samples using a RED Extract-N-Amp kit (Sigma–Aldrich, www.sigma.com).

A fragment of the COI gene was amplified and sequenced with the specific primers MgCOI_F 5' TCTCATATTTGGAGCTTGAG 3' and MgCOI_R 5'TAGGTGTTGAAAGAGAATGG 3' (Pérez-Portela et al., 2010). The first nuclear internal transcribed spacer (ITS1) was amplified and sequenced with the primers: ITS1 5' TCC GTA GGT GAA CCT GCG G 3' and ITS 2 5' GCT GCG TTC TTC ATC GAT GC 3' as described in White et al. (1990). PCR amplification reactions were performed in a 20 µl total-reaction volume with 10 µl of REDExtract-N-ampl PCR reaction mix (Sigma–Aldrich), 0.8µl of each primer (10 µM), 4.4 µl of ultrapure water (Sigma–Aldrich) and 4 µl of DNA template. A single step at 94⁰C for 7 min was followed by 35 cycles (94⁰C for 30 s, annealing at 48⁰C for 30s for the COI and a touchdown from 68⁰C to 55⁰C for the ITS1, and extension at 72⁰C for 35 s), and a final extension at 72⁰C for 7 min on a thermal cycler (BioRad Mycycler, www.biorad.com). The same primers were used for the sequencing reaction, and the PCR products were purified, and sequenced with an ABI Big-Dye Ready-Reaction Perkin Elmer kit on an ABI Prism 377XL automated sequencer (Scientific and Technical Services of the University of Barcelona). All the sequences were edited and aligned using Bioedit Sequence Alignment Editor (Hall, 1999) and Clustal X, and the alignments confirmed by eye.

Phylogenetic analyses were separately performed for both markers by computing NJ trees based on p-distance and Kimura 2-parameter (K2p). Bootstrap analyses (1000 replicates) were used to assess the robustness of the nodes, and sequences of *Asterias forbesi* were included as an out-group.

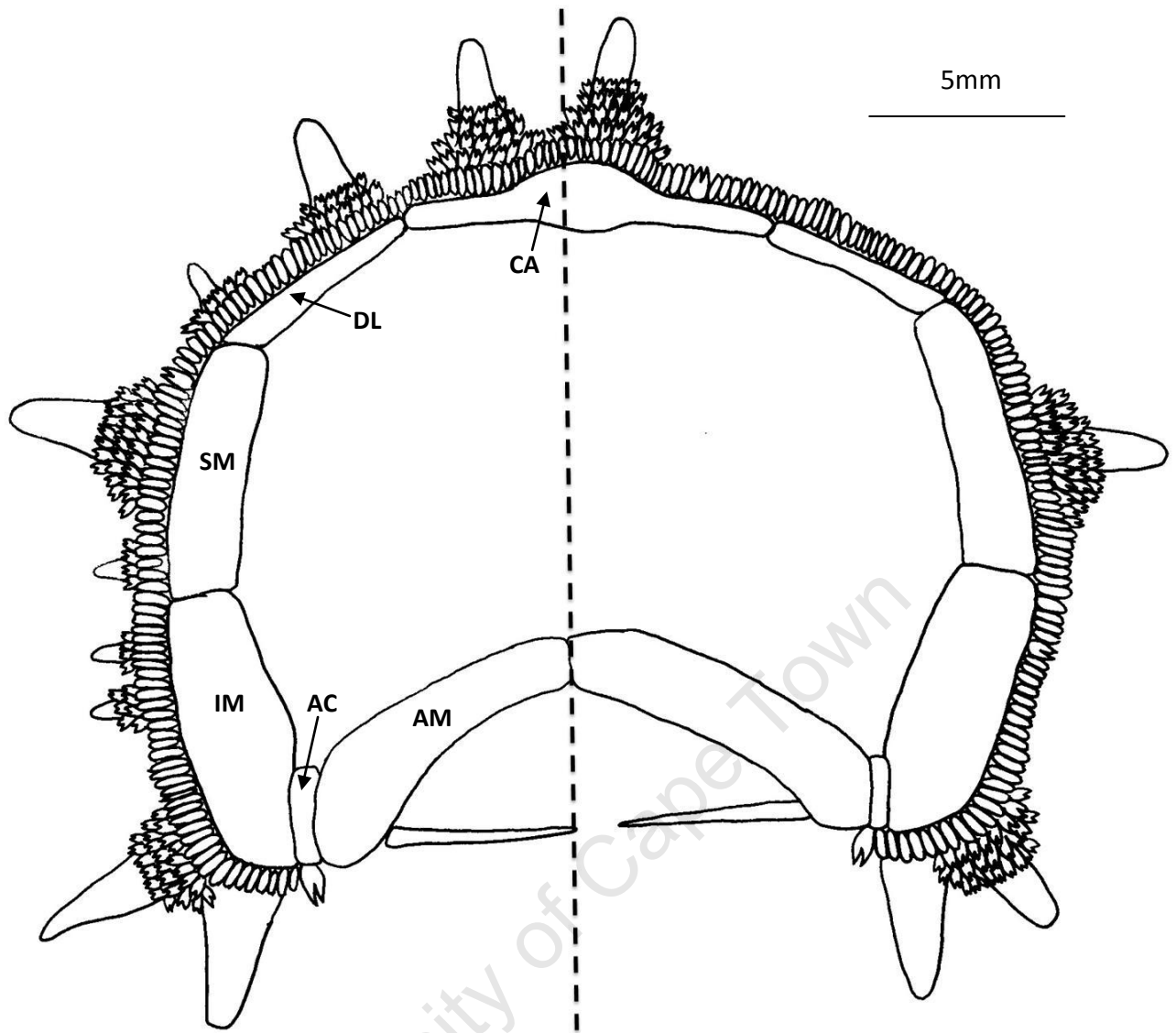
Morphological Analysis

Photographs were taken of the dorsal and ventral side of each specimen. Each individual starfish was examined macroscopically and under a dissecting microscope and, in order to determine whether lineage separation could also be distinguished morphologically, measured and scored based on a characteristics table (Table 1). This characteristics table was constructed using known distinguishing features for asteroid species (Clark and Coutman-Stock, 1976; O'Loughlin and Waters, 2004). Quantitative measurements were taken with Vernier callipers (mm) and analysed in ratios to control for the effect of size. These included measurements of the length of the arm relative to that of the disc. Measurements of the disc were taken from the leading pentagonal edge following from the madreporite. The longest arm was measured to ensure arm loss and subsequent regrowth did not influence the measurements. Arm length was measured from the pentagonal edge closest to the madreporite to the start of the eye-spot. The ratio of arm diameter and that of the adambulacral funnel was also included, with arm diameter measured three-quarters up the arm from the eye-spot tip. The width of the adambulacral groove was measured in proportion at the same position. An estimate of the depth of pedicellariae around the dorsal spines was taken and the spines on the leading edge of the disc following the madreporite were counted.

A number of qualitative measurements were also incorporated into the analysis, in the form of categorical data (Table 1). Colour was assessed on the basis of four colours (orange, brown, red and blue/grey). The location of spines was noted as being randomly distributed all over the body, only on the carnial, superomarginal and inferomarginal plates or intermediately distributed (Figure 3). Inferomarginal spines were categorised as monacanthid, bicanthid or tricanthid. The number of spines per plate was categorised as single or multiple and the number of rows of adambulacral plate spines was also assessed as having one or two rows. Pedicellariae shape and distribution of both large and small pedicellariae visible were assessed (Figure 4), as were the location of the pedicellariae on the inferomarginal spines.

Table 1: The 13 characteristics used for morphological analysis of South African collected *Mathasterias* individuals

Characteristic	State
1 Colour	(1) Orange (2) Orange with dark bands (3) Brown (4) Brown with dark bands (5) Red (6) Red with dark bands (7) Blue/grey (8) Blue/grey with dark bands (9) White
2 Arm to disc length (mm)	_____
3 Radius of arm to radius of groove (mm)	_____
Spines	
4 Number of Spines	_____
5 Spine location	(0) Only on the dorsal, superomarginal, inferomarginal plates (1) A few in between (2) Everywhere (on and in between plates)
6 Number of Spines per plate	(0) Single (1) Multiple
7 Inferomarginal Spines	(0) One row (1) Two rows (2) Three rows
8 Adambulacral plate spines	(0) One layer (1) Double layer
Tube Feet	
9 Number of rows	(0) Two (1) Four
Pedicellariae	
10 Shape	Large: (0) None (1) Straight (2) Crossed Small: (0) None (1) Straight (2) Crossed
11 Number (depth around carnial spines)	_____
12 Distribution	(0) Conspicuous between spines (1) Not conspicuous between spines
13 Presence on inferomarginal spines	(0) Everywhere (1) Outer wreathed, none on inner spines (2) Outer wreathed, a few large on inner spines



M. glacialis forma africana (Clark, 1974)

M. glacialis forma rarispina (Fisher, 1940)

Figure 3: Cross sectional structural form of a *Marthasterias* arm, showing the positioning of the plates and spines for each form where CA = carnial plate; DL = dorsolateral plate; SM = superomarginal plate; IM = inferomarginal plate; AC = actinal plate; AM = adambulacral plate

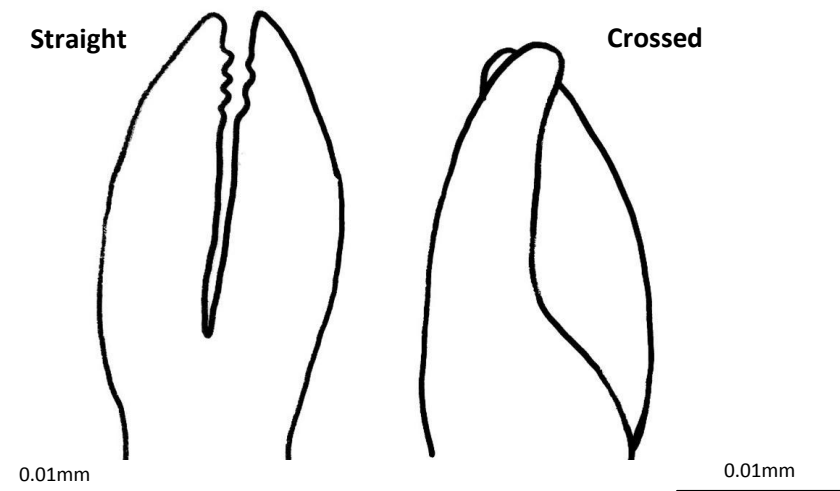


Figure 4: Two distinct pedicellariae types that characterise the South African *Marthasterias* (36X magnification)

Statistical Analysis

The morphological characteristics measured for both the Simon's Town samples (being the site from which all genetic samples were taken) and the samples from all the sites around the Peninsula were analysed using PRIMER version 6 (Clark and Gorley, 2006). Morphological characteristics that produced a variance of 0 were removed. Both qualitative and quantitative characteristic values were pre-treated through the standardisation of the variables based on their maximum value. A resemblance matrix was created for the samples based on Euclidean distance, which assigns equal weighting to all characteristics measured. Multidimensional scaling (MDS) tests were used to construct ordination plots, and cluster analyses were used to assess whether the morphological analysis revealed any distinct clustering of traits. SIMPROF tests were conducted to determine significance between the clustered samples. These MDS plots were used to visually assess any differences in distinguishing morphological traits, namely, if any groupings were formed based on the characteristics measured (Edkins et al., 2007). The factor of "Site" was tested for to see if there was distinct clustering of sample morphology based on location. ANOSIM tests were conducted on the data based on this factor to determine if any groupings of data were significant or random.

Results

Genetic Analysis

a) Mitochondrial COI Sequence

A p-distance value of 4% revealed distinct lineages in the NJ tree analysis separating the South African *Mathasterias* samples and the two European *M. glacialis* lineages (Figure 5). There is a clear break from the sequences of the out-group *Asterias forbesi*. The single South African *Mathasterias* clade was monophyletic and strongly supported by a bootstrap value of 95%. There was no distinct separation of the *africana* and *rarisipina* forms within the South African clade in this genetic analysis.

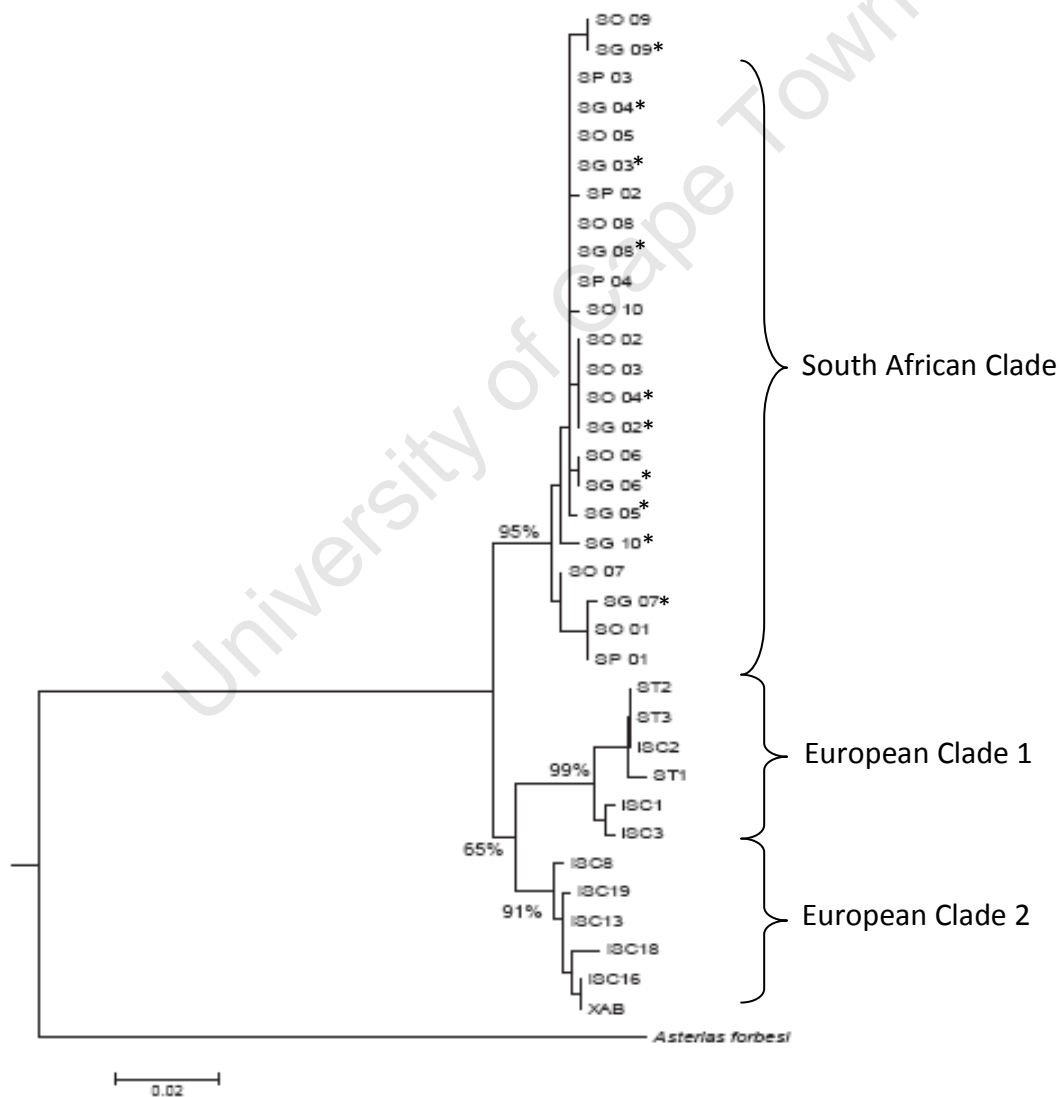


Figure 5: NJ tree based on p-distance showing the relative genetic divergence of the mitochondrial COI sequence of the South African and European *Marthasterias* samples with an outgroup genus *Asterias* (* indicate the position of the *africana* form)

b) Nuclear ITS1 Sequence

There is a clear separation of the South African and European lineages evident in the NJ tree analysis of the nuclear sequence (Figure 6), with a p-distance separation value of 3%. Bootstrap sequences support these results at a level of 59% and 92% respectively. The outgroup *Asterias forbesi* showed a clear separation from the *Marthasterias* genus. The South African *Marthasterias* lineage was monophyletic and no distinct separation of the *africana* and *rarisipina* forms within the single South African clade exist based on this analysis.

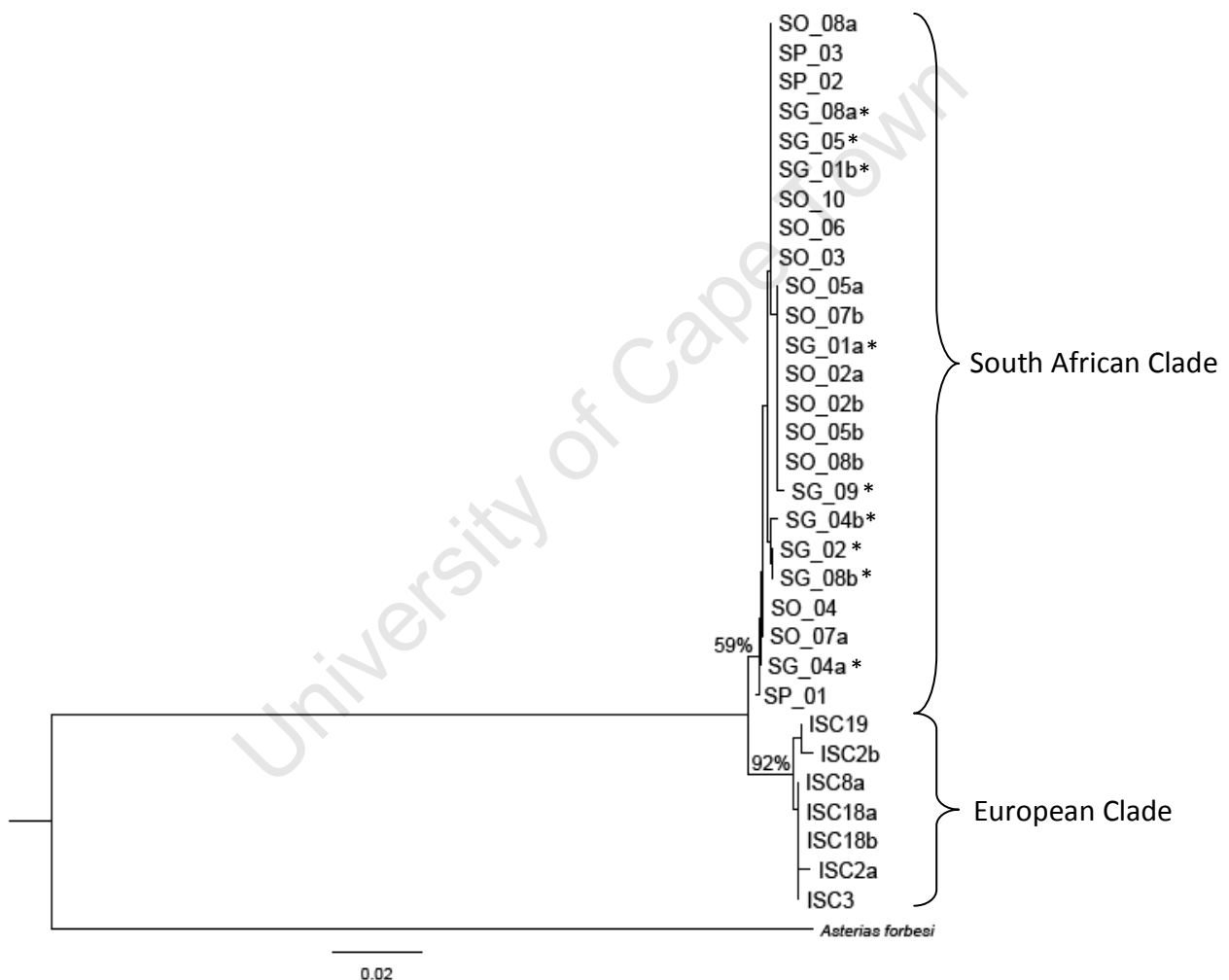


Figure 6: NJ tree based on p-distance showing the relative genetic divergence of the nuclear ITS1 sequence of the South African and European *Marthasterias* samples with an outgroup genus *Asterias* (* indicate the position of the *africana* form). The “a” and “b” codes indicate that there were two alleles within the same individual

Morphological Analysis

a) Simon's Town Samples

An MDS ordination plot of the morphological data for the Simon's Town samples showed some apparently clustering of samples (Figure 7), but the associated dendrogram and SIMPROF plot (Figure 8) showed that this clustering of samples was not significant.

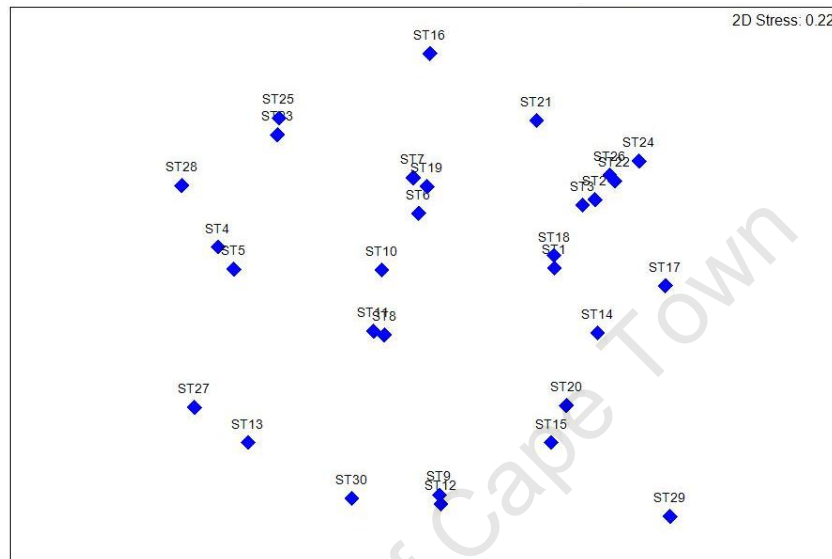


Figure 7: MDS ordination plot for Simon's Town samples constructed for morphological characteristics measured. Standardise variables by maximum; Euclidean distance (2D stress = 0.22)

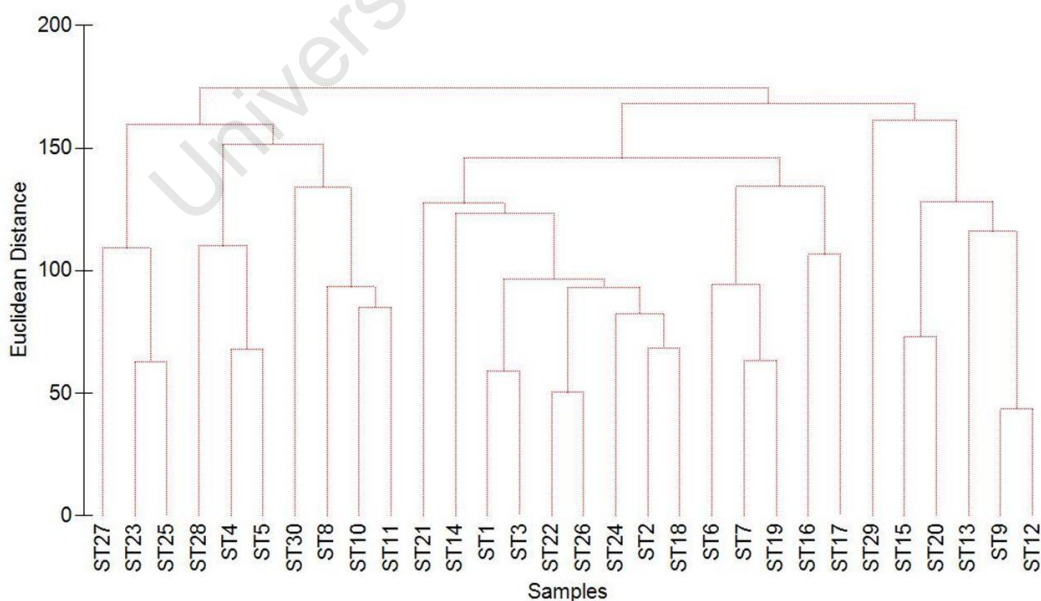


Figure 8: SIMPROF cluster analysis for Simon's Town samples based on group average, showing the degree of similarity (Euclidean Distance) for measured morphological characteristics. The single colour of the branches indicates that no significant differences were found between the samples

b) All Samples

An MDS ordination plot of the morphological data for all the South African *Marthasterias* individuals sampled showed high levels of overlap between the samples, with some outliers (Figure 9). The associated dendrogram and SIMPROF plot (Appendix C, Fig. 1) showed no significant clustering of samples.

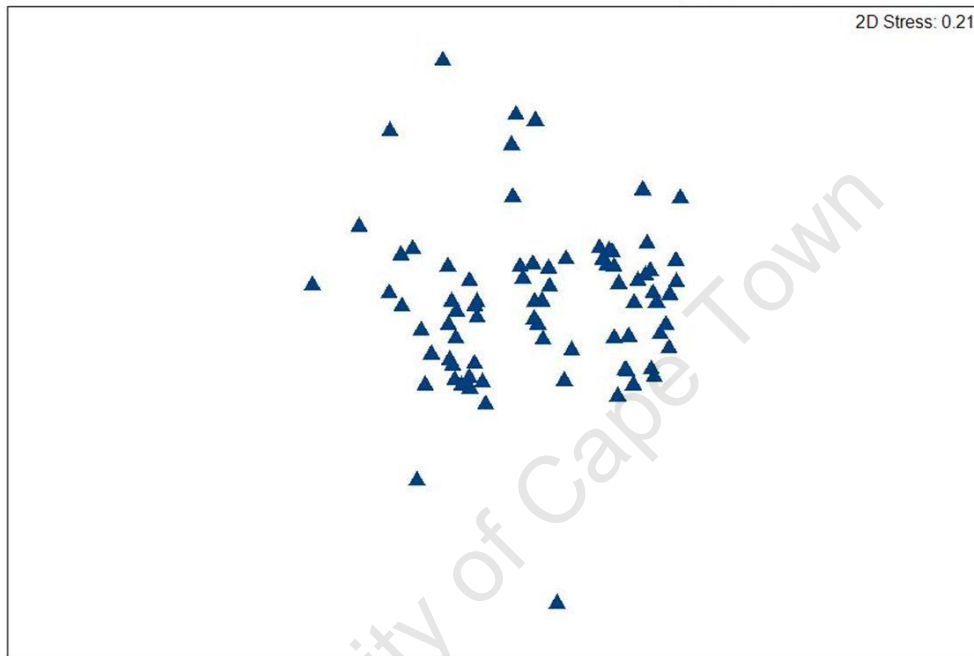


Figure 9: MDS ordination plot for all collected South African samples constructed from morphological characteristics measured. Standardise variables by maximum; Euclidean distance (2D stress = 0.21)

A high degree of overlap was observed between the morphological traits of all samples collected at the different sites around the Cape Peninsula (Figure 10). No significant morphological groupings were revealed specific to location (Appendix C, Fig. 2) although some outliers were noted. This was confirmed by the ANOSIM test results ($R = 0.094$, $p = 0.07$).

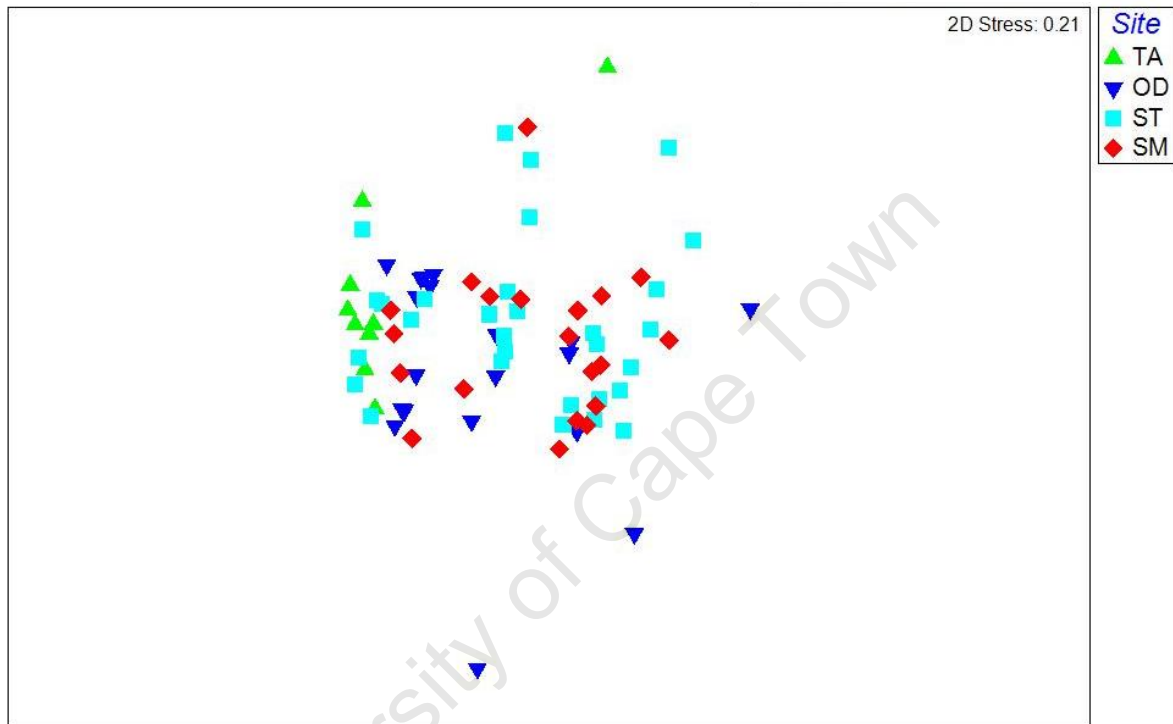


Figure 10: MDS ordination plot for all collected South African samples constructed from morphological characteristics measured. The different geometric symbols indicate the samples defined by the factor “Site”, where TA = the Twelve Apostles site; OD = Oudekraal; ST = Simon’s Town and SM = Smitswinkel. Standardise variables by maximum; Euclidean distance (2D stress = 0.21)

Discussion

The aim of this study was to resolve the century-old debate around the taxonomic classification of the well known South African spiny sea-star, *Marthasterias "glacialis"*, and specifically to test whether the two morphotypes found in South African waters are two separate species or not.

The genetic analysis of *Marthasterias* individuals from around the Cape Peninsula, South Africa, reveals that the *africana* (Clark, 1974) and *rarispina* (Fisher, 1940) forms are genetically indistinguishable. Despite the diversity in colour, spine arrangement and size, the genetic results of both the mitochondrial and nuclear sequence tests show a monophyletic grouping, indicating the presence of a single South African *Marthasterias* species. The genetic work was based on two sequences widely used in phylogenetics and taxonomy, and thus can be accepted as accurate, especially since it confirms work already conducted by Pérez-Portela (unpublished). The mitochondrial CO1 gene has been used successfully in sea-star genetics by Smith et al. (1990) and Waters and Roy (2003), while the ITS nuclear sequence has been employed by Colgan et al. (2005). The mitochondrial CO1 gene sequence is used as a universal eukaryote "barcode of life", because the mutation rate of the gene is fast enough to distinguish closely related species (Dawnay, 2007). The ITS region is widely used in taxonomy and molecular phylogeny because it is easy to amplify, even from small quantities of DNA (due to the high copy number of rRNA genes), and it has a high degree of variation even between closely related species (Chen et al., 2001).

The morphological results overall show no significant clustering of samples, indicating that the traits measured did not separate out the forms into distinct species, but rather represent a gradation of form. Although the genetic results came from collections from a single site, the morphological analysis looking at all specimens across the four sites provided no evidence for the existence of separate species. This may be accounted for by the close proximity of the sites, as well as the similar conditions found at each site. This may also be attributed to the generalist nature of *Marthasterias* – it is a predator capable of exploiting a wide range of prey resources (Tuya and Duarte, 2012).

Some outliers were detected in the analysis, and can be explained by human error in terms of mistaking certain "novel" traits for common, already described features. For example, the

outliers in the analysis of samples from all the sites were individuals who had been mistakenly identified as having a third row of inferomarginal spines. Rather than being an entirely novel trait, this trait is due to the presence of an actinal spine in large individuals that mimics the presence of a third row of inferomarginal spines, as previously identified by Clark (1974).

This study has also confirmed that this South African species is genetically distinct from the European *M. glacialis* and thus, should be described and named as a new species. Currently, the taxonomic classification of the South African *Marthasterias* sea-star is as two “forms”, *africana* (Clark, 1974) and *rarispina* (Fisher, 1940). This work provides the morphological and genetic evidence required to amalgamate the two forms into one and raise the current *forma* to species level. The name proposed is *Marthasterias africana* sp. nov., raising the old name to species level and hence requiring the use of the existing “*africana*”. This proposed new South African species *M. africana* sp. nov. will be formally described elsewhere.

As noted by Fisher (1940), there are also small but consistent differences in morphology between the European and the South African *Marthasterias* that allows their separation as different species. A predominant difference between the two species is locality, *M. glacialis* being found in the north-east Atlantic, from north Finland across the Mediterranean basin and the Adriatic Sea to the Guinean Gulf. In contrast, *M. africana* sp. nov. has only been found in the South Western portion of Southern Africa.

The colour pattern of *M. glacialis* is variable, from grey through green, to yellow-red; the spines are usually white, sometimes with purple tips. *M. africana* sp. nov. is predominantly orange, but may vary from red, brown or blue to white. It may have dark circular bands on the rays perpendicular to the direction of the ray. Spines can be bright orange to red and brown. Previous literature has identified size differences between the European and South African *Marthasterias*, such as that the *rarispina* form did not seem to grow as big as the European *M. glacialis*, instead only attaining half the average size (Mortensen, 1933). This study, however, has found that both the *africana* and *rarispina* forms have a diverse range of sizes, from a diameter of 64 mm up to 186 mm, attaining an average diameter of specimens collected here of 120 mm. Although the European *M. glacialis* can grow larger, Clark and Downey (1992) confirm that the largest specimens attain a diameter of 700 mm,

the average diameter of 250-300 mm is notably smaller than the average size of *M. africana* sp. nov. as measured by this study.

Furthermore, a distinction can be drawn between the two species based on the distribution of spine armament. *M. glacialis* has numerous slightly spaced blunt dorsolateral and cranial spines arranged in three regular longiseries down each ray, with one spine per plate (Figure 11). In contrast, the spine armament patterns of *M. africana* sp. nov. range from an extraordinarily bare surface with spines only on the carnial and superomarginal plates, to many irregularly scattered spines, often with multiple spines to each plate. The inferomarginal plate for both species has two spines. However, large *M. africana* sp. nov. may have a spine of the actinal plate which simulates the presence of a third inferomarginal spine series (as noted by Clark, 1974). There are also differences between *M. glacialis* and *M. africana* sp. nov. based on pedicellariae distribution. Both species has pedicellariae that wreath around the other inferomarginal spine, but some *M. africana* sp. nov. specimens also have large pedicellariae present on the innermost spines as well.



Figure 11: European *M. glacialis* dorsal view. Note the regular longitudinal series of spines along the carnial, superomarginal and inferomarginal plates. A few scattered spines are present on the dorsolateral plates. A key feature of *M. glacialis* is the presence of only one spine per plate (no scale available)

The unusual distribution of these two *Marthasterias* species, in similar temperate environments in different hemispheres, leads to questions about how these species diverged geographically. Observed patterns of diversity reflect the influence, both historical and contemporary, of ecological, genetic, behavioural, climate and tectonic processes (Benzie, 1999) and it is the interaction of these processes that drive population differentiation in marine species (Lessios et al., 2001 and Hedgecock et al., 2007). It is well established that *M. glacialis* is a broadcast spawner, its planktonic larvae allowing for a long dispersal capability (Pérez-Portela et al., 2010). The planktonic larvae remain in the water column for more than three months, passing through several larval stages until metamorphosis occurs with the development of adhesive structures allowing the larvae to attach to a substrate (Barker and Nichols, 1983). Therefore, it is possible that the planktonic larvae of *M. glacialis* found their way out of the Mediterranean and down the western coast of Africa to southern-most western Africa, which has very similar conditions to the Mediterranean. This is likely given reports (Plos, pers. com.) that species were found at 20 m depth at Walvis Bay (22°57'S, 14°30' E).

There are other species who share this strange distribution of occurring in temperate seas in both hemispheres, and nowhere else. For example, *Ecklonia* kelps are “antitropical” (Hubbs, 1952) species that occur only in cooler temperate seas (Steneck et al., 2002). *Ecklonia* are hypothesised to have initially evolved in the northern hemisphere, and colonised southwards across the tropics during historical climatic events that presented “corridors” of cooler waters across the tropics, allowing their movement south (Lindberg, 1991). The same may have occurred with these two *Marthasterias* species, and there may be fossil evidence of their movement south along the western African coast. This enormous area has been severely under-sampled, and if sampled, may potentially reveal further *Marthasterias* species. For example, a south Angolan *Marthasterias* has been recorded by Clark and Downey (1992). A more intensive genetic analysis of specimens from different sites around the entire South African coast is needed to distinguish whether unique clades exist within the *M. africana* sp. nov. group as has been identified with *M. glacialis* by Pérez-Portela (2010). Lindberg (1991) shows that the process of vicariance in the Pacific Ocean (the separation of organisms by a physical barrier) is not supported by available geologic and paleontological evidence, and instead that bi-hemisphere, “antitropical” (Hubbs, 1952)

distributions are based on biotic interchange. These events, such as changes in current direction and strength, impact the dispersal of planktonic larvae, allowing the dispersal of an individual over thousands of kilometres (Lindberg, 1991). The timing of these events (such as during Pleistocene glaciations) suggests several breaches (both northward and southward) of the tropics, rather than a single event.

Physical and historical factors that act on a population's connectivity may hinder potential long-distance movements of a species. In this case, the development of the Benguela upwelling system off the west southern African coast in the Late Miocene and the Pliocene (Marlow et al., 2000) resulted in a "blockage" of the southward movement of organisms. It is therefore hypothesised that the arrival of *Marthasterias* in South Africa occurred prior to this event. Further phylogenetic work is required to determine when the divergence between the *M. glacialis* and *M. africana* sp. nov. lineages occurred. It is hypothesised that the original population of *Marthasterias* occurred in European waters, and a portion of that population consequently moved south to the current location in South Africa. The original population is expected to have a broader distribution and higher levels of diversity than any subsequently distributed population. Both these traits are exemplified in this case. The distribution of *M. africana* sp. nov. is relatively limited compared to that of the European *M. glacialis*, ranging from East London and the Agulhas Bank, around the Cape Peninsula and Table Bay and perhaps even further north. One specimen has been described from Natal by Müller and Troschel (1842), but has been flagged as dubious by Clark and Courtman-Stock (1976). In contrast, *M. glacialis* is found over a huge range of the north-east Atlantic, from north Finland across the Mediterranean basin and the Adriatic Sea to the Guinean Gulf.

Work by Pérez-Portela (unpublished) has shown that there are two distinct lineages of *M. glacialis* in European waters, the second of which still remains to be described and named, whilst this study has shown that the South African *Marthasterias* represents only one lineage. The mitochondrial COI tree showed two distinct European lineages due to population isolation as a result of Pleistocene glaciations that allowed the two different lineages evolved independently. The populations were reconnected after the glaciations, and both European lineages already formed overlapped within the Mediterranean (Pérez-Portela, unpublished). Since mitochondrial material is maternally inherited and does not recombine, two distinct European clades were noted. However, the nuclear ITS1 tree does

not separate out two European lineages because of the reconnection of the European populations (Pérez-Portela, unpublished), while the South African clade was separated out easily due to large differences in nuclear markers resulting from reproductive isolation.

Clear signals emerge from this work that allow the amalgamation of the two South African morphotypes into one species, *M. africana* sp. nov., and include it as a separate species in the subgenus *Marthasterias* alongside the European *M. glacialis* lineages.

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Appendix A Technical Glossary

Table 1: Technical glossary of terms relating to sea-star taxonomy and structure

Term	Definition
Adambulacral	Relating to, or involving the ventral furrow where the tube feet are found. Includes plates, spines
Superomarginal	Relating to, or involving the upper sides, below the cranial plates and jointed to the inferomarginal plates. Includes plates and spines
Inferomarginal	Relating to, or involving the lower sides generally above the adambulacral and actinal plates, joining the superomarginal plates. Includes spines, plates
Monacanthid	Spine from plate does not split, only one spine visible
Actinal	Relating to, or involving area between the inferomarginal and adambulacral areas.
Carnial	Relating to, or involving the area on the upper dorsal side
Dorsolateral	Relating to, or involving both top and side. Includes plates and spines
Basal	End or towards the end
Distal	Furthest from the centre disc/body; usually refers to a position along the rays
Proximal	Closest to the central disc
Pedicellariae	Minute, pincer-like structures used for cleaning of surface
Rays	“Arms”, pentagonal in cross section
Madreporite	Circular structure on the dorsal side of the disc/body; part of the vascular system
Ocella	Photosensitive eye-spot tips at the end of each arm

Appendix B Morphological Features

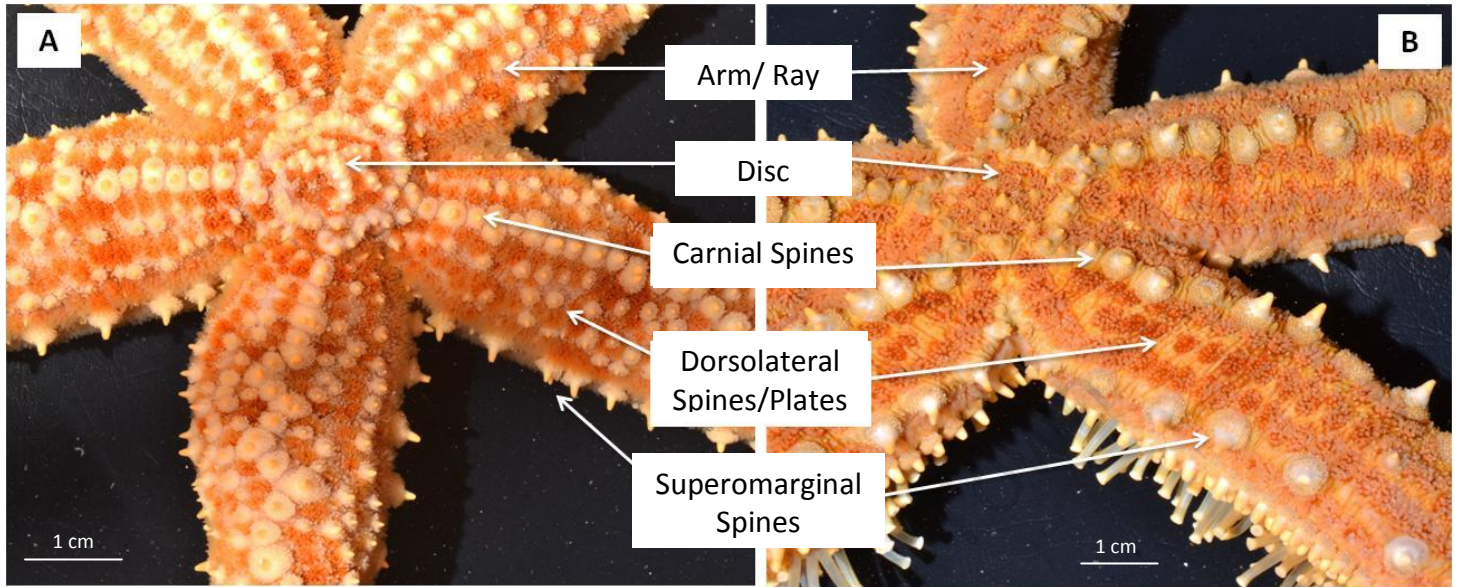


Figure 1: Photographs of the dorsal side of (A) *M. glacialis* forma *africana* (Fisher, 1940) and (B) *M. glacialis* forma *rarispinga* (Fisher, 1940) indicating the location of key morphological features

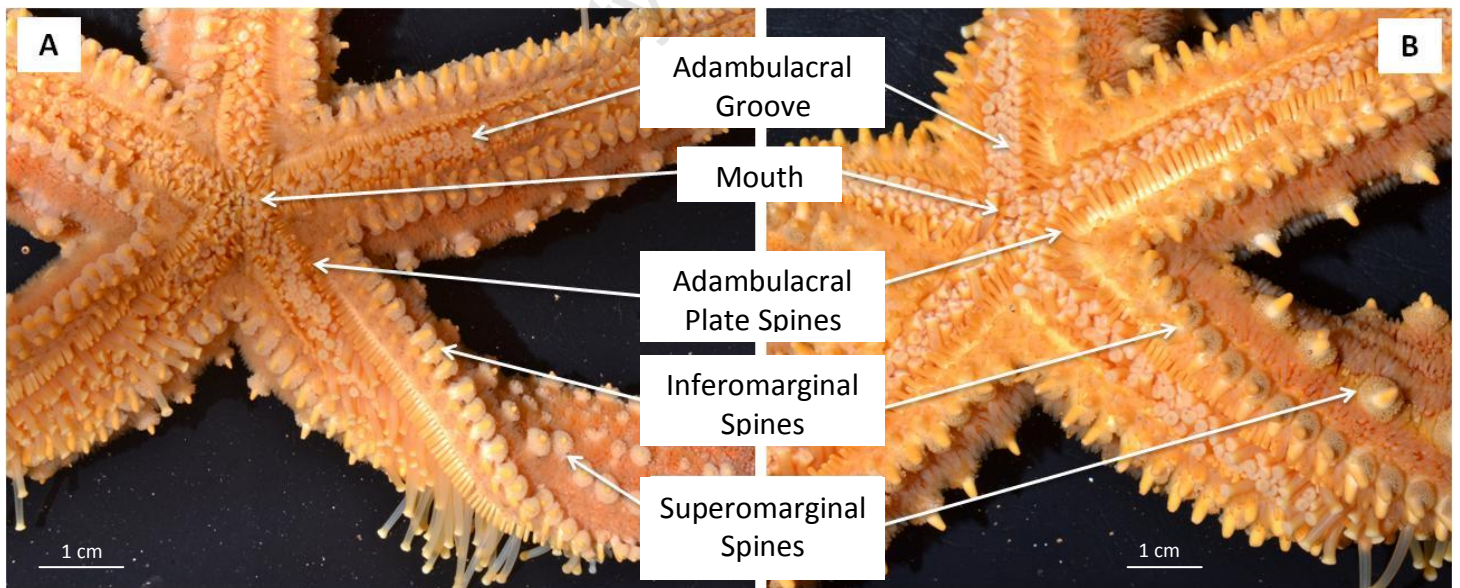


Figure 2: Photographs of the ventral side of (A) *M. glacialis* forma *africana* (Fisher, 1940) and (B) *M. glacialis* forma *rarispinga* (Fisher, 1940) indicating the location of key morphological features

