Distribution and phylogenetic inference within the flightless spring katydids (Tettigoniidae: *Brinckiella* Chopard, 1955) in the Greater Cape Floristic Region

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ABSTRACT

The genus *Brinckiella* is an endemic group from the Greater Cape Floristic region (GCFR) with several undescribed species. Some of them are of conservation concern, categorized by IUCN as Endangered (EN) and Vulnerable (VU) due to their restricted distribution caused by livestock overgrazing, agriculture, and urbanization. However, data are still missing to fully assess their distribution pattern and conservation status. Moreover, although the phylogeny of katydids was recently inferred, *Brinckiella* was not included in that analysis. Thus, its closest relatives and evolutionary history are still unknown. This study aimed to investigate the distribution and phylogenetic inference within the genus *Brinckiella*, map the distribution of its species, delimit the species through taxonomy and genetics, and elucidate the phylogenetic relationship and divergence time within the genus. Specimens were collected by hand and sweep netting. Phylogenetic inference was done using the COI gene and analysed under Maximum likelihood and Bayesian inference. This study identified 13 morphospecies of *Brinckiella*, of which five are new, undescribed species. The genus is currently distributed in 27 vegetation types in three biomes, and apart from the Fynbos and Succulent Karoo biomes, the genus also occurs in the Azonal vegetation biome. Fynbos had the highest number of species of *Brinckiella*, which is likely related to the vast diversity of plant that they are associated with and probably feed on. In the Succulent Karoo the distribution of *Brinckiella* might be associated with seasonal plants that occur in the spring and at the beginning of summer. The genus may be monophyletic, and it split from the common ancestor shared with *Holochlora fruhstorferi* Carl, 1914 about 14.2 Mya, and it split again from the common ancestor shared with *Isophya major* Brunner von Wattenwyl, 1878, *Phaneroptera gracilis* Burmeister, 1838 and *Kuwayamaea chinensis* (Brunner von Wattenwyl, 1878) at 10.16 Mya and diverged in the late Miocene around 8.79 Mya, producing two main clades (A and B). Clade A diverged 7.85 Mya and is composed by two closely related species: *B. aptera* and *B. mauerbergerorum,* while Clade B diverged 7.24 Mya and gave rise to two lineages (L1 and L2). *Brinckiella wilsoni* constitutes L1, and L2 split later in the early Pliocene around 4.74 Mya, and is represented by two sister species, *B. arboricola* and *B.* sp. n. 5.

Keywords: *Brinckiella*, Flightless spring katydids, distribution, COI, Phylogeny, Fynbos, Succulent Karoo, Azonal vegetation

1. INTRODUCTION

Diversity within the Greater Cape Floristic Region (GCFR)

Global patterns of species distributions result from the interactions of many factors, including climatic, edaphic, topographic, land use, inter- and intraspecific interactions and evolutionary factors (Gaston, 2000; Criddle *et al*., 2003). These factors are also responsible for spatial heterogeneity in species diversity and endemism in certain areas (Scarano *et al*., 2021). The Greater Cape Floristic Region (GCFR) in South Africa is one of the most diverse plant regions in the world (Rutherford *et al*., 2006; Bergh *et al*., 2014; Bradshaw and Cowling, 2014). The high plant species diversity, turnover, and endemism are attributed to the relatively stable climate and geology after the establishment of the Mediterranean climate at the beginning of the Pliocene (Suc, 1984; Moll, 1990; Chen *et al*., 2014; McCourt, 2016). Furthermore, a constant local climatic variation and frequent orographic rainfall are caused by mountains, while edaphic diversity is derived from the mosaic of soils blended by local gradients of precipitation. These factors created conditions for an extraordinary variety of habitats and ecological gradients with unique flora and fauna (Moll, 1990; Goldblatt, 1997). The GCFR comprises two main biodiversity hotspots: the Cape Floristic Region (CFR) and Succulent Karoo (Myers *et al*., 2000; Snijman, 2013; Bergh *et al*., 2014).

The CFR is located at the southwestern tip of Africa (Goldblatt and Manning, 2002; Liu *et al*., 2020) and is one of the five biodiversity hotspots in the Mediterranean climate and one of the only two hotspots that comprise an entire floral Kingdom, the other being New Caledonia (Cowling and Pierce, 2004). It is characterized by a warm and temperate climate with winter rainfall. The winter season is characterized by cyclonic fronts and north-westerly winds that bring plentiful rain. Summer is hot, influenced by a high frequency of Southeast winds, and the mean annual precipitation (MAP) is 480 mm (Bradshaw and Cowling, 2014).

With approximately 9,000 plant species, of which 80% are endemic, the hotspot is dominated by the unique flora that corresponds to the large Fynbos Biome, characterized by an exceptional diversity of Ericaceae, Proteaceae, Restionaceae, Rutaceae and Iridaceae (Cowling and Pierce, 2004; Manning, 2018). These occur in the three major vegetation types, the renosterveld,

strandveld and fynbos (Rebelo *et al*., 2006; Bergh *et al*., 2014). In contrast to the high plant diversity, the CFR has low diversity and endemism of vertebrates, with only 127 native mammals, of which four are endemic, and 324 species of birds, of which 22 are endemic. The reptiles are moderately diverse, with 100 species and 22 endemics. The diversity of amphibians is relatively low, with 51 native species and 16 endemics and, lastly, fish with 34 native species and 14 endemics (Rundel and Cowling, 2013b).

The Succulent Karoo is one of only two arid regions in the world considered a true biodiversity hotspot due to the high diversity and endemism of succulent plant species, the other being the Horn of Africa (Desmet and Cowling, 2004). It is composed of two bioregions, the western Namaqualand–Namib from Vanrhynsdorp all along the west coast to Southern Namibia in Lüderitz, and the Southern Karoo from Hantam, Tanqua, Roggeveld and eastwards into the Little Karoo (Rutherford *et al*., 2006; Allsopp *et al*., 2014). It is characterized by extremely arid summers with temperatures above 40ºC and winters with low rainfall. The average annual rainfall varies from 20 to 290 mm, resulting from cyclonic rains. The region is dominated by the Berg winds that bring masses of hot and desiccant air (Mucina *et al*., 2006). The region has 6,356 recorded species of plants, with the highest diversity of succulent dwarf shrubs among the families Crassulaceae, Asteraceae, Asparagaceae, Aizoaceae, Euphorbiaceae, Orchidaceae, Liliaceae and other geophytes, of which 40% are endemic (Desmet and Cowling, 2004; Scholtz *et al*., 2021). The diversity and endemism of vertebrates are low, with roughly 75 mammal species and two endemics, while reptiles are moderately high with about 90 species, 15 endemic. The amphibians are the least diverse group with approximately 20 species (one endemic species), and 26 indigenous freshwater fish species (Desmet and Cowling, 2004).

Insect diversity in the GCFR

Although the plant diversity is well known in both hotspots, the diversity of invertebrates, mainly insects, were considered to be poor (Goldblatt, 1997; Giliomee, 2003). The latter concluded that the richness of herbivorous insects in CFR is relatively lower than endophagous insects due to poor food sources for herbivores. Meanwhile, Kemp *et al* (2017) agreed that insect herbivore diversity is associated with plant distribution and is high at the local scales but not at the regional scales. For example, in the Cape Peninsula Region some insect species such as *Speleaiacris*

tabulae Peringuey, 1916, *Colophon westwoodi* Gray & Griffith, 1832, *Thestor yildizae* Koçak, 1983, *Poeciloblatta angusta* Saussure & Zehntner, 1895, *Dipteretrum brinckae* Princis, 1963, *Platylimnobia montana* Wood, 1952 and *Cephaleus ivyae* Davies, 1988 are endemic, similar to local plant diversity (Picker and Samways, 1996). Therefore, the CFR has higher diversity and endemism than other regions with similar environmental conditions (Procheş and Cowling, 2006; Kemp and Ellis, 2017).

The Succulent Karoo has a relatively high diversity of invertebrates and more than 50% of insect species are endemic, including exclusive pollinators of 75% of plant species. For example, monkey beetles (Hopliini), bee flies (Bombyliidae), long-tongued flies (Nemestrinidae) and pollen wasps (Masarinae) are among the common pollinators in this region (Colville *et al*., 2002; van Kleunen *et al*., 2007; Rundel and Cowling, 2013a; Scholtz *et al*., 2021). Additionally, frequent discoveries of new endemic species of insects in both CFR and SK suggests that both hotspots constitute a biological laboratory to study and understand the evolution of insects (Picker *et al*., 2002; Naskrecki and Bazelet, 2009; Bilton *et al*., 2015). This high diversity and endemism of insects and other invertebrates in CFR and Succulent Karoo are attributed in part to higher endemism of plants that certain insect groups co-evolved with, and localized radiation of certain groups of insects (Scholtz, Scholtz and Klerk, 2021). For example, a remarkable discovery of species radiation among the flightless spring katydids in the genus *Brinckiella* was made (Naskrecki and Bazelet, 2009).

Description of *Brinckiella*

The genus *Brinckiella* Chopard, 1955 belongs to the subfamily Phaneropterinae (Tettigoniidae: Orthoptera). It has eight species described and several undescribed ones (Naskrecki and Bazelet, 2009; Cigliano *et al*., 2022). They are differentiated from other Phaneropterinae by having a very small cylindrical body; a hypognathous head with the antennae longer than the body and globular to oval eyes; thorax with the pronotum with a smooth surface, without lateral carinae, with the metazona flat, the prosternum unarmed, and the sternum flat; the thoracic auditory spiracle are small, exposed, or slightly hidden under lateral lobes of the pronotum; legs are long to extremely long and slender, front coxa unarmed and the front femur are unarmed; wings are reduced in males,

generally shorter than pronotum (brachypterous) or absent (apterous) in females and the male of *B. aptera* (Chopard, 1955; Naskrecki and Bazelet, 2009).

Similar to other katydids, species of the genus *Brinckiella* produce sound by rubbing the scraper at the distal edge of the right forewing (tegmina) against the file, a modified vein covered with teeth on left tegmen (Xiao *et al*., 2013). *Brinckiella* species produce ultrasonic calls of 36 kHz and higher Naskrecki and Bazelet (2009), making them inaudible to humans as human hearing frequency ranges from 16Hz to 20 kHz (Møller and Pedersen, 2004). This genus is endemic to the Fynbos and Succulent Karoo in the Western and Northern Cape provinces. It is seasonal, reaching adulthood in September or October when many other Tettigoniidae of this region are still in their early nymphal or egg stages (Naskrecki and Bazelet, 2009). Some species are of conservation concern: *B. arboricola* is listed as Endangered (EN), while *B. karooensis*, *B. aptera* and *B. mauerbergerorum* are considered Vulnerable (VU) due to their restricted distribution, a consequence of habitat decline caused by livestock overgrazing, agriculture, and urbanization (Naskrecki and Bazelet, 2014). Thus, data are needed to assess the conservation status of *B. elegans*, *B. serricauda* and *B. viridis*.

Evolution of flightlessness

Species of the genus *Brinckiella* are brachypterous and apterous, which make them flightless. Indeed, the reduction of wings and flightlessness are common in Phaneropterinae of sub-Saharan and tropical Africa. These conditions are predominant in high-elevation areas, where all species of the genus *Atlasacris* Rehn, 1914, *Peropyrrhicia* Brunner von Wattenwyl, 1891, *Monticolaria* Sjöstedt, 1910, *Odonturoides* Ragge 1890, *Austrodontura* Fontana & Buzzetti, 2004, and some species of macropterous genera such as *Peronura* Karsch, 1889 and *Ducetia* Stål 1874 are flightless. However, *Brinckiella* may represent an independent origin of flightlessness in the Phaneropterinae as it differs from others in the complete absence of wings in the females and in the male of *B. aptera* (Naskrecki and Bazelet, 2009, 2011). Although the phylogeny of katydids was inferred by Mugleston *et al* (2013), the genus *Brinckiell*a was not included. Thus, its closest relatives are unknown, hence is crucial that its phylogeny should be studied.

Aims and objectives

Given that the genus *Brinckiella* is of conservation concern and that their distribution data are lacking to assess the conservation status of the group, and also that the phylogeny of the group is unknown, this thesis aims to:

1) Study the distribution of the genus *Brinckiella* by mapping the distribution of its species, and
2) To determine species delimitation through molecular data, elucidating the phylogenetic relationship and divergence within the genus.

2. METHODS

2.1. Study area

The fieldwork was conducted from September to December 2021 in two biodiversity hotspots: CFR and Succulent Karoo in the GCFR localized in the Western Cape and Northern Cape of South Africa. The choice of the sampling sites and collection period were based on the pilot study by Naskrecki & Bazelet (2009), who highlighted that the genus *Brinckiella* is endemic to these regions and likely seasonal, reaching their adult stage at the beginning of spring (September/October) (see Fig. 1 illustrating the study sites).

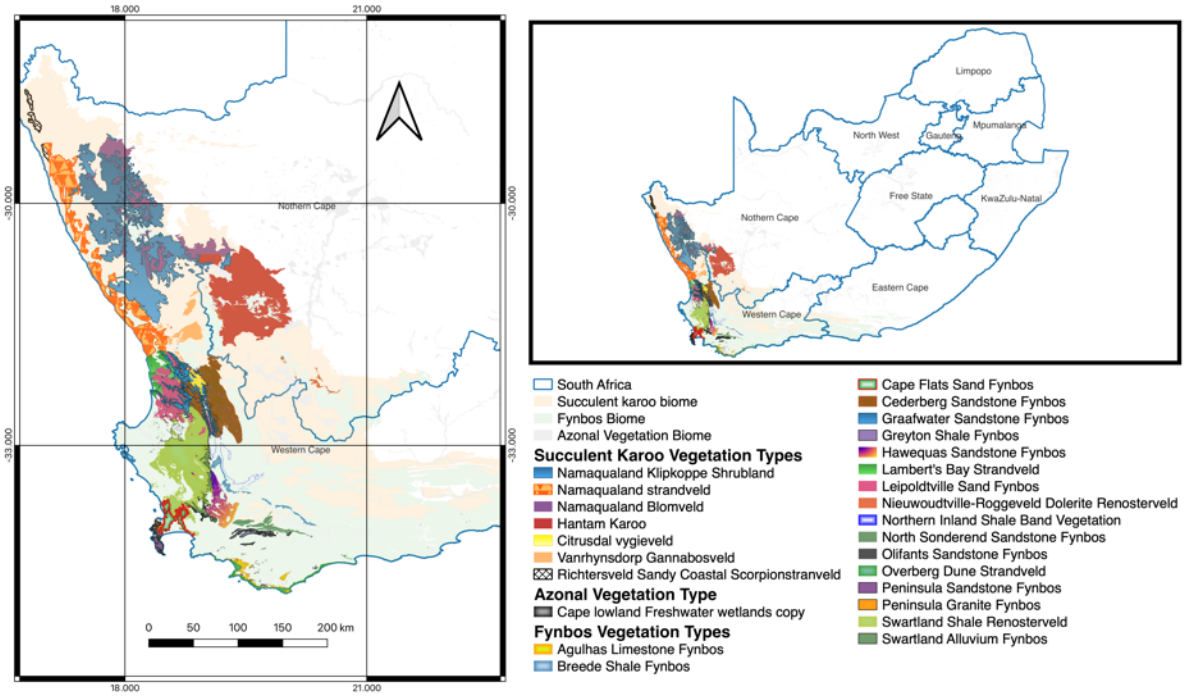

Figure 1. Maps indicating the vegetation types and biomes where species of the genus *Brinckiella* were collected

2.2. Data collection and species identification

The specimens were collected during the day and night, considering that species in the genus *Brinckiella* are active in both periods (Naskrecki and Bazelet, 2009). Two methods were employed

to collect them in the field: hand collecting and sweep netting. The methods were based on the biology and ecology of the group, mainly because these katydids are flightless with low dispersal capability, making them easy targets to catch by hand. Moreover, as the species are endemic to Fynbos and Succulent Karoo with several microhabitats, more than one collection method is needed (for example, in shrub vegetation with thorny, the sweeping net may not be efficient). Furthermore, the observation of these cryptic katydids in the field can be challenging since they are camouflaged among the host vegetation, hence the sweeping net is appropriate in this situation.

Species were identified morphologically using Naskrecki and Bazelet, (2009) identification key through the observation and comparison of the following characters: head, thorax, legs, wings, and abdomen (subgenital plate, ovipositor and cercus).

All specimens were preserved in 96% ethanol as the *Brinckiella* has soft and fragile body , but also to keep the tissue for molecular studies (Schauff, 1986; Gabrys et al., 2008). The voucher specimens will be deposited at Iziko South African Museum, Cape Town, South Africa. In addition to the specimens collected in this study, other specimens from several South African entomological collections were included for species distribution analysis (P. Naskrecki, unpublished data).

2.3. DNA extraction, amplification and sequencing

Only fresh specimens collected in 2021 were used for molecular analysis. DNA was extracted from 37 specimens representing nine *Brinckiella* morphospecies. All extraction were done at the University of Cape Town. Three individuals per species were processed by removing muscle tissue from one of the hind legs using the E.Z.N. A® Tissue DNA extraction kit (Omega Bio-tek, Norcross, GA, USA; Hemp et al., 2009; Guta, Macamo and Naskrecki, 2021). The extraction was performed following the kit manufacturer's protocol with slight modification based on the weight of the hind leg samples, which on average was 6 mg per specimen (protocol in appendix I).

The amplification of cytochrome oxidase subunit I (COI) was performed using the universal primers LCO1490/ HCO2198 that amplifies~658 base pairs (bp) (Folmer *et al*., 1994). The total volume of 20 µL for each specimen was acquired mixing together 10 µL of DreamTaq Green PCR Master Mix (2X) (ThermoFisher Scientific, Johannesburg, RSA), 2 µL forward primer and 2µL reverse primer, 4 µL of water and 2 µL of DNA. A negative control containing all essential

components of the amplification reaction except the template DNA were included to detect potential contamination of the reagents with foreign DNA. The PCR reaction was performed in a 96-Well PCR Thermal Cycler under the following temperature conditions: denaturation at 95 °C for 3 min; 35 cycles of 95 °C for 30 s; annealing at 55 °C for 30s, initial extension at 72 °C for 1 min and final extension at 72 °C for 10 min (Hemp et al., 2009; Mugleston et al., 2013).

The quality and quantity of PCR products were tested in a 1.8 percent agarose gel with ethidium bromide visualized under UV light. The PCR products were purified by adding 6.67 units Exonuclease I (ThermoFisher Scientific) and 0.67 units FastAP™ Thermosensitive Alkaline Phosphatase (ThermoFisher Scientific) to 10 µl of PCR product from the PCR and placed on 96-Well PCR Thermal Cycler under the following temperature conditions: 37°C for 15 min and 85°C for 15 min (modified from Werle et al., 1994; Thermo Fisher Scientific, 2012). The amplicons were sequenced in both directions (forward and reverse) using a BigDye Terminator V3.1 kit (Applied Biosystems), using manufacturers protocol with slight modifications. The same primers used in the PCR were used in sequencing. Sequencing was done by the Central Analytical Facilities (CAF) at Stellenbosch University.

The selected outgroups species are relatively distant but share a common ancestor with the ingroup (Wheeler, 1990). The following species of the Phaneropterinae were used as outgroups: *H. fruhstorferi* (KX057733.1), *I. major* (NC_042666.1), *P. gracilis* (KY316379.1) and *K. chinensis* (KX057735.1). The outgroups' COI sequences were downloaded from GenBank (accession number in the brackets).

2.4. Sequence alignment

The sequences were viewed pairwise, edited (trimming and ambiguities solving) and aligned using Geneious Prime® 2022.1.1 This was followed by translation to check for possible contamination by nuclear mitochondrial pseudogenes (numts), which can be coamplified with orthologous mtDNA, providing a wrong phylogenetic analysis (Song et al., 2008; Goios, Carvalho and Amorim, 2009). With numts detected in some sequences, only uncontaminated ones were used for phylogenetic inferences. The multiple sequence alignment (MSA) of the ingroup (*Brinckiella*) and the outgroups were performed together in Geneious Prime software using the Geneious alignment algorithm (http://www.geneious.com). All sequences were trimmed to be in the same length of 643 bp (see the sequences in appendix 2).

2.3.Data analysis

2.3.1. Species distribution maps

The map of species distribution and habitat preference of the flightless spring katydids was prepared in QGIS version 3.24.3 (http://qgis.osgeo.org/), using the following data: a CSV file containing coordinate and species of flightless spring katydids, and the spatial data of vegetation of South Africa downloaded from South African National Biodiversity Institute (2012).

2.3.2. Phylogenetic relationship

The haplotype networks to analyse and visualize the relationships among sequences were performed in PopART version 1.7 software using only ingroup species (Fig. 8) (Leigh and Bryant, 2015). The general time-reversible, model, with gamma distribution, proportion of invariable sites (GTR+G+I) was estimated in MEGA 11 as the best-fit model (Table 2) (Nei and Kumar, 2000; Stecher, Tamura and Kumar, 2020; Tamura, Stecher and Kumar, 2021). Phylogenetic relationship based on COI gene was estimated using maximum likelihood (ML) performed in RaxML V 8.2.12, under bootstrap value of 1000 (Kozlov et al., 2018).

2.3.3. Divergence time estimation

The divergence time within the genus *Brinckiella* was estimated using the Bayesian inference (BI) method and GTR model, Yule model, Markov Chain Monte Carlo (MCMC) at the length of 10000000. As there is no fossil record, trees were calibrated using strict clock with 0.0168 clock rate (Papadopoulou, Anastasiou and Vogler, 2010). The posterior probability to test the success of Bayesian MCMC runs was analysed in Tracer v1.7.2 (Rambaut et al., 2018), maximum clade credibility (MCC) tree was constructed using the TreeAnnotator program after discarding 10% of the first trees as burn-in. The tree was afterward visualized and edited in FigTree v1.4.4 (Rambaut, 2018).

3. RESULTS

3.1. Species richness and distribution

This study resulted in the identification of 13 morphospecies based on 177 specimens: *B. wilsoni* Naskrecki & Bazelet, 2009, *B. mauerbergerorum* Naskrecki & Bazelet, 2009, *B. aptera* Naskrecki & Bazelet, 2009, *B. arboricola* Naskrecki & Bazelet, 2009, *B. elegans* Naskrecki & Bazelet, 2009, *B. karooensis* Naskrecki & Bazelet, 2009, *B. serricauda* Naskrecki & Bazelet, 2009, *B. viridis* Chopard, 1955, and several yet undescribed species, tentatively named *B.* sp. "*tabulae*", *B.* sp. n. 1, *B.* sp. n. 2, *B.* sp. n. 3 and *B.* sp. n. 4 (Table 1).

Distribution

The genus *Brinckiella* is currently distributed in 27 vegetation types in three biomes, revealing that apart from the Fynbos and Succulent Karoo biomes, the genus also occurs in the Azonal vegetation Biome. In the Fynbos biome, 11 species were found in 19 vegetation types, while seven species were found in the Succulent Karoo biome in seven vegetation types. In addition, one species, *B. wilsoni,* was found in the Azonal vegetation Biome in one vegetation type (Fig. 2, Table 1).

Brinckiella wilsoni is the most widely distributed species as it occurs in 13 different vegetation types in all three biomes, with 84.6% of the population found in Fynbos (Fig. 3A). *Brinckiella mauerbergerorum* is the second most widely distributed species, found in eight vegetation types, with 62.5% of the population found in the Succulent Karoo and 37.5% in Fynbos (Fig. 3B). *Brinckiella aptera* occurs in six vegetation types, with 66.8% of the population in the Fynbos and *B. arboricola* in three vegetation types *(*Figs 3C, 3D*)*. Among the more narrowly distributed species is *Brinckiella* sp. n. 1 (Fig. 3E), which occurs in four vegetation types, all in the Fynbos Biome, and *B. karooensis* (Fig. 3F) which occurs in four vegetation types in the Succulent Karoo Biome. The species with the narrowest distributions are *B. viridis* and *B. serricauda* (each in two vegetation types), *B. elegans, B* sp. *"tabulae", Brinckiella* sp. n. 2, *Brinckiella* sp. n. 3 and *Brinckiella* sp. n. 5 (each in one vegetation type*)* (Fig. 3G).

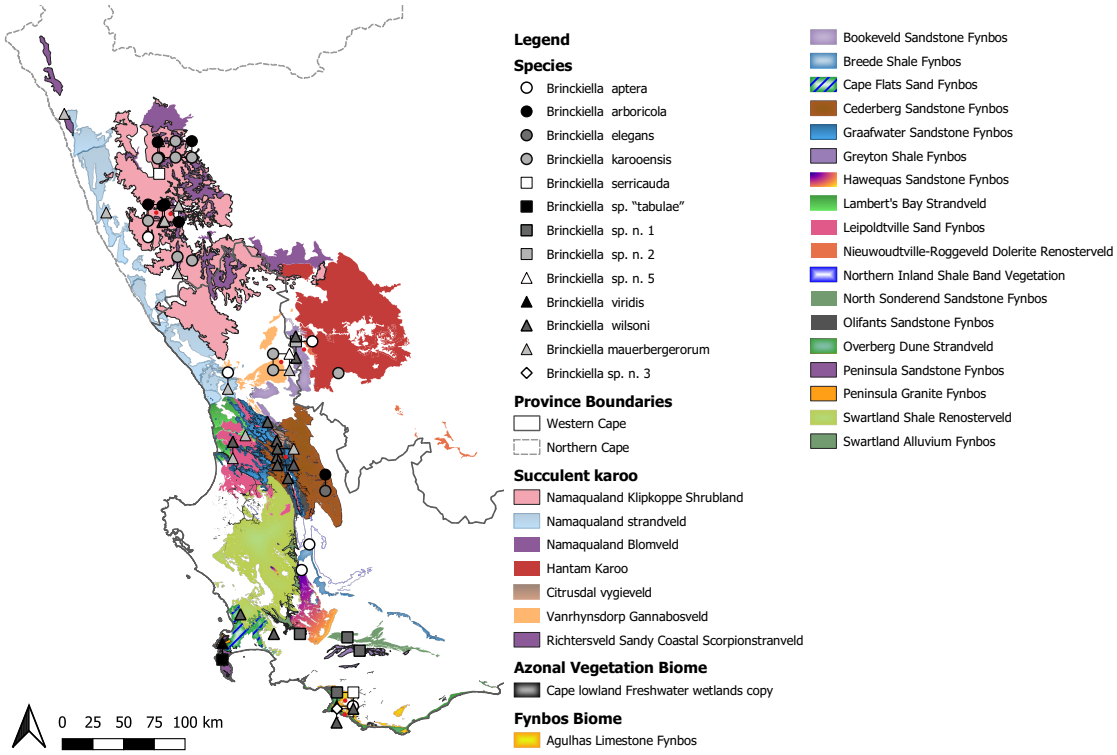

Figure 2. General distribution of the genus *Brinckiella* in Fynbos, Succulent Karoo, and Azonal Vegetation Biomes, in different vegetation types. The Fynbos Biome has more species of *Brinckiella*, found in 19 vegetation types, followed by the Succulent Karoo, with seven vegetation types and Azonal vegetation with only one.

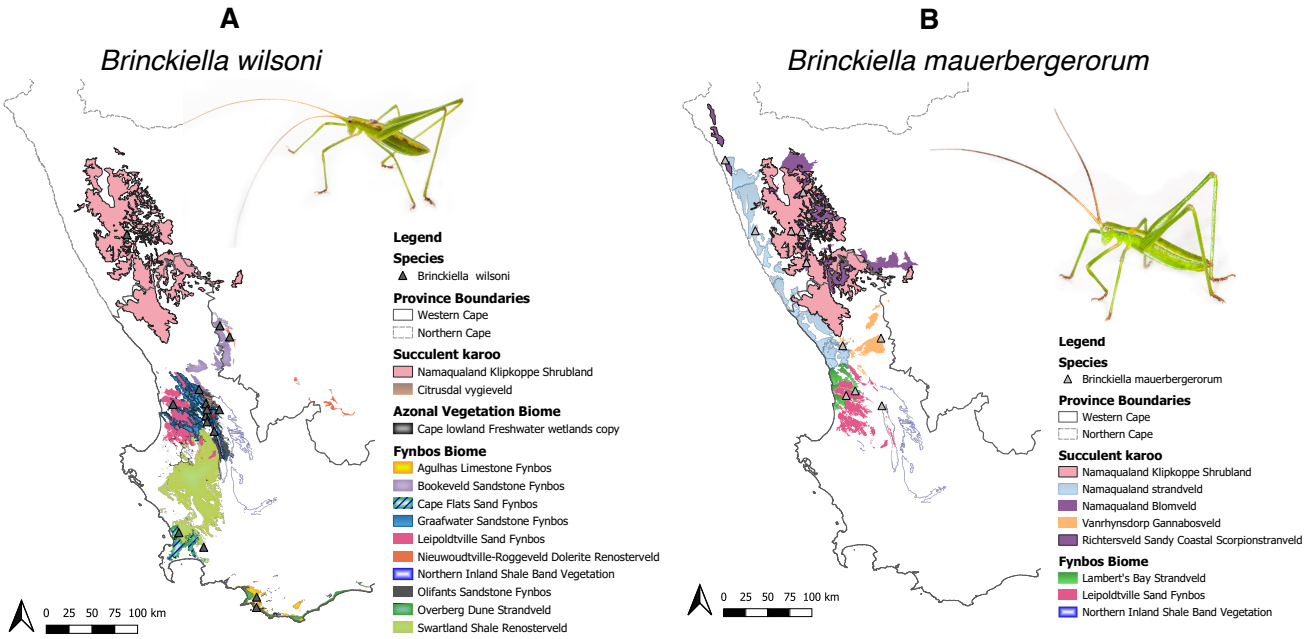

Figure 3A, 3B. Distribution of the first two widely distributed species across their vegetation type in GCFR. *Brinckiella wilsoni* (A) is the most widely distributed species, occurring in 13 vegetation types in Fynbos, Succulent karoo and Azonal vegetation Biome, while *B. mauerbergerorum* (B) is the most widely distributed species, found in eight vegetation types in Fynbos and Succulent Karoo.

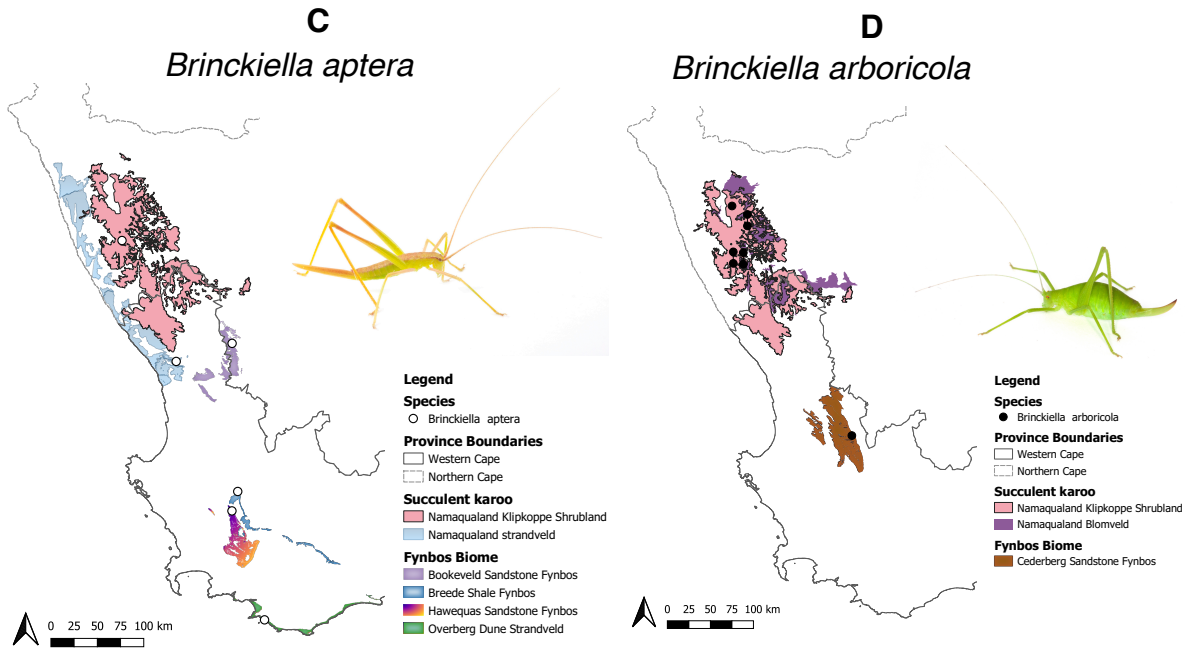

Figure 3C, 3D. Distribution of *B. aptera* which is found in six vegetation types and *B. arboricola* (D) found in three vegetation types in Fynbos and Succulent Karoo Biomes.

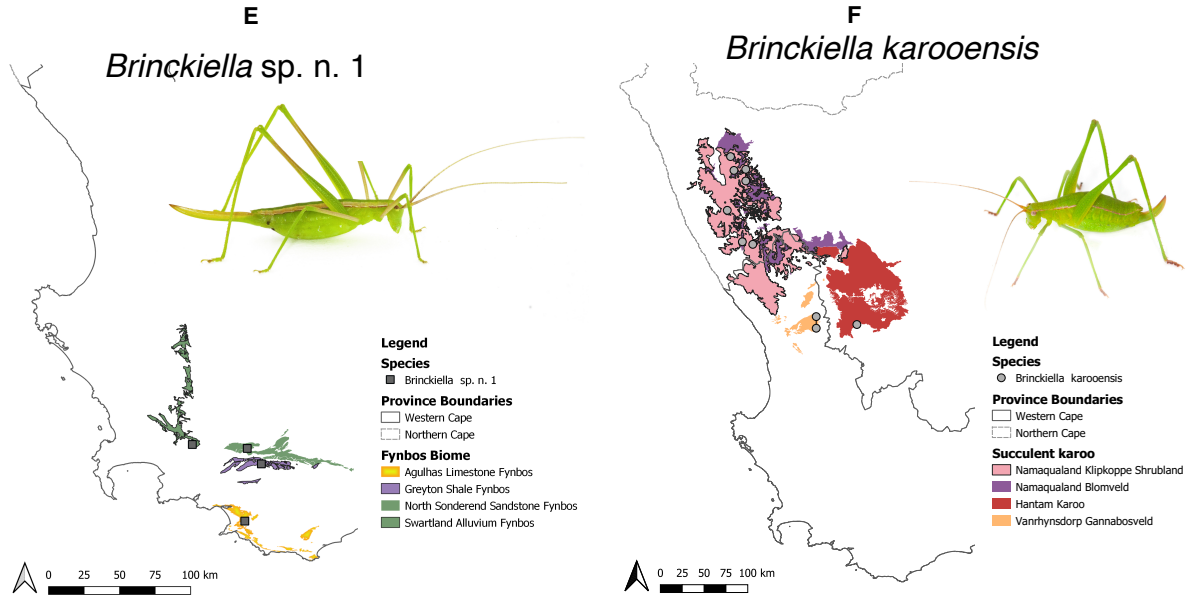

Figure 3E, 3F. Distribution of *B* sp. n. 1, with the entire population confined to four vegetation types of Fynbos and *B. karooensis* (F) restricted to four vegetation type of Succulent Karoo Biome.

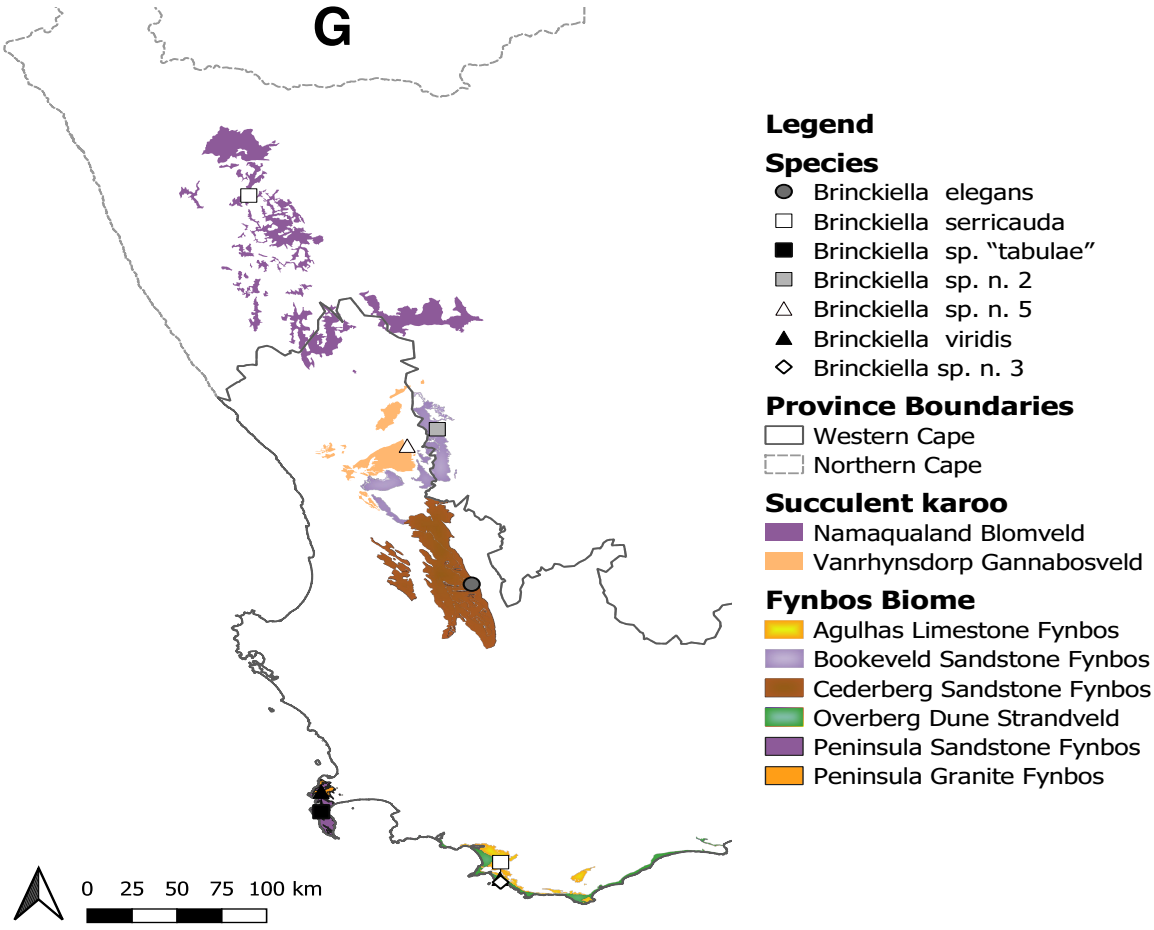

Figure 3G. The species with restricted distribution: *B. viridis* found in Overberg Dune Strandveld and Peninsula Granite Fynbos; *B. serricauda* in Agulhas Limestone Fynbos and Namaqualand Blomveld; *B. elegans* (Cederberg Sandstone Fynbos); *B* sp. "*tabulae*" (Peninsula Sandstone Fynbos); *B*. sp. n. 2 (Bookeveld Sandstone Fynbos); *B* sp. n. 3 (Agulhas Limestone Fynbos) and *B*. sp. n. 5 (Vanrhynsdorp Gannabosveld).

3.2. Phylogenetic relationship

Two different analyses, Maximum Likelihood (ML) and Bayesian inference (BI), produced similar tree topologies of the genus *Brinckiella*. Both ML and BI support a possible monophyly of the genus *Brinckiella* as all species analysed in this study, share the same ancestor and split into two main clades (A and B) (Fig 4-5). Clade A contains *B. aptera* and its sister species, *B. mauerbergerorum*. This clade, however, shows a very weak bootstrap support value of 24% using ML, making the sister arrangement of these two species tentative. However, the BI with 0.94 of posterior probability strongly supports that these two taxa are truly sister species. Clade B comprises three species, *B. wilsoni, B. arboricola,* and *B.* sp. n. 5 (65% bootstrap support and posterior probability of 1.0).

Both ML and BI trees complement the taxonomic identification. For example, although specimens B78 and B51 were previously identified in this study as *B. arboricola,* based on the morphologically features (head, thorax, legs, wings and abdomen), molecular data suggest that B78 is *B. mauerbergerorum* (posterior probability of 1.0 and bootstrap value of 100%) and B51 as a new species, tentatively named *B.* sp. n. 5 (posterior probability of 1.0 and bootstrap value 99%). Similarly, specimens B102 and B103 that were also identified as *B.* cf. *aptera* based on the morphological features, but molecular data placed them in *B. aptera* (posterior probability of 1.0 and bootstrap value of 100%) (Fig 4 and 5). The new morphospecies add five new species to the eight previously known ones and will be described in future work, using both morphological and molecular characters.

3.3. Divergence time

Results from the BI analyses show that the genus *Brinckiella* and the three outgroups represented in this study *Isophya major, Phaneroptera gracilis* and *Kuwayamaea chinensis* - split from the common ancestor shared with *Holochlora fruhstorferi* in the middle Miocene on the Langhian stage at about 14.2 million years ago (Mya). Moreover, around 10.16 Mya, the genus *Brinckiella* split again from the common ancestor shared with these three species, which was followed by diversification within the group in the late Miocene at the Tortonian age around 8.79 Mya, originating in two main clades A (two species) and clade B (three species) (posterior probability 1.0, Fig. 6). Clade A (*B. mauerbergerorum and B. aptera*) diverged 7.85 Mya (posterior probability 0.94), while Clade B diverged 7.24 Mya and gave rise to two lineages (L1 and L2). L1 is constituted by *B. wilsoni,* and L2 split later in the early Pliocene around 4.74 Mya, represented by two sister species, *B. arboricola* and *B.* sp. n. 5.

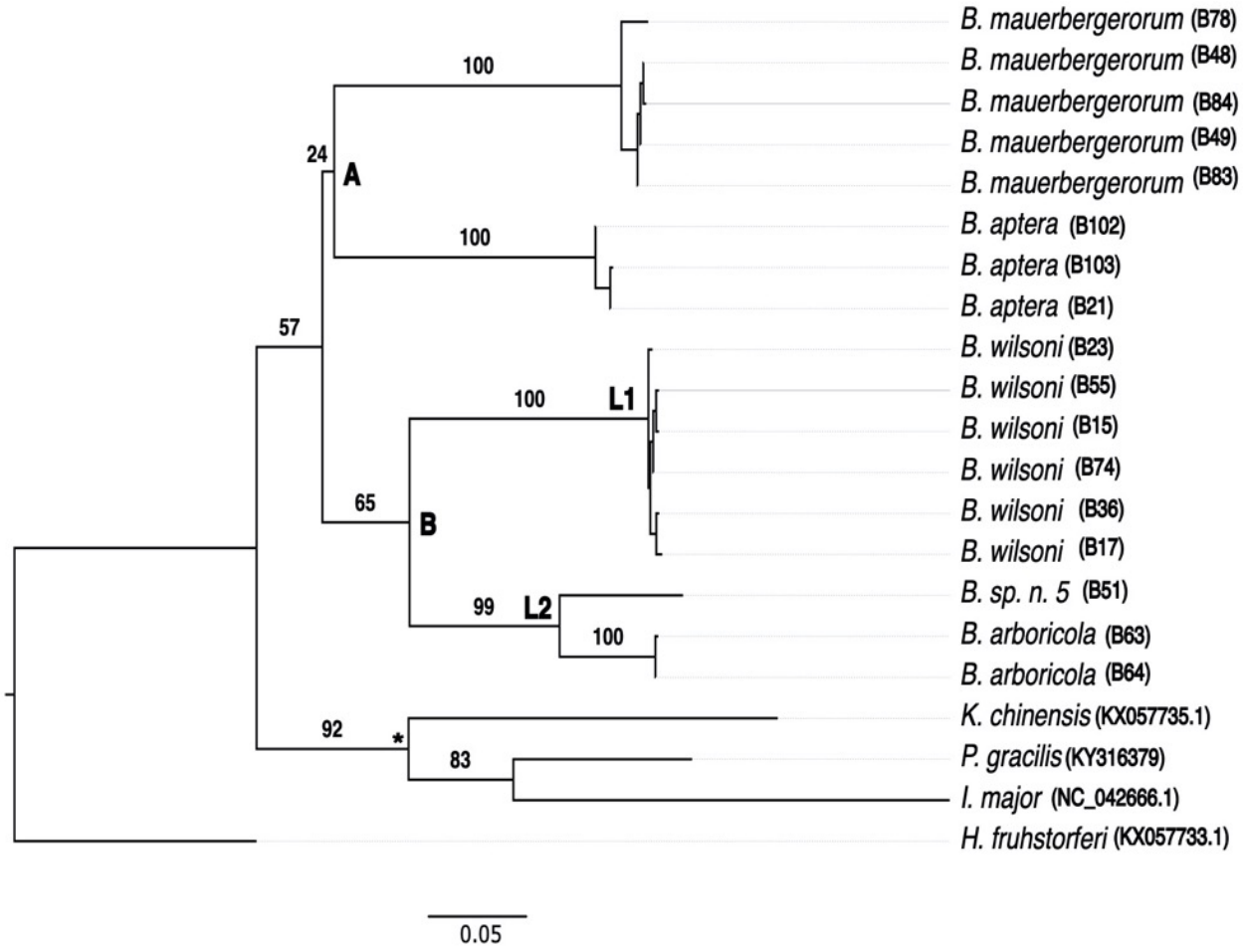

Figure 4. Phylogenetic relationship analyses of the genus *Brinckiella* based on COI gene inferred from Maximum Likelihood through GTR+G+I model. Bootstrap support values are shown above the branches, A and B on the nodes indicates the clades; L1 and L2 are lineages;* indicates the outgroups clade. Numbers shown after species names indicate the sample number.

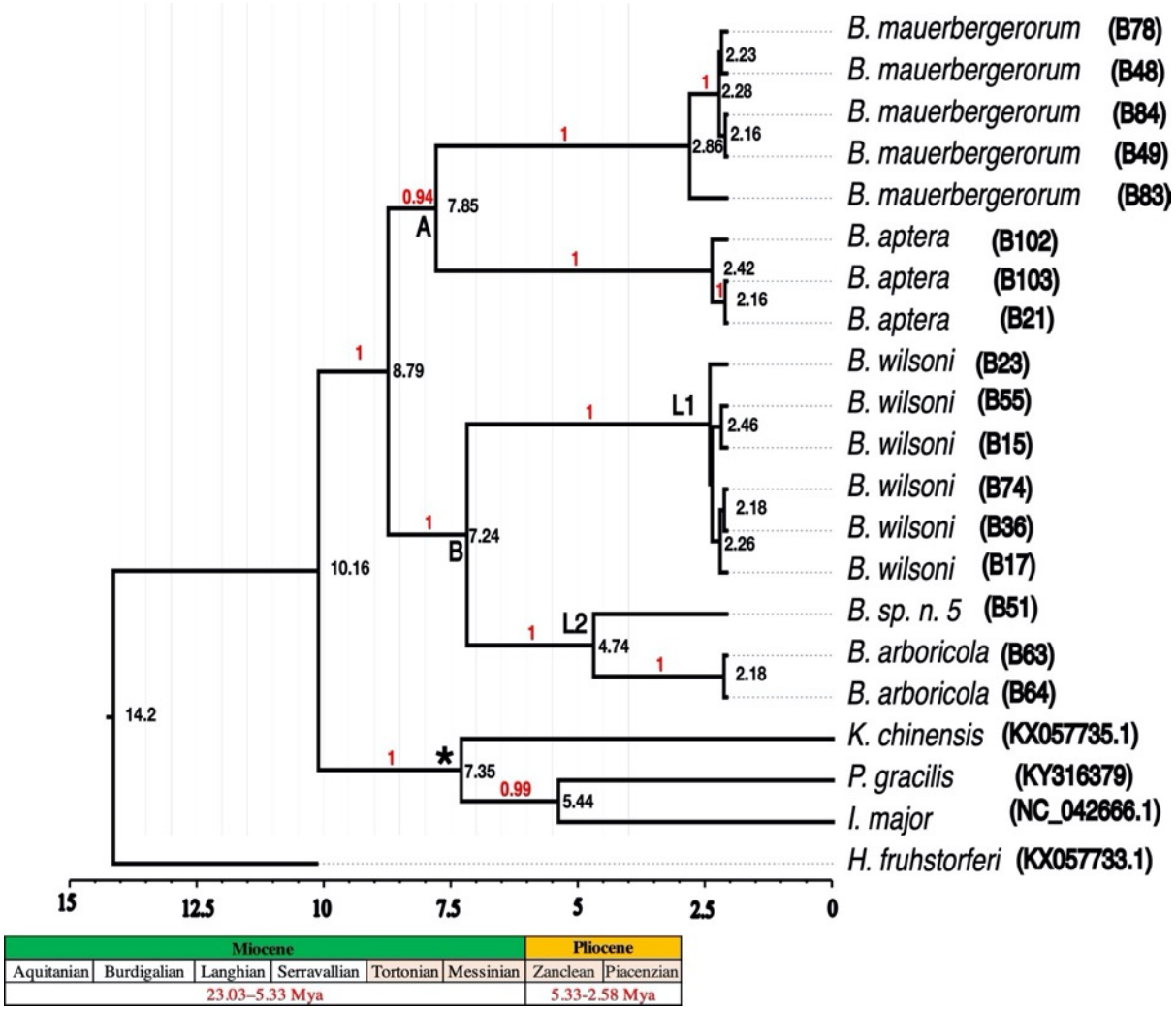

Figure 5. Divergence time estimation analyses of the genus *Brinckiella* based on COI gene inferred from BI. Numbers on red above the branches are posterior probability, on the nodes are the divergence time and in the brackets are the unique code of the specimens; * represents the outgroups clade.

4. DISCUSSION

4.1. Species richness

This study resulted in the identification of 13 morphospecies of the genus *Brinckiella*, which adds five new morphospecies to the eight previously described by (Chopard, 1955; Naskrecki and Bazelet, 2014). The five new species corroborate the predictions of Naskrecki and Bazelet (2009), who suggested the possibility of the existence of several undescribed species in this group. Previous species identification in this study, based on morphological features, such as the head, thorax, legs, wings, and abdomen (subgenital plate, ovipositor, and cercus), led to the misidentification of some specimens, such as B51 (female) and B78 (male) as *B. arboricola*. Also, B102 and B103 (females) were initially identified as *B* cf. *aptera*, since the females are completely wingless (Naskrecki and Bazelet, 2009) and hard to identify to the species level. Species misidentification is common for cryptic species, and it has several implications for evolutionary and biogeography analyses as well as conservation planning (Bickford *et al*., 2007). In many insects misidentification is common due to cryptic colouration and shapes, which may lead to the classification of several species as a single species (Schultz, 2018). In Orthoptera, females and juveniles of many species can be morphologically identical and indistinguishable (Hawlitschek *et al*., 2016; Schultz, 2018). For example, many species in the genus *Ruspolia* Schultess, 1898, show morphological similarity, making the identification based solely on external characters challenging (Naskrecki and Guta, 2019). Thus, Bickford *et al* (2007) and Hawlitschek *et al* (2016), strongly advise the use of combined methods of species identification based on morphology, genetics and acoustic, especially for cryptic species.

The inclusion of genetic data in this study helped to estimate the genetic distance between species in the genus *Brinckiella*, allowing to correct misidentified specimens. The specimen (B51) morphologically similar to *B. arboricola* is considered to be a new species (Fig. 6) and is tentatively named *B* sp. n. 5. This and other new species will be formally described in future taxonomic studies.

4.2. Distribution

The genus *Brinckiella* is distributed in 27 vegetation types in Fynbos, Succulent Karoo and Azonal vegetation biomes. Fynbos is the most diverse biome with 11 species, which may be attributed to the fact that Fynbos is the most widespread and diverse biome in Cape Province (Cowling and Pierce, 2004). Moreover, Fynbos is composed mainly of evergreen vegetation

that most insect groups feed on (Manning, 2018; Scholtz, Scholtz and Klerk, 2021). *Brinckiella* is part of Phaneropterinae, where most species are exclusively herbivorous (Naskrecki, 2009). Likely, its distribution is associated with that of its host plant species, as suggested by Naskrecki and Bazelet (2009). For example, during this study in Overberg Dune Strandveld, *B. aptera* and *B. wilsoni* were always found on the same plant species *Osteospermum moniliferum* (L.) Norl, *Olea capensis* L. and *Searsia laevigata* (L.) F. A. Barkeley.

Meanwhile, the distribution of *Brinckiella* in Succulent Karoo, the second most diverse biome, might be associated with some seasonal plants that occur in the spring and the beginning of summer, as observed in several insect groups (Colville *et al*.,2002; van Kleunen *et al*., 2007; Rundel and Cowling, 2013a; Scholtz *et al*., 2021). During this study, at the Namaqualand Klipkoppe Shrubland at Skilpad Restcamp, *B. arboricola* and *B. mauerbergerorum* were always observed feeding at night on several species of daisy flower, mainly *Osteospermum hyoseroide* (DC.) Norl and wild cabbage, *Trachyandra falcata* (L.f.) Kunth (Fig. 6).

Figure 6. A male of *B. mauerbergerorum* is standing on the daisy flower on which he was feeding.

While in Namaqualand Blomveld and Namaqualand Klipkoppe Shrubland, in Goegap National Reserve, Naskrecki and Bazelet (2009) found *B. karooensis* and *B. arboricola* on unidentified low shrubs and grasses. Both plant species bloom in midwinter and spring (Mucina *et al*., 2006;

Scholtz *et al*., 2021), the same period *Brinckiella* emerges (Naskrecki and Bazelet, 2014). During the day, *Brinckiella* was always found resting on the stem of the same grass species *Ehrharta longiflora* Eckl. Ex Schult & Schult. f. , which makes a great hiding spot as its panicle resembles *Brinckiella* (R. Guta, personal observation) (Fig. 8). In the Hantam Karoo at the Kliprug farm in Calvinia, *B. karooensis* was found mainly in the canopy of *Euphorbia mauritanica* Webb ex J. Gay and *Manochlamys albicans* (Aiton) Aellen. Although the plant species were randomly collected because of the short sample period, the plant species collected in this study seem to be seasonal, occurring in the midwinter and spring to the beginning of summer, the same period as the emergence of the genus *Brinckiella*. This may explain the host specificity and seasonality of many species of *Brinckiella* in the Succulent Karoo, as postulated by Naskrecki and Bazelet (2009).

Azonal vegetation is the least diverse biome, with only one species, *B. wilsoni*. This finding shows the occurrence *Brinckiella* apart from Fynbos and Succulent Karoo and reveals that the genus can adapt well to the waterbodies ecosystem. Azonal vegetation biome had long been considered as part of other biomes as it is essentially waterbodies constituted by seasonal or permanent water, that flow across all the South Africa Biomes. However, since this biome has its own environmental and vegetation conditions Mucina *et al* (2006) therefore, it is now categorized as a separate biome (SANBI, 2021).

Brinckiella wilsoni is the most widely distributed species, followed by *B. mauerbergerorum, B. aptera, and B. arboricola*. The distribution of these species might be linked to the microclimate condition and biological species adaptation, which enable them to survive under different temperature and habitat conditions. Moreover, their wide distribution can also be attributed to their tolerance of fragmented habitats. For example, *B. wilsoni*, *B. mauerbergerorum* and *B. aptera* can be abundant in different vegetation types, fragmented or not. The Overberg Dune Strandveld in Pearly Beach, where some of these species were found, is an urban ecosystem highly affected by invasive plant species, mainly *Eucalyptus*. The narrowly distributed species such as *B.* sp. n. 1, *B. karooensis*, *B. viridis, B. serricauda, B. elegans, B* sp. "tabulae", *B.* sp. n. 2, *B.* sp. n. 3 and *B.* sp. n. 5 might be more restricted to a specific type of habitat and may have their own environmental condition requirement. As there is not enough data to show this, a systematic study is necessary to determine the tolerance of

Brinckiella to environmental and climatic factors, such as temperature, wind, and habitat fidelity.

4.3.Phylogenetic relationship

The phylogeny suggest that the genus *Brinckiella* may be monophyletic group. *Brinckiella mauerbergerorum* is closely related to *Brinckiella aptera*. Although the group's phylogeny is incomplete, this relationship differs from that based on the morphological data discussed by Naskrecki and Bazelet (2009), who considered *B. mauerbergerorum* similar to *B. karooensis* based on the presence of minute denticles on the phallus. Therefore, the sequence data from *B. karooensis* are needed to give an accurate assessment of the relationships. The trees also show that *B. wilsoni* is closely related to *B. arboricola,* which is closely related to *B.* sp. *n. 5*. These results corroborate the previous study by Naskrecki and Bazelet (2009) who considered *B. wilsoni* morphologically similar to *B. arboricola*, differing in the unique shape of the male cercus and the female subgenital plate. However, for *B.* sp. *n. 5* the only specimen available was a female, which was very similar to the female of *B. arboricola*. Molecular data from this study shows that they are closely related but not to the point of being the same species. Thus, this study treats it as a putative new species that will be taxonomically described in future work.

4.4.Divergence time

The divergence time estimation and phylogenetic relationship of the genus *Brinckiella* represent the first study of this group as the phylogeny of katydids (Mugleston *et al*., 2013) did not include the genus *Brinckiell*a, and its closest relatives and divergence time were still unknown.

Although the divergence time is strongly supported (posterior probability of 1.0), the estimation was based on the COI gene, a fast-evolved gene. Since no fossils are available, the divergence time was calculated using the default model. Therefore, the divergence times in this study should be treated as approximations and may change as more data becomes available. Moreover, studies including nuclear and ribosomal genes are needed to accurately estimate the genus *Brinckiella*'s divergence time. Furthermore, trying to correlate the time of divergence with the historical events, the middle Miocene was marked by aridification and cooling of the GCFR that originated Renosterveld and Succulent Karoo (Verboom *et al*., 2014). Also, the progressive dryness and mild uplift in the GCFR in the Late Miocene–Early Pliocene have

promoted the diversification of several lineages of fauna and flora (Tolley *et al*., 2014; Scholtz *et al*., 2021).

5. CONCLUSION

In this study, 13 morphospecies of *Brinckiella* were identified, species distribution maps were updated and the group's phylogeny was inferred. This study adds five new morphospecies to the eight previous known ones. It also shows that when it comes to *Brinckiella* identification, combining methods such as morphology and genetics are essential since females and juveniles are generally uniform in their appearance and difficult to identify based only on morphology. The genus is distributed in 27 vegetation types in three biomes, and apart from the Fynbos and Succulent Karoo biomes, the genus also occurs in the Azonal vegetation biome. Fynbos is the most diverse biome and *B. wilsoni* the most widely distributed species, followed by *B. mauerbergerorum, B. aptera, and B. arboricola*. The general distribution pattern of the genus might be the plant species they host or feed on, as many species were always found on the same plants. Furthermore, the widely distributed species might be highly adapted to different temperatures and habitat conditions and are probably more tolerant to disturbed vegetation. In contrast, narrowly distributed species such as *B.* sp. n. 1, *B. karooensis*, *B. viridis, B. serricauda, B. elegans, B* sp. "tabulae", *B.* sp. n. 2, *B.* sp. n. 3 and *B.* sp. n. 5 might require specific habitat condition and are less tolerant of disturbed vegetation. However, further research is needed to systematically document the environmental and local climatic conditions such as temperature, wind, and habitat conditions and systematically observe host plants and the diet of different species within the group. Additional physiological data such as thermal limits might also shed light on their adaptability.

Molecular data suggest that *B. mauerbergerorum* is closely related to *B. aptera*. However, this result differs from the taxonomic correlation by Naskrecki and Bazelet (2009), who considers *B. mauerbergerorum* similar to *B. karooensis*. Therefore, as there are no molecular data on *B. karooensis*, it is essential to include all the species in future molecular studies to fully infer the phylogeny of the group. The phylogeny based on the COI gene suggests that the genus *Brinckiella* may be monophyletic group split in the middle Miocene about 14.2 Mya, originating in two clades. Although the high support value, the COI is a fast-involved gene and provides limited information, therefore further studies should include more nuclear and ribosomal genes. To summarise, this study provided new insight into the knowledge of insects, especially Tettigoniidae, in the Greater Cape Floristic Region.

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Table 1. Distribution of the genus *Brinckiella* within several vegetation types in Fynbos, succulent Karoo and Azonal vegetation Biomes.

Vegetation types	Species												
	B. aptera	***B. arboricola***	*B. elegans*	*B. karooensis*	*B. serricauda*	*B* sp. "tabulae"	*B* sp. n. 1	*B* sp. n. 2	*B* sp. n. 5	*B*. viridis	*B. wilsoni*	*B. mauerbergerorum*	*B* sp. n. 3
Agulhas Limestone Fynbos					x		x				x		x
Bokkeveld Sandstone Fynbos	x							x			x		
Breede Shale Fynbos	x												
Cape Flats Sand Fynbos											x		
Cape Lowland Freshwater Wetlands											x		
Cederberg Sandstone Fynbos		x	x										
Citrusdal Vygieveld											x		
Graafwater Sandstone Fynbos											x		
Greyton Shale Fynbos							x						
Hantam Karoo				x									
Hawequas Sandstone Fynbos	x												
Lambert's Bay Strandveld												x	
Leipoldtville Sand Fynbos											x	x	
Namaqualand Blomveld		x		x	x							x	
Namaqualand Klipkoppe Shrubland	x	x		x							x	x	
Namaqualand Strandveld	x											x	
Nieuwoudtville-Roggeveld Dolerite Renosterveld											x		
North Sonderend Sandstone Fynbos							x						
Northern Inland Shale Band Vegetation											x	x	
Olifants Sandstone Fynbos											x		
Overberg Dune Strandveld	x									x	x		
Peninsula Granite Fynbos										x			
Peninsula Sandstone Fynbos						x							
Richtersveld Sandy Coastal Scorpionstailveld												x	
Swartland Alluvium Fynbos							x						
Swartland Shale Renosterveld											x		
Vanrhynsdorp Gannabosveld				x					x			x	
Total	**6**	**3**	**1**	**4**	**2**	**1**	**4**	**1**	**1**	**2**	**13**	**8**	**1**

Table 2. Maximum Likelihood fits of 24 different nucleotide substitution models.

Model	Parameters	BIC	AICc	lnL	Invariant	Gamma	R	f(A)	f(T)	f(C)	f(G)	r(AT)	r(AC)	r(AG)	r(TA)	r(TC)	r(TG)	r(CA)	r(CT)	r(CG)	r(GA)	r(GT)	r(GC)
GTR+G+I	**49**	**6849.96**	**6482.30**	**-3191.97**	**0.563**	**2.030**	**1.843**	**0.36**	**0.26**	**0.17**	**0.21**	**0.12**	**0.00**	**0.21**	**0.18**	**0.04**	**0.00**	**0.00**	**0.06**	**0.01**	**0.37**	**0.00**	**0.01**
GTR+G	48	6855.00	6494.84	-3199.25	n/a	0.209	2.116	0.36	0.26	0.17	0.21	0.11	0.00	0.22	0.16	0.04	0.00	0.00	0.05	0.01	0.39	0.00	0.01
GTR+I	48	6857.64	6497.47	-3200.56	0.601	n/a	1.555	0.36	0.26	0.17	0.21	0.14	0.00	0.19	0.20	0.05	0.00	0.00	0.07	0.01	0.33	0.00	0.01
TN93+G+I	46	6964.06	6618.89	-3263.29	0.029	0.159	3.804	0.36	0.26	0.17	0.21	0.03	0.02	0.26	0.04	0.02	0.02	0.04	0.03	0.02	0.46	0.03	0.02
HKY+G+I	45	7013.29	6675.62	-3292.65	0.587	1.391	1.955	0.36	0.26	0.17	0.21	0.04	0.03	0.14	0.06	0.12	0.03	0.06	0.17	0.03	0.24	0.04	0.03
TN93+I	45	7014.76	6677.08	-3293.39	0.620	n/a	1.449	0.36	0.26	0.17	0.21	0.05	0.04	0.16	0.08	0.06	0.04	0.08	0.08	0.04	0.29	0.05	0.04
HKY+I	44	7028.69	6698.51	-3305.11	0.627	n/a	1.499	0.36	0.26	0.17	0.21	0.05	0.03	0.13	0.07	0.11	0.04	0.07	0.16	0.04	0.22	0.05	0.03
T92+G+I	43	7037.53	6714.86	-3314.29	0.576	1.075	2.388	0.31	0.31	0.19	0.19	0.04	0.03	0.14	0.04	0.14	0.03	0.04	0.22	0.03	0.22	0.04	0.03
TN93+G	45	7040.76	6703.09	-3306.39	n/a	0.435	1.795	0.36	0.26	0.17	0.21	0.05	0.03	0.20	0.07	0.04	0.04	0.07	0.05	0.04	0.34	0.05	0.03
T92+I	42	7057.14	6741.96	-3328.84	0.628	n/a	1.676	0.31	0.31	0.19	0.19	0.06	0.03	0.12	0.06	0.12	0.03	0.06	0.20	0.03	0.20	0.06	0.03
HKY+G	44	7091.77	6761.59	-3336.65	n/a	0.435	1.553	0.36	0.26	0.17	0.21	0.05	0.03	0.13	0.07	0.11	0.04	0.07	0.16	0.04	0.22	0.05	0.03
T92+G	42	7120.14	6804.96	-3360.35	n/a	0.430	1.676	0.31	0.31	0.19	0.19	0.06	0.03	0.12	0.06	0.12	0.03	0.06	0.20	0.03	0.20	0.06	0.03
K2+I	41	7212.52	6904.84	-3411.29	0.625	n/a	1.432	0.25	0.25	0.25	0.25	0.05	0.05	0.15	0.05	0.15	0.05	0.05	0.15	0.05	0.15	0.05	0.05
K2+G+I	42	7212.99	6897.81	-3406.77	0.604	3.273	1.540	0.25	0.25	0.25	0.25	0.05	0.05	0.15	0.05	0.15	0.05	0.05	0.15	0.05	0.15	0.05	0.05
K2+G	41	7223.65	6915.96	-3416.85	n/a	0.203	1.587	0.25	0.25	0.25	0.25	0.05	0.05	0.15	0.05	0.15	0.05	0.05	0.15	0.05	0.15	0.05	0.05
JC+I	40	7313.53	7013.35	-3466.55	0.623	n/a	0.500	0.25	0.25	0.25	0.25	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
JC+G+I	41	7319.68	7012.00	-3464.87	0.613	6.306	0.500	0.25	0.25	0.25	0.25	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
JC+G	40	7334.00	7033.81	-3476.79	n/a	0.208	0.500	0.25	0.25	0.25	0.25	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
GTR	47	7397.87	7045.21	-3475.43	n/a	n/a	1.282	0.36	0.26	0.17	0.21	0.14	0.00	0.18	0.20	0.04	0.02	0.01	0.07	0.01	0.31	0.03	0.01
TN93	44	7592.79	7262.61	-3587.16	n/a	n/a	1.248	0.36	0.26	0.17	0.21	0.06	0.04	0.16	0.08	0.04	0.05	0.08	0.06	0.05	0.29	0.06	0.04
HKY	43	7664.89	7342.21	-3627.97	n/a	n/a	1.220	0.36	0.26	0.17	0.21	0.06	0.04	0.11	0.08	0.10	0.05	0.08	0.14	0.05	0.20	0.06	0.04
T92	41	7698.93	7391.24	-3654.49	n/a	n/a	1.218	0.31	0.31	0.19	0.19	0.07	0.04	0.11	0.07	0.11	0.04	0.07	0.17	0.04	0.17	0.07	0.04
K2	40	7791.74	7491.56	-3705.66	n/a	n/a	1.202	0.25	0.25	0.25	0.25	0.06	0.06	0.14	0.06	0.14	0.06	0.06	0.14	0.06	0.14	0.06	0.06
JC	39	7880.22	7587.54	-3754.65	n/a	n/a	0.500	0.25	0.25	0.25	0.25	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08

NOTE: Models with the lowest BIC scores (Bayesian Information Criterion) are considered to describe the substitution pattern the best. For each model, AICc value (Akaike Information Criterion, corrected), Maximum Likelihood value (*lnL*), and the number of parameters (including branch lengths) are also presented [1]. Non-uniformity of evolutionary rates among sites may be modeled by using a discrete Gamma distribution (+*G*) with 5 rate categories and by assuming that a certain fraction of sites are evolutionarily invariable (+*I*). Whenever applicable, estimates of gamma shape parameter and/or the estimated fraction of invariant sites are shown. Assumed or estimated values of transition/transversion bias (*R*) are shown for each model, as well. They are followed by nucleotide frequencies (*f*) and rates of base substitutions (*r*) for each nucleotide pair. Relative values of instantaneous *r* should be considered when evaluating them. For simplicity, sum of *r* values is made equal to 1 for each model. For estimating ML

values, a tree topology was automatically computed. This analysis involved 21 nucleotide sequences. There were a total of 643 positions in the final dataset. Evolutionary analyses were conducted in MEGA11.

Abbreviations: GTR: General Time Reversible; HKY: Hasegawa-Kishino-Yano; TN93: Tamura-Nei; T92: Tamura 3-parameter; K2: Kimura 2-parameter; JC: Jukes-Cantor.

7. APPENDICES

Appendix 1: A modified DNA Extraction Protocol of the Genus *Brinckiella*.

Systematics Lab

Pearson Building, Dept. of Biological Sciences, UCT

LYSE

1. One hind leg of each specimen of *Brinckiella* was removed, weighed, and minced into small pieces with a scalpel blade and transferred into a 1.5 mL microcentrifuge tube.

2-3. Add into the tube the mixture of 100 μl of TL buffer +12.5 μl of proteinase K and vortex for 3-5 seconds.

Incubation

4. Incubate the sample at 55^{o}C for three hours in the heating block; afterwards, drop the temperature to 37^{o}C and leave it overnight.

5. Take the samples from the heating block and centrifuge 6.5 minutes under @12500.

6. Transfer the sample (supernatant) to the sterile 1.5mL microtube. Do not disturb or transfer an insoluble pellet.

BIND

7. Add the BL buffer (equal volume to the amount transferred from step 6) and vortex for 2-3s.

8. Incubate in the heat block for 10 min at 70^{o}C; (**Put elution buffer in the heat block**).

9. Add the equal amount of ethanol from step 7 and vortex 2-3s to mix thoroughly

10. Insert a HiBind DNA Mini into a 2mL collection tube (**label the collection tube**).

11. Transfer the entire sample from steps 6-9.

12. Centrifuge at maximum speed (@20238^{xg}) for 1 minute, discard the filtrate and reuse the collection tube.

WASH

13. Add into the sample 500 μl HBC Buffer diluted in 100% isopropanol (**see the bottle instruction**).

14. Centrifuge at maximum speed (@20238^{Xg}) for 30 seconds. Discard the old filtrate and collection tube.

15. Insect a HiBind DNA Mini into a new 2mL collection tube (provided).

16. Add 700 μl of DNA wash buffer diluted with 10mL of 100% ethanol. Centrifuge at maximum speed (@20238) for 30 seconds; discard the filtrate and reuse the collection tube.

17. Repeat step 16 for a second DNA wash buffer step.

18. Centrifuge the empty HiBind DNA Mini column at maximum speed for 2 minutes to dry the column. This step is critical for the removal of trace ethanol that may interfere with downstream applications. **Elute**

19. Transfer the HiBind DNA Mini column into a nuclease-free 1.5 mL microcentrifuge tube (**Label the final tube**).

20. Add 80 μl Elution buffer heated to 70 °c. Let sit at **room temperature for 2 minutes**. Centrifuge at maximum speed for 1 minute

21. Store eluted DNA at 20 °c.

Appendix 2: Sequences of the genus *Brinckiella* and four outgroups (*H. fruhstorferi*, *I major*, *P. gracilis*, *K. chinensis)*, based on 643 bp of cytochrome oxidase subunit I (COI), the numbers in the brackets represents the unique identification number for *Brinckiella* and accession number for the outgroups.

***K. chinensis* (KX057735.1)**

TAGGGTCACCTCCACCAGCAGGGTCGAAGAAGGAGGTATTTAAGTTTCGATCAGTTAAT
AATATTGTAATAGCTCCGGCTAAAACAGGGAGAGAAAGTAATAAAAGTAAAGCTGTAAT
AGCAACAGATCAAACAAATAGGGGTGTTTGATCTAAGGATATTCCAGGAGATCGTATAT
TAATAGTGGTAGTAATGAAGTTAACTGCCCCAAGAATAGAGGAAATACCGGCAAGGTGA
AGGGAAAAGATGGCTAAATCTACAGAAGCCCCTCCATGTGCAATTCCTCCGGATAGTGG
TGGATAGACTGTTCAACCAGTTCCAGCTCCATTTTCGACTAAGCTACTTGTTAAGAGTAG
AGTTAAAGAGGGGGGTAGTAGTCAAAAACTTATGTTATTTATTCGGGGGAATGCTATATC
AGGTGCTCCTAGTATTAAAGGGACTAATCAATTACCAAATCCCCCAATTATAATTGGTAT
TACTATGAAAAAAATTATTACGAAGGCGTGTGCAGTAACAATAACATTATAAATTTGATC
ATCTCCAATTAAATATCCGGGTTGTCCTAGTTCAGCTCGAATTAGTAAACTTAGTGATGTT
CCAACTATTCCAGCTCAAGCTCCAAAAATGAAATATAAGGTACCGAT

***I. major* (NC_042666.1)**

TCGGGTCACCTCCTCCTGCTGGGTCAAAGAAGGAGGTATTTAAATTACGGTCAGTTAATA
GTATAGTAATAGCACCTGCTAGGACAGGTAAGGAAAGTAGTAGTAACAATGCTGTGATT
GCTACGGATCATACAAATAAGGGGGTTTGGTCTAGTGATATACCAGGGGTTCGTATATTA
ATTGTTGTAGTAATAAAGTTTACCGCCCCTAAGATGGAGGAAATACCAGCTAAATGTAA
GGAGAAAATTGCTAAGTCTACAGAGGCACCTCCGTGAGCAATTCCGGAGGAAAGTGGAG
GGTAGACTGTTCAACCAGTGCCGGCCCCGTTTTCAACTAGACTGCTGGCTAGGAGGAGA
GTTAATGAAGGAGGGAGTAACCAAAAACTTATATTGTTTATTCGAGGGAAGGCTATATCT
GGGGCTCCTAATATTAAGGGGACTAATCAGTTTCCAAATCCTCCAATTATAATAGGTATA
ACTATAAAGAAGATTATTACAAATGCATGGGCTGTAACAATGACGTTGTAGATTTGGTCG
TCACCAATTAGGTACCCGGGTTGACCTAATTCAGCACGGATTAGTAGTCTTAAGGAGGTG
CCTACTATTCCTGCTCAGGCGCCAAAGATAAAATATAGTGTTCCGAT

***H. fruhstorferi* (KX057733.1)**

TTGGATCACCTCCACCCGCAGGATCAAAGAACGAAGTGTTAAGGTTTCGGTCAGTTAATA
GTATTGTAATAGCTCCTGCAAGAACTGGAAGAGAAAGAAGAAGAAGGAGAGCAGTAAT
TGCTACTGCTCAAACAAAGAGAGGAGTTTGATCAAGGGATATACCGGGGGCTCGTATAT
TAATTGTTGTAGTAATGAAGTTAACAGCACCTAGGATGGAAGAAATACCAGCAAGATGA
AGAGAGAAGATGGCTAAGTCGACAGAGGCTCCACCATGAGCAATATTTGCAGATAAAGG

GGGATAAACAGTTCATCCAGTTCCGGCTCCATTCTCTACAATTCTTCTAGTAAGAAGAAG
TGTTAGCGAAGGAGGTAACAATCAGAATCTTATATTGTTTATTCGAGGAAAAGCTATATC
AGGAGCCCCAAGTATTAAAGGTACTAATCAATTTCCAAACCCACCAATTATAATAGGTAT
TACTATAAAGAAGATTATTACAAATGCATGGGCTGTAACAATAACGTTATAAATTTGATC
ATCACCAATTAAATAACCTGGATTACCTAATTCAGCACGAATTAATATACTTAAGGAGGT
ACCAACTATACCTGCCCAAGCACCAAAAATAAAATATAAAGTTCCAAT

***B*. cf. *aptera* (B103)**

TAGGATCACCACCTCCAGCGGGATCGAAGAAGGAAGTATTAAGATTACGATCAGTGAGA
AGTATAGTAATAGCACCTGCTAGAACAGGAAGAGATAGAAGGAGAAGAAGAGCAGTGA
TGGCAACTGCTCATACAAATAAGGGGGTTTGATCTAAAGATATCCCAGGAGCTCGTATGT
TAATAGTAGTAGTAATGAAGTTAACTGCACCTAGAATTGAAGAAATTCCAGCTAAATGG
AGAGAGAAAATAGCTAAATCTACAGAAGCCCCAGCATGTGCAATCCCTGCAGATAAGGG
AGGGTAGACTGTTCACCCTGTCCCAGCTCCATTTTCTACTAAGCTGCTAGCCAGAAGAAG
AGTGAGTGAAGGGGGTAATAATCAAAAACTTATATTATTTATTCGAGGGAAGGCTATAT
CTGGGGCACCTAATATTAAAGGAACTAGTCAGTTTCCAAACCCTCCAATTATAATTGGTA
TAACTATAAAGAAAATTATAACAAAGGCATGAGCAGTTACAATAACATTATAAATTTGA
TCATCTCCAATTAAATATCCAGGTTGACCCAATTCAGCTCGAATTAGCAAACTAAGTGAA
GTACCAACTATGCCAGCCCACGCTCCAAAAATGAAGTATAATGTTCCAAT

***B*. cf. *aptera* (B102)**

TAGGATCACCACCTCCAGCAGGATCGAAGAAGGAAGTATTAAGATTTCGATCAGTGAGA
AGTATAGTAATAGCACCTGCTAGAACAGGAAGAGATAGAAGGAGAAGAAGAGCAGTGA
TGGCAACTGCTCATACAAATAAGGGGGTTTGATCTAAAGATATCCCAGGAGCTCGTATGT
TAATAGTAGTAGTAATGAAGTTAACTGCACCTAGAATTGAAGAAATTCCAGCTAAATGG
AGAGAGAAAATAGCTAAATCTACAGAAGCCCCAGCATGTGCAATCCCTGCAGATAAGGG
AGGGTAGACTGTTCATCCTGTCCCAGCTCCATTTTCTACTAAGCTGCTAGCCAGAAGAAG
AGTAAGTGAAGGGGGTAATAATCAAAAACTTATATTATTTATTCGAGGGAAGGCTATAT
CTGGGGCACCTAATATTAAAGGAACTAGTCAGTTTCCAAATCCTCCAATTATAATTGGTA
TAACTATAAAGAAAATTATAACAAAGGCATGAGCAGTTACAATAACATTATAAATTTGA
TCATCTCCAATTAAATATCCAGGTTGACCCAATTCAGCTCGAATTAGCAAACTAAGTGAA
GTACCAACTATGCCAGCCCATGCTCCAAAAATGAAGTATAATGTTCCAAT

***B*. *mauerbergerorum* (B84)**

TAGGGTCACCTCCACCAGCAGGATCAAAGAAAGAAGTATTTAAATTACGGTCAGTTAGA
AGTATAGTAATAGCTCCAGCTAGAACAGGAAGAGAAAGAAGTAAAAGAAGAGCAGTAA
TTGCGACAGCTCAAACAAATAAAGGAGTTTGATCTAAGGATATTCCTGGTGCTCGTATAT
TGATTGTTGTTGTAATAAAGTTTACAGCTCCAAGAATTGAGGAGATTCCTGCTAGATGAA
GGGAGAAAATTGCCAAGTCAACTGAAGCACCGGCGTGAGCAATTCCAGCAGAAAGGGG

AGGGTAGACTGTTCATCCAGTTCCAGCACCATTTTCAACGAGACTTCTTGCAAGGAGGAG
GGTTAAAGAAGGGGGTAATAATCAGAATCTTATGTTATTTATTCGTGGAAAAGCTATATC
GGGAGCCCCAAGTATTAGAGGAACTAATCAATTACCGAATCCCCCAATTATAATAGGTA
TTACTATAAAGAAAATTATTACAAAAGCATGAGCAGTCACAATAACATTGTAAATTTGAT
CATCTCCAATTAAATATCCTGGTTGTCCCAATTCAGCACGAATTAGCAAACTAAGGGATG
TACCAACTATGCCGGCCCATGCTCCCAAAATAAAGTATAATGTTCCAAT

***B. mauerbergerorum* (B83)**

TAGGGTCACCTCCACCAGCAGGATCAAAGAAAGAAGTATTTAAATTACGGTCAGTTAGA
AGTATAGTAATAGCTCCAGCTAGAACAGGAAGAGAAAGAAGTAAAAGAAGAGCAGTAA
TTGCGACAGCTCAAACAAATAAAGGAGTTTGATCTAAGGATATTCCTGGTGCTCGTATAT
TGATTGTTGTTGTAATAAAGTTTACAGCTCCAAGAATTGAGGAGATTCCTGCTAGATGAA
GGGAGAAAATTGCCAAGTCAACTGAAGCACCGGCGTGAGCAATTCCAGCAGAAAGGGG
AGGGTAGACTGTTCATCCAGTTCCAGCACCATTTTCAACGAGACTTCTTGCAAGGAGGAG
GGTTAAAGAAGGGGGTAATAATCAGAATCTTATGTTATTTATTCGTGGAAAAGCTATATC
GGGAGCCCCAAGTATTAGAGGAACTAATCAATTACCGAATCCCCCAATTATAATAGGTA
TTACTATAAAGAAAATTATTACAAAAGCATGAGCAGTCACAATAACATTGTAAATTTGAT
CATCTCCAATTAAATATCCTGGTTGTCCCAATTCAGCACGAATTAGCAAACTAAGGGATG
TACCAACTATACCGGCCCATGCTCCAAAAATAAAGTATAATGTTCCAAT

***B. arboricola* (B78)**

TTGGGTCACCCCCACCAGCAGGGTCAAAGAAAGAAGTATTTAAATTACGGTCAGTTAGA
AGTATAGTAATAGCTCCAGCTAAAACAGGAAGAGAAAGAAGTAAAAGAAGAGCAGTAA
TTGCGACAGCTCAAACGAATAAAGGAGTTTGATCTAAGGATATTCCTGGTGCTCGTATAT
TGATTGTTGTTGTAATAAAGTTTACAGCTCCAAGAATTGAGGAGATTCCTGCTAGATGAA
GGGAGAAAATTGCCAAGTCAACTGAAGCACCAGCGTGAGCAATTCCAGCGGAAAGGGG
AGGGTAGACTGTTCACCCAGTTCCAGCACCATTTTCAACGAGACTTCTTGCAAGGAGGAG
GGTTAAAGAAGGGGGTAATAATCAAAATCTTATATTATTTATTCGTGGAAAAGCTATATC
GGGAGCCCCAAGTATTAAGGGAACTAATCAATTACCGAATCCCCCAATTATAATAGGTA
TTACTATAAAGAAAATTATTACAAAAGCATGAGCAGTTACAATAACATTATAAATTTGAT
CATCTCCAATTAAATATCCTGGTTGTCCCAATTCAGCACGAATTAGCAAACTAAGGGATG
TACCAACTATACCGGCCCATGCTCCAAAAATAAAGTATAATGTTCCAAT

***B. arboricola* (B74)**

TAGGATCTCCTCCTCCAGCAGGATCAAAAAAGGATGTATTTAGATTACGATCTGTTAAAA
GTATAGTAATTGCTCCTGCTAATACAGGAAGTGATAAGAGTAGGAGAAGAGCAGTAATT
GCTACTGCTCATACAAATAAAGGAGTTTGATCAAGAGACATACCTGGTGCTCGTATATTA
ATTGTAGTTGTGATAAAATTAACGGCCCCTAAAATAGAAGAAATTCCAGCAAGATGAAG
AGAGAAAATAGCTAGATCTACAGAAGCTCCGGCATGAGCAATTCCAGCTGAAAGAGGGG

GGTAAACAGTTCATCCAGTTCCTGCTCCGTTTTCAACTAAACTTCTAGCCAATAAAAGGG
TAAGAGAAGGGGGAAGAAGTCAAAATCTTATGTTGTTTATACGTGGAAAAGCTATATCA
GGGGCTCCTAATATTAAGGGAACTAATCAATTTCCGAATCCTCCAATTATAATAGGTATT
ACTATAAAGAAAATTATTACAAAAGCGTGGGCTGTCACAATTACGTTATAAATTTGATCA
TCACCAATTAAGTAACCAGGTTGACCCAATTCAGCTCGAATTAGTATACTAAGAGAAGTA
CCAACTATGCCGGCCCATGCTCCGAAAATAAAGTATAATGTTCCAAT

***B. arboricola* (B64)**

TAGGATCTCCTCCCCCAGCAGGGTCAAAGAAGGATGTATTTAAATTACGGTCTGTAAGAA
GTATTGTAATTGCTCCTGCTAATACGGGAAGAGAAAGTAGAAGTAGGAGTGCAGTGATT
GCTACTGCTCAAACAAATAAAGGAGTTTGATCAAGTGATATTCCGGGAGCTCGTATATTA
ATGGTTGTAGTAATAAAATTAACAGCTCCTAAAATAGATGAAATTCCAGCTAGATGAAG
GGAAAAAATAGCTAAATCTACTGAAGCACCAGCATGGGCAATCCCTGCAGAGAGTGGGG
GATAAACTGTTCATCCTGTTCCAGCTCCATTTTCGACTAAACTACTAGCGAGTAATAAGG
TAAGTGAAGGAGGTAATAATCAAAATCTTATGTTATTTATTCGTGGGAATGCTATATCTG
GGGCTCCTAGTATTAAGGGCACTAATCAGTTTCCAAATCCTCCAATTATAATAGGTATAA
CTATAAAAAAAATTATAACAAAAGCGTGGGCAGTAACGATAACGTTATAAATTTGATCA
TCACCAATTAAGTAACCAGGTTGCCCAAGTTCAGCTCGAATTAGTAGTCTAAGTGAAGTA
CCAACTATGCCGGCCCATGCACCAAAAATGAAGTATAATGTGCCAAT

***B. arboricola* (B63)**

TAGGATCTCCTCCCCCAGCAGGGTCAAAGAAGGATGTATTTAAATTACGGTCTGTAAGAA
GTATTGTAATTGCTCCTGCTAATACGGGAAGAGAAAGTAGAAGTAGGAGTGCAGTGATT
GCTACTGCTCAAACAAATAAAGGAGTTTGATCAAGTGATATTCCGGGAGCTCGTATATTA
ATGGTTGTAGTAATAAAATTAACAGCTCCTAAAATAGATGAAATTCCAGCTAGATGAAG
GGAAAAAATAGCTAAGTCTACTGAAGCACCAGCATGGGCAATCCCTGCAGAGAGTGGGG
GATAAACTGTTCATCCTGTTCCAGCTCCATTTTCGACTAAACTACTAGCGAGTAATAAGG
TAAGTGAAGGAGGTAATAATCAAAATCTTATGTTATTTATTCGTGGGAATGCTATATCTG
GGGCTCCTAGTATTAAGGGCACTAATCAGTTTCCAAATCCTCCAATTATAATAGGTATAA
CTATAAAAAAAATTATAACAAAAGCGTGGGCAGTAACGATAACGTTATAAATTTGATCA
TCACCAATTAAGTAACCAGGTTGCCCAAGTTCAGCTCGAATTAGTAGTCTAAGTGAAGTA
CCAACTATGCCGGCCCATGCACCAAAAATGAAGTATAATGTGCCAAT

***B. wilsoni* (B55)**

TAGGATCTCCTCCTCCAGCAGGATCAAAAAAGGATGTATTTAGATTACGATCTGTTAAAA
GTATAGTAATTGCTCCTGCTAATACAGGAAGTGATAAGAGTAGGAGAAGAGCAGTAATT
GCTACTGCTCATACAAATAAAGGAGTTTGATCAAGAGACATACCTGGTGCTCGTATATTA
ATTGTAGTTGTGATAAAATTAACGGCCCCTAAAATAGAAGAAATTCCAGCAAGATGAAG
AGAGAAAATAGCTAGATCTACAGAAGCTCCGGCATGAGCAATTCCAGCTGAAAGAGGGG

GGTAAACAGTTCATCCAGTTCCTGCTCCGTTTTCAACTAAACTTCTAGCCAATAAAAGGG
TAAGAGAAGGGGGAAGAAGTCAAAATCTTATGTTGTTTATACGAGGAAAAGCTATATCA
GGGGCTCCTAATATTAAGGGAACCAATCAATTTCCGAATCCTCCAATTATAATAGGTATT
ACTATAAAGAAAATTATTACAAAAGCGTGGGCTGTCACAATTACGTTATAAATTTGATCA
TCACCAATTAAGTAACCAGGTTGACCCAATTCAGCTCGAATTAGTATACTAAGAGAAGTA
CCAACTATGCCGGCCCATGCTCCGAAAATAAAGTATAATGTTCCAAT

***B. arboricola* (B51)**

TTGGATCTCCTCCTCCTGCGGGATCAAAGAAGGATGTATTCAAATTACGGTCTGTAAGAA
GTATTGTAATTGCCCCTGCTAATACAGGAAGAGAAAGTAAAAGAAGAAGAGCAGTAATT
GCTACTGCTCAAACAAATAAAGGAGTTTGATCAAGTGATATTCCAGGGGCTCGTATATTA
ATAGTTGTGGTAATAAAATTTACAGCCCCTAAAATAGATGAAATACCGGCTAGATGAAG
AGAAAAAATAGCCAAGTCTACTGACGCACCAGCATGAGCAATTCCTGCAGAGAGTGGAG
GGTAAACTGTTCAACCTGTTCCGGCACCATTTTCAACTAAACTACTAGCAAGTAAAAGAG
TAAGTGAAGGAGGTAATAATCAAAATCTTATGTTATTTATTCGTGGAAATGCTATATCTG
GAGCTCCTAATATTAATGGTACTAATCAGTTTCCAAATCCTCCGATTATAATAGGTATAA
CTATAAAAAAAATTATAACGAAAGCATGAGCAGTTACGATTACGTTATAAATTTGATCAT
CACCAATTAAATAGCCAGGTTGTCCTAGTTCAGCTCGAATTAGCAGACTAAGCGAAGTAC
CAACTATACCAGCCCATGCACCAAAAATGAAGTATAATGTGCCAAT

***B. mauerbergerorum* (B49)**

TAGGGTCACCTCCACCAGCAGGATCAAAGAAAGAAGTATTTAAATTACGGTCAGTTAGA
AGTATAGTAATAGCTCCAGCTAGAACAGGAAGAGAAAGAAGTAAAAGAAGAGCAGTAA
TTGCGACAGCTCAAACAAATAAAGGAGTTTGATCTAAGGATATTCCTGGTGCTCGTATAT
TGATTGTTGTTGTAATAAAGTTTACAGCTCCAAGAATTGAGGAGATTCCTGCTAGATGAA
GGGAGAAAATTGCCAAGTCAACTGAAGCACCGGCGTGAGCAATTCCAGCAGAAAGGGG
AGGGTAGACTGTTCATCCAGTTCCAGCACCATTTTCAACGAGACTTCTTGCAAGGAGGAG
GGTTAAAGAAGGGGGTAATAATCAGAATCTTATGTTATTTATTCGTGGAAAAGCTATATC
GGGAGCCCCAAGTATTAGAGGAACTAATCAATTACCGAATCCCCCAATTATAATAGGTA
TTACTATAAAGAAAATTATTACAAAAGCATGAGCAGTCACAATAACATTGTAAATTTGAT
CATCTCCAATTAAATATCCTGGTTGTCCCAATTCAGCACGAATTAGCAAACTAAGGGATG
TACCAACTATGCCGGCCCATGCTCCAAAAATAAAGTATAATGTTCC
AAT

***B. mauerbergerorum* (B48)**

TAGGGTCACCTCCACCAGCAGGATCAAAGAAAGAAGTATTTAAATTACGGTCAGTTAGA
AGTATAGTAATAGCTCCAGCTAGAACAGGAAGAGAAAGAAGTAAAAGAAGAGCAGTAA
TTGCGACAGCTCAAACAAATAAAGGAGTTTGATCTAAGGATATTCCTGGTGCTCGTATAT
TGATTGTTGTTGTAATAAAGTTTACAGCTCCAAGAATTGAGGAGATTCCTGCTAGATGAA

GGGAGAAAATTGCCAAGTCAACTGAAGCACCGGCGTGAGCAATTCCAGCAGAAAGGGG
AGGGTAGACTGTTCATCCAGTTCCAGCACCATTTTCAACGAGACTTCTTGCAAGGAGGAG
GGTTAAAGAAGGGGGTAATAATCAGAATCTTATGTTATTTATTCGTGGAAAAGCTATATC
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CATCTCCAATTAAATATCCTGGTTGTCCCAATTCAGCACGAATTAGCAAACTAAGGGATG
TACCAACTATACCGGCCCATGCTCCCAAAATAAAGTATAATGTTCCAAT

***B. wilsoni* (B36)**

TAGGATCTCCTCCTCCAGCAGGATCAAAAAAGGATGTATTTAGATTACGATCTGTTAAGA
GTATAGTAATTGCTCCTGCTAATACAGGAAGTGATAAGAGTAGGAGAAGAGCAGTAATT
GCTACTGCTCATACAAATAAAGGAGTTTGATCAAGAGACATACCTGGTGCTCGTATATTA
ATTGTAGTTGTGATAAAATTAACGGCCCCTAAAATAGAAGAAATTCCAGCAAGATGAAG
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TAAGAGAAGGGGGAAGAAGTCAAAATCTTATATTGTTTATACGTGGAAAAGCTATATCA
GGGGCTCCTAATATTAAGGGAACTAATCAATTTCCGAATCCTCCAATTATAATAGGTATT
ACTATAAAGAAAATTATTACAAAAGCGTGGGCTGTCACAATTACGTTATAAATTTGATCA
TCACCAATTAAATAACCAGGTTGACCCAATTCAGCTCGAATTAGTATACTAAGAGAAGTA
CCAACTATGCCGGCCCATGCTCCGAAAATAAAGTATAATGTTCCAAT

***B. wilsoni* (B23)**

TAGGATCTCCTCCTCCAGCAGGATCAAAAAAGGATGTATTTAGATTACGATCTGTTAAAA
GTATAGTAATTGCTCCTGCTAATACAGGAAGTGATAAGAGTAGGAGAAGAGCAGTAATT
GCTACTGCTCATACAAATAAAGGAGTTTGATCAAGAGACATACCTGGTGCTCGTATATTA
ATTGTAGTAGTGATAAAATTAACGGCCCCTAAAATAGAAGAAATTCCAGCAAGATGAAG
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GGTAAACAGTTCATCCAGTTCCTGCTCCGTTTTCAACTAAACTTCTAGCCAATAAAAGGG
TAAGAGAAGGCGGAAGAAGTCAAAATCTTATGTTGTTTATACGTGGAAAAGCTATATCA
GGGGCTCCTAATATTAAGGGAACTAATCAATTTCCGAATCCTCCAATTATAATAGGTATT
ACTATAAAGAAAATTATTACAAAAGCGTGGGCTGTCACAATTACGTTATAAATTTGATCA
TCACCAATTAAATAACCAGGTTGACCCAATTCAGCTCGAATTAGTATACTAAGAGAAGTA
CCAACTATGCCGGCCCATGCTCCGAAAATAAAGTATAATGTTCCAAT

***B.aptera* (B21)**

TAGGATCACCACCTCCAGCGGGATCGAAGAAGGAAGTATTAAGATTACGATCAGTGAGA
AGTATAGTAATAGCACCTGCTAGAACAGGAAGAGATAGAAGGAGAAGAAGAGCAGTGA
TGGCAACTGCTCATACAAATAAGGGGGTTTGATCTAAAGATATCCCAGGAGCTCGTATGT
TAATAGTAGTAGTAATGAAGTTAACTGCACCTAGAATTGAAGAAATTCCAGCTAAATGG

AGAGAGAAAATAGCTAAATCTACAGAAGCCCCAGCATGTGCAATCCCTGCAGATAAGGG
AGGGTAGACTGTTCACCCTGTCCCAGCTCCATTTTCTACTAAGCTGCTAGCCAGAAGAAG
AGTGAGTGAAGGGGGTAATAATCAAAAACTTATATTATTTATTCGAGGGAAGGCTATAT
CTGGGGCACCTAATATTAAAGGAACTAGTCAGTTTCCAAATCCTCCAATTATAATTGGTA
TAACTATAAAGAAAATTATAACAAAGGCATGAGCAGTTACAATAACATTATAAATTTGA
TCATCTCCAATTAAATATCCAGGTTGACCCAATTCAGCTCGAATTAGCAAACTAAGTGAA
GTACCAACTATGCCAGCCCACGCTCCAAAAATGAAGTATAATGTTCCAAT

***B. wilsoni* (17)**

TAGGATCTCCTCCTCCAGCAGGATCAAAAAAGGATGTATTTAGATTACGATCTGTTAAGA
GTATAGTAATTGCTCCTGCTAATACAGGAAGTGATAAGAGTAGGAGAAGAGCAGTAATT
GCTACTGCTCATACAAATAAAGGAGTTTGATCAAGAGACATACCTGGTGCTCGTATATTA
ATTGTAGTTGTGATAAAATTAACGGCCCCTAAAATAGAAGAAATTCCAGCAAGATGAAG
AGAAAAAATAGCTAGATCTACAGAAGCTCCGGCATGAGCAATTCCAGCTGAAAGAGGGG
GGTAAACAGTTCATCCAGTTCCTGCTCCGTTTTCAACTAAACTTCTAGCCAATAAAAGGG
TAAGAGAAGGGGGAAGAAGTCAAAATCTTATATTGTTTATACGTGGAAAAGCTATATCA
GGGGCTCCTAATATTAAGGGAACTAATCAATTTCCGAATCCTCCAATTATAATAGGTATT
ACTATAAAGAAAATTATTACAAAAGCGTGGGCTGTCACAATTACGTTATAAATTTGATCA
TCACCAATTAAATAACCAGGTTGGCCCAATTCAGCTCGAATTAGTATACTAAGAGAAGTA
CCAACTATGCCGGCCCATGCTCCGAAAATAAAGTATAATGTTCCAAT

***B. wilsoni* (B15)**

TAGGATCTCCTCCTCCAGCAGGATCAAAAAAGGATGTATTTAGATTACGATCTGTTAAAA
GTATAGTAATTGCTCCTGCTAATACAGGAAGTGATAAGAGTAGGAGAAGAGCAGTAATT
GCTACTGCTCATACAAATAAAGGAGTTTGATCAAGAGACATACCTGGTGCTCGTATATTA
ATTGTAGTTGTGATAAAATTAACGGCTCCTAAAATAGAAGAAATTCCAGCAAGATGAAG
AGAGAAAATAGCTAGATCTACAGAAGCTCCGGCATGAGCAATTCCAGCTGAAAGAGGGG
GGTAAACAGTTCATCCAGTTCCTGCTCCGTTTTCAACTAAACTTCTAGCCAATAAAAGGG
TAAGAGAAGGGGGAAGAAGTCAAAATCTTATGTTGTTTATACGTGGAAAAGCTATATCA
GGGGCTCCTAATATTAAGGGAACCAATCAATTTCCGAATCCTCCAATTATAATAGGTATT
ACTATAAAGAAAATTATTACAAAAGCGTGGGCTGTCACAATTACGTTATAAATTTGATCA
TCACCAATTAAGTAACCAGGTTGACCCAATTCAGCTCGAATTAGTATACTAAGAGAAGTA
CCAACTATGCCGGCCCATGCTCCGAAAATAAAGTATAATGTTCCAAT

***P. gracilis* (KY316379)**

TAGGGTCACCTCCTCCAGCAGGATCAAAGAATGATGTATTTAAATTTCGATCAGTTAATA
ATATTGTAATAGCACCCGCTAAAACAGGTAGAGAGAGTAGAAGTAGTAAAGCTGTAATA
CCTACAGATCAAACAAATAAAGGTGTTTGATCAAGAGATATACCTGGTGATCGTATATTA

ATAATAGTAGTAATAAAGTTTACTGCACCTAGAATAGAAGAAATTCCAGCTAAATGAAG
GGAGAAAATAGCTAAATCTACAGAAGCTCCACCATGAGCAATTCCAGCAGATAGTGGAG
GGTATACAGTTCATCCTGTACCGGCCCCATTTTCGACTAGGCTTCTTGCTAGTAATAAAGT
TAATGATGGAGGTAATAATCAAAAACTTATGTTATTTATTCGAGGGAAGGCTATATCTGG
AGCACCTAGTATTAAAGGGACTAATCAATTACCAAATCCTCCAATTATAATAGGTATAAC
TATAAAGAAGATTATTACAAAAGCGTGAGCAGTAACAATTACATTATAAATTTGGTCATC
ACCAATTAGGTATCCAGGTTGACCTAATTCAGCTCGAATTAAAAGTCTTAACGAAGTTCC
TACTATTCCTGCTCAGGCTCCAAAAATAAAATATAGTGTTCCAAT

Figure 7. A female *Brinckiella* moulting to adult stage.

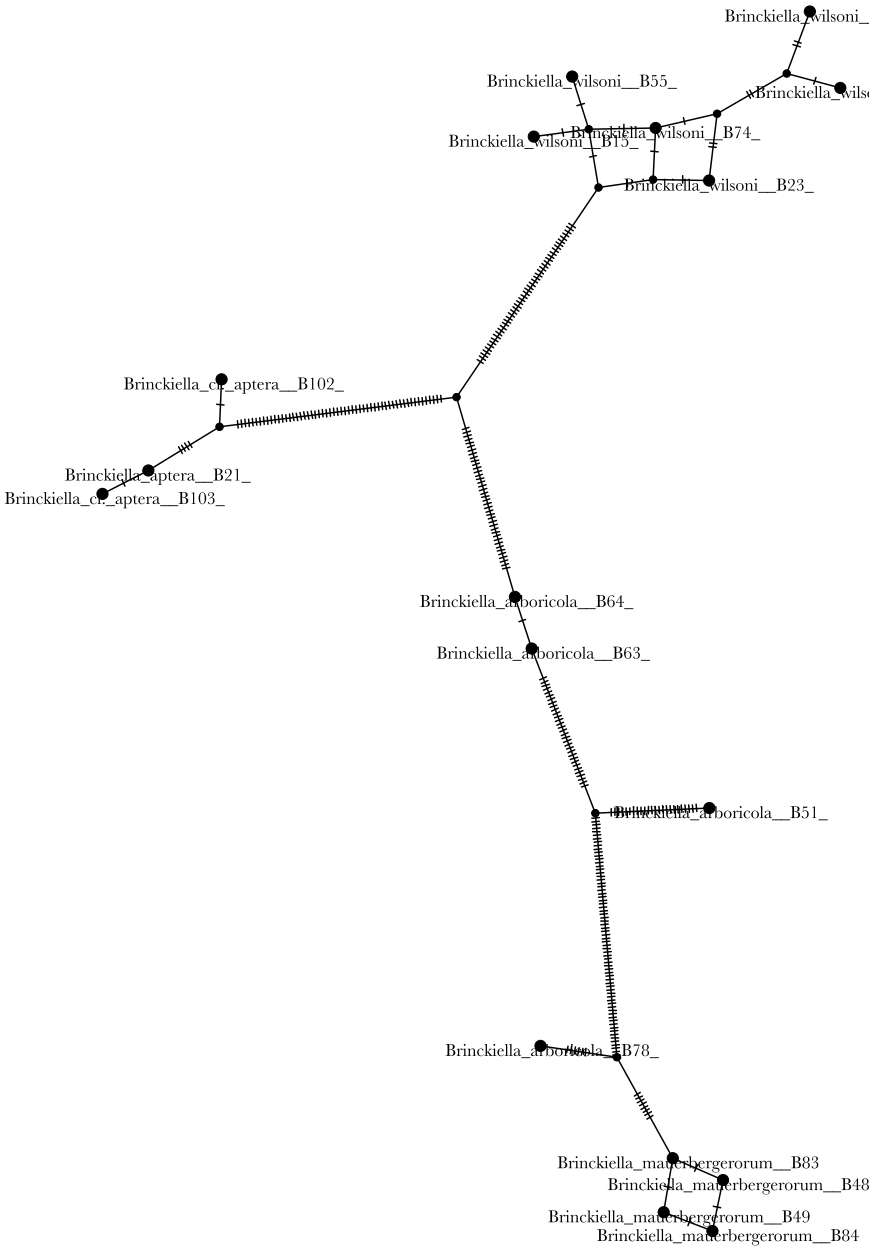

Figure 8. Haplotype networks analyse showing the relationship among different sequence of the genus *Brinckiella*.