Brittany Nicole Damron

Filogenética e a Evolução da morfologia na família Cosmetidae Koch, 1839 (Arachnida: Opiliones): O Curioso Caso de Cosmetidae.

Phylogenetics and morphology evolution of the family Cosmetidae Koch, 1839 (Arachnida: Opiliones): The Curious Case of Cosmetidae.

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Dedication

This thesis is dedicated to my younger siblings: Deven A. Damron- In this infinite and seemingly timeless universe, I am beyond grateful that our blips of existence on this pale blue dot were at the same time and together. Courtney R. Damron- even in your all to short life here you taught me more than any other human I know. Thank you for showing me that time alive does not equal life lived.

Epigraph

"The significance of our lives and our fragile planet is then determined only by our own wisdom and courage. We are the custodians of life's meaning. We long for a Parent to care for us, to forgive us our errors, to save us from our childish mistakes. But knowledge is preferable to ignorance. Better by far to embrace the hard truth than a reassuring fable. If we crave some cosmic purpose, then let us find ourselves a *worthy goal*."

Carl Sagan, Pale Blue Dot: A Vision of the Human Future in Space.

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Index

I. Background

The order Opiliones is a large and diverse arachnid order with approximately 6700 species worldwide, with the suborder Laniatores comprise approximately 70% of all of these species (4000 species), and a further 60% (about 2400 species) of suborder Laniatores are found only in the Neotropics (Kury, 2017). Even with this impressive diversity the Opiliones fauna is still poorly known in the Americas, with Central America and the Amazonian regions especially knowledge impoverished. Many reasons are given for this: lack of funding museums/institutions and researchers, difficulty with bureaucracy and political systems, and dangers posed to researchers (Grieneisen et al., 2014).

The two largest families of the Neotropical Opiliones are the Gonyleptidae (820+ spp), and the Cosmetidae (710+ spp) (Kury, 2017) both of which are found from Southern South America, into Central America. Though both are are the most diverse families in the Neotropics, the Gonyleptidae has received a higher share of modern taxonomy revision and phylogenetic work. Much of the subfamilial groupings in Gonyleptidae have been examined in various morphological and phylogenetic analyses (DaSilva & Pinto-da-Rocha, 2010; DaSilva & Gnaspini, 2010; Mendes, 2011; Pinto-da-Rocha & Bragagnolo, 2011; Pinto-da-Rocha et al., 2014; Bragagnolo et al., 2015; Gueratto et al., 2017; Carvalho & Kury, 2018). Even over many years and many scientists working on the hypotheses of relationships amongst Gonyleptidae clades, there is still much work to be accomplished with such a species rich family, and the relationships of clades and species

continues to change (Peres et al., 2019; Benedetti & Pinto-da-Rocha, 2019).

Cosmetidae have a geographic range from the Eastern United States to the Atlantic Rainforest of Brazil, and is recorded in all countries of this region except for Chile. Cosmetidae species richness is highest in Northern South America, Central America and Caribbean, and are one of the most commonly collected Opiliones families in Central American when sampling (Proud et al., 2012; Pinto-da-Rocha & Yamaguti, 2013). Unfortunately, Cosmetidae has not received the same amount of attention in resolving the internal relationships amongst its subfamilies and genera. Perhaps this is in part due to a lack of taxonomic experts in the Neotropical region where the family is especially diverse, and a general lack of interest from current opiliologists (Coronato-Ribeiro & Pinto-da-Rocha, 2015).

Adults of Cosmetidae are diagnosed and easily recognized by their spoon-shaped pedipalps, where the femora and tibia are laterally compressed to varying degrees and are held covering the chelicerae. The first Cosmetidae species were described by Perty (1833), and the species Cosmetus varius described in this work would later be designated as the type specimen for the family by Pickard-Cambridge (1904); though the first indication of the Cosmetidae as a group was made by Carl Ludwig Koch (1839). In the 19th century, taxonomists continued to describe some of the first species for Cosmetidae (Perty, 1833; CL Koch, 1839; Simon, 1879; Sørensen, 1884; and Banks, 1893), however the majority of taxonomic works began in the 20th century with Roewer's revision of the family (1912) and the creation of what would become known as the "Roewerian system" (Roewer, 1916; 1923; 1927; 1947). This system of

characters arises from the definitions of genera that C.F. Roewer created, which in the case of the Cosmetidae, relied significantly on a combination of dorsal scutum (DS) and free tergites armature patterns, and number of tarsal segments on legs. This quickly became problematic, because many of these characters can be variable, even intraspecifically (Mello-Leitão, 1933, Kury et al., 2007, Pinto-da-Rocha & Yamaguti, 2013), and the Roewerian system resulted in a substantial number of monotypic genera. In an attempt to rectify these issues, Clarence and Marie Goodnight (1953) synonymized a large number of species and genera from Central America, and Mexico, choosing to only use tarsal segments on leg I (5, 6 and + than 6 segments) in combination with DS armature, resulting in the 64 cosmetid genera of Roewer being reduced to only three genera. This was reversed in Kury (2003), when most genera were revalidated until a modern revision of the family could be made..

The two subfamilies of Cosmetidae (Cosmetinae and Discosomaticinae) were erected by Pickard-Cambridge (1904), and later amended by Roewer (1912). Each subfamily was defined by a single character: either the presence of pectination on the tarsal claws of legs III and IV (Discosomaticinae), or smooth tarsal claws (Cosmetinae). This character has been rejected since Ringuelet (1959) and smooth claws were proposed to be a plesiomorphic character, making Cosmetinae a paraphyletic subfamily. Modifications to the tarsal claw pectincation on legs III and IV character have been minimal, with simply presence being modified to a single or double row of teeth on the tarsal claws (Medrano & Kury, 2018). Studies of the discosomatine genera Gryne and Roquettea (Ferreira & Kury, 2010) indicated that they possess a form of pectination different from that observed in other genera of the subfamily

Discosomaticinae, but this was not explored further. A recent morphological analysis on the Discosomaticinae created two characters related to pectination, one simply absence/presence, and the other the number of rows of pectination (Medrano & Kury, 2018). In Ferreira and Kury (2010), the subfamily Cosmetinae was also proposed to be paraphyletic- though they did state that the monophyly of the subfamily Discosomaticinae was weak without further phylogenetic work. It is obvious from the problems related to the Roewerian system that new characters, especially those related to genitalia, be used to examine the relationships amongst the species of the Cosmetidae. It is expected that some genitalia characters and their forms would be highly species or genus specific, especially the male intromittent organ, which has a strong selection pressure on it from the female genitalia of a conspecific (Brennan & Prum, 2015).

The issues with taxonomy and interpretation of characters in Cosmetidae have been difficult to resolve, since many historical descriptions are short, without images or poorly illustrated, and for a short time juveniles were designated to their own family, due to the presence of raptorial pedipalps until their final instar (Palpinidae Pickard-Cambridge, 1904), which was later synonymized with Cosmetidae (Roewer, 1923). The authors Mello-Leitão (1933) and Sørensen (1932) contributed a substantial number of species to the family from South America, as well as the monograph of Venezuelan cosmetids by González-Sponga (1992), though none of these attempted to revise any pre-existing groups.

Despite this complex history, in the last 10-15 years there has been an increasing number of works attempting to rectify the problematic taxonomy of Cosmetidae using genitalia characters, and relatively robust

descriptions and designations for less diverse groups (see Taito, Kury & Barros, 2014; Ferkeria, Monteiro & Pinto-da-Rocha, 2015; Eulibitia, Medrano & Kury, 2017; Rhaucus García & Kury, 2017; and Metalibitia, Coronato-Ribeiro & Pinto-da-Rocha, 2017). The two most speciose genera of Cosmetidae, Cynorta Koch, 1839 and Paecilaema Koch, 1839, have had new characters and neotypes designated in an attempt to rectify the many taxonomic issues in the family (Kury et al., 2007 and Kury & Medrano, 2018, respectively). Townsend et al. (2010), created a key to Central American species of cosmetids, one of the few works focusing on the region, with substantial information about dimorphism, morphology and genitalia. However, many of these works focus on a single genus each with a low level of species richness (except Townsend et al., 2010), and no attempts have been made to examine many species across multiple genera spanning Cosmetidae richness and geographical range in a single analysis.

This thesis will examine morphological characters through a phylogenetic analysis, in an attempt to classify more accurately the subfamilial framework of Cosmetiade, and through a well sampled phylogeny explore the evolution of the family more fully. Due to Laniatores preferring warm wet forests, having generally low vagility, and being nocturnal and cryptic, many groups have high rates of endemism (Pinto-da-Rocha et al., 2005), and the Cosmetidae are no exception. These features also make Laniatores excellent model organisms to understand the shared history of terrestrial ecosystems (Sharma & Giribet, 2012). To date no analysis examining divergence events have been completed using the Cosmetidae as the model taxon. Since Cosmetidae are numerous and known to exist throughout the Caribbean

and Central America, any studies sampling both this region and South America can provide integral information about the history of this region. Additionally, analyses looking at divergences can provide additional information regarding the ages of clades, demonstrating over time the shared history of lineages, especially when morphological characters are found to be uninformative.

II. Introduction

A. Phylogenetic and Systematic History of the family Cosmetidae

To-date no work published has done any substantial investigation of the relationships within Cosmetidae. The few cosmetid species that have had molecular data collected were simply used as an outgroup in other analyses (Pinto-da-Rocha et al., 2014; Bragagnolo, Hara & Pinto-da-Rocha, 2015), or as exemplars in a larger analysis at order level (Sharma & Giribet, 2011; 2014). This is unlike the family Gonyleptidae, which has had multiple phylogenetic analyses, one for the entire family (Pinto-da-Rocha et al., 2014), and many examining the relationships within subfamilies (e.g, DaSilva & Pinto-da-Rocha, 2010; DaSilva & Gnaspini, 2010; Mendes, 2011; Bragagnolo & Pinto-da-Rocha, 2012). The relationships in Gonyleptidae have continued to evolve as more molecular data is collected and further analyses are completed (e.g. Pinto-da-Rocha, et al., 2014, Peres et al., 2019), and while many advances have been made in understanding the systematic relationships in Gonyleptidae, there are still groups within the family in need of revision (see Introduction in Pinto-da-Rocha et al., 2014).

Cosmetidae has not received the same level of interest beyond some cladistic analyses completed with only morphological characters, but these have focused mostly on the subfamily Discosomaticinae (Medrano & Kury, 2018) or a single genus with a limited number of species from across the family's richness (Coronato-Ribeiro & Pinto-da-Rocha, 2017). Given that many characters historically used to define clades have been shown to be highly homoplastic and therefore problematic (Ringuelet,

1959) and these analyses being limited in scope, no conclusions about relationships within the Cosmetidae in its entirety can be made with confidence. Homoplasy is especially evident in characters such as number of tarsal segments on legs, the armature of the DS, and pectination on claws III and IV. As mentioned, this is especially evident for the subfamily Discosomaticinae, with a single synapomorphy of pectinate tarsal claws on legs III and IV. To date it has been stated that this character had arisen in various forms, but this assertion has not been tested across the diversity of the family, only within a small proportion of species (Medrano & Kury, 2018).

The morphology of the Cosmetidae has in the past been considered conserved (Kury and Pinto-da-Rocha, 2007a) raising concerns regarding conducting strictly morphological analyses, especially when morphological characters may have a high level of homoplasy. This is only problematic due to the conserved patterns in external morphology in a single lineage, genus or species group, though perhaps not the case when the entirety of the family is taken into consideration. A relatively small number of characters, and the continued use of historic (and problematic) characters have led to hypotheses of relatedness that may not be supported by more robust and modern morphological characters and molecular data. Analyses to date have only addressed a very small proportion of the family's total species richness (~3%; Medrano & Kury, 2018), which has not allowed characters that have been proposed as diagnostic features to be tested as valid synapomorphies or diagnostic features across the richness of the family. Given a well sampled phylogeny the evolutionary history of Cosmetidae can be explored for the

first time, and morphology in the context of a phylogeny can be examined to attempt a subfamilial classification.

B. Use of Morphology in Phylogenetic Analyses and the Evolution of Morphology in the Family Cosmetidae.

Methods for collecting molecular data has advanced significantly, with difficulty of methods and costs falling (Giribet, 2015). With this, there is a fear that some phylogeneticists will ignore morphological data, when genetic data can easily be extracted and sequenced (Mooi & Gill, 2010). It has been proposed that this can be due to morphological data and how collecting these data are not using more modern methodology, such as that of molecular methods (Wiens, 2001). The most pressing problems being a lack of appropriate experts and taxonomists, and issues with "character analysis" such as translating anatomical data into codified characters, standardization of that coding, ordering, and weighting characters (Wiens, 2001). Some authors proposed that phylogenetic signal of morphological data could be lost when accompanied by molecular data, especially when consensus methods were employed and the data was not congruent with that of molecular data (Miyamoto, 1985). This was disputed by others on the principle that congruence between morphological characters and covariation of this data would still play a role in relationships that would be hypothesized (Donoghue, 1989; Eernisse and Kluge, 1993). Additionally, it is likely that the perceived benefits of using just DNA sequences, such as designating an associated sequence in a database with a holotype specimen (Tautz et al., 2003), come with their own host of similar issues, such as sequences being a subjective source of data. This is countered with the argument that

alignment methods, differentiation between paralogs and orthologs, and selection of genes to sequence are all issues that cannot easily be solved or standardized (Lipscomb et al., 2003).

Morphological data should not necessarily be excluded from being used in large phylogenetic analyses. They are still informative and important in understanding the evolution of lineages of taxa on Earth, both extant and extinct, the latter containing the vast majority of diversity of life on Earth (Edgecombe 2010). It has been shown in various studies in the last decade that there can be some phylogenetic signal for morphological characters, especially for genitalia characters of animals with internal fertilization, requiring intromittent male genitalia (Song & Bucheli 2010). Bieler et al. (2014) demonstrated that with bivalves almost half of their 210 morphological characters (99) had significant phylogenetic signal, and many of these characters could be easily identified and codified in fossil taxa, allowing fossils to be placed in the resulting phylogeny. Sánchez-Pacheco et al. (2018) had shown that though the morphological data in their dataset for Riama accounted for only 1.7% of the matrix, it still had an effect on the resulting topology when compared to a molecular only data-based phylogeny. A similar pattern had been presented in De Sá et al.'s (2014) study of Leptodactylus, where only 3% of their dataset contained morphological data, but according to SPR (Subtree Prune and Regraft) distance calculations between molecular and total evidence trees, there were differences in placement of derived clades between the two resulting topologies. These patterns were further supported in this work by the increased support of relationships for more than half of the clades examined, most of which were for more derived relationships, in one case

resolving placement of a species with no morphological coding (see The relevance of non-molecular evidence discussion in De Sá et al. 2014).

Avoiding coding for morphological data will only exacerbate the problems that exist for morphology coding in phylogenetic analyses. Additionally historical collections of taxa that can not have molecular data extracted from them (fossils, and some historical museum collections) may not be appropriately placed in phylogenetic analyses, and the only way of placing these taxa would be coding the morphological data available for them and placing them as terminal taxa (Lee & Palci 2015). Additionally, not all fields of biology are interested in the phylogeny itself, but the possible patterns of evolution the phylogeny conveys, such as the evolution of morphological adaptations in response to changes in the environment.

Cosmetidae present a unique challenge when conducting a total evidence analysis. While it is likely that many of the current and commonly used characters to define lineages (especially subfamilies and genera) have been proposed to be uninformative, this has not been explicitly tested in a phylogenetic analysis, and it is therefore important that these characters be analyzed in a study with molecular data. To date, many analyzes of lineages within the family have only used the morphological characters for a few chosen clades (representing at most 3% of species in the family, see Coronato-Ribeiro & Pinto-da-Rocha, 2017; Medrano & Kury, 2018), leading perhaps to the false assumption about the validity of currently used morphological characters, and their possible signal in a phylogenetic analysis.

Morphology of the Cosmetidae has been problematic historically. The Roewerian system (Roewer, 1912; 1923; 1947) and subsequent changes

and synonymizations (Goodnight & Goodnight, 1953) have obscured the valid taxonomic patterns of the family, and made understanding morphological characters and their ability to demonstrate relatedness difficult. An example of this was the continued use of the diagnostic character proposed to define the subfamilies Discosomaticinae and Cosmetinae; the presence/absence of pectination on tarsal claws of legs III and IV (Roewer, 1923; Ferreira & Kury, 2010), even though this character was thought to be uninformative as early as the mid-20th century (Ringuelet, 1959).

Male genitalia is a wellspring of morphological knowledge in Opiliones (Martens, 1976), with the genitalia of the Cosmetidae have only recently being explored in taxonomic works, though in Cosmetidae the general form is conserved when compared to Gonyleptidae, a family with similar species richness. Intromittent organs, though a simple concept, are massively diverse, usually attributed to coevolution of male and female reproductive organs; with male genitalia being shown to be an important source of characters to define species of arthropods (Eberhard, 1985; Hosken & Stockley, 2004; Brennan & Prum, 2015; Kelly & Moore, 2016). Many different mechanisms act on this evolutionary “arms race”, from sexual conflict and female choice to lock and key mechanisms to prevent hybridization (Brennan & Prum, 2015). Female genitalia, though likely to be a legitimate source of morphological data, tend to be more difficult to analyze relative to a male’s intromittent organ, which in Opiliones can easily be everted and removed from the individual. Female genitalia on the other hand, are not sclerotized and short in Laniatorids, making removal and preparation for microscopy difficult.

Evolution of the male intromittent organ in Opiliones is theorized to have evolved independently from those found in other arthropod groups: it is hypothesized that early Opiliones would have had genitalia that behaved as a way of placing spermatophores like the group Cyphophthalmi (Macías-Ordóñez et al., 2010), but quickly evolved to an intromittent organ, which appears in some of the earliest fossil records of Opiliones in the Devonian (Dunlop et al. 2003). However, this would indicate genitalia in Opiliones as having originated independently in the lineage of, and therefore different from that of other arthropods, though the evolution of genitalia in Opiliones are still strongly influenced by the same evolutionary mechanisms listed above. This is reflected in the complexity seen in structures of the penis, such as those seen on the glans in Laniatores, and in the complex system of sensilla and setae on the ventral plate (VP). The latter in Laniatores is believed to play an important role in copulation with females, acting as stimulation to guarantee deposit of immobile sperm into sperm receptacles within the lumen of the ovipositor (Macías-Ordóñez et al., 2010). It is believed this is why Laniatores males tend to be immobile during copulation, while species in Eupnoi are more mobile (which do not have penises with complex sensilla structures; Macías-Ordóñez et al., 2010). Though it has been hypothesized that structures on the glans and stylus have evolved due to male-male competition, such as ventral processes for removing sperm of a previous mate (Macías-Ordóñez et al. 2010), there is recent evidence of possible ovipositor plugs in certain species of Laniatores, including Cosmetidae species, which could explain the differences in structures on the glans amongst families (Townsend et al., 2018). Mating plugs are deposited in the reproductive tract of females to prevent

subsequent mating with males, acting as a form of sperm competition in male-male competition strategies in sexual selection models (Miller, 2013).

Male genitalia were not used in descriptions or generally discussed until later in the 20th century. In the 1960s, Forster (1962 & 1963) had written various guides to New Zealand's Opiliones fauna, including images of male genitalia as part of the diagnosis of species, due to many only being differentiated when male genitalia were examined. Martens (1976) would then contribute significantly to general knowledge of Opiliones genitalia with his work focusing on form and function of both male and female genitalia, suggesting the importance of genitalia for systematics of Opiliones. With the exception of a single species in Martens (1976), and the substantial work of Goodnight and Goodnight (1976) focusing on morphology and development of the species Erginulus clavotibialis, Cosmetidae genitalia were not examined or used in diagnosis as common practice until more recent and modern taxonomic works. The first works including more than a single species being that of Avram & Soares (1983) and Gonzalez-Sponga (1992), the former included female genitalia, still a rarity in modern literature.

While male genitalia have been studied in depth for many years, but female genitalia have been mostly ignored until recently (Bennett & Townsend, 2013; Walker & Townsend, 2014; Brooks et al., 2017). It is postulated that the reduced complexity in Laniatores ovipositors is likely related to cryptic female choice, and male sperm competition (Machado & Macías-Ordóñez 2007), which perhaps is why the ovipositor has been ignored as a source of phenotypic characters. This does not mean that there are no possible characters to be used, as the large mechano-sensilla

on the external portion of the ovipositor exhibit interspecific variation in their distal tip shape, and texture in the family Cosmetidae (Walker & Townsend 2014). It is likely that many informative morphological characters would correspond to internal structures of the ovipositor, where much of cryptic female choice will occur. Though limited in characters, female genitalia in Laniatores, and therefore Cosmetidae, should not be entirely overlooked.

III. Objectives

The primary objective of this thesis is to conduct phylogenetic analyses of species from across the geographic range of the family Cosmetidae to assess its monophyly, and to examine possible subfamilial frameworks, therefore creating a backbone for the internal relationships of the family using both morphological and molecular data. This work will build the groundwork future endeavours to understand and further examine relatedness amongst clades within the family. Divergence events will be explored in this analysis to further understand the evolutionary history of the Cosmetidae.

Additional objectives are to examine the influence of morphology in a total evidence analysis of the family Cosmetidae, and examine the informativeness of morphological characters presented here for the first time, as well as those presented in the taxonomic literature for the family. Examining the patterns of evolution can assist in understanding the relationships and history of the family, further supporting the framework established in the phylogenetic analyses of this work.

IV. Materials and Methods

A. Sample Selection

a. Ingroup terminal selection

Species for the family Cosmetidae were selected to encompass as many genera holotype species, and exemplars as possible across the majority of the geographical distribution of the family. Individuals were used based on availability of fresh biological material, if males were available in the sample to examine male genitalia, and there was a representative in the samples of the Arachnological Lab IBUSP. Other samples were made available to be used in this analysis from the Museum of Comparative Zoology of Harvard University (MCZ), the Federal University of Piauí (UFPI), and from colleagues at Toulouse University in France (CEBA project).

This resulted in 125 specimens of Cosmetidae, 17 belonging to indeterminate genera, and 33 belonging to unknown species. Forty genera (~35% of all), and 17% of known species are represented in this sample, across the geographical range from Mexico to Brazil. Of the genera included in this work, 14 have more than 10 species, and this work sampled anywhere from 3% to 30% of the species from any given genus (TAB 01). Almost half of the genera studied were represented by a specimen of their respective type species. (TAB. 1).

Table 1: Genera represented from the Cosmetidae in this analysis, number of species of each, and whether the type specimen is represented. The symbol '--' in the table are for cases where no individuals included were described species, and proportion can not be calculated.

Genera (~30% of family)	**Number of species**	**Proportion of total species in genus**	**Type represented**
Arucillus	1	0.5	no
Bokwina	2	1	yes
Cosmetus	4	0.19	no
Cynorta	22	0.13	yes
Cynortula	1	0.03	no
Cynortellula	1	1	yes
Cynortellana	1	0.14	no
Discosomaticus	3	--	no
Erginulus	5	0.14	no
Eucynorta	4	0.11	no
Eucynortella	1	0.07	no
Cynortoides	1	0.11	no
Eucynortula	1	0.08	no
Eulibitia	2	0.11	no
Eupoecilaema	3	0.38	no
Ferkeria	2	1	yes
Flirtea	4	0.16	no
Gnidiella	1	1	yes
Gryne	4	0.36	yes
Heterovononones	1	--	no
Holovononones	1	1	yes
Metacynorta	1	0.17	yes
Metacynortoides	1	0.2	no
Metalibitia	4	0.4	yes
Metavononoides	1	0.25	no
Meterginus	5	0.24	yes
Paecilaema	11	?	yes
Paecilaemana	2	--	no
Paracynorta	1	1	yes
Paraprotus	2	0.5	no
Paravonoes	2	--	no
Pebasia	1	--	no

Genera (~30% of family)	**Number of species**	**Proportion of total species in genus**	**Type represented**
Platygndes	1	1	yes
Platymessa	1	0.33	no
Reimoserius	1	1	yes
Rhaucus	1	--	
Roquettea	2	0.29	yes
Taito	4	0.14	no
Vonones	1	0.08	no
Unknown	18	--	

b. Outgroup terminal selection

Outgroup terminals were selected based on availability of molecular data, as well as their relation to the family Cosmetidae. The root taxon Triaenonychoides cekalovic Soares 1968 (Triaenonychidae, Opiliones) is a commonly used species for rooting Gonyleptoidea phylogenies due to its position in the suborder in Insidiatores, sister to the suborder to which Gonyleptoidea belongs, the Grassatores (Pinto-da-Rocha et al. 2014). Another ten terminals were chosen based on their relationship to the family Cosmetidae from a phylogeny of the superfamily Gonyleptoidea (Pinto-da-Rocha et al. in preparation). These specimens represent the families Cranaidae, Gonyleptidae, Gerdesiidae, Stygnidae and most importantly Metasarcidae (the sister family to Cosmetidae; Pinto-da-Rocha et al. 2014) (TAB. 2).

Table 2: Species used as the outgroup in the analyses of Cosmetidae relationships.

Species	**Family**
Triaenonychoides cekalovic	Triaenonychidae
Gonyleptes horridus	Gonyleptidae
Mischony cuspidatus	Gonyleptidae
Ampheres luteus	Gonyleptidae

Species	**Family**
Eusarcus insperatus	Gonyleptidae
Eutimesius simoni	Stygnidae
Gerdesius mapringuari	Gerdesiidae
Gerdesius paruensis	Gerdesiidae
Ayacucho spielbergi	Metasarcidae
Ayacucho querococha	Metasarcidae

B. Morphology character coding methods

Specimens that were used for coding of somatic and genitalic characters for analyses were male, with the exception of a single species Cynortula stellata Roewer 1912 (OP0453), which was initially wrongly identified as male and sequenced. When more than one male was available, the most robust male would be selected for character coding, since there is evidence of major and minor males in species (Solano-Brenes et al., 2018), if only one male was available this male was used. This was followed for all specimens when possible, due to perceived differences between the sexes and in some cases differences between males within a single species. Individuals were then immersed in ethanol and viewed using a dissecting light microscope (Leica EZ4) to code for most somatic characters. For level of DS granulation and presence of granulation on free tergites, individuals would be superficially dried in order to more easily observe these characters (as discussed in Coronato-Ribeiro & Pinto-da-Rocha, 2017).

The selected male would be dissected and the penis removed and prepared for SEM (scanning electron microscopy). Removal of the penis involved pulling back the genital operculum, and making small incisions with a dissecting scalpel on either side of the ventral plate to pull back and expose the penis. The penis was then removed in its entirety (from

the base) with sheath still intact. The penis would have excess sheath removed to expose the distal end (where the important characters are found) and then placed in a small vial in 70% EtOH and placed back into the sample tube, until they were prepared for SEM.

To be cleaned and prepared for SEM, the penis would be placed in a container with deionized (DO) water, and then into an ultrasonic cleaning machine for one minute. The penis would then be placed into a container with detergent and DO water, placed back into the machine for an additional minute, rinsed one last time before being subjected to an ethanol gradient dehydration.

The ethanol gradient process used started with 70% ethanol, and would rest for 10 minutes, if the penis had not already been stored in ethanol. Then the penis would be removed from the container, and moved to a vial with a percentage that was higher by approximately 5-10%, and rest for another ten minutes. This process was repeated until the penis had rested in 100% ethanol for two-three cycles (approximately 30 minutes). The penis would then be removed from the ethanol, and placed in a petri dish to completely dry. No other chemicals would be used to help in the desiccation process, due to previous instances of the penis drying too quickly and becoming shriveled, obscuring important characters.

SEM stubs were prepared by painting an adhesive (usually red nail polish) on the top of the stub, and placing two pieces of wire on the stub to act as a platform for the penis. Colloidal silver was used to adhere the penis to the end of the wire-platform, the mounted specimen was then dried in an incubator until sputter coated with a Balzer sputtercoater to a thickness of approximately 10-20μm and photographed with a Zeiss DSM940 electronic microscope at Instituto de Biociências, Universidade

de São Paulo. At least four photos were taken; ventral, dorsal and both lateral views, when appropriate photos of unique features were also taken.

In a few cases ovipositors were examined and photographed. Some were photographed using SEM protocols similar to the ones described above for male genitalia. The exception to this protocol is the amount of time the ovipositors were cleaned using an ultrasonic cleaner. Since they are far more fragile than penises their cleaning time was usually reduced to one minute in total. Other ovipositors were photographed using a Zeiss Axioskop 2plus with a mounted AxioCam MRc camera, after being cleared for at least an hour in glycerine. Photographs were examined from the literature when available for species in this analysis. No characters were created or scored for ovipositors in this work since only a few were examined, and only examined in combination with trees for possible shared morphological patterns.

Ninety-five Morphological characters, were scored either by direct observation of the specimen (as described above) or by examining photos, and drawings from literature, typically in the case of some outgroup species. Fifty-four of the morphological characters were modified from the literature, and 41 new characters were examined in this thesis (see morphological character list in appendix), all characters were treated as unordered. There were six iterations of morphological characters before the final character list was used, each iteration being used in an analysis and informativeness examined. The scoring was maintained in Mesquite v 3.6 (Maddison & Maddison 2018), and exported as a TNT-format morphological matrix. Additional formatting was needed to make the morphological matrix compatible with RAxML, which was completed using text editor.

All plates have been created using Inkscape (Harrington, 2005).

C. Molecular methodology

Two main forms of DNA extraction methods were used for the samples in this analysis. DNA was extracted for some samples used the Agencourt® DNAdvance System kit (Beckman Coulter, California, USA) following protocols in Pinto-da-Rocha et al. (2014).

This protocol was inefficient for specimens less than 5mm in length due to limited amount of muscular tissue, so DNA was extracted using a modified protocol based on the InstaGene matrix (Bio-Rad Laboratories, Hercules, CA, USA). Muscular tissues would be extracted from an individual, which would be completely desiccated then suspended in 50µl InstaGene matrix. Then a simple process in a thermocycler was followed to complete the extraction methods; one hour and thirty minutes at 40˚C, eight minutes at 90˚C and finally 4˚C until samples could be analyzed using Nano-drop to determine if the extraction was successful.

Five loci were amplified for this analysis: the nuclear ribosomal gene 28S, mitochondrial ribosomal genes 12S and 16S, mitochondrial Cytochrome c oxidase subunit I (COI), and the nuclear histone protein gene H3. Amplifications for 12S, 16S, H3, and COI were performed using GoTaq G2 Flexi DNA Polymerase (Promega) in a 25 µl volume containing 5 µl of DNA, 5µL 5X GoTaq Flexi Buffer, 2µL MgCl2, 1µL DNTP set, 1µL of each primer and 0.05µL of GoTaq. 28S samples were amplified using Phusion High-Fidelity (New England Biolabs, Thermo Scientific) using the same mastermix recipe. PCR conditions for 12S, 16S, H3, 28S, and some COI samples comprised initial denaturation for 5

min at 95˚C, 35 cycles of denaturation for 30 at 95˚C, annealing for 30-60 seconds at primer-specific temperature (see below), extension for 1 min to 1 min and 10 sec at 72˚C, and a final extension for 7 min at 72˚C. Problematic COI samples used the PCR Touchdown protocol as proposed in Astrin et al. 2016.

Amplifications were performed with following primer sets: 28S rDNA with 28SRD1AF (5'-CCCSCGTAAYTTAGGCATAT-3') and 28SRD4B (5'-CCTTGGTCCGTGTTTCAAGAC-3') at 50˚C (55˚C with Phusion High Fidelity) overlapping with 28SD3AP (5'-CAAGTACCGTGAGGGAAAGTTG-3') and 28SB (5'-TCGGAAGGAACCAGCTACTA-3') at 55˚C (60˚C with Phusion High Fidelity; Nadler et al., 1999; Reyda and Olson, 2003; Edgecombe and Giribet, 2006; Arango and Wheeler, 2007); H3 with H3A-F (5'-ATGGCTCGTACCAAGCAGACVGC-3' and H3A-R (5'-ATATCCTTRGGCATRATRGTGAC-3') at 55˚C (Colgan et al., 1998); 12S with 12SAIN (5'-AAAAACWAGGATTAGATACCCT-3') and 12SOP2RN (5'-CCCTTAAAYYTACTTTGTTACGACC-3') at 45˚C (Pinto-da-Rocha et al., 2014); 16S with 16SpotFN (5'-GACTGTGCAAAGGTAGCATAATC-3') and 16SBR (5'-CCGGTCTGAACTCAGATCACGT-3') at 45˚C (Palumbi, 1996; Pinto-da-Rocha, 2014); and COI with either LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCOoutout (5'-GTAAATATATGRTGDGCTC-3') at 45˚C (Folmer et al., 1994) under PCR protocols as described above, or LCO1490-JJ (5'-CHACWAAYCATAAAGATATYGG) and HCO2198-JJ (5'-AWACTTCVGGRTGVCCAAARAATCA) at 45˚C (Astrin & Stüben, 2008) with the Touchdown PCR protocol. PCR products were

inspected by gel electrophoresis and transillumination and purified using Agencourt AMPure XP (Beckman Coulter Inc.). Purified products were then quantified using a Thermo Scientific NanoDrop spectrophotometer. Once this data was collected, the purified samples would be cycle-sequenced directly in both forward and reverse directions using ABI Big-Dye Sequence Terminator (v.3.1), precipitated by sodium acetate (CH3COONa) and analyzed on an ABI Prism 3100 Genetic Analyzer/HITACHI automated sequencer (Applied Biosystems).

Contiguous sequences were assembled using quality thresholds with either Consed/PhredPhrap (Ewing and Green, 1998; Ewing et al., 1998; Gordon et al., 1998, 2001) or Geneious R 9.1.8 (https://www.geneious.com, Kearse et al., 2012).

D. Dataset organization

Two total evidence datasets were created from the above specimens. One dataset with missing data, which includes 125 terminals that are Cosmetidae, and 11 that are the outgroup (ATMD all taxa missing data), and another data set with no missing data containing all of the 11 outgroup species and 98 species of Cosmetidae (MTAD missing taxa all data). Both datasets were analyzed using the methodology as outlined below.

E. Phylogenetic analysis methods

a. Maximum Parsimony Analysis implementing Dynamic Homology in the program POY

COI and H3 sequences were aligned using MAFFT (version 7.271, Katoh et al., 2013) using global pair alignment, with opening cost of 3, adn

closing cost of 1, visualized in Aliview (Larsson 2014), and inspected for stop codons using the Invertebrate Mitochondrial coding, and the standard code (respectively) in Aliview. All sequences were then trimmed using Aliview so that the first base corresponded to the first codon position. Three internal partitions were annotated for 12S, two for 16S, and four for 28S. This was based on assessing possible homologous regions within each gene as described in Giribet (2001).

Maximum Parsimony phylogenetic analyses were conducted using the program POY v5.1.1 (Wheeler et al., 2015) using a 16 core Ryzen 1700X processor with 16GB of RAM on the IB-USP Arachnology laboratory computer.

Nucleotide sequences and morphological data were then subjected to a direct optimization (DO, Wheeler 1996) search process. This process would run DO for a specified time frame (2 to 10 hours in five searches, a total of 30 hours), and report the most parsimonious trees (MPTs) and their costs. This would allow for exploration of optimal max_time for the DO search for the current dataset. For the datasets of this research it was found that the lowest tree cost was with max_time of 00:08:00 for MTAD, and 00:10:00 for ATMD.

After the initial search process, all sequence data and morphological data were analyzed using DO in POY. Sequences for H3 and COI were read in to POY as pre-aligned sequences, while 12S, 16S, and 28S were aligned using dynamic homology alignment methods implemented in POY (with the transform command). All transformations were equally weighted, and all morphological characters were considered unordered. Five rounds of searches were completed with the max_time. Each search round (with the search command) implements Wagner tree

building, Subtree Pruning and Regrafting (SPR), Tree Bisection and Reconnection (TBR), Branch Swapping (RAS+swapping, as described in Goloboff 1996), Parsimony Ratchet (Nixon 1999), and Tree Fusing (Goloboff 1999). The final trees from the DO analysis were then used in an iterative pass (IP) analysis (Wheeler, 2003a), and costs for all the previous optimal trees were calculated and the implied alignment was generated (Wheeler, 2003b). Goodman-Bremer values were calculated for trees in TNT v 1.5 (Goloboff & Catalano 2014) using the bremer.run script (Goloboff et al. 2008a).

b. Maximum Likelihood Analyses using the program RAxML

Analyses conducted using RAxML were performed on the CIPRES Science Gateway (https://www.phylo.org/portal2/) using RAxML HPC2 on XSEDE (version 8.2.10, Stamatakis 2014). For Maximum Likelihood analyses in RAxML, all sequences were aligned using global pair alignment in MAFFT, with an gap-opening cost of 3 and closing cost of 1 with a -maxiterate value of 1000. All sequences and morphological data were then concatenated into a super matrix using SequenceMatrix 1.8 (Vaidya et al., 2011) and edited in a text editor to follow input format for RAxML.

Data was partitioned by molecular marker and morphological characters (multi-state). Phenotypic characters were analyzed under a MK multi-state data model using a Lewis ascertainment correction (Lewis 2001) for the morphological characters, while molecular data were analyzed using the GTR+GAMMA model. Random seed was set to 12345 (-p), following script is commands for RAxML as reported on CIPRES;

raxmlHPC-HYBRID -T 4 -n result -s infile.txt -q part.txt -K MK -p 12345 -m ASC_MULTIGAMMA -f a -N 1000 -x 12345 -o opilio_0969,opilio_0362,opilio_0092,opilio_1589,opilio_0952,opilio_0499,opilio_0046,opilio_0280,opilio_1579,opilio_0139,opilio_0960 --asc-corr lewis

Bootstrap values were calculated for phylogenies in RAxML, using 1000 iterations

c. Bayesian Analyses and Divergence Events

The appropriate models for Bayesian analyses for the MAFFT aligned sequences were determined using jModelTest2 on XSEDE implemented on CIPRES Science Gateway (https://www.phylo.org/portal2/). The Akaike information criterion (AIC) (Posada & Buckley 2004) scores were used to select the best fitting models for Bayesian analyses, with the exception of COI sequences. The best model for COI was GTR+G, however initial analyses did not converge for COI parameters, and so the less complicated model of HKY+G was used for COI in further analyses. The best fit models for 12S and 16S both were GTR+I+G, 28S was GTR+G, and H3 was HKY+I+G.

Divergence analyses were completed using multi-gene data in the program BEAST 1.10.0 (Drummond et al. 2012) under a relaxed clock model on CIPRES Science Gateway (https://www.phylo.org/portal2/). The Markov chain Monte Carlo (MCMC) search ran with four chains for 100,000,000 generations sampling the Markov chain every 10,000 generations, and the sample points of the first 20,000,000 generations were discarded as ‘burnin’, as determined by Tracer v1.7.1 (Rambaut et al. 2018). Cosmetidae were constrained as a monophyletic clade, as was

the outgroup that belonged to Gonyleptoidea. Divergence events were based on dates provided for Gonyleptoidea from previous analyses of Opiliones (Sharma & Giribet 2011; Giribet & Sharma 2014), using a Uniform distribution with a minimum age for the Gonyleptoidea clade of 100 million years, and a Yule process tree speciation model.

F. Differences in topology between POY and RAxML

Weighted SPR distance and topology similarity were calculated using a script in the program TNT v 1.5 (Goloboff & Catalano 2016) to calculate a measurable difference in topologies between trees created in POY and RAxML.

G. SPR distance calculations for effect of Morphology on Topology

The effect of morphological data on the total evidence topology was examined using SPR distance (Goloboff et al., 2008a; Goloboff & Catalano, 2016), calculated in the program TNT v1.5. These comparisons were done for all datasets, comparing a molecular only analyses to a total evidence analysis. This was done with a simple script that reported the weighted SPR distance (see Goloboff et al. 2008a), and the proportion of similar topology.

H. Morphological Character Optimization and History

The program Mesquite v. 3.6 was used to determine the Retention Index (RI) and Consistency Index (CI) for each character in each of the datasets for analyses (see morphological character list in appendix).

Character history was mapped and visualized in Mesquite to determine possible synapomorphies for groups, and to explore generally

observed patterns across all datasets and analyses. This was done for both the Maximum Parsimony and the Maximum Likelihood analyses, where in the former characters were mapped according to the Maximum Parsimony method in Mesquite, and the latter with the Maximum Likelihood methods implemented in Mesquite. For the purposes of model simplicity and discussion of general overall patterns of evolution, the Parsimony analyses are discussed here.

I. Geographic Distribution of Clades

Images of the geographic distributions of species and clades for all datasets and analyses were created using the R package phytools (Revell 2012). All tree figures with maps were created as figures and images using R and exported.

V. Results

A. Phylogenetics of the family Cosmetidae under Parsimony optimality criterion, Maximum Likelihood, and Bayesian.

a. Results of POY with ATMD set.

The parsimony analysis using DO and IP resulted in a tree length of 26363 (FIG. 1 poy_atmd). Cosmetidae is maintained as a monophyletic clade with a Goodman-Bremer score of 25, with their closest sister family from the Gonyleptoidea being Metasarcidae (Goodman-Bremer score 34). This relationship is expected due to findings in previous analyses of Gonyleptoidea families (see Pinto-da-Rocha et al., 2014), as well as some morphological characters, such as a generally rectangular shaped ventral plate (VP) of the male genitalia.

The current subfamilies within the family Cosmetidae are not monophyletic in this tree. The character state of smooth tarsal claws is plesiomorphic, since it is also shared with much of the outgroup. In phylogenetic analyses here, the Cosmetinae as they are currently defined are not monophyletic, but a paraphyletic group. Though this analysis includes the type for the subfamily Discosomaticinae, the type species of *Discosomaticus* Roewer, 1923 (*D. cinctum*) was not included in this analysis, however the species *D. sturmi* is remarkably similar to *D. cinctus*. It is possible that the species related to *D. sturmi* are more similar to the type than that of *D. distinctus*, which was demonstrated in Avram and Soares (1983) to have a very distinct penis morphology. Since the species herein are not monophyletic within the scope of this analysis,

both the Cosmetinae and Discosomaticinae should be viewed as synonymous until further work can be completed.

The genera of *Metalibitia* Roewer, 1912, *Platygyndes* (Roewer, 1943), and *Ferkeria* Roewer, 1947 are grouped into a sister clade to the rest of the family (Goodman-Bremer score 13, clade 1; FIG 1). The species of Cosmetidae most closely related to the *Metalibitia+Ferkeria+Playtgndes* (clade 1), is a group of species mostly from Ecuador and Peru, from various genera, such as *Cynorta* and *Flirtea* Koch, 1839 (clade 2; FIG 1). The species of these two genera (*Cynorta albituber*, and *Flirtea erxlinea*), will need to be placed in other genera according to modern definitions (Kury et al., 2007; Kury & García, 2016). Another lineage near the *Metalibitia+Ferkeria+Playtgndes* clade contains species from Colombia and Panama, from various genera (clade 3; FIG 1).

Some genera that have recently been reviewed maintain monophyly of species, such as *Taito* (clade 9; FIG 1; Kury & and Barros 2014), *Gryne*, and *Roquettea* (clade 10; FIG 1; Ferreira & Kury 2010; Medrano & Kury 2018). These more modern definitions, especially using characters associated with male genitalia, has improved small parts of the taxonomy of the family.

The subfamily Discosomaticinae is polyphyletic, split into three distinct lineages in this analysis, each associated loosely with a form of pectination of tarsal claws on legs III and IV, with the exception of one *Discosomaticus* species (*D. distinctus*, clades 6, 9, and 10; FIG 1).

b. Results of POY with MTAD set

The total tree cost for this dataset was 20222 (FIG. 2), with Cosmetidae again maintained as a monophyletic clade (Goodman-Bremer score 25), with their sister family being Metasarcidae (Goodman-Bremer score 28). Though in general clades 1, 2, and 3 maintain a very similar position to those in Figure 1, others have moved considerably (see species of clade 8; FIG 2). Additionally clade 9 (FIG 2) has moved closer to the clade 3 than in figure 1, but this can in part be due to sampling density.

c. Results of RAxML with ATMD set

Similarly to the POY analysis above, RAxML analyses recovered the family Cosmetidae as a monophyletic clade (Bootstrap of 90; FIG. 3) with their sister clade being the family Metasarcidae (Bootstrap of 100).

The subfamily of Cosmetinae is paraphyletic in this RAxML analysis as well. This analysis does not improve understanding of the obscured relationships in the deeper nodes of the tree (see clade 8; FIG 3), however the clades of Discosomaticinae groups observed in the Parsimony analysis (*Roquettea* + *Gryne*- clade 10, *Paraprotus* + some *Discosomaticus* species- clade 6, and finally *Discosomaticus distinctus* + *Taito*- clade 9) are maintained with the same species as that in figure 1 (see table 5 for species). A significant difference being that clades 9 and 10 in figure 2 have more species separating them in the phylogeny than in figure 1. This lends additional support to splitting these species groups apart, and no longer recognizing them as belonging to the same subfamily.

Clades 1, 2 and 3 are maintained in much the same position as in figure 1, with the *Metalibitia+Ferkeria+Platygyndes* clade recovered as the sister clade of the remaining Cosmetidae (Bootstrap 100).

d. Results of RAxML with MTAD set

As observed in all analyses already presented above, the Cosmetidae as a whole is maintained as a monophyletic clade, however again neither Cosmetinae or the Discosomaticinae are monophyletic, and Cosmetinae is a paraphyletic group (FIG. 4). The *Metalibitia+Ferkeria+Platygyndes* clade is recovered as the sister clade to the rest of the Cosmetidae, and as mentioned above, many genera are not monophyletic within the family, with the exception of a few recently reviewed and erected genera. The taxonomic chaos of the family is especially evident in the deeper nodes of the family, especially those from Central America and the Caribbean (clades 12, 13, 14, and 8; FIG4), where speciose genera do not form monophyletic lineages. In general, clades in figure 4 are like that of figure 3, with the exception of clades 12 and 13, which have been split.

e. Results of BEAST analysis with ATMD set and Divergence Events.

Cosmetidae is maintained as a monophyletic family with a posterior probability value of 1 (FIG 5). This analysis is only based on molecular data, which was used to determine the divergence events of the family Cosmetidae, and was completed to explore the possible relationships in Cosmetidae further. Though clades 1 and 2 are relatively in the same location in figure 5 as they are in figure 1, *Metalibitia+Ferkeria+Platygyndes* (clade 1), as well as the Peru/Ecuador groups of species containing *Rhaucus* sp. 953, and *Cynorta albituber*

(clade 2) are sister to the rest of the family. Clades 3 and 4 are in the same position as those in figures 1 and 3, but the more derived clades are different than that observed in either the POY or RAxML analyses (see positions of all other caldes; FIG 1, 3 & 5).

In this BEAST analysis, the age of Cosmetidae is older than has been proposed in other analyses, at approximately 47 million years old. Clades 1 and 2 diverged from the rest of the Cosmetidae almost immediately after the family diverged. Interestingly clade 5 (a Central American clade) diverged from the rest of the family almost 10 million years before other lineages of Central American species (such as clades 8, 12, 13, and 14).

B. Geographic Distribution of species of Cosmetidae

Figures 6-10 show the geographic distributions of terminals from figures 1-5, without the outgroup terminals. As demonstrated in figures 1-5, many genera that have not had recent revisions are not monophyletic, but as can be seen in figures 6-10, the vast majority form clades that tend to correlate strongly with the geographical region from which the species were collected, rather than the current taxonomic classification.

C. Differences in POY and RAxML topologies

Though the two optimality criteria (Maximum parsimony and Maximum Likelihood) necessarily treat data differently, the phylogenies obtained from their analyses are still ~86% similar to one another, with an unweighted SPR distance of 18. The SPR distance values are as high due to the distance that some lineages have moved. While the differences between sister species, and the small changes would not have a high

impact on the SPR distance, the fact that some clades have moved significantly in their position, increases the SPR distance score.

Some major lineages are the same, like that of the Metalibitia and Peruvian lineages that are sister to the rest of the Cosmetidae (caldes 1 and 2, FIG. 1-5). As you move to more derived positions in the trees, differences become more apparent (FIG. 11 and FIG. 12; see Table 5 for species included in collapsed branches). Some clades were not collapsed in these topologies because the inner relationships were not the same, though the clade still contains the same species (see branch highlights FIG. 11; FIG. 12). The lineage with the biggest differences is the most recently diverged in both trees, dominated by Central American species, with a green highlighted clade and group G. It is likely that the relationships here are not concordant between the POY and RAxML topologies for the same reasons many of these nodes have low Goodman Bremer and Bootstrap values; low taxon sampling from the region, and perhaps the genetic markers used in this work do not resolve these relationships.

D. Effects of Morphological Characters on the topology of Phylogenetic analyses.

In this study, morphological characters represented approximately 3% of the total matrix, represented as 95 characters. Of these 95 characters, only 25 had a Retention Index approximately/greater than 0.500 in Parsimony analyses. Of these 25, 12 were somatic characters, the other 13 were characters related to male genitalia. SPR distances calculated by the program TNT show that in the case of Maximum Parsimony analyses,

morphological characters had some influence on topology in both Parsimony and Maximum Likelihood analyzes (TAB. 3).

Table 3: SPR distance and similarity between molecular tree and total evidence tree for POY and RAxML datasets.

	ATMD dataset	**MTAD dataset**
POY analyses	8.5579 weighted (30 unwt) SPR moves (similarity: 0.9357)	9.1885 weighted (34 unwt) SPR moves (similarity: 0.9133)
RAxML analyses	10.8199 weighted (28 unwt) SPR moves (similarity: 0.9186)	8.1774 weighted (23 unwt) SPR moves (similarity: 0.9243)

There were approximately 6% missing data in the ATMD data set (mostly molecular data), and in the POY analysis the addition of morphology made the topology more stable, although some were likely to have substantial amount of missing data. While in the MTAD (which has no missing molecular data) the addition of morphology had a slightly larger effect on more derived clades. This is demonstrated by the larger number of SPR moves and lower percent similarity observed between the molecular only and total evidence MTAD trees (TAB. 5).

The opposite is observed in the RAxML analyses when looking at the weighted and unweighted SPR distance. Adding morphology into the ATMD Maximum Likelihood analysis appears to move a terminal further away from its position in a molecular only analysis, than in the MTAD dataset. This could be the result of a few taxa with a large proportion of missing data, acting as wild card taxa.

E. Evolution of Morphology in the family Cosmetidae

Morphology was coded for 95 characters (see appendix for character list), and Consistency and Retention indices are presented in table 6. Character

history was traced for 57 of the morphological characters (FIG 13-70?). Images for examples of somatic character variation is presented in figures 71-75, and SEM of genitalia for clades of interest are in figures 76-94. Figure demonstrating the ovipositor variation for some species of Cosmetidae is in Figure 95.

F. NEW SUBFAMILY Metalibitiinae subfam. Nov.

The clade containing *Metalibitia* (Type-genus), *Ferkeria*, and *Platygyndes* is resolved as the sister clade to the rest of the Cosmetidae in POY and RAxML analyses, and again in BEAST as part of a clade with Peruvian/Ecuadorian species (posterior probability 0.7729) , which warranted a closer look at this lineage as a potential subfamily. This general relationship of the genus *Metalibitia* being sister to the rest of the Cosmetidae has support in other analyses that have included Metalibitia as terminals in their analyses (Pinto-da-Rocha et al 2014; Derkarabetian et al. 2019). This monophyly of the genus, as well as its relationship with *Ferkeria* and *Platygyndes* species, is recovered in all analyses in this work. This clade is further supported by possessing many features that are not consistently found in the rest of the family, if at all. The Metalibitiinae subfam. nov. clade in general, though they exhibit the same pedipalp compression as all Cosmetidae, possess many other characters setting them apart as distinct from the rest of the family.

Subfamily Metalibitiinae new

Cosmetinae Koch, 1839 (part)

Included genera: *Metalibitia* Roewer, 1912 (type genus), *Platygyndes* (Roewer, 1943), and *Ferkeria* Monteiro & Pinto-da-Rocha, 2015.

Diagnosis: Family Cosmetidae, with gamma or alpha dorsal scutum (DS) outline, with multiple parachelicerate projections. Males with monomorphic chelicerae, separated tubercles on pedipalp femur. Area IV of DS always armed with either tubercles or spines, and a line of granules on the posterior margin. Coxae IV in all species have a small apical prolateral apophyses, and no clavi inguines present on coxae IV. Genital glans does not possess a ventral process, stylus is cylindrical in shape.

Description: Prosoma and Opithosoma. Either a high rounded ocularium that is armed (*Ferkeria*+*Platygyndes*), or a low unarmed ocularium (*Metalibitia*), area I, II, and III typically armed, though in is absent in a few species (*Metalibitia brasiliensis*, *Platygyndes titicaca*, and *Platygyndes* 0738). All individuals in this novel subfamily have DS dense granulation, with *Ferkeria* 0739 being an exception with a smooth DS.

Legs. Males in this lineage (except for *Platygyndes* 0739) have armature on the dorsal surface of femur III and IV.

Genitalia. *Ferkeria*+*Platygyndes* have a sulci at the base of the VP in ventral view, curved apical margin, one pair of MS-A, two pairs of MS-B, two pairs of MS-C, two pairs of MS-D, two pairs of sunken MS-E, and a small amount of type 4 microsetae in lateral fields in ventral view. Stylus short, does not surpass apical margin of VP, seminal opening of stylus with “finger like projections.

Metalibitia possess defining mid-VP membranous extensions, all macrosetae reduced and cylindrical, one pair of MS-A, one pair of MS-B, two pairs of MS-C, one pair of MS-D (except in M. borelli), and three-four pairs of sunken MS-E. No microsetae are observed on the VP

of Metalibitia. Stylus in *Metalibitia* is far exceeds the apical margin of the VP, seminal opening with either no armature, or a frayed appearance.

Remarks: Free tergites are armed in some species within this new subfamily, though not a pattern in all species included, and patterns seem to be genera specific, as rounded tubercles in *Metalibitia*, and pointed tubercles in *Platygyndes* and *Ferkeria*. This is a rare character typically only found in this new subfamily, and shared predominantly with the outgroup analyzed in this work.

Femur and tibia of the pedipalp are compressed as found in the rest of the family Cosmetidae, but not to the same extreme as that observed in some other species. Initially the species *Platygyndes* titicaca was identified as a Gonyleptid (Roewer, 1943), and it wasn't until the work of Pinto-da-Rocha and Hara (2013) that this species was synonymized with *Praelibitia titicaca*, and moved to the family Cosmetidae. This is also evident in the other species included in this new subfamily, where the compression is not so extreme, though still evident (*Metalibitia* being more like the rest of the Cosmetidae; Coronato-Ribeiro & Pinto-da-Rocha, 2017).

Genitalia in this clade are clearly unique for the family Cosmetidae, and demonstrate what could be viewed as "intermediate" forms between the rest of the family Cosmetidae, and the outgroup. Characters associated with the VP in this lineage are different between the genera. While individual genera groups have differing patterns of characters associated with the VP, there are a number of characters that all genera in this lineage share associated with the glans. Most species in this lineage do no possess a dorsal process on the glans, and no ventral process, but no wattle or caruncle like that observed in the rest of the Cosmetidae.

Distribution: Found in Bolivia, Uruguay, and the Southernmost part of Brazil, closest to this region (state of Rio Grande do Sul). This is especially important to note, as geographic region of species is especially informative in the family Cosmetidae, and reflects relatedness pattern more closely than that of morphology.

G. Synonymization of Cosmetinae and Discosomaticinae

COSMETINAE Koch, 1839

Cosmetinae Koch, 1839: 19; Kury, 2003: 36.

Discosomaticinae Roewer, 1923: 388; Kury, 2003: 86. NEW SYNONYM

Diagnosis: Subfamily Cosmetinae, have alpha, beta, and delta DS shape. Tarsal claws on legs III and IV can be smooth, or have pectination. VP of penis have one pair of MS-B, two pairs of MS-E, no membranous extensions. Glans is projected from the base of the VP, has no ventral process, possesses a dorsal process, and stylus has a wattle/caruncle, with armature.

Remarks: See Kury, 2003 for extensive list of species and genera included in each subfamily, as they are synonymous. As demonstrated by all phylogenetic analyses in this current work, neither of these subfamilies are monophyletic, and until further work and modern diagnoses can be done they should be synonymized.

VI. Discussions

A. Discussions Regarding Phylogenetic relationships

a. *Discussion of Monophyly of the family Cosmetidae*

The family Cosmetidae is maintained as a monophyletic group in both POY and RAxML analyses. This would indicate that the defining features of the pedipalp femur and tibia shape can be used as a synapomorphy for the family, as it is supported by analyses using only molecular data. In addition, there are a suite of other characters exhibited by all Cosmetidae that is found in many of the lineages in the family.

The internal relationships of some parts of the family are questionable, which may in part be due to the sampling density of specimens being low (about 17% of the described species richness; TAB. 1), and an artifact of the sequences used in this study. I believe this is evident in all trees, where there are nodes that have lower Goodman-Bremer, bootstrap values, or posterior probabilities than those observed elsewhere in the tree. This is especially evident in the RAxML analysis- where some nodes have Bootstrap values lower than 50 (FIG 3 & 4). This may be a result of the "missing" samples that would help to resolve these nodes, a high level of homoplasy associated with morphological characters, or the fact that the loci chosen in this analysis do not help resolve this hierarchical level. For example, COI is a sequence that may help to resolve recent divergences and the relationships that are the result of those events, and the other more highly conserved sequences, like 12S and 16S, resolve the deeper relationships in the tree (Combosch et al., 2017). This could lead to the intermediate

relationships not being resolved adequately, reflected as low support values in topologies presented here. This is a known issue discussed around the “work-horse” sequences from Sanger sequences (i.e. 16S, COI, H3, 28S, 18S), which though used together may resolve relationships, the resolution can be weak (Combosch et al., 2017).

In the relationships of the clades from Central America and the Caribbean, poor taxa sampling is perhaps the cause for the patterns observed. This is especially evident in the location of clades 8, 12, 13, and 14 in all phylogenies, where their placement was not consistent, other than most of them being restricted to younger lineages (see FIG 5). This is perhaps in part because this region is where the species richness for the family is fairly large, and almost entirely unknown, and though the terminal sampling in this work covers localites across the region, it is quite paltry in species number considering the diversity. Sister species, especially those collected from the same locality or localities in close proximity, will necessarily have higher Goodman-Bremer and Bootstrap values, however nodes that contain conspecific samples from multiple distant localities (i.e., in other countries) have values that are much reduced.

b. *Discussion of Monophyly of the two subfamilies, Cosmetinae and Discosomaticinae*

F.O. Pickard-Cambridge, in his 1904 work, did little to address the subfamily Discosomaticinae, only listing it as a subfamilial group of the Cosmetidae with pectination on tarsal claws III and IV. Discosomaticinae was renamed and species further diagnosed and described by Roewer (1923), though more than half of the genera he described were

monotypic. The subfamily has been the focus of a systematic review, and a single cladistic analysis (Ferreira & Kury, 2010; Medrano & Kury, 2018), though these only use morphological data, and do not address adequately the question of whether pectination can be considered a synapomorphy in the family Cosmetidae. These works mainly focused on the genera *Gryne* and *Roquettea*, and maintained that these, as well as the genera *Discososomaticus*, *Protus*, *Paraprotus*, and *Sibambea*, form a monophyletic group (see Ferreira & Kury 2010; Medrano & Kury 2018). Their patterns are only partially supported here, where *Roquettea* and *Gryne* form a clade (clade 10), but *Paraprotus speciousus* was not recovered as related to them but to some species of *Discosomaticus* (clade 6). Additionally this study found that the latter clade of genera did not form a clade with *Roquettea*+*Gryne*, but their own in another part of the phylogeny. This current study demonstrates that the character of pectination on tarsal claws is uninformative as a simple absence/presence character (see Ringuelet 1959), especially since no other characters were proposed to define the subfamily. The results of Medrano and Kury (2018) implying monophyly is likely due to their analyses relying on only morphological data. It is possible that the analysis would resolve that the character states of pectination observed to have arisen one time only, since it is the most parsimonious reconstruction. However, it is evident that when molecular data is used in an analysis, many of the features proposed, such as pectination of tarsal claws III and IV (in all its forms), and even some characters proposed in this analysis such as attenuate ventral plate of the penis, are homoplastic arising in various independent lineages throughout the family.

It is apparent from this work that the two current subfamilies of Cosmetidae are not monophyletic, demonstrating again the dire need for a new subfamilial framework for this family.

c. *Discussion of Relationships within and among the genera of Cosmetidae*

Most of what is known about the relationships in and amongst genera in the family Cosmetidae have been the result of cladistic analyses of morphological characters, or the taxonomic diagnosis and rediagnoses of groups of species and/or type species (Coronato-Ribeiro & Pinto-da-Rocha 2017; Medrano & Kury 2018). With no comprehensive analyses examining these relationships incorporating molecular data as well as morphological data, it was unknown whether the hypotheses of relatedness proposed in these works would be maintained.

In this analysis, some of these patterns are still maintained, such as the monophyly of the genera *Taito* (clade 9; Kury & Barros 2014), *Metalibitia* and *Ferkeria* (clade 1; Coronato-Ribeiro & Pinto-da-Rocha 2017 and Monteiro & Pinto-da-Rocha 2015 respectively). For *Taito*, the authors had proposed that this genus was most closely related to the genera *Cynortopyga* Roewer, 1947, *Eucynortella* Roewer, 1912 and *Vononoides* Roewer, 1912. Only *Eucynortella* was included in this current work, and therefore only a statement can be made about the relationship with that genus. In the analyses presented here, *Taito* is not closely related to the species of *Eucynortella* included here (*E. longa*), however, this species is from a locality in Panama, which would not have a closely shared history with the region of Amazonas where the genus *Taito* is located.

Some recent rediagnoses of genera are not supported in this study, such as the proposed monophyly of various genera of the Discosomaticinae (see the placement of *Paraprotus speciosus* in Medrano & Kury 2018). In Medrano and Kury (2018), the distribution of the subfamily and relationships were briefly discussed, but these phylogenetic relationships are far more complex, especially in a region with dynamic biogeographical history. The species in Medrano & Kury (2018) and additionally the species in this work from Discosomaticinae, span a region with a complex history over the course of various divergence events in the family Cosmetidae, some shortly after the family diverged from the rest of Gonyleptoidea. While the analyses here support a clade of *Paraprotus*+*Discosomaticus* (clade 6) as presented in Medrano & Kury (2018), *Discosomaticus* it not recovered as monophyletic due to the placement of *D. distinctus* as a sister species to the genus *Taito* (clade 9). The *Paraprotus*+*Discosomaticus* is also more closely related to lineages from Amazonas, the Cerrado, and the Northern Atlantic Rainforest of Brazil, such as *Flirtea batman*+*F. valida* in POY (clade 6; FIG 1 & 2), RAxML (FIG. 3 & 4), and BEAST analyses (FIG. 5), and in RAxML and BEAST analyses are the sister clade to the type species for *Paecileama*. The *Gryne*+*Roquettea* clade (10), recovered as a distinct lineage in the analyses here are distant from that of *Paraprotus*+*Discosomaticus,* and more closely related to lineages from French Guiana (see species of clade 11; FIG 1-5). This region of the South American continent has a complex biogeographical history, and given that the lineage containing *Paraprotus*+*Discosomaticus* diverged earlier in the history of the family Cosmetidae than that of the lineage containing *Gryne*+*Roquettea* (FIG 5), this would imply that the shared

history were shaped by different geological events. Morphology of pectination of tarsal claws III and IV further divides these lineages. *Roquettea* and *Gryne* both exhibit two rows of pectination of more or less equal height, and *Paraprotus* and *Discosomaticus* species in this analysis have two rows of pectination where the medial row is taller than the ectal row (FIG 43). This latter group is still split in the phylogeny, furthering the idea that pectination is not an informative character for subfamilial classification, and needs considerable work.

The inclusion of certain species in the genus *Flirtea* is questionable, Kury and García (2016) had proposed that the genus *Flirtea* only contained three species; *F. picta* (holotype), *F. valida*, and *F. batman*, all of which are Brazilian species and included in the present analyses. However, my results suggest that the specimen that is identified as *Flirtea picta* (clade 4) is not closely related to *F. valida* or *F. batman* (clade 6), though in all phylogenies, these two clades are closely related (FIG 1-5) just not enough to be one genus. The authors' reasoning for this was it is possible that speciation in this group is due to allopatric speciation. However in no analyses here do all three of these species create a clade; instead they are polyphyletic, where *F. batman*+*F. valida* are more closely related to the *Paraprotus*+*Discosomaticus* group, which is additionally supported by genitalia characters (FIG. 76). *F. picta* is more closely related to *P. u-flavum*, the type species for the genus *Paecilaema*, which when compared to the distribution of this species in Kury and García (2016), and that of the recent work on *Paecilaema* (Kury & García, 2018), their geographic ranges overlap. The definition of *Flirtea* and its validity as a genus is questionable, due to *F. picta* being so closely related to that of the type of *Paecilaema* (*P. u-flavum*).

Another small genus included in this work that has seen a recent revision is *Eulibitia* (Medrano & Kury, 2017). The type species *E. maculata* was not included here, but two species from the range of the genus in Colombia were, *E. scalaris*, and one undescribed species. The genera that were proposed to be most closely related to *Eulibitia* were not analysed here (*Ambatoiella*, *Libitia*, and *Libitiella*) and therefore no statements can be made about the validity of these relationships. Medrano and Kury did suggest that *Eulibitia* were related to *Taito*, which in the case of RAxML analyses the *Eulibitia* species (clade 7) included here are close to the *Taito* clade (clade 9; FIG. 2). However, the position of the two *Eulibitia* species included in this work could be unstable, because in the POY and BEAST analyses they are sister to a nested Central American clade, and not *Taito* (FIG 1, 2 & 5). Interestingly, *Eulibitia* included here are not closely related to other Colombian species that are included in this analysis. As mentioned above for the Discosmaticinae, the biogeographical history of this region is quite dynamic, and it appears that the lineage that includes *Eulibitia* diverged after that of the *M. serratus* group (clade 3).

In the case of *Flirtea* and many other genera in the family Cosmetidae, perceived shared phenotypic characters do not necessarily equate to a shared common history. There are many genera that as they were defined, have especially large geographic ranges (like historically, *Cynorta* and *Paecileama*), some of which encompassed almost the entire geographic range of the family. This is counterintuitive to expectations based on the biology of these organisms because it is known that Laniatores have low dispersal ability and high site fidelity (Pinto-da-Rocha et al., 2005). This has been demonstrated in a recent

study of the genus *Meterginus* (García & Damron 2019). Historically, this genus was known from Central America, Brazil, Ecuador and Colombia, but its range is now restricted to only Mexico, Guatemala, Belize, and Honduras. Similarly, demonstrated in this work, that one of the most commonly collected *Meterginus* species from Colombia (*M. serratus*), is not closely related to the type species *M. basalis*, and that the *M. serratus* clade diverged quite early in the history of the family (clade 3; FIG 5), has morphologically distinct genitalia, and should be synonymized with or described as another genus.

d. *Divergence events in the family Cosmetidae.*

The patterns observed in divergence events in the family Cosmetidae paint a compelling story about the diversity of the family, as well as their biology being closely associated with the terrestrial landscapes of which they are a part.

The most interesting patterns of divergence are the clades from Central America and the Caribbean. There are two distinct lineages in the BEAST analysis, one of which diverged before the other (clade 5), and is closely related to a group of species from Colombia, Venezuela, Brazil and French Guiana. This pattern could correspond with a land bridge hypothesis referred to as GAARlandia (Iturralde-Vinent & Macphee 1999), which additionally has had some support in arachnid groups (Tong et al. 2019). The GAARlandia hypothesis suggests that an additional land bridge existed into the Carribean from South America, before the formation of the Isthmus of Panama. This is demonstrated by some lineages in the Caribbean being more closely related to South American lineages and being older, than lineages in the mainland of Central

America and North America (Tong et al., 2019). This analysis however does not have a dense enough sampling of the Northern part of South America (in Colombia and Venezuela especially), and of the Lesser Antilles to provide a clear image of the history of the region. More robust sampling is necessary to see if the family Cosmetidae also exhibit a pattern associated with the GAARlandia hypothesis.

The current distribution of Cosmetidae is better at predicting the relationships that are observed in the analyses, especially in groups that were paraphyletic or polyphyletic (Hedin et al., 2012). This is especially obvious in speciose genera of the Cosmetiade, such as *Cynorta, Paecilaema,* and *Eucynorta* species, where though the *Cynorta* and *Paecileama* have been rediagnosed and their ranges severely restricted, *Eucynorta* still is speciose (+35 species) with a range from Central America to South America, with various species arising in different lineages at different times over the course of the family's history. These patterns further support to the dismantling of these genera (Kury et al., 2007; Kury & Medrano, 2018). This is even observed in the genus *Flirtea* which had received a recent revision to the species that should be included in the genus (Kury & García, 2016). This genus did not represent a monophyletic clade in any of the analyses here, and represent, in the case of POY and RAxML a paraphyletic relationship, and an apparent polyphyletic relationship in BEAST analyses.

B. Discussion of SPR analysis demonstrating influence of Morphology in Topology

In these analyses, the inclusion of morphological characters did have a small influence on overall topology between a molecular tree and total

evidence tree. In the study on the *Leptodactylus* group in De Sá et al. (2014), an unweighted SPR value for differences between total evidence and molecular topologies were 23-25, which was considered relatively small, with many differences being in just derived nodes. This is similar to what is observed in the RAxML analyses of the current work, where the addition has changed little in the topology- though the impact of change is more obvious in the ATMD set, which is likely due to the molecular data missing for various terminals- some of which are missing up to three of the markers. However, it should not be assumed that the placement of taxa with missing data in RAxML analyses are necessarily incorrect, as demonstrated by Wiens (2006) who was able to show that a terminal could be accurately placed in a Maximum Likelihood analysis with up to 50% of their data missing.

In POY analyses the opposite was observed, where the ATMD set had less of a difference between the molecular and total evidence topologies. This, in part, is due to the fact that in a parsimony analysis there is stability in a topology when more terminals are introduced into an analysis, even if a portion of their data is missing (Wiens 2005), and the addition of morphological characters is likely to only have an impact on the more derived nodes of a clade, while the nodes closer to the base of the tree are less likely to change.

Though stated in other studies that the inclusion of morphological characters helped increase support for nodes (De Sá et al. 2014; Sánchez-Pacheco et al. 2018), this was not a universal pattern found for the Cosmetidae- though some clades did see an increase, many would see a decrease in the support of node. For example, in the RAxML analyses, bootstrap values were lower in the total evidence analysis. This could be

due to the placement of another clade (clade 2) in the molecular analysis as sister to the rest of the Cosmetidae with the *Metalibitia+Platygyndes+Ferkeria* clade (clade 1). However, clade 2 has morphology that is more like that are the rest of the Cosmetidae, while clade 1 is more like the outgroup phenotypically, which is possibly why when morphology was added, it moved a slightly more derived position. The values within the *Metalibitia+Ferkeria+Platygyndes* also saw a decrease in bootstrap values for the *Metalibitia*, likely due to the change in position of *M. paraguayensis* and *M. brasiliensis*.

These results suggest that many of the somatic characters currently used in the classification of supraspecific lineages in the family Cosmetidae are problematic at best, and appear to have high levels of homoplasy. This is due to a combination of characters proposed by Roewer still being used to determine relatedness, and new characters proposed that have yet to be explicitly examined across the diversity of the family in a phylogenetic context (e.g. DS armature, coloration, see section below *Discussion of Evolution of morphological Character states in the family Cosmetidae*). Though some species groups in the family Cosmetidae exhibit a high level of conservation in many characters, including genitalia characters (Medrano & Kury 2017), extrapolating these patterns to define groups across the family is difficult without a more dense species sample.

The concern with developing more complex characters for this research was in creating compound characters- which still occurred with some characters (such as DS coloration, and microsetae fields and types on VP). There are some authors that believe that the use of compound characters, especially when unordered and unweighted in Parsimony (as

done in this work) can confound true relationships where phylogenetic signal of a state is lost (Brazeau 2011). This is a known issue in phylogenetics and a discussion done in depth by various authors about the problems with morphological character coding (see Wiens 2001 for review of these issues). However, in this work, there were approximately six iterations of a morphological character list, that were then analyzed as part of a total evidence POY analysis. The CI and RI of characters and their character history were explored after each run to advise on moving forward with the character. In many cases, it made more sense in this work to code complex morphological patterns as a compound character to reflect evolutionary history more accurately, than to do a reductionist approach to the character and its states (see below discussion on pectination of tarsal claws on legs III and IV).

C. Discussion of the Evolution of morphological Character states in the family Cosmetidae.

The family Cosmetidae, as shown in all phylogenetic analyses here, is a monophyletic clade originally defined by the compressed femur and spoon-shaped tibia of the pedipalps, held covering the chelicerae. The analyses here show that additional morphological characters can be used to define lineages within the family, though many should be explored further.

a. *Characters of the Prosoma (1-7)*

Of the characters related to the prosoma, few were especially informative in an absolute sense (low CI and RI; see morphological character list in appendix), though a few are promising characters that should be explored further.

Parachelicerae projections (named “guards of protoglyphs” in Medrano & Kury 2018), seem to be seen predominantly in Cosmetidae, though the character states of multiple projections and a single pointed projection are shared with a few terminals in the outgroup. The Cosmetid lineages that possess these states are either proximate to the outgroup, or restricted to a small clade in the family (FIG. 13). Multiple projections are restricted to *Metalibitia+Ferkeria+Platygyndes* clade, but within the family Cosmetidae it is also observed in the few species associated with *Flirtea valida*. The rest of the family possess a single projection of various forms. However, in clades that are associated with Central America, it appears that some species have a modified form, that appears to be lobed or “warty” in appearance. This pattern evolved in a few lineages, and in one case is closely associated with another character of a single low square projection. When examining FIG. 13, it is possible that either states three and four are either the same/closely associated, or need to be further examined for micro-anatomy that may be more informative than the states presented in this work. Currently, some of these forms may be informative in regards to genera or suprageneric level (i.e. lobed/warty form).

Ocularium shape in the family Cosmetidae is a mix of forms, from low and wide, to “dome-shaped”, and high with a median depression (FIG. 14). Since all of these states are also shared with the various terminals of the outgroup, it can not be said that any of these states are only found in the Cosmetidae. Though in general, the family possess a low and wide ocularium, the extent of the qualifiers “low” and “wide” need to be explored in more depth, as the words alone are not informative of the actual observed forms. Rarely other forms of ocularium shape were

observed in the family, and due to the general pattern being very similar, this character would not serve much purpose in defining clades within the family. For example, the species *Bokwina sandersoni*, and *Vonones circumlineatus* (clade 8) both have high domed ocularium, unlike any of the other species in their clade. Another unidentified species from Peru, Cosmetidae sp 1065, has a high ocularium with a median depression, which is not observed in any species in the same lineage, or even in the rest of the family.

Ocularium armature (tubercle, spine or protuberance) is rarely observed within the family Cosmetidae (FIG. 15). It should be noted here that the genus *Roquettea* does possess in some species armature forms, though in this analysis the species included do not possess armature. In general, possessing armature is a plesiomorphic state rarely observed in clades within the family. The only exception being the *Ferkeria+Platygyndes* clade where all species included in this analysis possess spines. For the genus *Roquettea* the armature is likely to be an autapomorphic form, perhaps associated with major and minor male forms found in the species of the genus (Medrano & Kury 2018). The specific type of armature will be important as a character to define small species groups, and perhaps genera as work is completed on lineages within the family.

b. *Dorsal Scutum (DS), Opithosoma, and Venter characters (8-24)*

Many characters proposed as diagnosable features in the Roewerian system are found on the dorsal scutum (DS, e.g. armature). Additionally characters, such as coloration pattern (Kury & Barros 2014; Kury &

García 2016; Kury & Medrano 2018) overall shape of DS (Kury et al. 2007; Kury & Medrano 2016), and granulation presence and level (Coronato-Ribeiro & Pinto-da-Rocha 2017) are characters presented in recent literature as diagnostic characters of the DS.

In general coloration on the DS of the Cosmetidae poses a unique challenge. While there is a general amount of intraspecific variation in some species (Goodnight & Goodnight, 1976), there are other genera that seem to have generally conserved patterns (Kury & Barros, 2014). There is evidence that the waxy yellowish coloration is secreted by glands in the cuticle (Kury & Pinto-da-Rocha, 2007), and though in much of the literature coloration is used to define a genus or species, there is no general pattern across the family to define any suprageneric groups. For example, coloration patterns proposed as a defining characteristic of the *Taito* genus (Equuleus-pattern), is also observed in a few other terminals from Peru and Brazil (*Cynortula stellata*, *Platymessa transversalis*, *Flirtea batman*; FIG. 16), that are not closely related to the genus in any of the analyses here (i.e. *Platymessa transversalis* in clade 5 of FIGS 1-4). The same is true for the coloration pattern presented in the re-diagnosis of *Flirtea* (Scaramuccia-pattern), and the Lyre-pattern used to define the rediagnosed *Paecileama*. Though some of these patterns can be found in multiple closely related species, it is not typically restricted to a single group or clade. An additional concern about using DS coloration as a morphological character in phylogenetic analyses, is the potential that the coloration itself is not determined by genetics, but by environmental factors. Even within a single species, *Erginulus clavotibialis*, it was shown that regionally the coloration on the DS was different, even though genitalia from males all indicated they were the

same species (Goodnight & Goodnight, 1976). Given that a single species can experience extreme variation (or none), and that we are still uncertain of how or why this excretion is created, it is hard to say if it is something that can be said to be under natural selection. This is an important distinction if a morphological character is to be used in a phylogenetic analysis. Until further studies can be completed about how this coloration is made in Cosmetidae, using the coloration on the DS should be used with extreme caution.

Originally presented as a diagnostic character in Kury et al. (2007), the DS outline has been refined over the years to include many intermediate forms of the original four (Kury & Medrano 2016). In this analysis only the original four states were used, since the updated forms in Kury and Medrano (2016) have potentially more than seven forms for the family Cosmetidae, which would create a large complex compound character. When just using the four forms, the most common for the family Cosmetidae is the beta DS outline (FIG. 17), though all forms are observed within the family. Within the clades that are dominated by beta forms, or even the various Central America clades that exhibit alpha, the more specific forms presented in Kury and Medrano (2016) can be explored further to determine if the lineages, especially the distantly related ones, exhibit the same or unique forms. For example, this is possible in the genus *Taito* (see Kury & Barros, 2014), since they tend to exhibit a very modified version of the beta outline. Similarly, it could be that alpha outline exhibited in *Erginulus clavotibialis* (clade 13) is different than that observed in *Arucillus armasi* (clade 14), but until more focused work can be done with these lineages, no statements can be made at this time.

Sulci separating the areas of the DS are conspicuous in most of the Gonyleptoidea, though there are exceptions in some groups, including the Cosmetidae. The family predominantly have very shallow sulci between the DS areas, and in some cases are not visible at all. Within the family, only a few examples in this analysis have sulci that are obviously visible, such as *Platygyndes titicaca* Roewer, 1943, *Arucillus armasi* Vasconcelos and Pérez González, 2003, and *Metacynortoides transversalis* Goodnight and Goodnight, 1942 (FIG. 18). These individuals are far removed from one another in the phylogeny, and the presence of obvious DS sulci is a plesiomorphic character, and should not indicate relatedness amongst taxa that present this character state.

Granulation on the DS in the family Cosmetidae is observed throughout the family, though the most common state in the species included in this analysis do not present any granulation on the DS, essentially being mostly smooth (FIG. 19). When granulation is present, it is usually found in a few species that are closely related, though at times a single member of a clade will possess granulation, even if no other species in that same clade have any granulation present. Perhaps it can be used to define a clade (single genus), or provide an autapomorphy for a species, but at a suprageneric level, granulation on the DS is not informative of relationships.

Characters commonly used to define genera in the Rowerian system were the presence of armature and its general form on the DS (Roewer, 1923). Here, simple forms were examined for all DS areas, where the presence of tubercles/spine/protuberances, and their absence were coded. In the case of almost all areas, there is a high level of homoplasy, so using the current character states do not provide a viable

framework for the family in its entirety. Each area has presence observed through out the family, though some have stronger potential patterns than others. For example, while the presence of Area I armature seems homoplastic (FIG. 20), there is merit to the presence of highly modified states, such as that observed in the genus *Roquettea*. Area II seems to overwhelmingly have no armature observed (FIG. 21), and when present, spines were never observed in the species included in this analysis only tubercles. This state is shared with the outgroup and it is likely, unless microanatomy can show otherwise, that this is a plesiomorphic character within the family.

Areas III and IV (FIG. 22 & FIG. 23) are more promising in possessing states that would be informative of relatedness among lineages. Area III has spines present in the overwhelming majority of species presented in this analysis, with a modified version being present in the genus *Roquettea* as a protuberance, which has various forms depending on the species examined. This is potentially the pattern also presented in the many clades that have presence of spines. For example, when examining the clade that contains *Meterginus serratus* Roewer, 1912, *M. marmoratus* (Roewer, 1912), and *Cosmetus tamboritos* Coronato-Ribeiro & Pinto-da-Rocha 2015, the spines exhibit different forms. *M. mamoratus* and *C. tamboritos* are sister species and both exhibit spine(s) where the base is covered in fine granules, though *C. tamboritos* spines have a joined base (Coronato-Ribeiro & Pinto-do-Rocha 2015). *M. serratus* has two separate spines like that of *M. marmoratus*, however *M. serratus* has smooth spines that are retro-dorsally directed than that seen in *M. marmoratus* (see A & B; FIG. 71). This would indicate that there appears to be little association between

absence and presence of forms, as was used in the Roewerian system, but perhaps more detailed forms can be used-as in *Roquettea*- to diagnose a species.

Area IV is an interesting case in the family Cosmetidae, because for the *Metalibitia+Ferkeria+Platygyndes* clade (clade 1), there is a general presence of spines on area IV (FIG. 23), though this presence is observed elsewhere in the family, it is interspersed throughout the tree, and therefore it may be that these are independent forms that have arisen and are distinct from this clade- which is evident in other species, such as *A. armasi* (FIG. 71), where the form is distinct from that observed in species of *Metalibitia* (Coronato-Ribeiro & Pinto-da-Rocha, 2017). This is not observed in the outgroup terminals included here, and therefore the form that has arisen in the *Metalibitia* may be considered as a distinct form that arose in that lineage, and can be considered a synapomorphy.

Armature on the free tergites is rare amongst the Cosmetidae, exhibiting a plesiomorphic pattern in phylogenies (FIG. 24, FIG. 25, and FIG. 26), though it is likely autapomorphies, due to the fact that this character is coded as absence/presence. It may be that each observation of presence are actually distinct forms, and therefore have arisen various times within the family, that are different than the forms observed in the outgroup.

The presence of granules on the posterior margin of the DS appears to be restricted to a few lineages in the family Cosmetide (FIG. 27). This form is shared with the outgroup, but it is the absence of these rows that seems to arise various times in the family. The *Metalibitia*/*Ferkeria*/*Platygyndes* clade appears to be like the outgroup, as well as a few lineages throughout the family, possibly making the

presence of granules something that can be used as a feature that can diagnose genera or suprageneral groups within the family- in combination with other important features. The forms of presence in lineages should be examined to see if they are distinct from other forms, and perhaps micro-anatomy can help detect these differences. Though the presence is not an autapomorphy for these lineages, they appear so infrequently, that the presence with other characters could help in a suite of characters that are more robust for diagnosing a lineage when more works are completed on these lineages.

c. *Pedipalps and Chelicerae Characters (24-32)*

The family Cosmetidae have been defined by their characteristically shaped femur and tibia of the pedipalp and the way that the pedipalp is held covering the chelicerae. Currently, it is uncertain why Cosmetidae have evolved the pedipalp shape they have (Ferreira Pagoti et al. 2019), with no added benefits to sensing their environment, feeding, or benefiting interactions with conspecifics. These characters are maintained as defining features of the family (FIG. 29), and the shape of the tibia of the pedipalp (FIG. 30), while the arrangement of tubercles on the femur of the pedipalp may be informative for subfamilial groups (FIG. 28). The separate tubercles on the femur of the pedipalps observed in the outgroup and also in the *Metalibitia+Ferkeria+Platygyndes*, do not demonstrate any specific patterns. Occasionally within these groups you will see that the tubercles are arranged in a dorsal row, however this state is also shared with the majority of the rest of the Cosmetidae. An interesting state is the reduction of these tubercles to a single ridge, as if the tubercles all merged and have created a crest on the dorsal margin of the femur.

However this final form has arisen multiple times within the family, and therefore it is likely that this feature is distinct in each lineage and could use a closer examination to see if there are multiple forms for the various clades, which could be used for diagnostic purposes.

All Cosmetidae that were sampled for this analysis, did not have setae on the pedipalp tarsus that were more than 50% of the length of the tarsus (FIG. 31). Long tarsal setae are a common feature in other Gonyleptoidea, like in the families Cranaidae, Stygnidae, and Gonyleptidae, and are reduced in Cosmetidae. This may be due to the way that the Cosmetidae hold their pedipalps against their chelicerae, instead of in the raptorial pose that many Gonyleptoidea hold their pedipalps.

Chelicerae in the family Cosmetidae can come in predominantly two forms, monomorphic where males and females are essentially the same, or where males will have enlarged chelicerae as a sexually dimorphic feature (FIG. 32). Various species of the family Cosmetidae exhibit patterns that would imply that there are major and minor males in populations (Ferreira & Kury, 2010; Solano-Brenes et al., 2018; Medrano & Kury, 2018), however, it was hard to code for this or account for it when determining whether males had monomorphic or enlarged chelicerae. In some cases, only a single male was available to examine for the terminal species represented in this work. When a population was available, the most “robust” male would be selected and his characters coded. Though enlarged chelicerae are restricted to various clades all over the family, it would be important to explore exactly how enlarged chelicerae are, and if this is separated into forms of major and minor males. That could not be accomplished within the scope of this work,

because it was not possible to sample a large enough number of specimens from a species, and this would be necessary in order to understand these patterns.

d. *Legs Characters (33-60)*

In the majority of literature, characters associated with the legs of Opilones have been used to diagnose and define clades (Roewer, 1912; 1923; 1947; Goodnight & Goodnight, 1953). Many times these groupings were determined by the number of tarsal segments, however this was not explored here, as it is evident from taxonomic definitions and phylogenetic works, that this can vary within a single species (and individual), and therefore is not an informative feature for representing true relationships amongst species (Mello-Leitão 1933, Hickman, 1958; Kury et al. 2007, Pinto-da-Rocha & Yamaguti 2013; Damron et al. 2018).

Armature and structures associated with other parts of the legs were examined to see if any patterns were apparent within the family. Coxae in the family Cosmetidae have pro- and retrolateral dorsal apophyses, though only the form of prolateral dorsal apophyses for coxae II show any patterns that were not highly homoplastic (FIG. 33). A simple apophysis seems to be plesiomorphic in nature, as it is the only form observed in the outgroup terminals used in this analysis. The bilobed form seems to be the most common form in the family Cosmetidae, with one half of the tree being mostly dominated by this form. Rarely, various lineages or species (mostly derived nodes), would exhibit a bifid apophysis, following the pattern of perhaps a more complexity evolving along that lineage. If this is the case, then the specifics of these forms should be explored in more detail, as they may

represent an autapomorphy, and can be used for defining the terminal or a species group.

In the family Cosmetidae, the apical prolateral armature on femur IV is not as pronounced as that found in many families of Gonyleptoidea. For the vast majority of the Cosmetidae this armature has been reduced from an apophyses to a simple or "warty" tubercle (FIG. 34). Though the genera *Metalibitia*, *Ferkeria*, and *Platygyndes* exhibit an apophysis, it is not as extreme in form as some of the outgroup (e.g. *Gonyleptes horridus*, or *Mischonyx cuspidatus*). Notably, an apophysis also appears in the majority of taxa in a derived Central American clade, for example species of *Erginulus*, *Paravonones* and *Heterovonones* (clade 13). Closer examination of these apophyses are necessary to determine if they are plesiomorphic or an autapomorphy. For example, if you look at the apophyses of *Metalibitia* species (Coronato-Ribeiro & Pinto-da-Rocha 2017), and *Erginulus clavotibialis* and *E. subserialis* (FIG. 72), one can see that their forms appear to be remarkably similar. The modified tubercles observed in the family Cosmetidae will provide more promising patterns that could help to distinguish the clades of the family. *M. serratus*, *M. marmoratus*, and *C. tamboritos* have tubercles that almost have the appearance of a "divided" tip (Coronato-Ribeiro & Pinto-da-Rocha 2015; FIG. 71), while those of some species of *Gryne* (*G. coccineloides* and *G. dimorpha* FIG. 72) appears to be a cluster of tubercles. While the form found in *C. tamboritos* is similar to that found in *Gryne*, the latter seems to be more a cluster of tubercles, while in *C. tamboritos* is a joined base, where the apical tip of the tubercle appears to be split. Again, like that observed in Gonyleptoidea, it is likely that each

group of species will exhibit their own form that is lineage specific, but coding for such a character would have been difficult in these analyses.

The term clavi inguines was first proposed in a work on *Taito* ("groin warts" in Kury & Barros 2014), though the term was also used in works on *Eulibitia* and *Rhaucus* (Medrano & Kury 2017; García & Kury 2017). They can appear as a group of tubercles, or as a single one seen in dorsal view on coxae IV, near to coxae III. The presence of this character seems to be found in various lineages of the family Cosmetidae (FIG. 35), but this is restricted to a few larger groups. The simple state of presence appears homoplastic in nature, and therefore the forms of this presence should be examined. For example, in *Eulibitia* (Medrano & Kury 2017) clavi inguines is generally present as a low cluster of tubercles, while in *Taito* only a single or couple tubercles are observed (Kury & Barros 2014). The presence of clavi inguines also appears in more recently diverged lineages from Central America (clades 12 and 13). Though the character states are more complex and likely associated with specific genera or species, here absence and presence states were proposed, since in the original use of this character it was proposed as a potentially "older" characteristic in the family (see comments related to the genus *Flirtea*, Kury & Barros 2014). Later in the work on *Eulibitia* (Medrano & Kury 2017) it was suggested that this genus and *Taito* were closely related due to the presence of clavi inguines, while in the *Rhaucus* work it was suggested that there are distinct forms across the family (García & Kury 2017). The latter work was more accurate for the patterns across the Cosmetidae, but this could not have been determined without examining a more thorough taxon sample from across the geographic range of the family. These genera are not closely related to one another in this work,

as was implied by the previous studies. If this character is to be developed further, it would be best to examine the specific pattern associated with presence. Further complicating this character is the fact that many species in the family Cosmetidae have strongly granulated coxae, and differentiating between this and the potential presence of clavi inguines could be a difficult task.

Armature of femur IV is a commonly used diagnostic feature for many groups within Gonyleptoidea, and the Cosmetidae are no exception. However, femur III is not typically examined or thought to possess informative characters in the same way as femur IV. It should be noted, that even if femur IV possess armature, that does not mean that femur III will also have armature (FIG. 36; FIG. 38, and FIG. 39). Presence of armature on femur III presents a distinct pattern, since it is a feature shared with clades 1 and 2, and a few select lineages, and then a large clade that typically contains clades 12, 13, and 14. The vast majority of species that exhibit armature on femur III are restricted to early-diverging lineages within Cosmetidae, and again in a more derived group that is predominantly Central American species. This warrants deeper inspections, and works focusing on these clades would benefit examining the forms more deeply.

Retrolateral armature on femur IV is observed in various lineages spread throughout the family, with little pattern associated with the mere presence of armature (FIG. 38). This is likely because the actual forms of armature are species specific, and coding for all the forms present in the family would lead to a composite character that could have many states, making coding especially difficult and complicated. This is especially evident in the genus *Erginulus*, whose species are diagnosable by the

armature on femur IV (Pickard-Cambridge 1904; Bank 1906; Rower 1912; Rower 1947; Goodnight & Goodnight 1947). Though in some species, like those in *Metalibitia* (Coronato-Ribeiro & Pinto-da-Rocha, 2017), the legs in both males and females are covered in granules, this granulation is more pronounced in males, but does not necessarily have a distinct pattern of spination. This is not as much the case for prolateral armature on femur IV (FIG. 39), which is not as prevalent in the family as retrolateral armature. Many times both or only retrolateral armature is present, though when both are present, it is mostly observed in the early-diverging lineages , such as *Metalibitia+Ferkeria+Platygyndes*, the group associated with *Gnidiella picta* (clade 5), and the large Central American lineage (clades 12-14). Rarely is only prolateral armature observed, and when it is present, it is restricted to single species/terminals in this analysis, such as in the lineage that includes *Taito*. It is likely that both forms of armature should be viewed as diagnostic for species as a very specific form, but perhaps not as informative across the family.

Shape of femur IV shows a compelling pattern (FIG. 37), that upon further examination is not as simple as presented in some previous works of simply being curved or straight (Coronato-Ribeiro & Pinto-da-Rocha 2017). Though there is some similarity in curvature in the *Metalibitia+Ferkeria+Platygyndes* clade and that of the outgroup, the vast majority of the family has a mostly straight femur IV, with the exception of a few terminals and a small recently diverged group from Central America dominated by *Erginulus*, *Paravonones*, and *Heterovonones* species (clade 12). The former group had curvature that is retrolateral in dorsal view (Coronato-Ribeiro & Pinto-da-Rocha 2017), while that of the Central American clade is a bit mixed, exhibiting either

sinuous curvature, or a dorsally curved femur IV (FIG. 73). The forms exhibited in this latter group are possibly the result of a more complex form evolving along the lineage over time, which is especially evident in *Holovonones compressus* (Pickard-Cambridge 1904), where the femur is compressed laterally as well (FIG. 73).

Patella (FIG. 40) and tibia (FIG. 41) follow patterns similar to those described above for armature of femur IV. If a male has evolved large spination patterns on femur IV, then it is likely that the production of spines will be increased for all of the leg, not just the femur. However, further research is needed to know exactly what genes may be dictating this, and whether these genes produce a phenotype for the whole leg or just parts, makes this purely speculation.

Metatarsus of some species of Cosmetidae exhibit sexually dimorphic modifications, though no outgroup individuals were coded to possess any (FIG. 42). Two forms were coded for in these analyses, but there were three observed within the family Cosmetidae, though more may be present within the state of spination presence with a higher sampling density of species. The presence of spines on the metatarsus, are observed as low spines/tubercles in *Ferkeria* (Monteiro & Pinto-da-Rocha 2015) and *Platygyndes titicaca* (see images in Pinto-da-Rocha & Hara 2011), and a cluster of spines as the basal end of the metatarsus in *Rhaucus* sp. 953 (FIG. 73) . However, the genus *Gryne* also has a modified metatarsus that is described in Ferreira & Kury (2010) as “swollen… with polygonal cross-section”. This is true to varying degrees for all individuals examined in this thesis (FIG. 74). Sexual selection commonly can select for very specific autapomorphies in males of species, which is especially evident in vertebrate studies (van

den Elzen et al. 2001; Gilbert et al. 2009), but has been explored in Opiliones (Buzatto & Machado, 2014) and other arachnids as well, especially in the spider family Salticidae (Masta & Maddison 2002).

An infamous diagnostic character in the family Cosmetidae has been the absence/presence of pectination on tarsal claws of legs III and IV. Though the presence of pectination was suggested as a defining feature for the subfamily Discosomaticinae (Roewer 1923), it was viewed as uniformative in the 1950s (Ringuelet 1959). In previous works on the Discosomaticinae, the presence of pectination was maintained as a somewhat informative character, though only as an apomorphy initially (Ferreira & Kury 2010), and hinted at being independently arising forms more recently (Medrano & Kury, 2018). Analyses here however demonstrate that the character is far more complex than a simple presence or absence character, and that various forms have arisen in a few distantly related lineages in the family Cosmetidae (FIG. 43), all currently considered discosomaticines. There are two distinct forms, and another that is typical of the genus *Bodunius* (single row of pectination) that was not explored here, as that genus was not included in analyses. The two distinct forms explored were two rows of pectination, one with the teeth roughly equal in size, the other with the mesal row with larger, more obvious teeth (FIG. 75). The latter character is seen in two separate clades containing *Discosomaticus* species, where the ectal row of teeth in *Discosomaticus distinctus* is not as reduced as those in *Discosomaticus sturmi*. A more recent analysis of the subfamily Discosomaticinae (Medrano & Kury 2018) discusses the potential of a non-monophyletic subfamily, where the genera *Bodunius* and *Fortalezius* were suggested to belong to a clade within the non-monophyletic Cosmetinae, while the rest

of the genera included in the work formed a clade. The relationships presented there are still problematic due to very low sampling density (as noted by the authors) and the coding of the pectination characters. Given that they did a reductionist coding, with one character as absence/presence and the other forms of presence, this could force a monophyletic relationship in a cladistic parsimony analysis. The coding used here implies the true evolutionary history of the character, that pectination has arisen multiple times, and therefore the different forms should be coded with an absence state in a single character.

e. *Male genitalia Characters (61-95)*

The connection of the ventral plate (VP) to the truncus of male genitalia has been explored before (Kury & Barros 2014). In this current work the only state that was especially informative was that of the sulci at the base of the VP observed in *Ferkeria* and *Platygyndes* species (FIG. 44). The "calli" proposed in Kury and Barros (2014) was found to not be informative across the family in previous morphological character list iterations, and in some cases, appeared to be an artifact of preparation, and not an actual structure of the penis. Another informative state was the VP and truncus being aligned and straight, with no lateral widening of the truncus (state 0, FIG 44). This is restricted to a few clades and associated with other characters of the VP (such as attenuate VP and rectangular overall shape, see below). At times, coding for such characters, like this one, was difficult since the preparation process in some cases would destroy or warp genitalia to a point where the true form was obscured. Working carefully with the penis, and making sure that the ethanol

gradient was not done too quickly- and therefore desiccation was slow- will prevent this from happening in the future.

Usually the VP of the penis was approximately 50+% of the width of the truncus in the family Cosmetidae, and amongst species of Gonyleptoidea. Occasionally a very attenuate form was observed within the family Cosmetidae (FIG. 45), and while this seems to have arisen in various lineages in the family, there is a strong case for this being informative in one group in particular (FIG. 75), showing that in some cases the attenuate form has arisen in an ancestor and all individuals in that lineage exhibit that state. The forms that arise in other lineages are different, as can be seen in *Roquettea* and a single *Gryne* species (FIG. 77 & FIG. 78). This character would serve as an informative form to be used with other genitalia characters to provide a robust diagnosis for lineages within the family.

Generally, the family Cosmetidae exhibits a curved apical margin, with two other forms being found in various lineages from deeper nodes in the trees (FIG. 46). A large cleft, or a convex apical margin is also observed regularly in the outgroup species sampled here, though each of these are viewed in a few lineages in the Cosmetidae. Due to this restriction of existing in only a few lineages, and being rare in the family, apical margins that are straight, convex, or sinuous are likely to only be informative at a specific or generic hierarchical level, and unlikely to be informative at a suprageneric level without substantially more dense sampling of species, for example in *Bokwina*, which is from a poorly sampled region.

The overall shape of the VP in the family Cosmetidae is either trapezoidal or rectangular, with a few lineages with a more true square

shape (FIG. 47). About half of cosmetids, where genitalia are known, exhibit a rectangular shape of the VP, which is especially evident in lineages like that of *Paraprotus* and *Flirtea batman* (FIG 76), and the other a more trapezoidal shape like that seen in *Meterginus basalis* (FIG. 79; FIG. 80). The terminology referring to the overall shape of the VP is inexact and further complicates morphology characters associated with this structure. Various terms have been used to refer to VP shape- such as a "sub-rectangular" shape (Kury et al., 2007; Kury & Barros, 2014; Medrano & Kury, 2016: Medrano & Kury, 2017; Kury & Medrano, 2018), and though this is not an incorrect statement, it also does not convey enough information about the shape. It is suggested here that trapezoidal be a term now used for any VP shape that is not a true rectangle or square, where one pair of corners (either apical or basal) are closer to one another creating angles that are greater than 90°. However, caution should be used again defining an overall shape, if care is not used to prepare the penis for SEM, many of these states can be interchanged for the same species if preparation methods cause collapsed lateral margins.

Macrosetae (MS) have been proposed to be sources of informative characters in Gonyleptoidea (Kury & Villarreal 2015; Medrano & Kury 2016), though in the Cosmetidae this is not always the case for all of the MS, as polymorphism is common in the terminals sampled in this work. MS-A is one set that seems to be evolving in a predictable pattern, where the number of pairs were reduced to a single pair, then in the more derived clades, an additional pair was regained (FIG. 48). Though various forms were explored for MS-A, such as length, distal tip shape, and position of MS-A1 on the VP, only position of MS-A1 seemed to have

any informative pattern (FIG. 49), where various clades would have basally positions MS-A (see *Flirtea* in FIG. 81), or at mid-length of VP (see FIG. 82). However, this is further complicated when there are two pairs of MS-A, and while in this work it was MS-A1 that was coded for in this character, there are cases where MS-A2 is basally located on the VP. In this latter case, there is a pair of MS located at the base of the VP, and though it is not MS-A1, there is still an MS located at the base of the VP. It may be better to explore this character as one that depends on whether there are any MS located on the basal half of the VP.

MS-B and MS-E are more conserved in the family than many of the other

MS analysed here. MS-B are usually a single pair observed towards the base of the VP or even on the truncus (FIG. 50), though *Ferkeria* and *Platygyndes* are an exception to this (FIG. 83). Number of MS-E pairs are also highly conserved, with only two pairs being observed outside of the genus *Metalibitia* (FIG. 57; FIG. 83; Coronato-Ribeiro & Pinto-da-Rocha 2017), though the form of MS-E and its position on the VP are likely to be informative for particular lineages (FIG. 59 and FIG. 58, respectively). This is perhaps due to the placement of this MS, compared to that of MS-B. MS-B at the base of the VP is far less likely to come into meaningful contact with female genitalia, and is less likely to play a major role and hence be under any selection pressure. MS-E tends to be closer to the apex of the VP, and is more likely to come in contact with internal structures of the ovipositor, and therefore may play a role in copulation, although no studies to date have examined interactions between male and female genitalia in Cosmetidae.

MS-C seems to exhibit the most informative features regarding shape, with only 2-3 pairs being observed (FIG. 51). A third MS-C is observed in *Gryne marginalis*, and *Roquettea carajas*, but these genera are known for possessing what has also been defined recently as a supernumerary MS (MS-SN; Medrano & Kury 2018). The shape of MS-C in the family Cosmetidae is a modified compression/helicoidal, though cylindrical forms are still found within the family (state 1; FIG. 52; FIG. 84). The apical tip of MS-C also shows modification in some clades (state1; FIG. 53; FIG. 76), where there are multiple projections at the tip, instead of the commonly observed "fan-like" shape as a result of compression. The modified and autapomorphic character states of MS-C could be expected as they are likely to be the MS that interacts the most with the ovipositor of females, and so would be under selective pressure from the female genitalia. The presence of the MC-SN (supernumerary) in the family Cosmetidae is not restricted to the *Roquettea* and *Gryne* genera, as implied by Medrano and Kury (2018), and is most likely the presence of polymorphism in the number of MS-C, as they are the same shape.

The definition of MS-D in Cosmetidae has changed slightly over the years (compare Kury & Villarreal 2015 to the statements in Medrano & Kury 2016), and the specific definitions between MS-D and MS-A have recently been questioned by Proud and Townsend (2019). Based on the comments made in Medrano and Kury (2016), the majority of Cosmetidae have two pairs of MS-D, however, there are a few clades (FIG. 54) which exhibit the homoplastic state of only one pair of MS-D, a character state shared with the majority of the outgroup sampled in this analysis. The length of MS-D was explored, but does not provide much

information beyond that of MS-D2 mostly being reduced when present (and following the definition of Medrano & Kury 2016; FIG. 56), and MS-D1 in *Metalibitia* are always reduced (FIG. 55).

The majority of Cosmetidae possess mats of microsetae on the ventral surface of the VP, usually present only in the lateral fields with microsetae type 4 (FIG. 60) with a few exceptions where the microsetae also are present in a midfield. Type 1 and type 4 are the most common forms of microsetae present, though in some cases there is another form observed very rarely that is scale-like in its appearance (see *Taito* OP2677, FIG. 80). In a few cases, microseteae mats of type 4 are found on the dorsal surface of the VP (FIG 85), though it is not consistent, and is observed occasionally in a few species throughout the tree, and only one clade exhibits this in all of the terminals (FIG. 61; clade 2, FIG 85). Though microsetae patterns are restricted to mostly the Cosmetidae in this analysis, patterns do not strongly support a clear pattern for the whole family, or even suprageneric level. The outgroups sampled in this analysis are mostly glabrous, like that of *Metalibitia*, but patterns of microsetae fields and other types are observed in other groups of Gonyleptoidea (Kury 2016).

A strange structure observed in the genus *Metalibitia* is the membranous extension on the lateral median portion of the VP (FIG. 62; FIG. 83). This feature is not shared with any other genus in the family Cosmetidae, though a form of membranous extensions are observed in the sister family Metasarcidae. However, these are distinct from that of *Metalibitia* (Pinto-da-Rocha et al. 2014) since in Metasarcidae the membranous extensions are strongly projected and covered by spines. Though there is the potential of a highly modified “membrane” in *E.*

subserialis (FIG. 62), this could as well be a modified truncus and was proposed to be a way of everting the glans for copulation (Proud & Townsend 2019).

The presence of a "podium" under the glans structure is commonly observed through the family Cosmetidae (FIG. 63). This character is a modification of a character state proposed in Medrano and Kury (2018), though the absence is restricted to only a few small clades like *Metalibitia* (FIG. 83), *Flirtea batman* and *F. valida* (FIG. 81), and *Roquettea* (FIG. 77). This seems to be associated with a very specific VP plate shape in the *Flirtea* and *Roquettea* species, and is perhaps due to the evolution of this VP shape. When used with other characters, it may be used to diagnose at the genus level.

Characters related to the glans of the penis are likely to be the most informative for Opiliones than other parts of the penis, due to its interaction with the female genitalia (Macías-Ordóñez et al. 2010). There are a few characters used here that are demonstrated to be synapomorphies for various lineages. For example, the glans of *Metalibitia* are inserted at mid-VP, while all other taxa including outgroups have the glans positioned at the base of the VP (FIG. 64). Another example is the "bump scales" (Kury & Barros 2014), being restricted to most of the species of the genus *Taito* (FIG. 65; FIG. 80).

Some features of the glans can provide family wide patterns, such as the presence of a dorsal process on the glans, and no ventral process on the stylus (FIG. 66 and FIG. 68, respectively). The dorsal process is not observed in the *Metalibitia* and *Ferkeria* group (FIG. 83), though it appears in all other species. In general the dorsal process is triangular in shape (FIG. 67), though a few clades have a dorsal process that is more

conical and attenuate (FIG. 76), or has a concave apex, creating an almost "heart-like" apex (FIG. 80; FIG. 82).

The stylus of the glans in the Cosmetidae typically possess what has been referred to as a caruncle or wattle, which can take on various forms (Medrano & Kury 2018 discuss this in detail related to *Roquettea* and *Gryne*). The lineage that includes *Metalibitia+Ferkeria+Platygyndes* has the most simplistic stylus in the family (cylindrical with little to no armature; FIG. 83). This character state is shared with much of the outgroup, and while some species in this clade have a "pseudo" wattle, they all have simplistic styli (FIG. 69). The presence of a wattle/caruncle is observed in most of the Cosmetidae. In some lineages the wattle is reduced, and does not extend the full length of the stylus (clade 2 FIG 1-5: see FIG. 78; FIG. 85), while the rest of the family exhibit a large wattle that can extend the full length of the stylus (clade 3 FIG 1-5; FIG 86; FIG. 69). In general, Cosmetidae have a large wattle/caruncle that extends the full length of the stylus, the armature on this wattle is varied, and the forms would be species or genera specific, and should be explored more fully in future diagnoses of clades.

Armature on the apex of the stylus is a more complex character than presented here. Without creating a character with many states, it is hard to encompass the entirety of the diverse forms observed in the family. Though there are distinct patterns in states analyzed in this work (FIG. 70), the forms of each state are likely to be different. For example, the filamentous projections at the seminal opening in *Ferkeria* and *Platygyndes*(FIG. 83) are different than that observed in *Taito* (FIG.80). The projections in the former are more finger-like, while the ones in *Taito* are more true to the term filamentous. The same can be said for the

combination of stylus barbs and projections (state 2- FIG. 70). For example, the combination of these two look different in the *Gnidiella picta* Roewer, 1957 clade (FIG. 87), compared to the *Cynorta quinquesignata* Franganillo Balboa, 1926 (FIG. 86) clade. Each form is likely to be genus specific, and therefore quite variable in the Cosmetidae, and should be examined in more detail in the future when reviewing and diagnoses lineages.

Characters like those of the wattle and armature of the stylus are oversimplified for the structure being examined. Brazeau (2011) addresses this when discussing character states and their "all encompassing statements". In general states will not necessarily be as dichotomous as a 0 or 1 (missing or not, etc), and are in fact shades of grey, demonstrating a distance of relation. This pattern is evident in the presence and form of armature and a caruncle/wattle on the stylus. There is a general progression seen from the outgroup through the *Metalibitia+Ferkeria+Platygyndes* clade, where the ventral process has been lost, yet a caruncle/wattle has yet to reach the same extent as seen in the rest of the Cosmetidae, though some species in this subfamily do exhibit what could be a "proto-wattle" (see *Platygyndes* 0738 in FIG. 83). This can be evidence of a possible use of the structure, such as a ventral process or the wattle/caruncle in Cosmetidae being used to remove sperm of a competitor from the ovipositor of a female (Macías-Ordóñez et al. 2010), though recent research has shown that there are potentially mating plugs being used in various species of Laniatores, Cosmetidae included (Townsend, Pérez-González & Proud 2019). Unfortunately no current work exists explicitly examining the interactions between male and female genitalia internally to know the 'true' function of these structures.

f. *Discussion of the Evolution of ovipositors in the family Cosmetidae*

Ovipositors were not extensively prepared as part of this analysis due to lack of time, and no morphological characters were coded for female genitalia. Descriptions, light microscopy images, and SEM images were examined from the literature to understand the evolutionary history of the ovipositor in a phylogenetic context (FIG. 95 ovipositor). In general Laniatores have ovipositors that are fleshy, short, and cylindrical, divided apically into two or four lobes with an interspecifically variable number of peripheral setae. The root terminal of this analysis, *Triaenonychoides cekalovic*, has only two lobes, and the distal surface is free of setae/sensilla, though it is wrinkled. This species possesses a larger number of peripheral setae (14-16) than that observed in the rest of the outgroup and the Cosmetidae. Two other outgroup families, Cranaidae and Stygnidae, have been examined recently (Townsend et al., 2015). They have two or four lobes with as few as 10 peripheral setae, and as many as 16. In the case of the Cranaidae the surface of the ovipositor had deep wrinkling, though no microsetae were observed, while the Stygnidae specimen had a smooth distal surface (Bennett & Townsend, 2013).

The Cosmetidae tend to have less peripheral setae than those mentioned above, with a range of 8-12 depending on the species. Species could have either two or four lobes, and in general the distal surface of the ovipositor is smooth, though there is evidence in the literature of some species possessing microsetae (Walker & Townsend, 2014). Cosmetidae tend to have split or modified tips of peripheral setae, while some species (FIG. 96 ovipositor) have accuminate tips, so this is not a consistent

pattern across all Cosmetidae, or even within a single clade (See *Erginulus* clade in FIG. 96).

In general there are some key differences Cosmetidae have from other groups in Gonyleptoidea, such as less overall number of peripheral setae. However, the Laniatores ovipositors has a relatively high intraspecific level of variation (Townsend et al., 2015), though general patterns are highly conserved across the suborder. Cosmetidae ovipositors are a complicated example, as they tend to exhibit patterns that are shared in many other groups and are not consistent across the family. For example, Cosmetidae have examples of both 2 and 4 lobes within the family (as does the family Phalangodidae; see Townsend et al., 2015). The same is true for surface texture and whether the peripheral setae are curved or straight (both are observed in Cosmetidae). What seems to be an informative source of characters is in the distal tips of the peripheral setae. As shown in FIG 96., the distal tip of the setae is noticeably different interspecifically, however the images shown are all species that are closely related to one another, with the exception of *Platygyndes titicaca*. Therefore it is likely that this character may only be appropriate as an apomorphy for the species, and can not be used as a general pattern for a higher taxon ranking. In samples examined in this work, there is no obvious patterns that allow any comments to be made about suprageneric level classification using external characters of the ovipositor. It is likely that more informative characters will be found in the internal structures of the ovipositor that interact directly with male genitalia. Currently only specific level characters can be created, focusing on the structures of the apical surface of the ovipositor, and the peripheral setae.

D. Biogeography of the family Cosmetidae

No biogeographical analyses examining ancestral ranges were completed as part of this thesis, but the distribution findings presented here are compelling and warrant discussion. As demonstrated in figures 6-10, it is apparent that species that are from localities near to one another are more likely to be closely related. The maps depict five different phylogenetic hypotheses related to geographical distribution records. Even using an arbitrary geographical division (e.g., Mexico+Northern Central America Countries, Panama+Costa Rica, Yucatan+Great Antilles) in order to simply visualize, we observe a great correlation with biogeographic region independently of which phylogenetic hypothesis is chosen. There are very few exceptions to this general pattern. This is not unexpected, as demonstrated by the many ways speciation occurs (i.e vicariance events and barriers, immigration to new niches, genetic mutations), but it does provide a warning about diagnoses of species and groups in the family Cosmetidae. In the diverse family Sclerosomatidae, Hedin et al., (2012) found that the majority of subfamilial and genera based taxonomic groups were not monophyletic. Morphological classification was not supported, but clades were strongly associated with biogeographic regions, and clades that were maintained were typically associated with a specific region and relied on this as part of their classification (Hedin et al., 2012), this appears to be the case with the Cosmetidae as well.

When examining the relationships of the lineages that contain species of the Discosomaticinae (clades 6, 9 and 10; FIG 1-5), the lineages are not closely related to one another, though they diverged at the same time as one another (FIG 5). The relationship of the lineages is not one of sister groups, and though geographic distribution of each group is generally

Amazonas and Northern Brazil (FIG 6-10), the divergence events for each of these lineages perhaps were influenced in part by events in this region approximately 30 mya. As suggested in Stampar et al., (2012) before and during this time there was a large inland sea and lake in the Northern part of South America, as well as repeated massive flooding in Amazonas after this time. These events could have lead to rapid radiation events for species in Amazonas (FIG. 4 & 5).

Other lineages of this region, such as the genus Taito, could have been strongly influenced by these events. This genus was said to be closely related to Eucynortella in the work of Kury and Barros (2014), but analyses here show that Taito diverged almost 10 my before that of E. longa (FIG 5): and Panama, where E. longa is found, would have had little contact with mainland South America from 25 mya, to about 7 mya (Bacon et al., 2015). Perhaps the type species of Eucynortella (E. spectabilis) which is from a locality in French Guiana, would be more closely related to the genus Taito. When examining the map figures presented here (FIG 6-10), it becomes apparent that the genus Taito is more closely related to species from Peru and the Andes, the latter of which was suggested in Kury and Barros (2014), than that of species in Amazonas.

Many genera in Cosmetidae superficially appear to be morphologically similar, however the patterns observed in the analyses here are as observed in Sclerosomatidae (Hedin et al., 2012). The authors found in the family Sclerosomatidae, that the taxonomy, based considerably on shared morphology, did not reflect the true relationships amongst the genera and lineages in the family when assessed using modern molecular methods, however geographic region did. This would suggest that future

taxonomic work with Cosmetidae take into consideration the geographic range of species and the regions they are associated with, and do not rely alone on morphological data to imply relatedness. This “biogeographic regionalism” is especially evident in figures 6-10.

Additionally, due to the Cosmetidae being from the Americas, geographic range combined with morphology may not be enough to make accurate placements taxonomically of groups, especially from Central America and the Caribbean. Hedin et al., (2012) had said of the Sclerosomatidae that a taxonomic “divide and conquer” method would be inadequate for that family, and the same would be true for the Cosmetidae. In regions poorly studied, where the fauna is essentially unknown, sequencing individuals collected from a single region, would be substantially more informative for phylogenetic placement in the family as a whole. Then shared morphology can be inferred from these analyses, and coupled with geographic region, assist with robust and accurate taxonomic descriptions of clades.

VII. Conclusions

This was the first attempt to provide a phylogenetic hypothesis for the family Cosmetidae using a total evidence approach with a large representative species set. This study had a sample of species of Cosmetidae representing approximately 17% of the family's species, from regions across the entirety of the geographic range of the Cosmetidae. Though many types and genera are not included in this work, the family Cosmetidae remains a clade, but the two current subfamilies (Cosmetinae and Discosomaticinae) are not. The character of pectination on tarsal claws of legs III and IV, used for defining Discosomaticinae, has arisen at least three times in the family Cosmetidae, making the character state of smooth pectinate claws plesiomorphic.

It is clear from the analyses in this work that many morphological characters used to diagnose and define genera and species within the family are problematic and homoplastic, and alone are not enough to accurately reflect the evolutionary history of the family. Even characters, such as DS armature, and coloration, have very low CI and RI values (see appendix), showing that when a phylogenetic perspective is taken into consideration across the family, these characters are uninformative beyond being a diagnostic feature to define a single species. Though a new subfamily has been proposed, this is a result of the lineage being repeatedly resolved monophyletic, as well as possessing clear morphological characters which can define the lineage and the groups

within it. Many genera are not monophyletic, and it is likely that many more will not be pending phylogenetic analysis with larger sample sizes.

Genitalia characters are informative, even when scaled back in the specificity of the character states. While the characters related to stylus shape and the armature on the wattle and stylus are not very detailed, they still show patterns amongst species groups that can provide direction for further examination. The wattle/caruncle in Cosmetidae can be hypothesized to play an important role in the interaction with the ovipositor, lending additional support to the importance of examining and proposing more characters associated with this structure of the penis. When examining all of the possible forms for Cosmetidae, it is difficult to not create large composite morphological characters trying to encompass all the diversity. In the case of this project the forms were reduced to some basic 'bins' that may have a more detailed form associated with a species group as a synapomorphy. These forms should be examined in more detail as more analyses with lineages when new genera and subfamilies are proposed for the Cosmetidae in the future.

Future work with the Cosmetidae would benefit from denser taxon sampling, especially from Central America and the Caribbean, as well as testing new morphological characters for use in the diagnosis of species. The inclusion of more of the monotypic genera of the family would also help in resolving the relationships amongst groups in the family. Caution should be used when diagnosing suprageneric groups based solely on morphology, and geographic range of the species and genera should be carefully considered and presented in descriptions. Completing analyses with molecular data will assist in determining monophyletic lineages. This is especially important for species found in localities with complex

and dynamic biogeographical history, especially when statements are made about potential ranges of clades. It is not enough to say that a species or genus is found in a single country, since some are large and possess a complex biogeographical history, it would be better to refer to a specific region. As is suggested in Hedin et al. (2012), a robust sample of species as exemplars should be sequenced and a large molecular phylogenetic hypothesis presented, then a "post-hoc search of morphological synapomorphies" (Hedin et al., 2012) be employed and used to taxonomically define groups.

In the case of Cosmetidae caution should be used when conducting total evidence analyses using morphological characters, since many characters, especially somatic characters, have high levels of homoplasy. Unless the characters are revisited prior to being included in analyses against a molecular based analysis. Moving forward, a focus on genitalia will provide additional support for clades, and will be especially informative at the genera level. Unfortunately female genitalia at this time do not provide any sound characters that can add support to clades, and obvious differences seem to be associated with a species, and not at higher taxonomic rankings.

X. Resumo

A família Cosmetidae (Arachnida: Opiliones) é a família de Opiliones mais diversificada da América Central, e depois da família Gonyleptidae nos Neotrópicos. Continuam sendo um grupo pouco estudado e se beneficiam de qualquer trabalho sistemático. Esta pesquisa de PhD é uma tentativa de entender as relações dentro da família. Foram utilizados múltiplos critérios de otimalidade, evidência total usando otimização direta em POY e máxima verossimilhança em RAxML, e molecular apenas usando Bayesiano em BEAST, datação por divergência foram calculadas em BEAST. Foram utilizados cinco marcadores (28S rRNA, 12S rRNA, 16S rRNA, COI e H3) e 95 caracteres morfológicos (i.e. caracteres genitais, contorno do escuto dorsal, armadura do escuto dorsal) para determinar os clados. As análises demonstram que as subfamílias atuais (Discosomaticinae e Cosmetinae) não são válidas e a subfamília Cosmetinae é um grupo parafilético. Enquanto parte da monofilia de gêneros está implícita (*Taito*, *Metalibitia*, *Ferkeria*), outros gêneros diagnosticados na literatura recente não são (*Cynorta*, *Paecileama*, *Flirtea*). Foi demonstrado aqui que o alcance geográfico e, portanto, o histórico compartilhado são uma característica importante a ser considerada na definição de clados. Uma nova subfamília é proposta aqui, apoiada por hipóteses filogenéticas e morfologia. Análises preliminares de datação por divergência mostram que a família tem mais de 10 my a mais do que o inicialmente estimado, com uma idade de 47±5 my. Novos diagnósticos de espécies, gêneros e subfamílias da família devem se concentrar nos caracteres da genitália e na região biogeográfica das espécies nos grupos.

XI. Abstract

The family Cosmetidae (Arachnida: Opiliones) is the most diverse harvestmen family in Central America, second to the family Gonyleptidae in the Neotropics. They remain a poorly studied group, and benefit from any systematic work. This PhD research is an attempt at understanding the relationships within the family Cosmetidae. Multiple optimality criteria were used, total evidence using direct optimization in POY and Maximum Likelihood in RAxML, and molecular only using Bayesian in BEAST, divergence dating were also calculated in BEAST. Five molecular markers were used (28S rRNA, 12S rRNA, 16S rRNA, COI, and H3), and 95 morphological characters (i.e. genitalia characters, dorsal scutum outline, dorsal scutum armature) to determine clades. Analyses demonstrate that the current subfamilies of Cosmetidae (Discosomaticinae and Cosmetinae) are not valid, and the subfamily Cosmetinae is a paraphyletic group. While some monophyly of genera is implied in this analysis (see *Taito*, *Metalibitia*, and *Ferkeria*), other genera rediagnosed in recent literature are not (*Cynorta*, *Paecileama*, and *Flirtea*). It was demonstrated in these analyses that geographic range and therefore shared history is an important characteristic to consider when defining clades. A new subfamily is proposed here, supported by phylogenetic hypotheses and morphology. Preliminary divergence dating analyses shows that the family Cosmetidae is more than 10 million years older than originally estimated, with an age of approximately 47±5 myr. New diagnoses of species, genera, and subfamilies within the family Cosmetidae should focus on genitalia characters, and biogeographic region of species in the groups.

XII. References

Astrin, J.J. and Stüben, P.E., 2008. Phylogeny in cryptic weevils: molecules, morphology and new genera of western Palaearctic Cryptorhynchinae (Coleoptera: Curculionidae). Invertebrate Systematics, 22(5), pp.503-522.

Astrin, J.J., Höfer, H., Spelda, J., Holstein, J., Bayer, S., Hendrich, L., Huber, B.A., Kielhorn, K.H., Krammer, H.J., Lemke, M. and Monje, J.C., 2016. Towards a DNA barcode reference database for spiders and harvestmen of Germany. PLoS One, 11(9), p.e0162624.

Arango, C.P. and Wheeler, W.C., 2007. Phylogeny of the sea spiders (Arthropoda, Pycnogonida) based on direct optimization of six loci and morphology. Cladistics, 23(3), pp.255-293.

Bacon, C.D., Silvestro, D., Jaramillo, C., Smith, B.T., Chakrabarty, P. and Antonelli, A., 2015. Biological evidence supports an early and complex emergence of the Isthmus of Panama. Proceedings of the National Academy of Sciences, 112(19), pp.6110-6115.

Banks, N., 1893. The phalanginae of the United States. The Canadian Entomologist, 25(8), pp.205-211.

Bennett, M.K. and Townsend Jr, V.R., 2013. Reproductive morphology of three species of Neotropical harvestmen (Opiliones, Laniatores, Gonyleptoidea). Journal of Morphology, 274(12), pp.1415-1424.

Bieler, R., Mikkelsen, P.M., Collins, T.M., Glover, E.A., González, V.L., Graf, D.L., Harper, E.M., Healy, J., Kawauchi, G.Y., Sharma, P.P. and Staubach, S., 2014. Investigating the Bivalve Tree of Life–an

exemplar-based approach combining molecular and novel morphological characters. Invertebrate Systematics, 28(1), pp.32-115.

Bragagnolo, C. and Pinto-Da-Rocha, R., 2012. Systematic review of Promitobates Roewer, 1913 and cladistic analysis of Mitobatinae Simon, 1879 (Arachnida: Opiliones: Gonyleptidae). Zootaxa, 3308(1), pp.1-48.

Bragagnolo, C., Hara, M.R. and Pinto-da-Rocha, R., 2015. A new family of Gonyleptoidea from South America (Opiliones, Laniatores). Zoological Journal of the Linnean Society, 173(2), pp.296-319.

Brazeau, M.D., 2011. Problematic character coding methods in morphology and their effects. Biological Journal of the Linnean Society, 104(3), pp.489-498.

Brennan, P.L. and Prum, R.O., 2015. Mechanisms and evidence of genital coevolution: the roles of natural selection, mate choice, and sexual conflict. Cold Spring Harbor perspectives in biology, 7(7), p.a017749.

Brooks, E.A., Townsend Jr, V.R., Allen, E.A. and Tuthill, M.A., 2017. Interspecific variation in ovipositor morphology among manaosbiid and nomoclastid harvestmen (Arachnida, Opiliones, Laniatores). Acta Zoologica, 98(1), pp.56-65.

Buzatto, B.A. and Machado, G., 2014. Male dimorphism and alternative reproductive tactics in harvestmen (Arachnida: Opiliones). Behavioural processes, 109, pp.2-13.

Carvalho, R. and Kury, A., 2018. Further dismemberment of Discocyrtus with description of a new Amazonian genus and a new subfamily

of Gonyleptidae (Opiliones, Laniatores). European Journal of Taxonomy, (393).

Colgan, D.J., McLauchlan, A., Wilson, G.D.F., Livingston, S.P., Edgecombe, G.D., Macaranas, J., Cassis, G. and Gray, M.R., 1998. Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. Australian Journal of Zoology, 46(5), pp.419-437.

Combosch, D.J., Collins, T.M., Glover, E.A., Graf, D.L., Harper, E.M., Healy, J.M., Kawauchi, G.Y., Lemer, S., McIntyre, E., Strong, E.E. and Taylor, J.D., 2017. A family-level tree of life for bivalves based on a Sanger-sequencing approach. Molecular Phylogenetics and Evolution, 107, pp.191-208.

Coronato-Ribeiro, A. and Pinto-da-Rocha, R., 2015. Four new species of Cosmetus from Panama, with comments on the systematics of the genus (Opiliones: Cosmetidae). Zoologia (Curitiba), 32(5), pp.409-422.

Coronato-Ribeiro, A. and Pinto-Da-Rocha, R., 2017. Taxonomic revision and cladistic analysis of the genus Metalibitia Roewer, 1912 (Opiliones, Cosmetidae, Cosmetinae). Zootaxa, 4291(2), pp.201-242.

Damron, B.N., Pinto-da-Rocha, R.I.C.A.R.D.O. and Longhorn, S.J., 2018. Description of a new species of Eucynorta (Opiliones, Cosmetidae) from Cortés, Honduras. Zootaxa, 4450(1), pp.125-134.

DaSilva, M.B. and Pinto-da-Rocha, R., 2010. Systematic review and cladistic analysis of the Hernandariinae (Opiliones: Gonyleptidae). Zoologia, 27(4).

DaSilva, M.B. and Gnaspini, P., 2010. A systematic revision of Goniosomatinae (Arachnida: Opiliones: Gonyleptidae), with a cladistic analysis and biogeographical notes. Invertebrate Systematics, 23(6), pp.530-624.

de Sá, R.O., Grant, T., Camargo, A., Heyer, W.R., Ponssa, M.L. and Stanley, E., 2014. Systematics of the neotropical genus Leptodactylus Fitzinger, 1826 (Anura: Leptodactylidae): phylogeny, the relevance of non-molecular evidence, and species accounts. South American Journal of Herpetology, 9(s1).

Donoghue, M.J., 1989. Phylogenies and the analysis of evolutionary sequences, with examples from seed plants. Evolution, 43(6), pp.1137-1156.

Drummond, A.J., Suchard, M.A., Xie, D. and Rambaut, A., 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Molecular biology and evolution, 29(8), pp.1969-1973.

Dunlop, J.A., 2003, August. A spiny harvestman (Arachnida: Opiliones) from the Upper Carboniferous of Missouri, USA. In Proceedings of the 21st European Colloquium of Arachnology (Vol. 4, p. 9).

Eberhard, W.G., 1985. Sexual selection and animal genitalia (Vol. 244). Cambridge, MA: Harvard University Press.

Edgecombe, G.D. and Giribet, G., 2006. A century later–a total evidence re-evaluation of the phylogeny of scutigeromorph centipedes (Myriapoda: Chilopoda). Invertebrate Systematics, 20(5), pp.503-525.

Edgecombe, G.D., 2010. Arthropod phylogeny: an overview from the perspectives of morphology, molecular data and the fossil record. Arthropod Structure & Development, 39(2-3), pp.74-87.

Eernisse, D.J. and Kluge, A.G., 1993. Taxonomic congruence versus total evidence, and amniote phylogeny inferred from fossils, molecules, and morphology. Molecular biology and evolution, 10(6), pp.1170-1195.

Ewing, B. and Green, P., 1998. Base-calling of automated sequencer traces using phred. II. Error probabilities. Genome research, 8(3), pp.186-194.

Ewing, B., Hillier, L., Wendl, M.C. and Green, P., 1998. Base-calling of automated sequencer traces usingPhred. I. Accuracy assessment. Genome research, 8(3), pp.175-185.

Ferreira, C.P. and Kury, A.B., 2010. A review of Roquettea, with description of three new Brazilian species and notes on Gryne (Opiliones, Cosmetidae, Discosomaticinae). Zoological Science, 27(8), pp.697-708.

Flomer, O., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotech, 3, pp.294-299.

Flórez, D.E. and Sánchez, H., 1995. La diversidad de los arácnidos en Colombia, aproximación inicial. Colombia, Diversidad Biótica, I. Inst. Ciencias Naturales, UN, Inderena, Fes, Fen'.(Ed. O. Rangel.) pp, pp.327-372.

Forster, R.R., 1962. A key to the New Zealand Harvestmen-part 1. Tuatara: Journal of the Biological Society, 11, pp.28-40.

Forster, R.R., 1963. A key to the New Zealand Harvestmen-part 2. Tuatara: Journal of the Biological Society, 11, pp.28-40.

Franganillo, B.P., 1926. Aracnidos nuevos o poco conocides de la Isla de Cuba. Aracnidos de Cuba., 24.

García, A.F. and Kury, A.B., 2017. Taxonomic revision of the Andean harvestman genus Rhaucus Simon, 1879 (Arachnida, Opiliones, Cosmetidae). Zootaxa, 4338(3), pp.401-440.

García, A.F. and Damron, B., 2019. Giving attention to the oldies: redescription of Meterginus basalis (Arachnida: Opiliones: Cosmetidae) with some notes on the genitalia. Revista Mexicana de Biodiversidad, 90(1).

Gilbert, C.C., Frost, S.R. and Strait, D.S., 2009. Allometry, sexual dimorphism, and phylogeny: a cladistic analysis of extant African papionins using craniodental data. Journal of Human Evolution, 57(3), pp.298-320.

Giribet, G., 2015. Morphology should not be forgotten in the era of genomics–a phylogenetic perspective. Zoologischer Anzeiger-A Journal of Comparative Zoology, 256, pp.96-103.

Goloboff, P.A., 1996. Methods for faster parsimony analysis. Cladistics, 12(3), pp.199-220.

Goloboff, P.A., 1999. Analyzing large data sets in reasonable times: solutions for composite optima. Cladistics, 15(4), pp.415-428.

Goloboff, P.A., Farris, J.S. and Nixon, K.C., 2008. TNT, a free program for phylogenetic analysis. Cladistics, 24(5), pp.774-786.

Goloboff, P.A. and Catalano, S.A., 2011. Phylogenetic morphometrics (II): algorithms for landmark optimization. Cladistics, 27(1), pp.42-51.

Goloboff, P.A. and Catalano, S.A., 2016. TNT version 1.5, including a full implementation of phylogenetic morphometrics. Cladistics, 32(3), pp.221-238.

González-Sponga, M.A., 1992. Arácnidos de Venezuela: Opiliones Laniatores II familia Cosmetidae. Academia de Ciencias Físicas y Matemáticas y Naturales.

Goodnight, C.J. and Goodnight, M.L., 1942. Phalangids from Central America and the West Indies. Falángidos de Centroamérica y las Indias Occidentales. American Museum Novitates., (1184), pp.1-23.

Goodnight, C.J. and Goodnight, M.L., 1947. Phalangida from Tropical America. Chicago Natural History Museum.

Goodnight, C.J. and Goodnight, M.L., 1953. The opilionid fauna of Chiapas, Mexico, and adjacent areas (Arachnoidea, Opiliones). American Museum of natural history.

Goodnight, M.L. and Goodnight, C.J., 1976. Observations on the systematics, development, and habits of Erginulus clavotibialis (Opiliones: Cosmetidae). Transactions of the American Microscopical Society, pp.654-664.

Gordon, D., Abajian, C. and Green, P., 1998. Consed: a graphical tool for sequence finishing. Genome research, 8(3), pp.195-202.

Gordon, D., Desmarais, C. and Green, P., 2001. Automated finishing with autofinish. Genome Research, 11(4), pp.614-625.

Grieneisen, M.L., Zhan, Y.U., Potter, D. and Zhang, M., 2014. Biodiversity, taxonomic infrastructure, international collaboration, and new species discovery. Bioscience, 64(4), pp.322-332.

Harrington, B. et al (2004-2005). Inkscape. http://www.inkscape.org/.

Hedin, M., Tsurusaki, N., Macías-Ordóñez, R. and Shultz, J.W., 2012. Molecular systematics of sclerosomatid harvestmen (Opiliones, Phalangioidea, Sclerosomatidae): geography is better than

taxonomy in predicting phylogeny. Molecular Phylogenetics and Evolution, 62(1), pp.224-236.

Hickman, V.V., 1958. Some Tasmanian harvestmen of the family Triaenony-Chidae (suborder)(Laniatores). In Papers and proceedings of the Royal Society of Tasmania (Vol. 92, pp. 1-116).

Hosken, D.J. and Stockley, P., 2004. Sexual selection and genital evolution. Trends in ecology & evolution, 19(2), pp.87-93.

Iturralde-Vinent, M. and MacPhee, R.D., 1999. Paleogeography of the Caribbean region: implications for Cenozoic biogeography. Bulletin of the AMNH: no. 238.

Katoh, K. and Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular biology and evolution, 30(4), pp.772-780.

M. Kearse, R. Moir, A. Wilson, S. Stones-Havas, M. Cheung, S. Sturrock, S. Buxton, A. Cooper, S. Markowitz, C. Duran, T. Thierer, B. Ashton, P. Meintjes, and A. Drummond. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics. 28(12): 1647-1649.

Kelly, D.A. and Moore, B.C., 2016. The morphological diversity of intromittent organs. Integrative and comparative biology, 56(4), pp.630-634.

Kirby, W., 1819. XXVII. A Century of Insects, including several new Genera described from his Cabinet. Transactions of the Linnean Society of London, 12(2), pp.375-453.

Koch, C.L., 1837. Die Arachniden: Getreu nach der Natur abgebildet und beschrieben (Vol. 4). In der CH Zeh'schen Buchhandlung.

Kury, A.B., 2003. Annotated catalogue of the Laniatores of the New World:(Arachnida, Opiliones). Revista Ibérica de Aracnología, (7), pp.5-337.

Kury, A.B. and Pinto-da-Rocha, R., 2007. Cosmetidae Koch, 1839. Harvestmen, the biology of Opiliones. Harvard University Press, Cambridge, pp.182-185.

Kury, A.B., Manzanilla, O.V. and Sampaio, C., 2007. Redescription of the type species of Cynorta (Arachnida, Opiliones, Cosmetidae). The Journal of Arachnology, 35(2), pp.325-334.

Kury, A.B. and Barros, C.M., 2014. A new genus and eight new species of Amazonian cosmetines (Opiliones, Laniatores, Cosmetidae). Zoological Studies, 53(1), p.24.

Kury, A.B. and Villarreal M, O., 2015. The prickly blade mapped: establishing homologies and a chaetotaxy for macrosetae of penis ventral plate in Gonyleptoidea (Arachnida, Opiliones, Laniatores). Zoological Journal of the Linnean Society, 174(1), pp.1-46.

Kury, A. B., and García, A. F., 2016. On the identity of Flirtea C.L. Koch, 1839 (Arachnida, Opiliones, Cosmetidae). Zootaxa 4093, 231–247.

Kury, A.B. and Medrano, M., 2016. Review of terminology for the outline of dorsal scutum in Laniatores (Arachnida, Opiliones). Zootaxa, 4097(1), pp.130-134.

Kury, A.B., 2016. A classification of the penial microsetae of Gonyleptoidea (Opiliones: Laniatores). Zootaxa, 4179(1), pp.144-150.

Kury, A.B., 2017. Classification of Opiliones. Museu Nacional/UFRJ website. Online at:

http://www.museunacional.ufrj.br/mndi/Aracnologia/opiliones.html

Kury, A.B. and Medrano, M., 2018. A whiter shade of pale: anchoring the name Paecilaema CL Koch, 1839 onto a neotype (Opiliones, Cosmetidae). Zootaxa, 4521(2), pp.191-219.

Larsson, A., 2014. AliView: a fast and lightweight alignment viewer and editor for large datasets. Bioinformatics, 30(22), pp.3276-3278.

Lee, M.S. and Palci, A., 2015. Morphological phylogenetics in the genomic age. Current Biology, 25(19), pp.R922-R929.

Lewis, P.O., 2001. A likelihood approach to estimating phylogeny from discrete morphological character data. Systematic biology, 50(6), pp.913-925.

Lipscomb, D., Platnick, N. and Wheeler, Q., 2003. The intellectual content of taxonomy: a comment on DNA taxonomy. Trends in Ecology & Evolution, 18(2), pp.65-66.

Machado, G. and Macías-Ordóñez, R., 2007. Social behavior. Harvestmen: The Biology of Opiliones.(R. Pinto-da-Rocha, G. Machado & G. Giribet, eds.). Harvard University Press, Cambridge, pp.400-413.

Macías-Ordóñez, R., Machado, G., Pérez-González, A. and Shultz, J.W., 2010. Genitalic evolution in Opiliones. The evolution of primary sexual characters in animals. Oxford University Press, New York, pp.285-306.

Maddison, W.P. and Maddison, D.R., Mesquite: a modular system for evolutionary analysis. Version 3.51 (2018).

Masta, S.E. and Maddison, W.P., 2002. Sexual selection driving diversification in jumping spiders. Proceedings of the National Academy of Sciences, 99(7), pp.4442-4447.

Medrano, M. and Kury, A.B., 2016. Characterization of Platymessa with redescription of the type species and a new generic synonymy (Arachnida, Opiliones, Cosmetidae). Zootaxa, 4085(1), pp.52-62.

Medrano, M. and Kury, A.B., 2017. Taxonomic revision of the Andean genus Eulibitia Roewer, 1912 (Arachnida, Opiliones, Cosmetidae), with the description of five new species. European Journal of Taxonomy, (357).

Medrano, M. and Kury, A.B., 2018. Relationships and cladistic analysis of Roquettea with description of two new species and notes about evolution of stylus in Cosmetidae (Opiliones, Grassatores). Invertebrate systematics, 32(5), pp.1206-1233.

Mello-Leitão, C.D., 1933. Notas sobre os opiliões do Brasil descritos na obra póstuma de Sörensen:" Descriptiones Laniatorum. Boletim do Museu nacional, 9(1), pp.99-114.

Mello-Leitão, C.D., 1941. Alguns opiliões novos da Colômbia. Anais da Academia Brasileira de Ciências, 13(3), pp.165-171.

Mendes, A.C., 2011. Phylogeny and taxonomic revision of Heteropachylinae (Opiliones: Laniatores: Gonyleptidae). Zoological Journal of the Linnean Society, 163(2), pp.437-483.

Miller, C.W., 2013. Sexual selection: male-male competition. The Princeton guide to evolution, pp.641-646.

Miller, M.A., Pfeiffer, W. and Schwartz, T., 2010, November. Creating the CIPRES Science Gateway for inference of large phylogenetic

trees. In 2010 gateway computing environments workshop (GCE) (pp. 1-8). Ieee.

Miyamoto, M.M., 1985. Consensus cladograms and general classifications. Cladistics, 1(2), pp.186-189.

Monteiro, Y.F. and Pinto-Da-Rocha, R., 2015. Revision of the genus Ferkeria Roewer, 1947, with the description of a new species (Opiliones: Laniatores: Cosmetidae). Zootaxa, 4006(2), pp.361-373.

Mooi, R.D. and Gill, A.C., 2010. Phylogenies without synapomorphies—a crisis in fish systematics: time to show some character. Zootaxa, 2450(1), pp.26-40.

Nadler, S., Felix, M.A., Frisse, L., Sternberg, P., De Ley, P. and Thomas, W.K., 1999. Molecular and morphological characterisation of two reproductively isolated species with mirror-image anatomy (Nematoda: Cephalobidae). Nematology, 1(6), pp.591-612.

Nixon, K.C., 1999. The parsimony ratchet, a new method for rapid parsimony analysis. Cladistics, 15(4), pp.407-414.

Palumbi, S.R., 1996. Nucleic acids II: the polymerase chain reaction. Molecular systematics, pp.205-247.

Peres, E.A., Benedetti, A.R., Hiruma, S.T., Sobral-Souza, T. and Pinto-da-Rocha, R., 2019. Phylogeography of Sodreaninae harvestmen (Arachnida: Opiliones: Gonyleptidae): Insights into the biogeography of the southern Brazilian Atlantic Forest. Molecular phylogenetics and evolution, 138, pp.1-16.

Perty, M., 1833:Brasilianische Spinnen.InJ. B. de Spix& F. F.Martius, DelectusAnimaliumArticulatorumquaein itinereper Braziliamann.1817et 1820 colligerunt: 191–209.Monachii

Pickard-Cambridge, F.O., 1904. Opiliones. Biologia Centrali-Americana, Arachnida, Araneidea and Opiliones, 2, pp.545-560.

Pinto-da-Rocha, R., da Silva, M.B. and Bragagnolo, C., 2005. Faunistic similarity and historic biogeography of the harvestmen of southern and southeastern Atlantic Rain Forest of Brazil. The Journal of Arachnology, 33(2), pp.290-300.

Pinto-da-Rocha, R. and Bragagnolo, C., 2011. Systematic revision and cladistic analysis of the Brazilian subfamily Sodreaninae (Opiliones: Gonyleptidae). Invertebrate Systematics, 24(6), pp.509-538.

Pinto-Da-Rocha, R. and Hara, M.R., 2011. Redescription of Platygyndes Roewer 1943, a false Gonyleptidae,(Arachnida, Opiliones, Cosmetidae). ZooKeys, (143), p.1.

Pinto-da-Rocha, R. and Yamaguti, H., 2013. Paecilaema batman, a new species of Brazilian troglophilous harvestman that exhibits a remarkable color patches variation (Opiliones: Cosmetidae). Zoologia (Curitiba), 30(4), pp.441-446.

Pinto‐da‐Rocha, R., Bragagnolo, C., Marques, F.P. and Antunes Junior, M., 2014. Phylogeny of harvestmen family Gonyleptidae inferred from a multilocus approach (Arachnida: Opiliones). Cladistics, 30(5), pp.519-539.

Posada, D. and Buckley, T.R., 2004. Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. Systematic biology, 53(5), pp.793-808.

Proud, D.N., Felgenhauer, B.E., Townsend, V.R., Osula, D.O., Gilmore, W.O., Napier, Z.L. and Van Zandt, P.A., 2012. Diversity and

habitat use of Neotropical harvestmen (Arachnida: Opiliones) in a Costa Rican rainforest. ISRN Zoology, 2012.

Proud, D.N. and Townsend, V.R., 2019. Unusual penis morphology among cosmetid harvestmen (Arachnida: Opiliones) from Mesoamerica. Zoomorphology, 138(2), pp.233-247.

Rambaut, A., Drummond, A.J., Xie, D., Baele, G. and Suchard, M.A., 2018. Posterior summarization in Bayesian phylogenetics using Tracer 1.7. Systematic biology, 67(5), pp.901-904.

Revell, L.J., 2012. phytools: an R package for phylogenetic comparative biology (and other things). Methods in ecology and evolution, 3(2), pp.217-223.

Reyda, F.B. and Olson, P.D., 2003. Cestodes of cestodes of Peruvian freshwater stingrays. Journal of Parasitology, 89(5), pp.1018-1025.

Ringuelet, R.A., 1959. Los arácnidos argentinos del Orden Opiliones. Revista del Museo Argentino de Ciencias Naturales" Bernardino Rivadavia", 5.

Roewer, C.F., 1912. Die Familien der Assamiiden und Phalangodiden der Opiliones-Laniatores.(= Assamiden, Dampetriden, Phalangodiden, Epedaniden, Biantiden, Zalmoxiden, Samoiden, Palpipediden anderer Autoren). Archiv für Naturgeschichte, 78(3), pp.1-242.

Roewer, C.F., 1913. Die Familie der Gonyleptiden der Opiliones-Laniatores. Archiv für Naturgeschichte A, 79, pp.1-256.

Roewer, C.F., 1916. 7 neue Opilioniden des Zoolog. Museums in Berlin. Archiv für Naturgeschichte, Berlin, Abt. A, Original-Arbeiten, 81(12), 6–13.

Roewer, C.F., 1923. Weberknechte der Erde, systematische Bearbeitung der bisher bekannten Opiliones, von Dr. Carl-Friedrich Roewer.

Roewer, C.F., 1927a. Weitere Weberknechte I. (1. Ergänzung der: "Weberknechte der Erde," 1923). Abhandlungen der Naturwissenschaftlichen Verein zu Bremen, Bremen, 26(2) [“1926”], 261–402, pl. 1. [Issued January 1927].

Roewer, C.F., 1943. Über Gonyleptiden. Weitere Webernechte (Arachn., Opil.) XI. Senckenbergiana, 26(1-3), pp.12-68.

Roewer, C.F., 1947. Diagnosen neuer Gattungen und Arten der Opiliones–Laniatores. Weitere Weberknechte XII. Cosmetidae. Senckenbergiana, 28(1-3), pp.1-58.

Roewer , C. F. 1952. Neotropische Arachnida Arthrogastra zumeist aus Peru. Senckenbergiana, 33(1-3): 37-58.

Roewer, C.F., 1957. Uber Oligolophinae, Caddoinae, Sclerosomatinae, Leiobuninae, Neopilioniae (Phalangiidae, Opiliones, Palpatores).(Weitere Weberknechte XX). Senckenbergiana Biol., 38, pp.323-358.

Roewer, C.F., 1963. Opiliones aus Peru und Colombia. [Arachnida Arthrogastra aus Peru V]. Senckenbergiana Biologica, 44, 45–72.

Sánchez‐Pacheco, S.J., Torres‐Carvajal, O., Aguirre‐Peñafiel, V., Nunes, P.M.S., Verrastro, L., Rivas, G.A., Rodrigues, M.T., Grant, T. and Murphy, R.W., 2018. Phylogeny of Riama (Squamata: Gymnophthalmidae), impact of phenotypic evidence on molecular datasets, and the origin of the Sierra Nevada de Santa Marta endemic fauna. Cladistics, 34(3), pp.260-291.

Sharma, P.P. and Giribet, G., 2011. The evolutionary and biogeographic history of the armoured harvestmen–Laniatores phylogeny based on ten molecular markers, with the description of two new families

of Opiliones (Arachnida). Invertebrate Systematics, 25(2), pp.106-142.

Sharma, P.P. and Giribet, G., 2011. The evolutionary and biogeographic history of the armoured harvestmen–Laniatores phylogeny based on ten molecular markers, with the description of two new families of Opiliones (Arachnida). Invertebrate Systematics, 25(2), pp.106-142.

Sharma, P.P. and Giribet, G., 2012. Out of the Neotropics: Late Cretaceous colonization of Australasia by American arthropods. Proceedings of the Royal Society B: Biological Sciences, 279(1742), pp.3501-3509.

Sharma, P.P. and Giribet, G., 2014. A revised dated phylogeny of the arachnid order Opiliones. Frontiers in genetics, 5, p.255.

Simon, E., 1879. Essai d'une classification des Opiliones Mecostethi. Remarques synonymiques et descriptions d'espèces nouvelles. In Annales de la Société entomologique de Belgique (Vol. 22, pp. 183-241).

Soares, H.E.M., 1968. Contribuição ao estudo dos opiliões do Chile (Opiliones: Gonyleptidae, Triaenonychidae). Papéis Avulsos de Zoologia, 21, pp.259-272.

Solano-Brenes, D., García-Hernández, S. and Machado, G., 2018. All the better to bite you with! Striking intrasexual differences in cheliceral size define two male morphs in an Amazonian arachnid. Biological Journal of the Linnean Society, 125(3), pp.521-534.

Song, H. and Bucheli, S.R., 2010. Comparison of phylogenetic signal between male genitalia and non‐genital characters in insect systematics. Cladistics, 26(1), pp.23-35.

Sørensen, W.E. (1884b) Opiliones Laniatores (Gonyleptides W. S. Olim) Musei Hauniensis. Naturhistorisk Tidsskrift, Kjøbenhavn, (series 3) 14(3), 555–646.

Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics, 30(9), pp.1312-1313.

Stampar, S.N., Maronna, M.M., Vermeij, M.J., d Silveira, F.L. and Morandini, A.C., 2012. Evolutionary diversification of banded tube-dwelling anemones (Cnidaria; Ceriantharia; Isarachnanthus) in the Atlantic Ocean. PLoS One, 7(7), p.e41091.

Tautz, D., Arctander, P., Minelli, A., Thomas, R.H. and Vogler, A.P., 2003. A plea for DNA taxonomy. Trends in ecology & evolution, 18(2), pp.70-74.

Tong, Y., Binford, G., Rheims, C.A., Kuntner, M., Liu, J. and Agnarsson, I., 2019. Huntsmen of the Caribbean: Multiple tests of the GAARlandia hypothesis. Molecular phylogenetics and evolution, 130, pp.259-268.

Townsend Jr, V.R., Viquez, C., Vanzandt, P.A. and Proud, D.N., 2010. Key to the species of Cosmetidae (Arachnida, Opiliones) of Central America, with notes on penis morphology and sexual dimorphisms. Zootaxa, 2414(1), pp.1-26.

Townsend, V.R., Bertram, M.S. and Milne, M.A., 2015. Variation in ovipositor morphology among laniatorean harvestmen (Arachnida: Opiliones). Zoomorphology, 134(3), pp.487-497.

Townsend Jr, V.R., Pérez-González, A. and Proud, D.N., 2019. Putative mating plugs of harvestmen (Opiliones, Laniatores). Zoologischer Anzeiger, 278, pp.101-109.

Walker, E.A. and Townsend Jr, V.R., 2014. Ovipositor morphology of cosmetid harvestmen (Arachnida, Opiliones, Laniatores): A new source of informative characters. Journal of Morphology, 275(12), pp.1376-1385.

Wheeler, W., 1996. Optimization alignment: the end of multiple sequence alignment in phylogenetics?. Cladistics, 12(1), pp.1-9.

Wheeler, W.C., Lucaroni, N., Hong, L., Crowley, L.M. and Varón, A., 2015. POY version 5: phylogenetic analysis using dynamic homologies under multiple optimality criteria. Cladistics, 31(2), pp.189-196.

Wheeler, W.C., 2003. Iterative pass optimization of sequence data. Cladistics, 19(3), pp.254-260.

Wheeler, W.C., 2003. Implied alignment: a synapomorphy‐based multiple‐sequence alignment method and its use in cladogram search. Cladistics, 19(3), pp.261-268.

Wiens, J.J., 2001. Character analysis in morphological phylogenetics: problems and solutions. Systematic Biology, 50(5), pp.689-699.

Wiens, J.J., 2005. Can incomplete taxa rescue phylogenetic analyses from long-branch attraction?. Systematic Biology, 54(5), pp.731-742.

Wiens, J.J., 2006. Missing data and the design of phylogenetic analyses. Journal of biomedical informatics, 39(1), pp.34-42.

Vaidya, G., Lohman, D.J. and Meier, R., 2011. SequenceMatrix: concatenation software for the fast assembly of multi‐gene datasets with character set and codon information. Cladistics, 27(2), pp.171-180.

Van den Elzen, R., Guillen, J., Ruiz-del-Valle, V., Allende, L.M., Lowy, E., Zamora, J. and Arnaiz-Villena, A., 2001. Both morphological

and molecular characters support speciation of South American siskins by sexual selection. Cellular and Molecular Life Sciences CMLS, 58(14), pp.2117-2128.

Vasconcelos, E. and González, A.P., 2003. A new species of Arucillus Silhavy, 1971 from the Dominican Republic (Opiliones: Laniatores: Cosmetidae). Revista Ibérica de Aracnología, (7), pp.135-140.

XIII. Appendix

MORPHOLOGY CHARACTER LIST

SOMATIC CHARACTERS

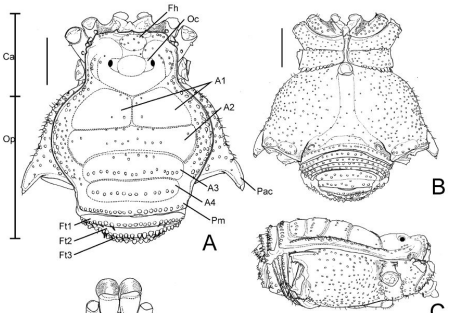
DORSAL SCUTUM

1. Dorsal scutum—width (ratio prosoma/maximal scutum width) (modified from Bragagnolo & Pinto-da-Rocha 2012) (CI= 0.041; RI= 0.439):

0. prosoma slightly narrower than opisthosoma (ratio 0.51- 0.70);
1. prosoma and opisthosoma with similar widths (ratio + 0.71).

2. DS-Prosoma: Frontal hump (modified from Hara et al. 2012) (CI= 0.25; RI= 0.4):

0. Present;
1. Absent.



3. DS. Prosoma. Shape of posterior margin of the cheliceral socket (CI= 0.033; RI= 0.275):

0. Straight shallow squared sockets;
1. Rounded deeper sockets, creating a “w” shape.

4. DS. Prosoma. Anterior margin. Shape of paracheliceral projections/tubercles (CI= 0.171; RI= 0.540):

0. Absent;
1. Multiple projections, either distinct from one another or bases united;
2. Single pointed projection;
3. Single rounded projections (can be low/reduced);
4. Single pyriform projection;
5. Single low square projection;
6. Single projection, lobed or warty in appearance.

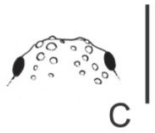
5. DS. Prosoma. Pair of tubercles on anterior margin (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.2; RI= 0.33):

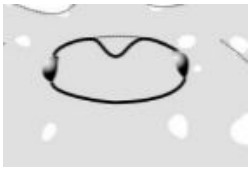
0. Present (Pinto-da-Rocha et. al., 2012: fig.6A);
1. Absence of larger tubercles on anterior margin.

0 1

6. DS. Prosoma. Ocularium shape (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.20; RI= 0.466):

0. Dome-shaped (Pinto-da-Rocha & Hara, 2011: fig. 1C);
1. Moderately high with median depression (Kury et. al., 2007: fig. 1);
2. Low and wide (Ferreira & Kury, 2010: fig. 12A).

0 

1 

2 

7. DS. Prosoma. Ocularium armature (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.143; RI= 0.667):

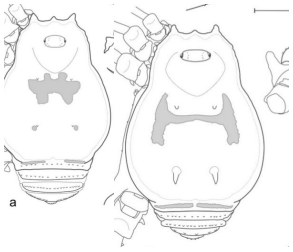
0. Absent;
1. Larger tubercles or spines.

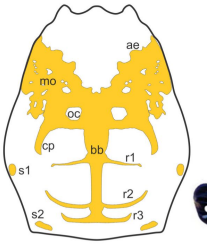
8. DS. Cuticle color in ethanol (CI= 0.043; RI= 0.154):

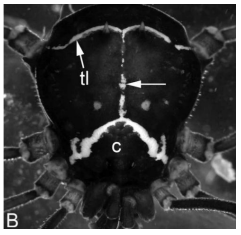
0. Single color or darker regions and veining in cuticle;
1. Lighter regions or spots in cuticle.

9. DS. Opithosoma. Coloration on cuticle (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.281; RI= 0.461):

0. Without 'dry markings' or yellow-white markings on DS;
1. Equuleus pattern (see Kury & Barros 2014);
2. Scaramuccia pattern (see Kruy & García 2016);
3. Lyre mask (As defined in Medrano & Kury 2018);
4. Reticulation on/along lateral and posterior margins of DS and/or midline/transverse lines "backbone";
5. Spots (see some *Roquettea*, *Gryne* amd *Cynorta conspera*).

Equuleus pattern 

Scaramuccia pattern 

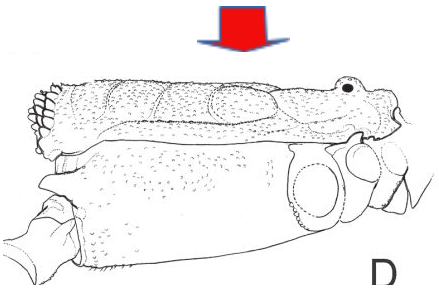
Midline-transverse lines 

10. DS. Outline (Kury et. al., 2007: fig 12) (CI= 0.130; RI= 0.474):

0. Alpha;
1. Beta;
2. Gamma;
3. Delta.

11. DS. Opisthosoma. Sulci separating areas (from Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.125; RI= 0.533):

0. Shallow, indistinguishable;
1. Conspicuous.



12. DS. Granulation (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.061; RI= 0.262):

0. Dense (Fig. 1B);
1. Sparse (Fig. 1A);
2. Absent (Ferreira, 2006: fig 21).

13. DS. Opisthosoma. Area I, paramedian armature (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.086; RI= 0.385):

0. Armed with tubercles;
1. Armed with spines;
2. Armed with protuberances (as described in Ferreira & Kury 2010);
3. Absent.

14. DS. Opisthosoma. Area II, paramedian armature (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.083; RI= 0.353):

0. Armed with tubercles;
1. Armed with spines;
2. Absent.

15. DS. Opisthosoma. Area III, armature type (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.068; RI= 0.268):

0. Armed with tubercles;
1. Armed with spines;
2. Armed with protuberances;
3. Absent.

16. DS. Opisthosoma. Area IV/posterior margin, armature type (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.074; RI= 0.265):

0. Armed with tubercles;
1. Armed with spines/protuberances;
2. Absent.

17. DS. Opithosoma. Free Tergites. Granulation (CI= 0.033; RI= 0.275):

0. Present;
1. Absent.

18. DS. Opithosoma. Free tergite I armature (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.100; RI= 0.182):

0. Unarmed;
1. Armed with tubercles/spines.

19. DS. Opisthosoma. Free tergite II armature (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.091; RI= 0.167):

0. Unarmed;
1. Armed with tubercles/spines.

20. DS. Opisthosoma. Free tergite III armature (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017)(CI= 0.027; RI= 0.133:

0. Unarmed;
1. Armed with tubercles/spines.

21. DS. Lateral margins armature (modified from Kury & Villarreal, 2015) (CI= 0.027; RI= 0.345):

0. Without any armature or special forms;
1. Special forms such as armed with tubercles or wrinkling of DS margins.

22. DS. Opithosoma. Posterior margin (CI= 0.033; RI= 0.561):

0. With a line of granules along margin;
1. Absence of granules in a line.

23. Venter. Spine on anal operculum (modified from Kury & Villarreal, 2015) (CI= 0.067; RI= 0.125):

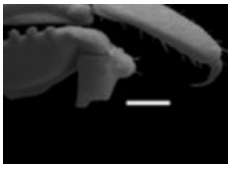
0. Absent in male and female;
1. Present only in male.

PEDIPALPS AND CHELICERAE

24. Pedipalp. Trochanter. Number of apical ventral tubercles (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.111; RI= 0.238):

0. One (Fig. 4E);
1. Two (Fig. 3C);
2. Three (Pinto-da-Rocha & Hara, 2011: fig. 1B);
3. Absence of tubercles.

0 1 2


25. Pedipalp. Femur. Arrangement of dorsal tubercles (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.057; RI= 0.313):

0. Tubercles separated;
1. Projection of tubercles contiguous or absent;
2. Tubercles replaced with a ridge on dorsal surface.

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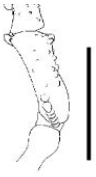
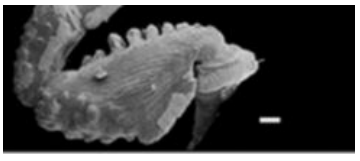



26. Pedipalp. Tibia. Dorsal-ventral shape (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 1.00; RI= 1.00):

0. Cylindrical;
1. Flattened, spoon-shaped.

27. Pedipalp. Tibia. Shape in lateral view (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.286; RI= 0.667):

0. Base wider than apex;

1. Apex larger than base;

2. Rectangular (dorsal and ventral margins parallel in lateral view) (Pinto-da-Rocha & Hara, 2011: figs1E–G).

1  2 

28. Pedipalp. Ectal surface of tibia (CI= 0.111; RI= 0.111):

0. With granules/tubercles on surface;

1. Smooth ectal surface.

29. Pedipalp. Tarsus. Size of setae (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 1.00; RI= 1.00):

0. Long, ±0.5 tarsus length;

1. Short, < 0.5 of tarsus length.

30. Cheliceral hand (Segment II) shape (CI= 0.037; RI= 0.447):

0. Monomorphic;

1. Hypertelic in males, with or without elbow like projection dorsally.

31. Chelicera. Basichelicerate. Bulla Granulation in dorsal view (CI= 0.056; RI= 0.485):

0. Dorsal surface with granulation;

1. Dorsal surface mostly smooth.

32. Chelicera. Basichelicerate. Bulla basal armature in dorsal view (CI= 0.033; RI= 0.370):

0. Basal margin with many closely placed tubercles;

1. Basal margin with spread-out tubercles.

LEGS (TOTAL PARTS)

33. Legs. Coxa I. Prolateral-dorsal apophyses (modified from Ferreira thesis, 2006) (CI= 0.043; RI= 0.214):

0 Present, simple;

1 Present, bilobed;

2 Present, bifid.

34. Legs. Coxa I. Retrolateral dorsal apophysis (modified from Ferreira thesis, 2006) (CI= 0.167; RI= 0.00):

0. Absent;

1. Present.

35. Legs. Coxa II. Prolateral dorsal apophysis (modified from Ferreira thesis, 2006, and Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.053; RI= 0.471):

0. Present, simple;
1. Present, bilobed;
2. Present, bifid.

36. Legs. Coxa II Retrolateral dorsal apophysis (modified from Ferreira thesis, 2006) (CI= 0.024; RI= 0.293):

0. Absent;
1. Present.

37. Legs. Coxae II & III. Process fulci. In dorso-lateral view (CI= 0.027; RI= 0.265):

0. Absence;
1. Presence.

38. Legs. Coxa III. Prolateral dorsal apophysis (modified from Ferreira thesis, 2006) (CI= 0.026; RI= 0.383):

0. Absent;
1. Present.

39. Legs. Coxa III. Retrolateral dorsal apophysis (modified from Ferreira thesis, 2006) (CI= 0.091; RI= 0.000):

0. Absent;
1. Present.

40. Legs. Coxa IV. Apical Prolateral armature (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.054; RI= 0.500):

0. Apophyses;
1. Tubercles- warty in appearance or fused bases;
2. Smooth single tubercle.

41. Legs Coxa IV. Retrolateral armature (CI= 0.143; RI= 0.33):

0. Absent;
1. Apophyses.

42. Legs. Coxae IV. Clavi Ingiunes (CI= 0.048; RI= 0.545):

0. Absent;
1. Present.

43. Legs. Trochanter I. Retrolateral armature (CI= 0.037; RI= 0.257):

0. Absent;
1. Present.

44. Legs. Trochanter II. Retrolateral armature (CI= 0.045; RI= 0.300):

0. Absent;
1. Present.

45. Legs. Trochanter III. Retrolateral armature (CI= 0.027; RI= 0.280):

0. Absent;
1. Present.

46. Legs. Trochanter IV. Dorsal armature (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.125; RI= 0.222):

0. Present;
1. Absent.

47. Legs. Trochanter IV. Shape of retrolateral armature (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.064; RI= 0.362):

0. Single projection (Figs 5E, G);
1. Grouped projections, not in a row (Figs 5F, H; 6F);
2. Projections in a row (Fig. 6G);
3. Absent.

0  1  2 

48. Legs. Trochanter IV. Number of retrolateral apical tubercles (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.068; RI= 0.388):

0. None (Figs 5E; 6H);
1. One (Figs 5G; 6G);
2. Two (Figs 5F; 6F);
3. Four.

0  1  2 

49. Legs. Trochanter IV. Number of retrolateral median projections (tubercles or apophyses) (modified from Hara et al. 2010) (CI= 0.143; RI= 0.333):

0. None;
1. One–three;
2. Four–five.

50. Legs. Trochanter IV. Number of retrolateral proximal tubercles (modified from Hara et al. 2010) (CI= 0.100; RI= 0.400):

0. None;
1. One,

51. Legs. Femur III. Armature (CI= 0.04; RI= 0.429):

0. Smooth or same as in female;
1. Armed with granules, tubercles, spines or apophyses.

52. Legs. Femur IV. Shape (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.190; RI= 0.261):

0. Straight;
1. Curved retrolaterally (in dorsal view);
2. Curved dorsally (in lateral view);
3. Sinuous (in lateral view).

53. Legs. Femur IV. Retrolateral Armature (CI= 0.032; RI= 0.318).

0. Smooth, or femur IV same as in female;
1. Different than in female, with presence of armature (i.e. tubercles, apophyses, spines).

54. Legs. Femur IV. Prolateral Armature. Male (CI= 0.034; RI= 0.569):

0. Smooth as in female;
1. with armature.

55. Legs. Femur IV. Male modifications that are not armature (CI= 0.333; RI= 0.0):

0. Absent;
1. Present, apex of femur swollen and bulbous;
2. Present, apical end of femur compressed laterally;
3. Present femur compressed ventro-dorsally.

56. Legs. Patella IV. Armature (CI= 0.029; RI= 0.45):

0. Unarmed;
1. Armed with tubercles, spines.

57. Legs. Tibia IV, sexual dimorphism (CI= 0.030; RI= 0.475):

0. absent;
1. different than that observed in female.

58. Legs. Tibia IV. Retrolateral or prolateral apophyses (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.05; RI= 0.345):

0. Present, pointed;
1. Present, rounded or rombous;
2. Absent.

59. Legs. Metatarsus IV. Sexual dimorphism (CI= 0.40; RI= 0.571):

0. As in females, no modification;
1. Armed with tubercles/spines;
2. Prismatic/swollen (see Ferreira & Kury 2010, Medrano & Kury 2018).

60. Legs. Tarsus. Claws (CI= 0.667; RI= 0.90):

0. Smooth;
1. Pectination- two ventral rows, medial teeth more pronounced;
2. Pectination- two ventral rows, both rows of teeth similar appearance.

PENIS/GENITALIA CHARACTERS

61. Penis. Truncus. In relation to ventral plate (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.087; RI= 0.382):

0. Straight, both VP and truncus with similar width;

1. Truncus enlarged laterally, with sulci at base of VP in ventral view (Monteiro & Pinto-da- Rocha 2015; FIG 6B & D);

2 Truncus enlarged laterally (as proposed in Kury & Barros 2014; Coronato-Ribeiro & Pinto-da-Rocha 2017; FIG 11A-F).

62. Penis. VP. Distal edge of truncus overlapping VP (CI= 0.036; RI= 0.206):

0. no overlapping;

1. VP sunken into truncus (+10%- unlikely to be an artifact of prep).

63. Penis. VP. Thickness in lateral view (CI= 0.111; RI= 0.500):

0. Thickness +50% that of truncus;

1. Attenuate, extremely thin ("paper-thin").

64. Penis. VP. Shape of apical margin (CI= 0.16; RI= 0.30):

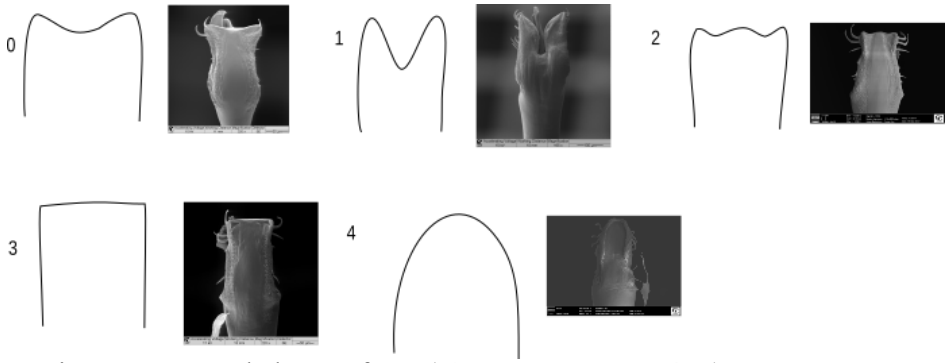
0. Curved (concave);

1. Cleft (20-50% of VP length);

2. Sinuous;

3. Straight;

4. Convex.



65. Penis. VP. General shape of VP (CI= 0.059; RI= 0.492):

0. Square, all sides equal length;

1. Rectangular, lateral sides longer than apical margin;

2. Trapezoidal, lateral margins converge apically or basally.

66. Penis. VP. Macrosetae (MS) A (modified from Kury & Villarreal 2015) (CI= 0.303; RI= 0.610):

0. One pair;

1. Two pairs;

2. Three pairs;

3. Absent.

67. Penis. VP. MS Length of MSA1 (CI= 0.036; RI= 0.325):

0. Reduced;

1. Long.

68. Penis. VP. MS Branching patterns of MSA (CI= 0.111; RI= 0.0):
    0. Simple, sometimes with acuminate tip;
    1. Bifid.

69. Penis, VP, MS-A,most apical pair position (CI= 0. 323; RI= 0.412):
    0. basal on VP (Ferreira and Kury 2010: fig. 8);
    1. at mid-length of VP.

70. Penis. VP. MS B (possibly only visible in ventral view SEM) (modified from Kury & Villarreal 2015; and Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.75; RI= 0.75):
    0. absent;
    1. one pair;
    2. two pairs- Synapomorphy for Ferkeria.

71. Penis. MS-B. Position on VP (CI= 0.05; RI= 0.457).
    0 Pair of MSB at place where truncus meets VP;
    1 Above truncus, well onto ventral surface of VP.

72. Penis. VP. MS C (modified from Kury & Villarreal 2015; and Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.25; RI= 0.571):
    0. One pair;
    1. Two pairs;
    2. Three pairs;
    3. Four or more.

73. Penis. VP. MS-C. Shape (CI= 0.125; RI= 0.462):
    0. Cylindrical;
    1. Compressed/helicoidal.

74. Penis. VP. MS-C. Apical tip (CI= 0.50; RI= 0.75):
    0. No modification, smooth/accuminate;
    1. With modification, bifid/with projections.

75. Penis. VP. MS-SN (from Medrano & Kury 2018) (CI= 0.333; RI= 0.333):
    0. Absent;
    1. Present,

76. Penis. VP. MS D (modified from Kury & Villarreal 2015; and Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.231; RI= 0.474):
    0. One pair;
    1. Two pairs.

77. Penis. VP. MS. Length of MSD1 (compare to the widest portion of VP) (CI= 0.068; RI= 0.305):

0. Reduced (less than 10%);
1. 10-25% of width of VP;
2. 25-50% width;
3. 50%+ of width.

78. Penis. VP. MS. Length of MSD2 (CI= 0.077; RI= 0.20):

0. Reduced;
1. 10-50% of VP width at widest point;
2. Long (+50%).

79. Penis. VP. MS Number of MSE (modified from Kury & Villarreal 2015; and Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 1.00; RI= 1.00):

0. Two pairs;
1. Three pairs;
2. Four pairs- Synapomorphy for Metalibitia.

80. Penis. VP. MSE (CI= 0.067; RI= 0.391):

0. MSE located on ventral surface of VP;
1. MSE shifted laterally, proximate to lateral MS.

81. Penis. VP. MSE. Appearance (CI= 0.30; RI= 0.588):

0. Simple, sunken in VP;
1. Simple and reduced- sometimes hidden by microsetae;
2. Large/raised and obvious base around MSE;
3. Not reduced, can be as long as some lateral macrosetae.

82. Penis. Macrosetae. Position on lateral margin (CI= 0.053; RI= 0.28).

0 Dorso-laterally, on the dorsal surface of VP;
1 Along lateral margin of VP.

83. Penis. VP. Texture and microsetae type on ventral surface (modified from Kury 2016, and Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.143; RI= 0.28):

0. Glabrous;
1. Textured with microsetae in just a lateral field, type 4;
2. Textured with microsetae in just lateral field, type 1;
3. Textured in lateral field and midfield, types 1 & 4;
4. Textured in lateral and mid-field, type 1;
5. Textured in lateral field, type 1&4;
6. Textured in lateral and mid field, type 4.

84. Penis. VP. Microsetae (type 4) on dorsal surface (CI= 0.083; RI= 0.214):

0. Absent;
1. Present.

85. Penis. Membranous extension of glans (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.75; RI= 0.80):

0. Present, placed on truncus apex (Benedetti, 2012: figs 49A–C);

1. Present, occupying lateral median region of plate;

2. Absent (Pinto-da-Rocha et. al., 2012: fig. 7);

3. Present, dorsal lip of truncus, scaled appearance (Only observed in *E. subserialis*).

86. Penis. VP. Glans. (modified from Medrano & Kury 2018) (CI= 0.167; RI= 0.75):

0. podium absent- truncus in lateral view is not enlarged dorsally, creating a pedastool below the glans;

1. podium present- truncus in lateral view enlarged dorsally, sometimes coming up around the glans.

87. Penis. Glans. Length relative to truncus width (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.047; RI= 0.163):

0. Long, length of glans 1.5-2 times the width of truncus;

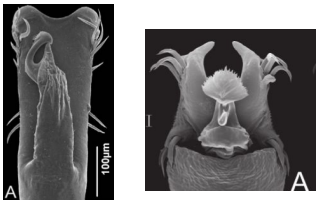
1. Short, length of glans 1-1.5 times the width of the truncus;

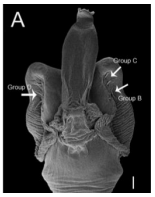
2. Short, less than the width of the truncus.

88. Penis. Glans. Position of base of glans on ventral plate (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 1.00; RI= 1.00):

0 Basal region (Ferreira & Kury, 2010: fig. 11; Pinto-da-Rocha et. al., 2012: fig. 7);

1 Middle region.

0 

1 

89. Penis. Glans. Bump scales (see Kury & Baros 2014-Taito work) (CI= 1.00; RI= 1.00):

0. Absent;

1. Present.

90. Penis. Dorsal process of glans (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017, and Gonyleptoidea project) (CI= 0.20; RI= 0.692):

0. Present;

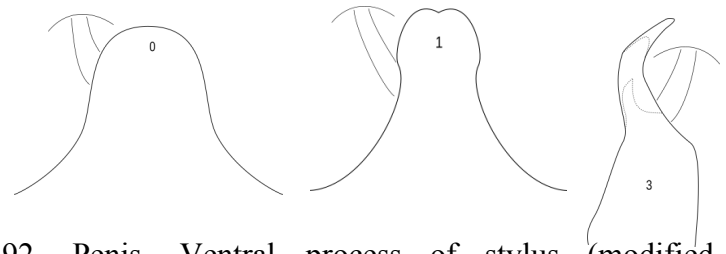
1. Absent.

91. Penis. Dorsal process. Shape (modified from Kury & Barros, 2014; Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.091; RI= 0.333):

0. Triangular- with varying degrees of narrowness at apical end. Base always wider than apex for this state;

1. Square-bilobed - apex has small dip, giving the apex of the dorsal process an almost heart shape form (modified from Kury & Barros, 2014);

2. Conical- very thin, varying in length.



92. Penis. Ventral process of stylus (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017, and various other publications) (CI= 0.333; RI= 0.60):

0. Present (Pinto-da-Rocha et. al., 2012: fig. 7);

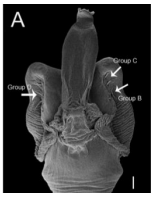
1. Absent .

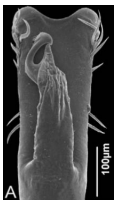
93. Penis. Stylus. Width of base of stylus, versus base of glans (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.125; RI= 0.00):

0. Narrower than glans at point of attachment;

1. Same width (Ferreira & Kury, 2010: fig. 11);

2. Wider.

0 

1 

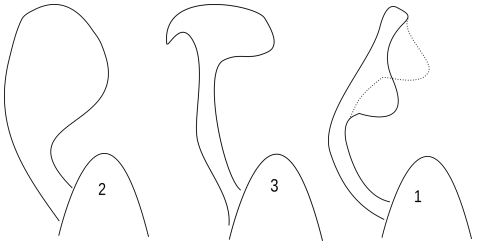
94. Penis. Stylus. Apex Shape (adapted from Gonyletpoidea characters) (CI= 0.115; RI= 0.511):

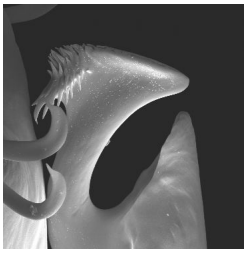
0. Cylindrical-typical of *Ferkeria* and *Metalibitia*with no modification around seminal opening;

1. Wattle present, reduced, not present full length of stylus;

2.Wattle present, large/obvious, fully extended along length of stylus (modified from Medrano & Kury 2018);

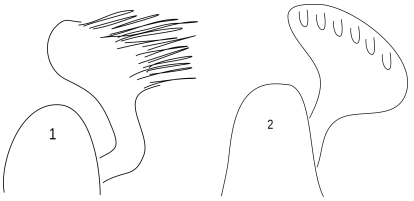
3. Axe-blade shape with dorsal beak (3&1 below, as described for some *Roquettea* in Ferreira & Kury 2010, and Medrano & Kury 2018).





95. Penis. Stylus. Apex ornamentation (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017 and Medrano Kury 2018) (CI= 0.0698; RI= 0.403):

0. Unarmed- with out filaments or barbs;
1. Armed only with filamentous projections at seminal opening;
2. Armed with stylus barbs and filamentous projections;
3. Armed with only barbs, cteniform.



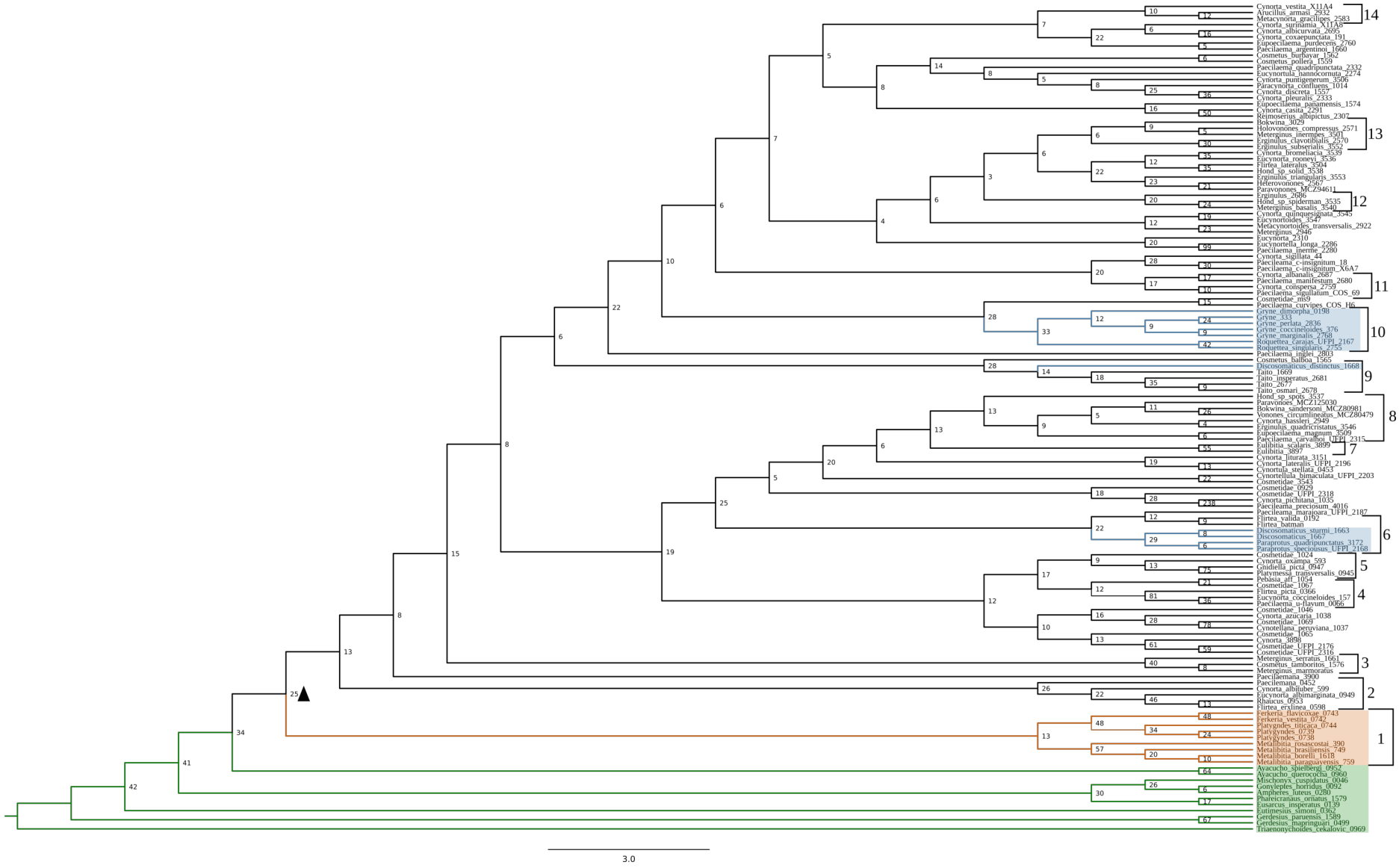


Figure 1: Total Evidence POY tree for ATMD dataset (tree cost 26363) with Bremer scores. Highlighted in green is the outgroup, the node for the family Cosmetidae is indicated with a black triangle, blue is the subfamily Discosomaticinae, and highlighted in orange is the new subfamily Metalibitiinae subfam.nov.

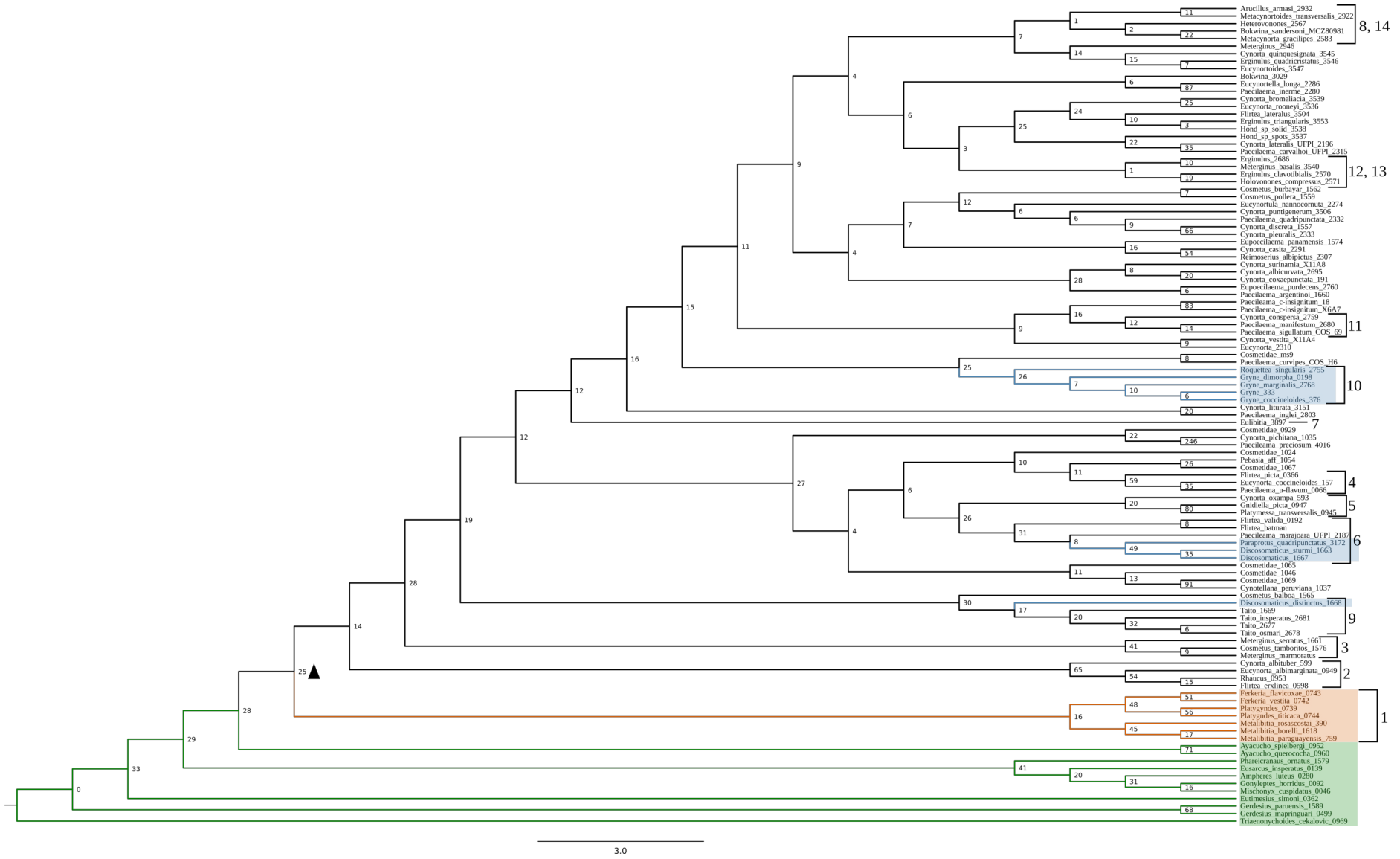


Figure 2: Total evidence POY tree for MTAD dataset (tree cost 20222). Highlighted in green is the outgroup, the node for the family Cosmetidae is indicated with a black triangle and in blue the subfamily Discosomaticinae. Highlighted in orange is the new subfamily Metalibitiinae subfam. nov.. Clade numbers correspond to POY ATMD tree.

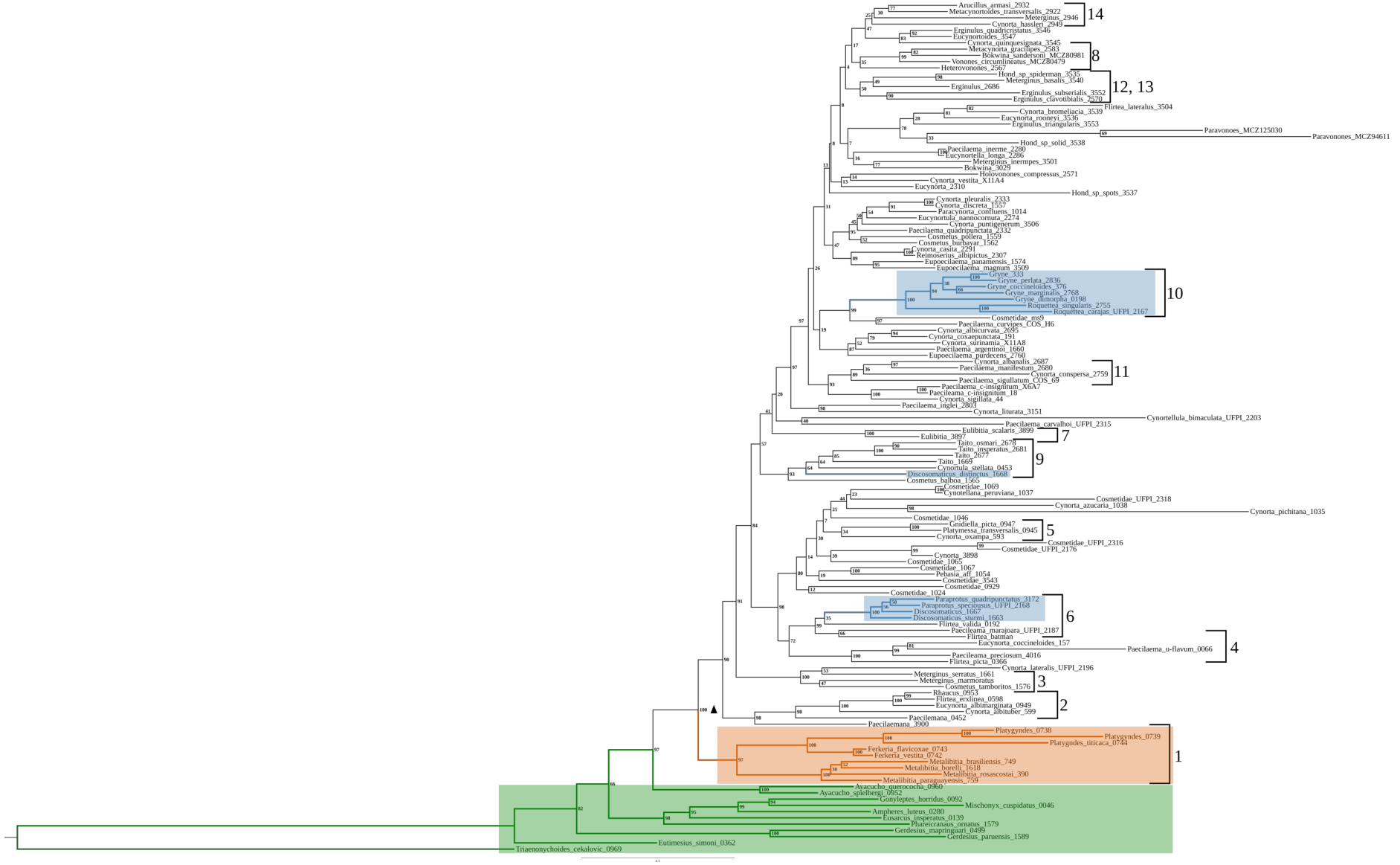


Figure 3: Total Evidence RAxML tree for ATMD set, with Bootstrap values. Highlighted in green is the outgroup, family Cosmetidae is indicated by black triangle. Highlighted in blue is species associated with the subfamily Discosomaticinae, and orange is the new subfamily Metalibitiinae subfam. nov.. Clade numbers correspond to those in Figure 1.

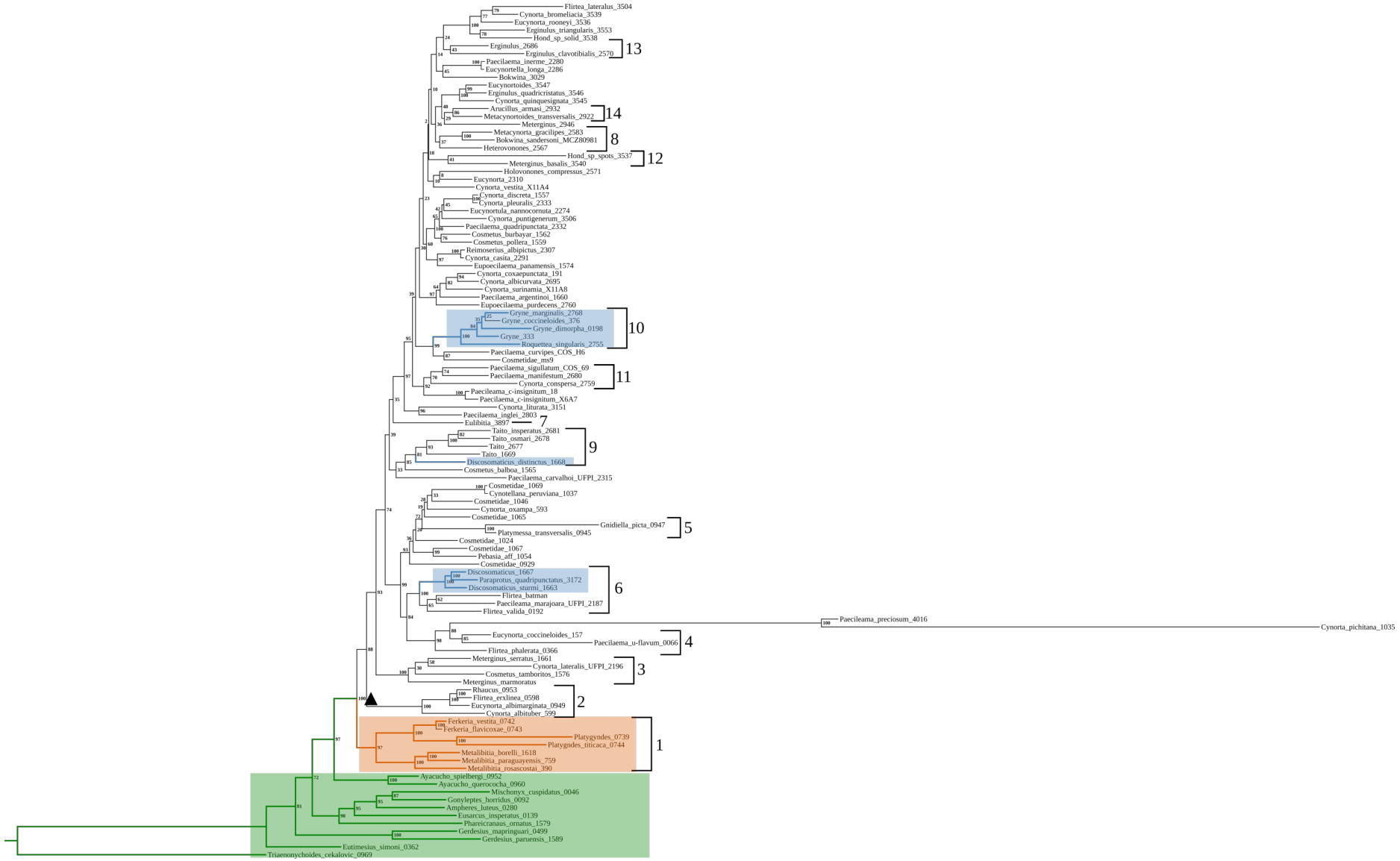


Figure 4: Total Evidence RAxML tree for MTAD set, with Bootstrap values. Highlighted in green is the outgroup, the family Cosmetidae is indicated with a black triangle. Highlighted in blue are species associated with the subfamily Discosomaticinae, orange is the new subfamily Metalibitiinae subfam. nov.. Clade numbers correspond to those in Figure 1.

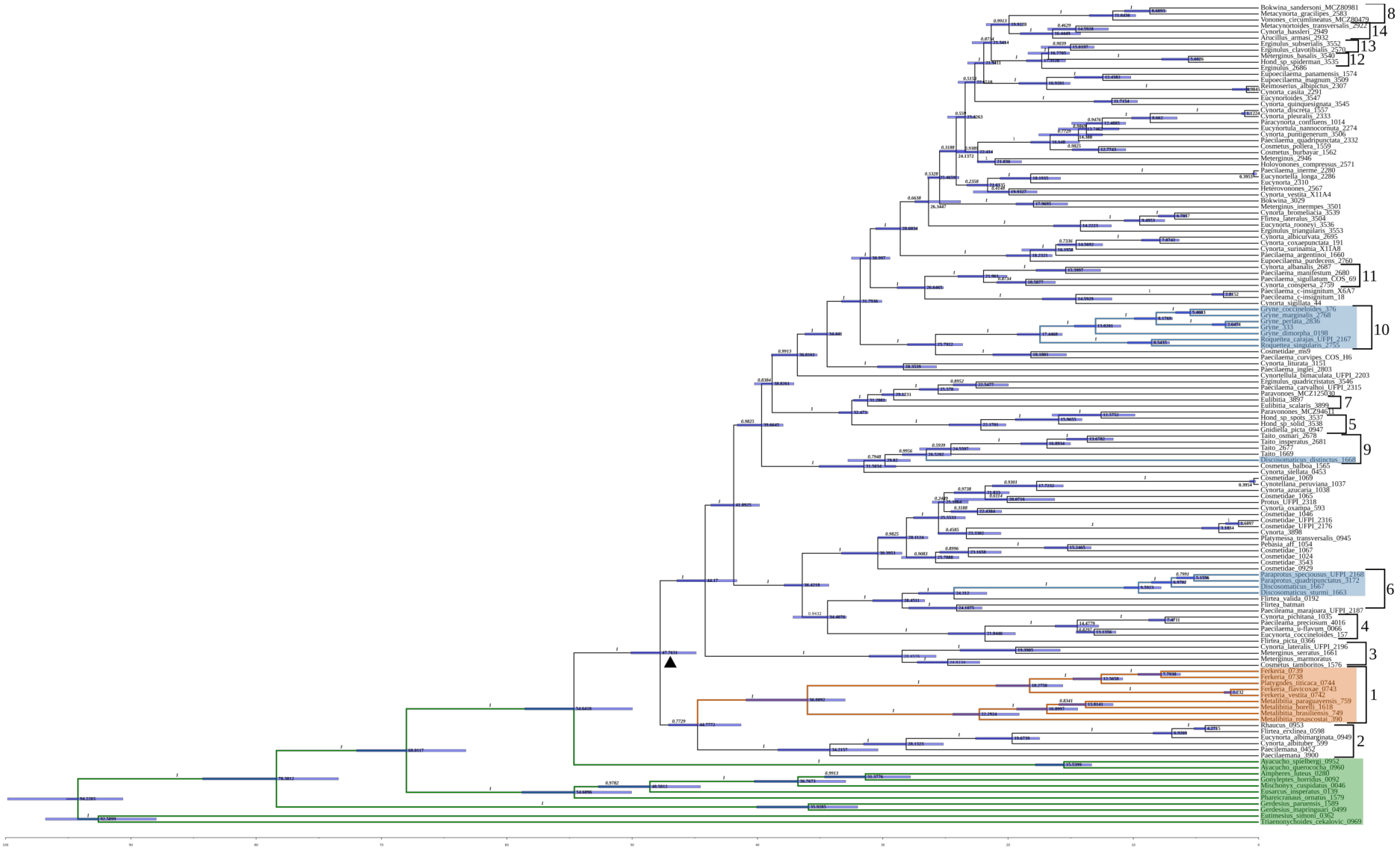


Figure 5: BEAST tree for ATMD set, Posterior probability in italics above branches, age of node in bold. Bars at nodes represent 95% credible intervals. Highlighted in green is the outgroup, family Cosmetidae indicated by black triangle. Highlighted in blue is species associated with the subfamily Discosomaticinae, orange is the the new subfamily Metalibitiinae subfam. nov.. Clade numbers correspond to those in Figure 1

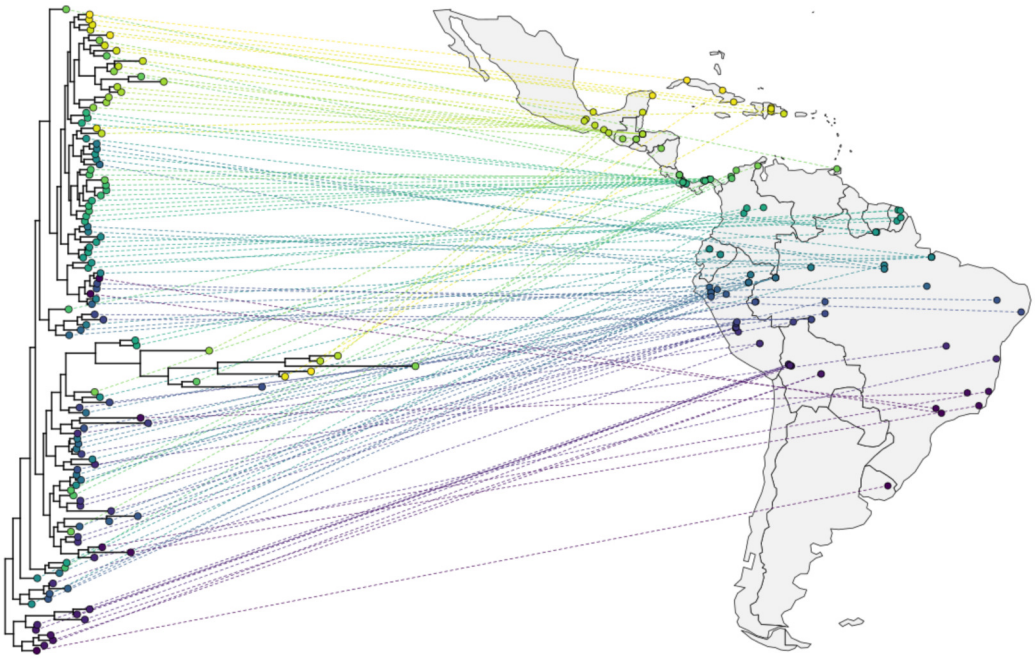


Figure 6: POY ATMD phylogeny with terminals mapped to their collection locality.

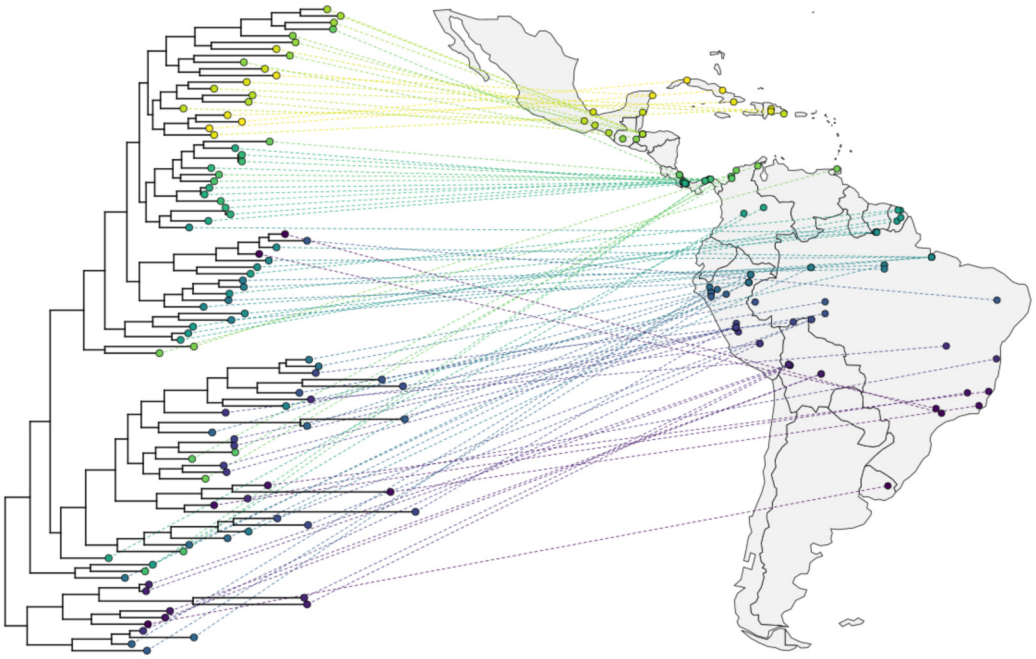


Figure 7: POY MTAD phylogeny with terminals mapped to their collection locality.

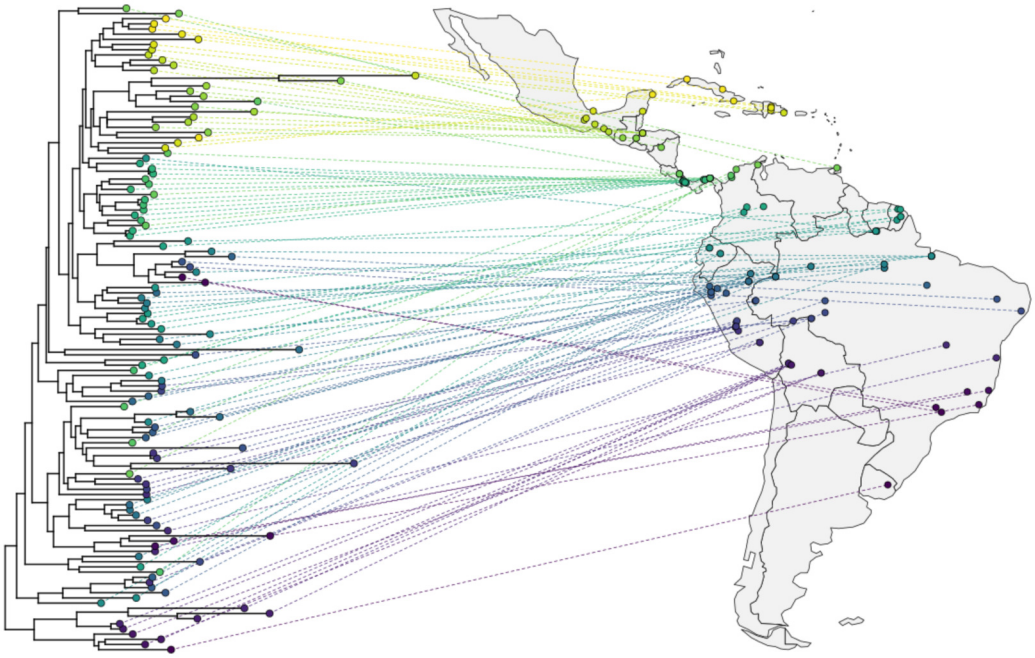


Figure 8: RAxML ATMD phylogeny with terminals mapped to their collection locality.

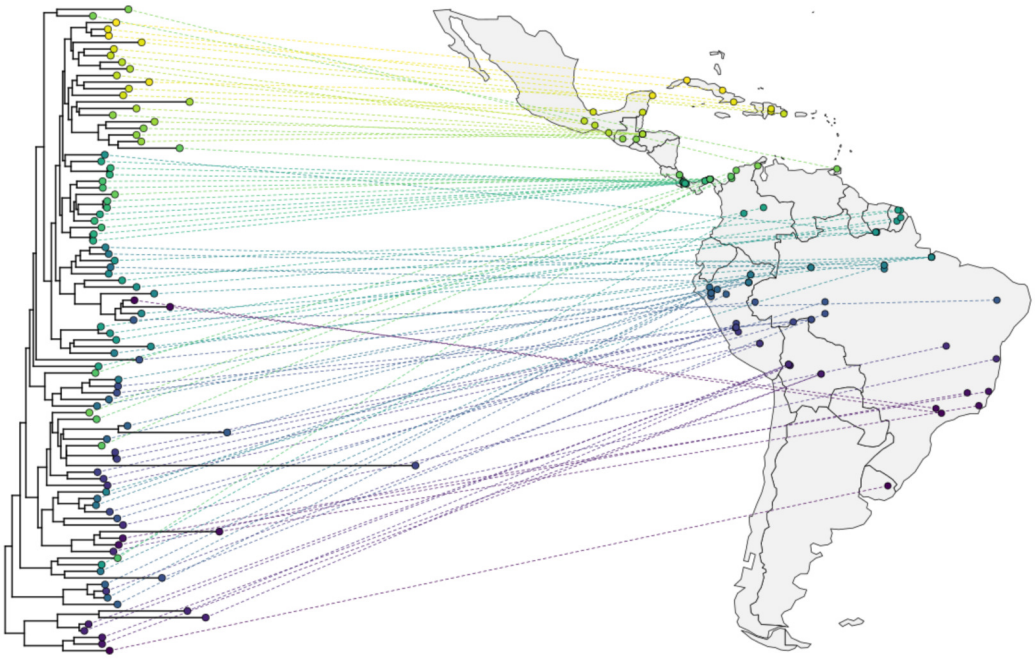


Figure 9: RAxML MTAD phylogeny with terminals mapped to their collection locality.

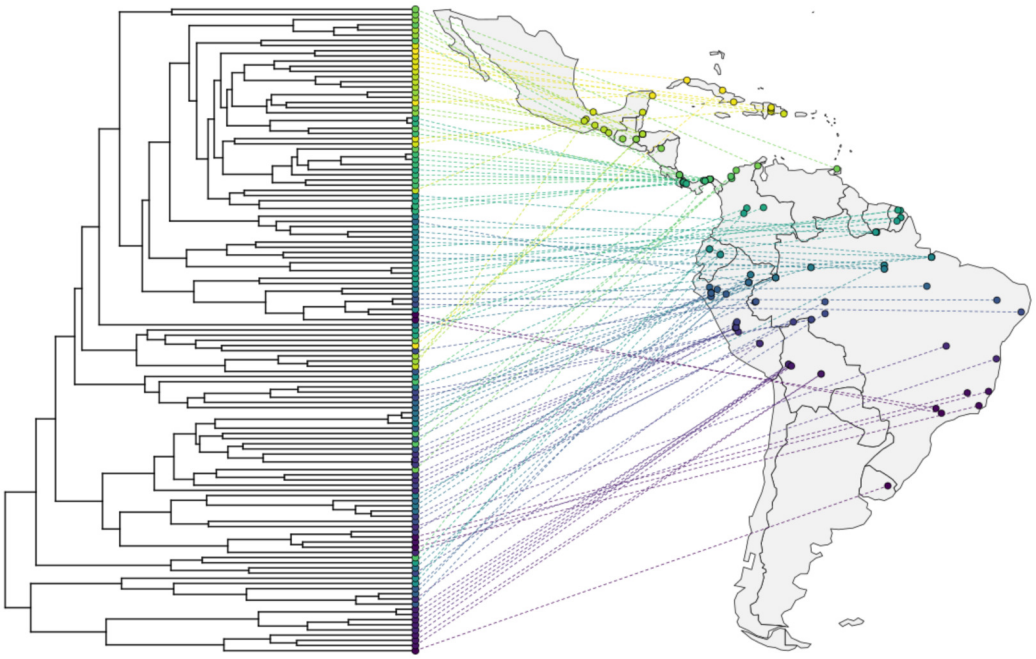


Figure 10: BEAST ATMD phylogeny with terminals mapped to their collection locality.

Table 4: Species in collapsed branches in Figures 11 and 12.

Clade in FIG 11 & 12	Clade number FIG 1-5	Species included
A	**1**	Metalibitia borelli 1618
		Metalibitia brasiliensis 749
		Metalibitia paraguayensis 759
		Metalibitia borelli 1618,
		Ferkeria vestita OP0742,
		Ferkeria flavicoxae OP0743,
		Platygndes titicaca 744,
		Ferkeria_0738
		Ferkeria_0739
B	**2**	Paecilemana Sp. OP452
		Cynorta albituber 599
		Eucynorta albimarginata 0949
		Flirtea erxlinea 598
		Rhaucus_0953
C	**N/A**	Cosmetidae sp. 1065
		Cynorta sp. 3898
		Cosmetidae sp (UFPI 2176)
		Cosmetidae sp (UFPI 2316)
D	**5**	Cynorta oxampa 593
		Platymessa transversalis 0945
		Gnidiella picta 0947
E	**7**	Eulibitia scalaris 3899
		Eulibitia sp. 3897
F	**10**	Gryne coccineloides 376
		Gryne dimorpha 0198
		Gryne marginalis 2768
		Gryne perlata 2836
		Roquettea carajas (UFPI 2167)
		Roquettea singularis 2755
		Paecilaema curvipes COS_H6
		Cosmetidae ms9 COS_X11A6
G	**12**	Meterginus basalis 3540
		Hond_sp_spiderman 3535
		Erginulus 2686

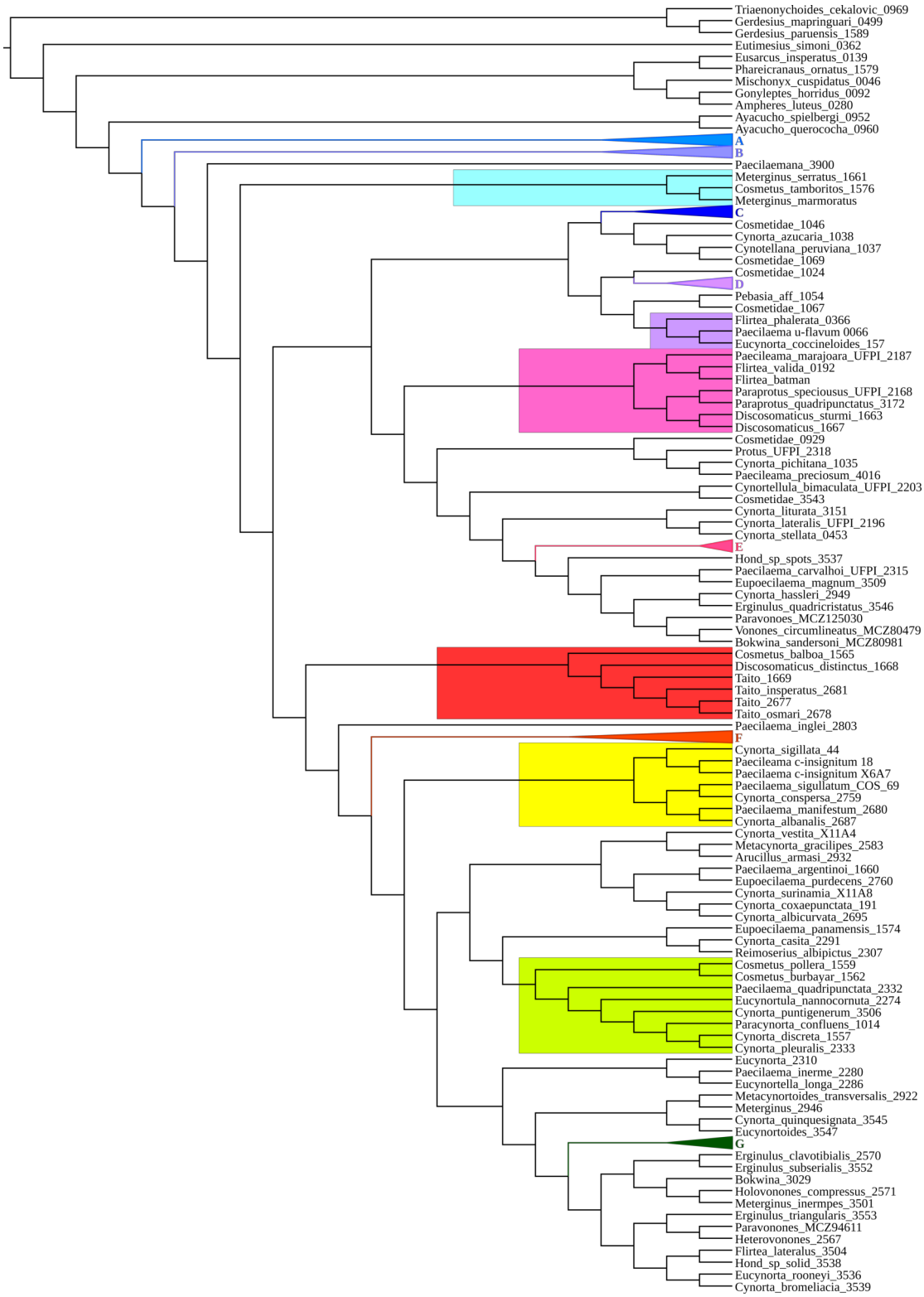


Figure 11: POY ATMD tree with clades shared with RAxML tree collapsed (species in Table 4), and similar clades highlighted.

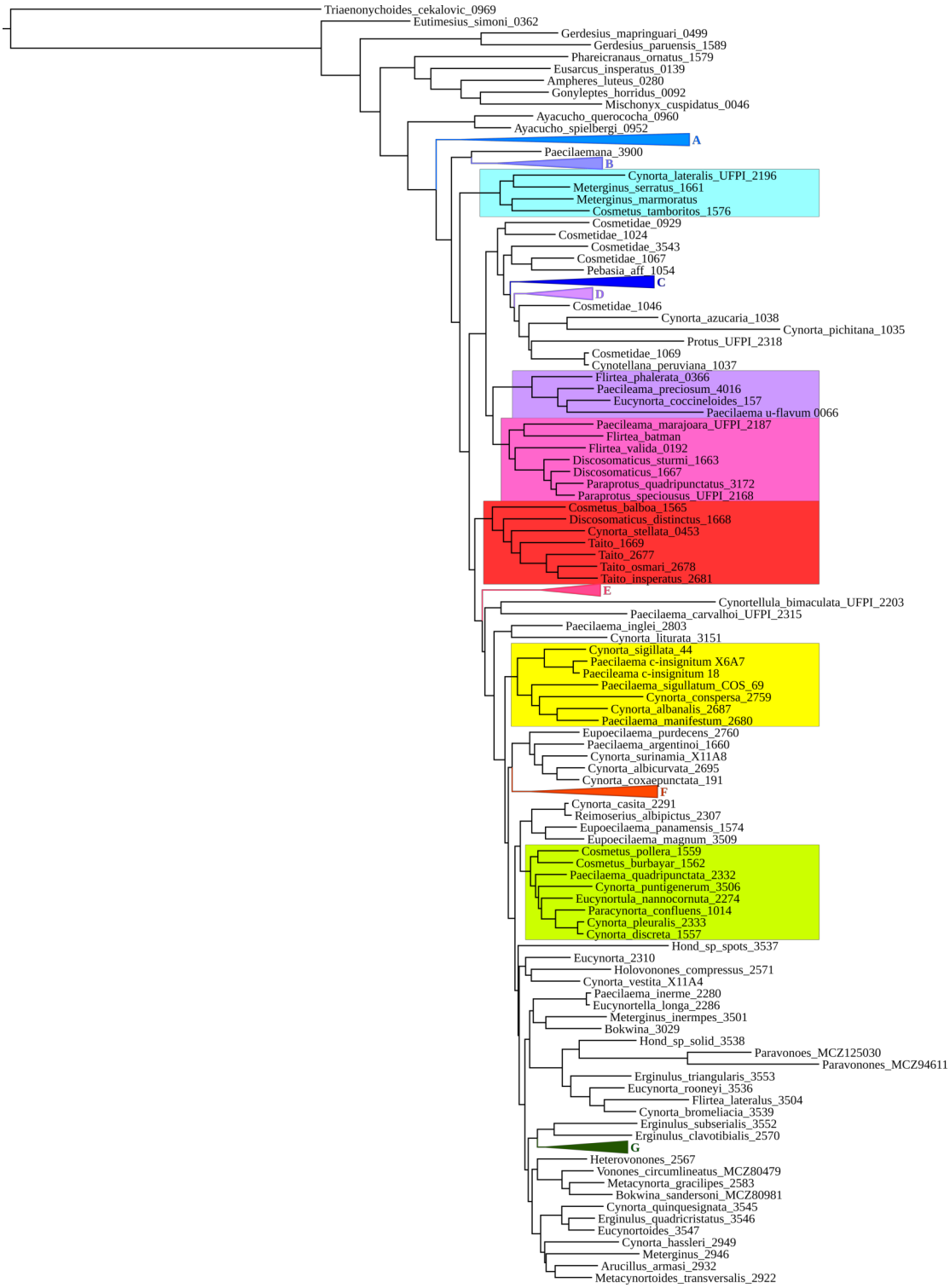


Figure 12: RAxML ATMD tree, with clades shared with POY tree collapsed (species in Table 4) and similar clades highlighted.

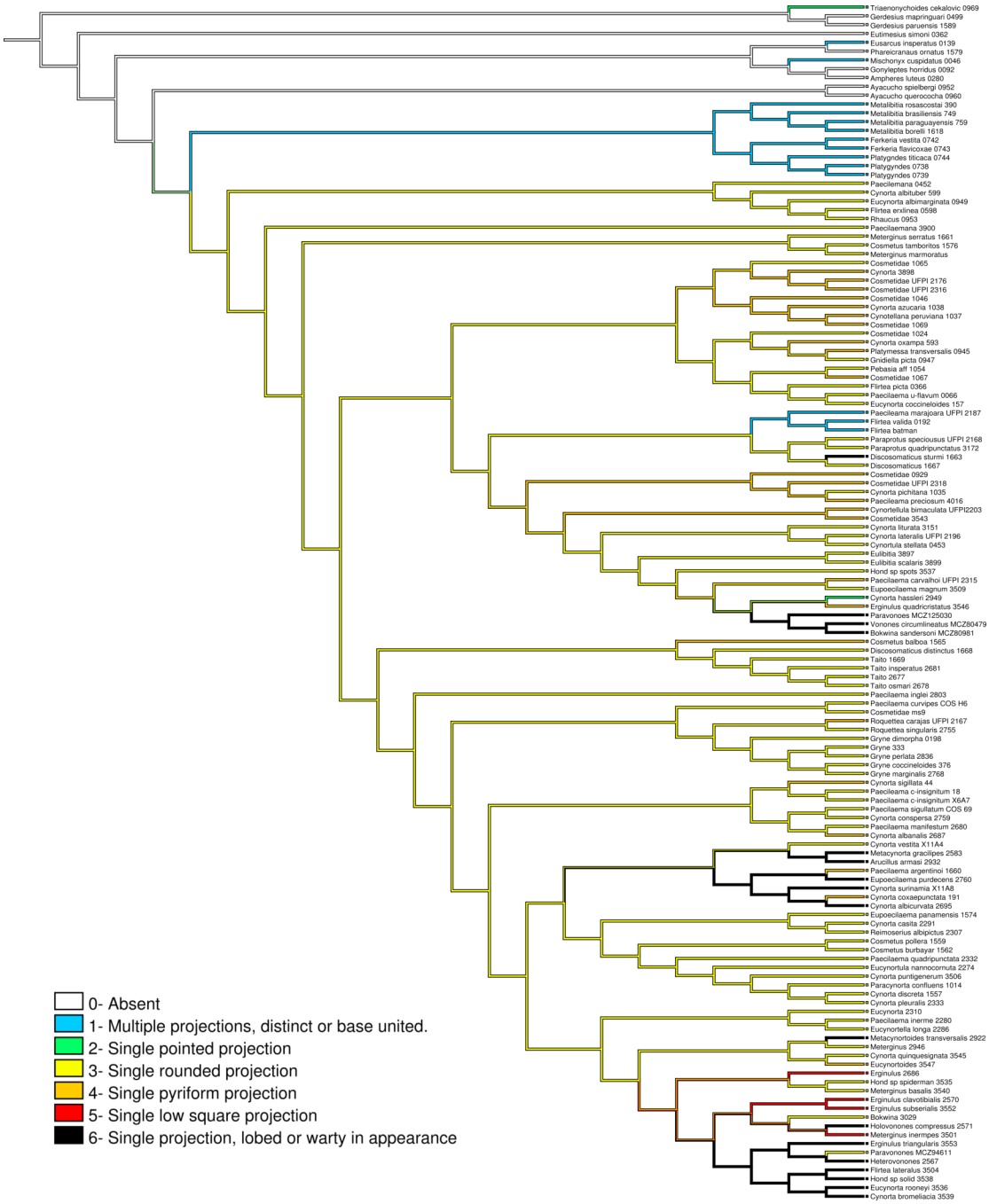


Figure 13: Character 4: DS. Prosoma. Anteriormargin. Shape of paracheliceral projections/tubercles: (CI= 0.171; RI= 0.540). Parsimony reconstruction(Unordered) [Steps:35]

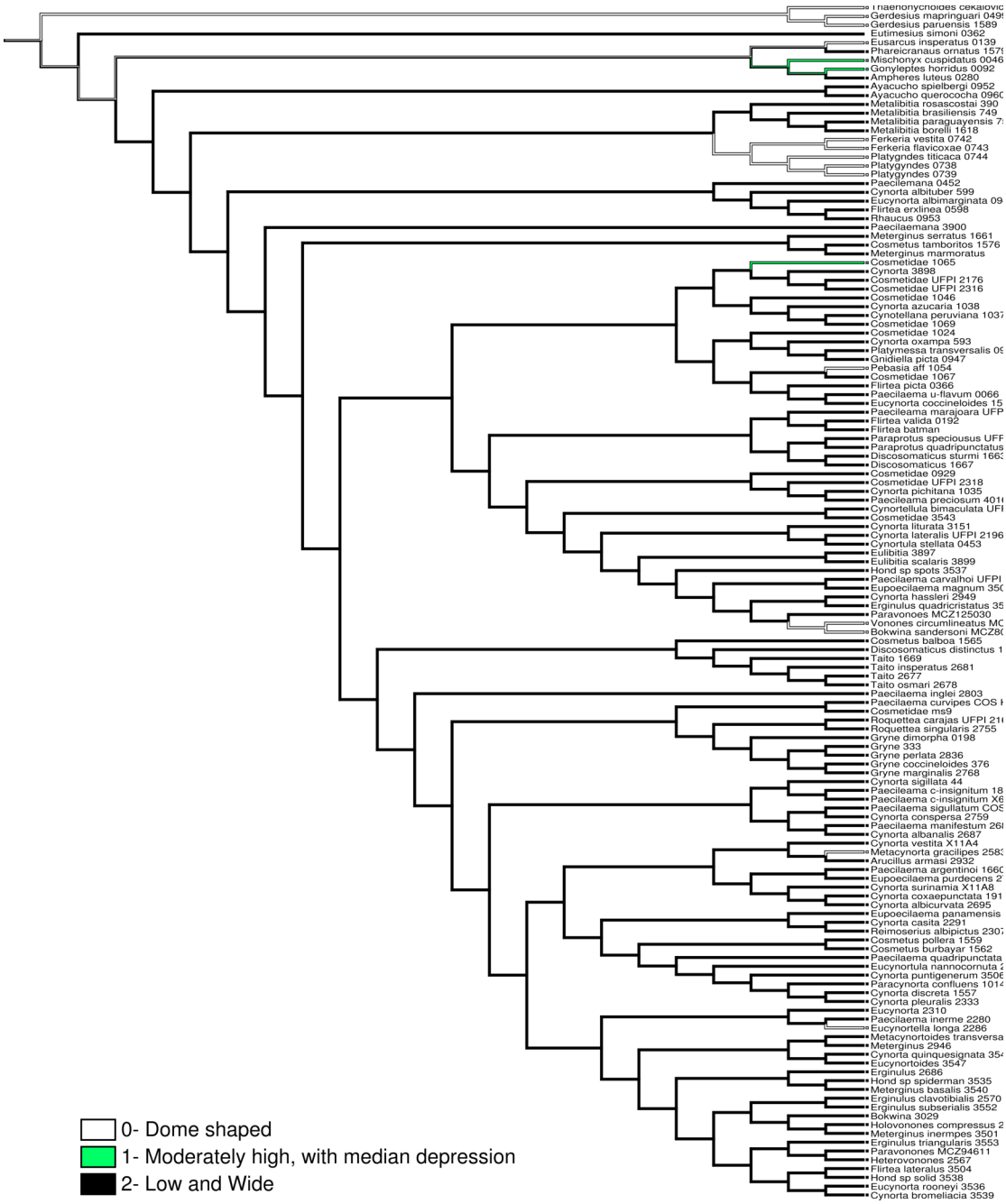


Figure 14: DS. Prosoma. Ocularium shape (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.20; RI= 0.466) Parsimony reconstruction (Unordered) [Steps: 10]

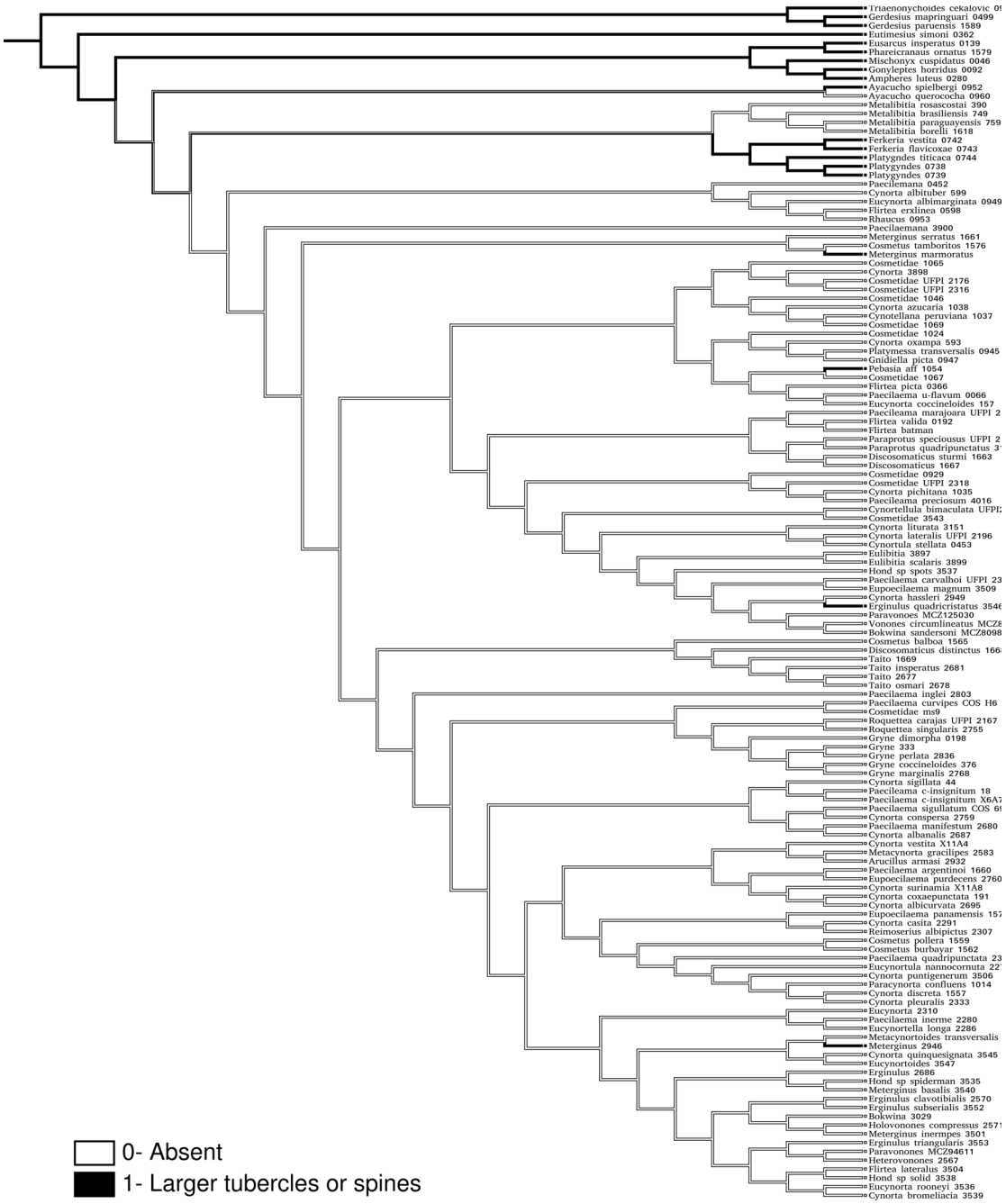


Figure 15: Character 7. DS. Prosoma. Ocularium armature (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.143; RI= 0.667): Parsimony reconstruction (Unordered) [Steps: 7]

Figure 16: Character 9. DS. Opithosoma. Coloration on cuticle (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.281; RI= 0.461): Parsimony reconstruction (Unordered) [Steps: 57]

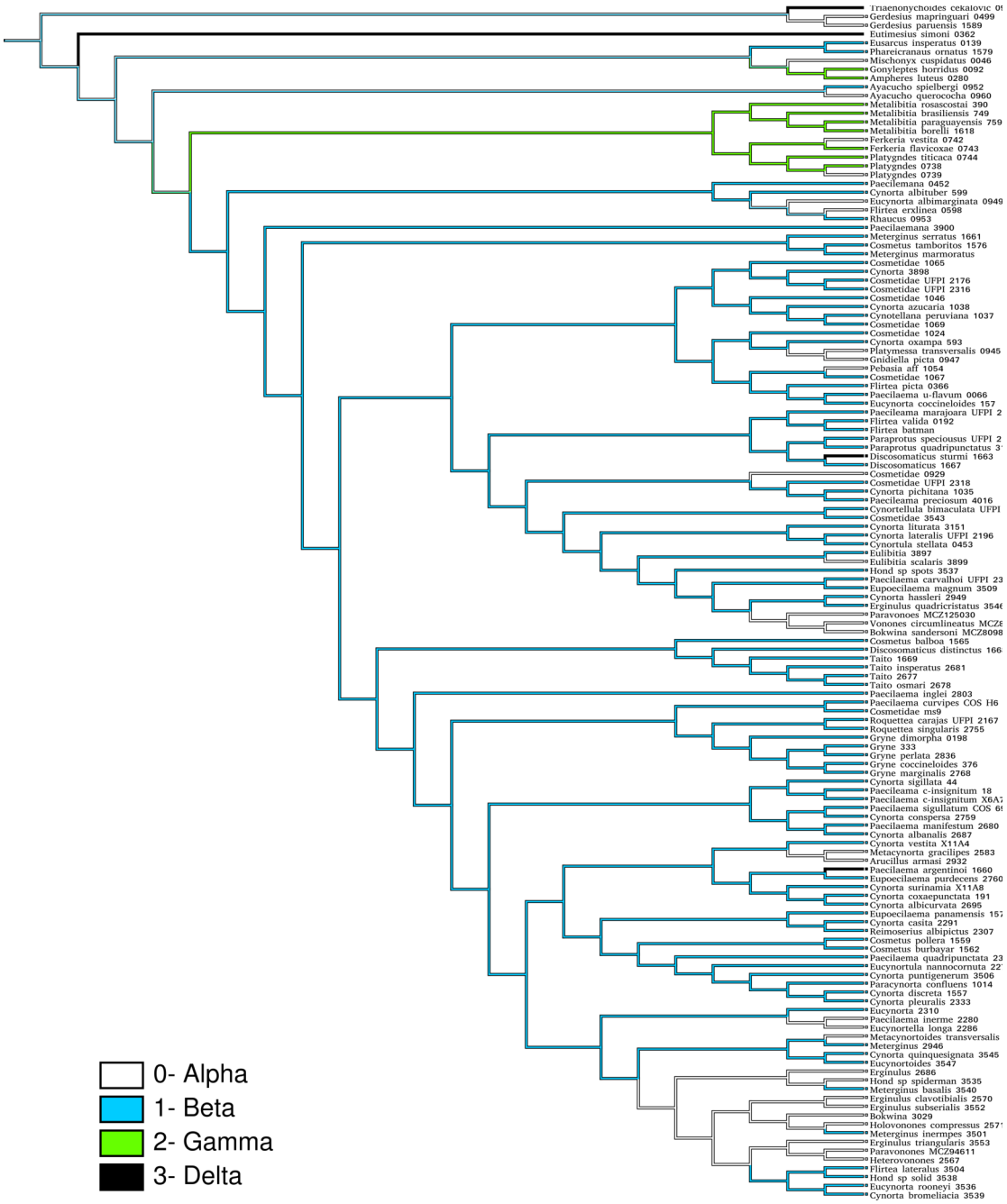


Figure 17: Character 10. DS. Outline (Kury et. al., 2007: fig 12) (CI= 0.130; RI= 0.474): Parsimony reconstruction (Unordered) [Steps: 23]

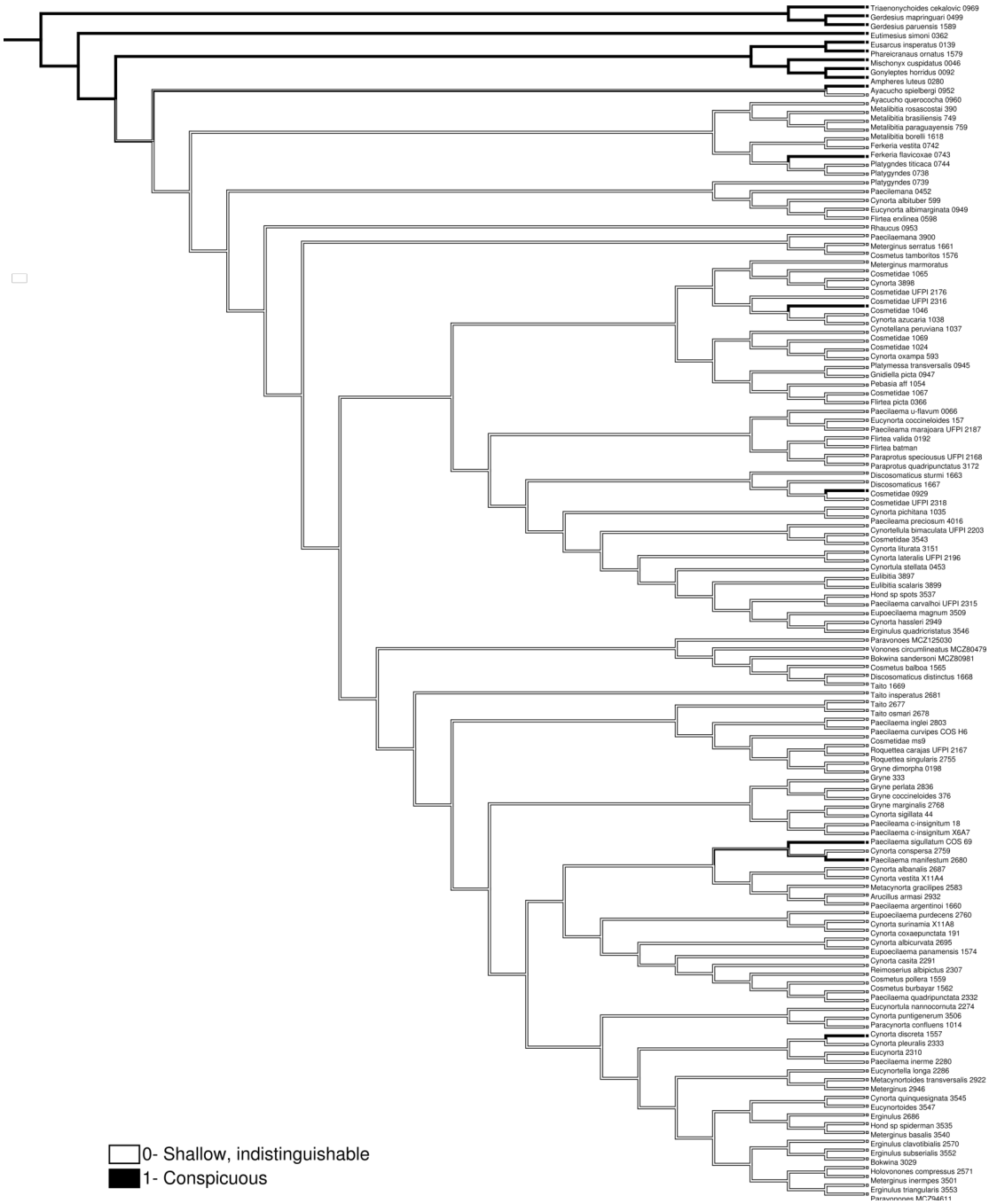


Figure 18: Character 11. DS. Opisthosoma. Sulci separating areas (from Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.125; RI= 0.533): Parsimony reconstruction (Unordered) [Steps: 8]

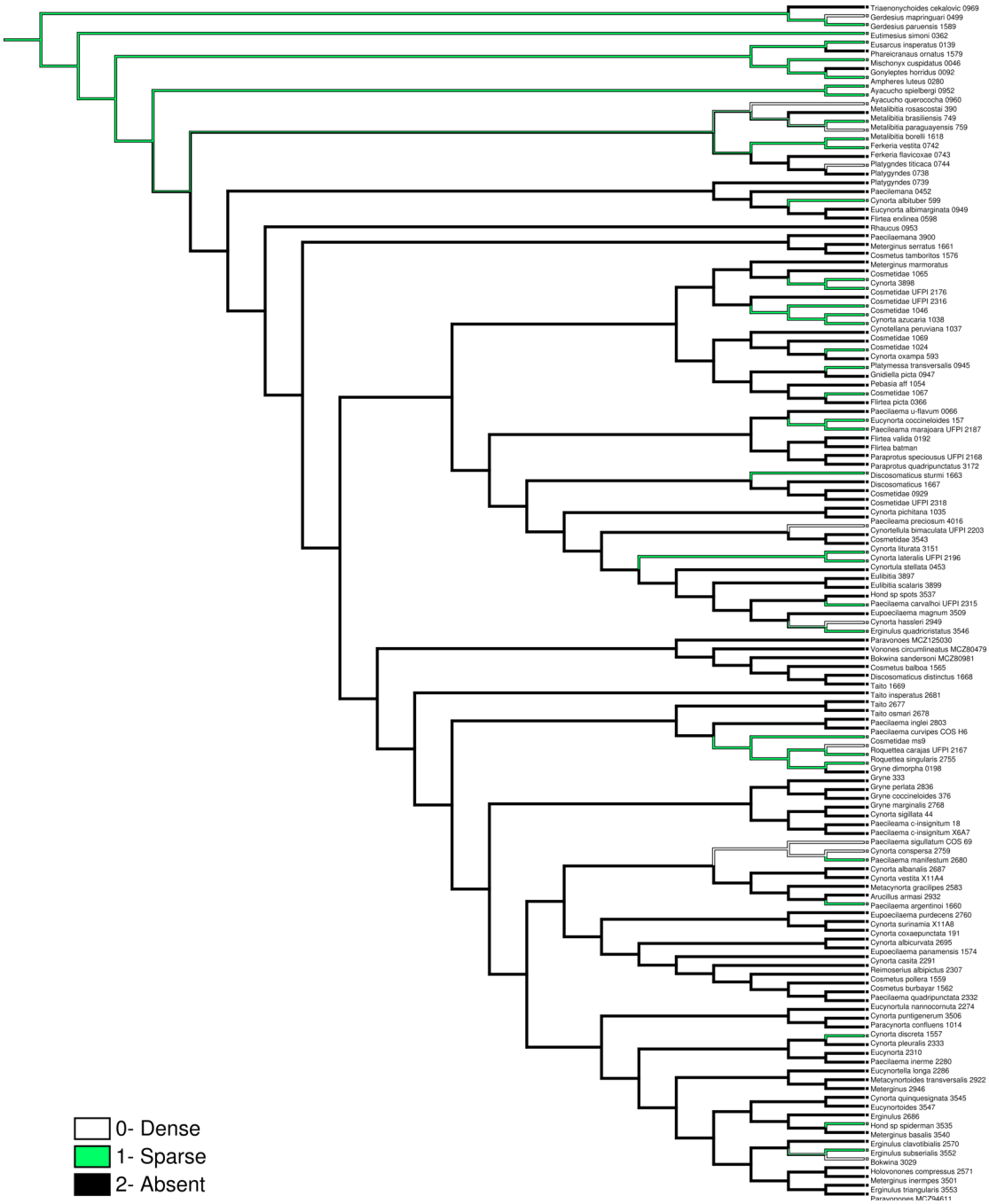


Figure 19: Character 12. DS. Granulation (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.061; RI= 0.262): Parsimony reconstruction (Unordered) [Steps: 33]

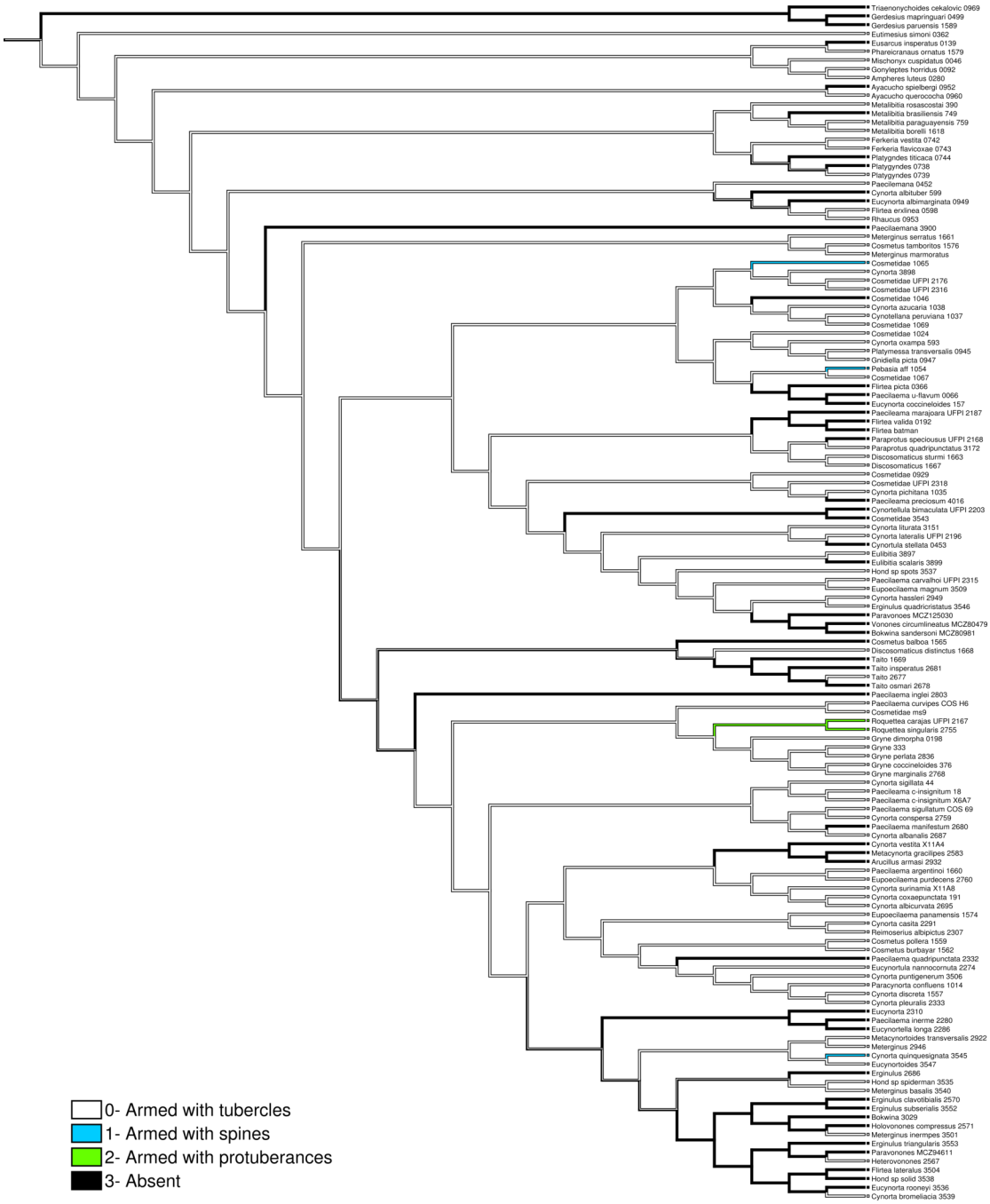


Figure 20: Character 13. DS. Opisthosoma.Area I, paramedian armature (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.086; RI= 0.385):Parsimony reconstruction (Unordered) [Steps: 35]

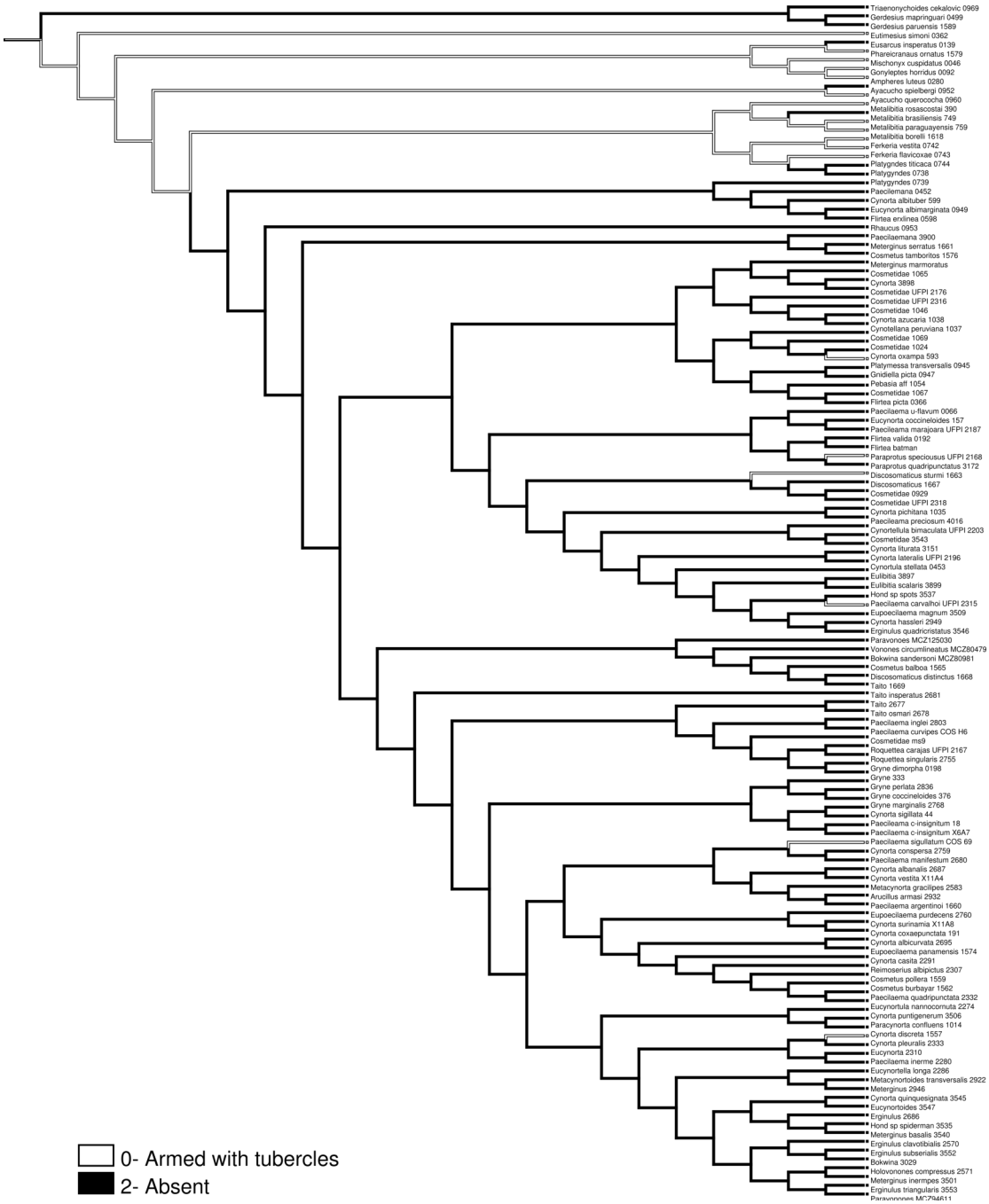


Figure 21: Character 14. DS. Opisthosoma. Area II,paramedian armature (modified fromCoronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.083; RI= 0.353):Parsimony reconstruction(Unordered) [Steps: 12

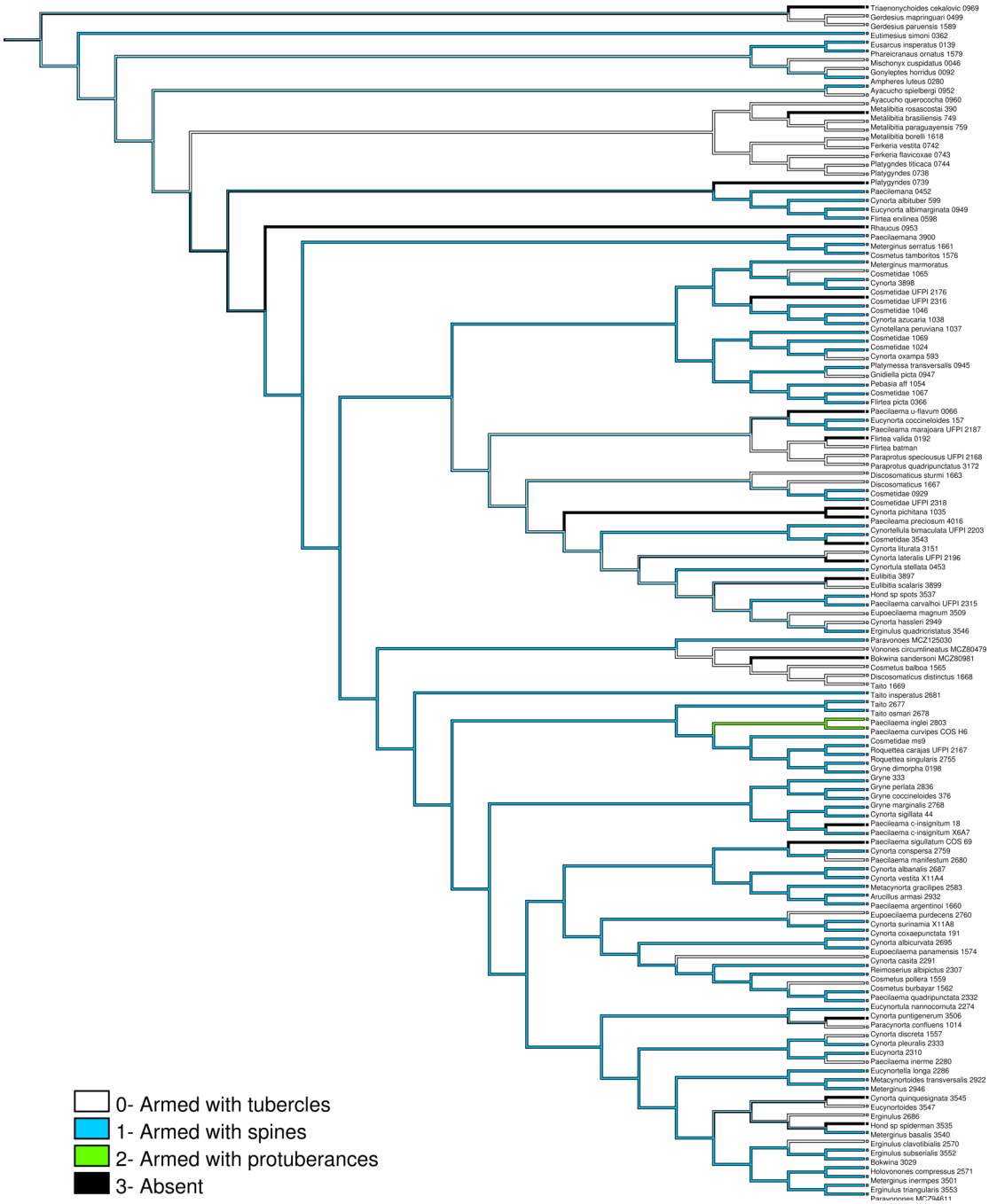


Figure 22: Character 15. DS. Opisthosoma. Area III, armature type (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.068; RI= 0.268): Parsimony reconstruction (Unordered) [Steps: 44]

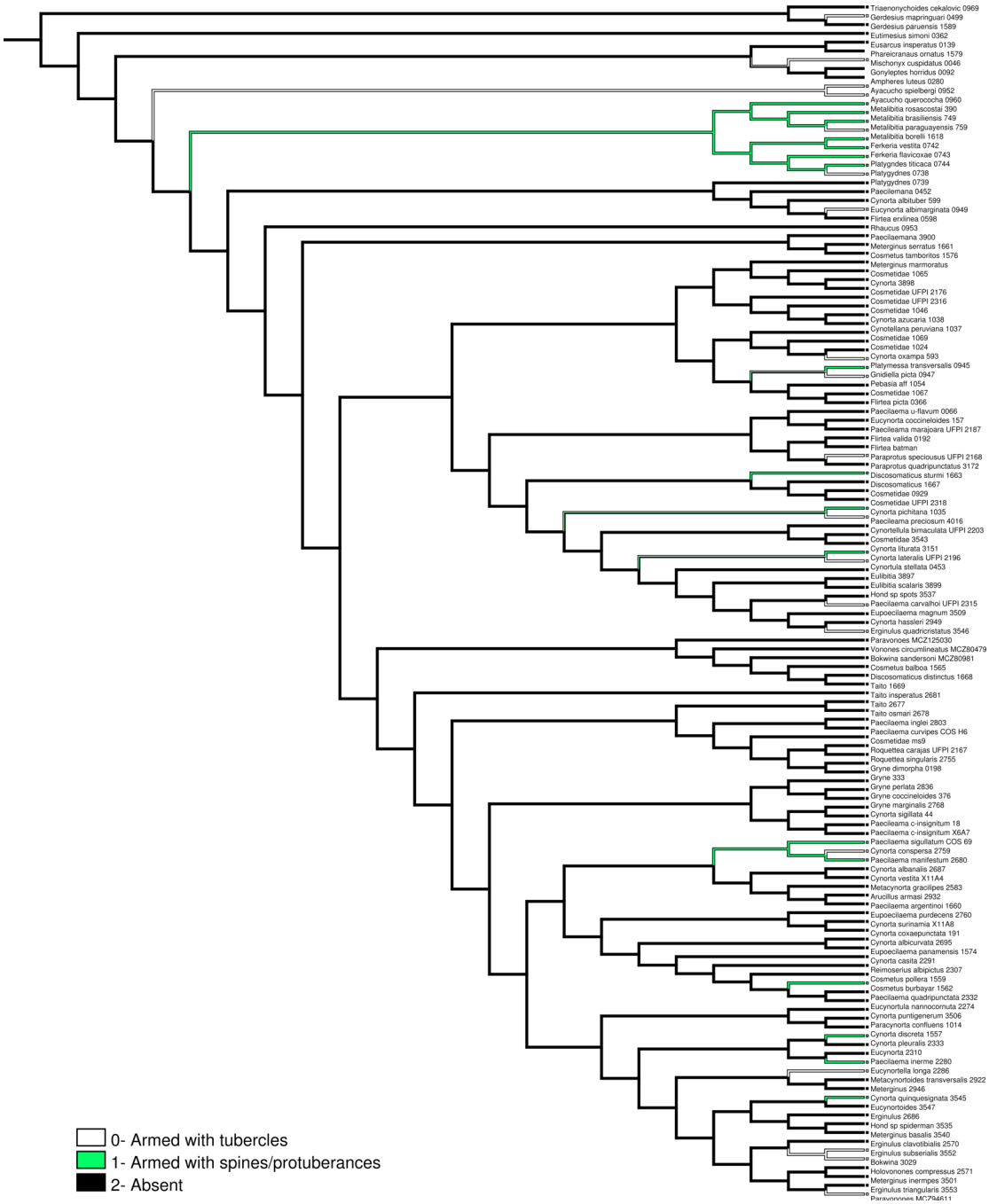


Figure 23: Character 16. DS. Opisthosoma. Area IV/posterior margin, armature type (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.074; RI= 0.265): Parsimony reconstruction (Unordered) [Steps: 27]

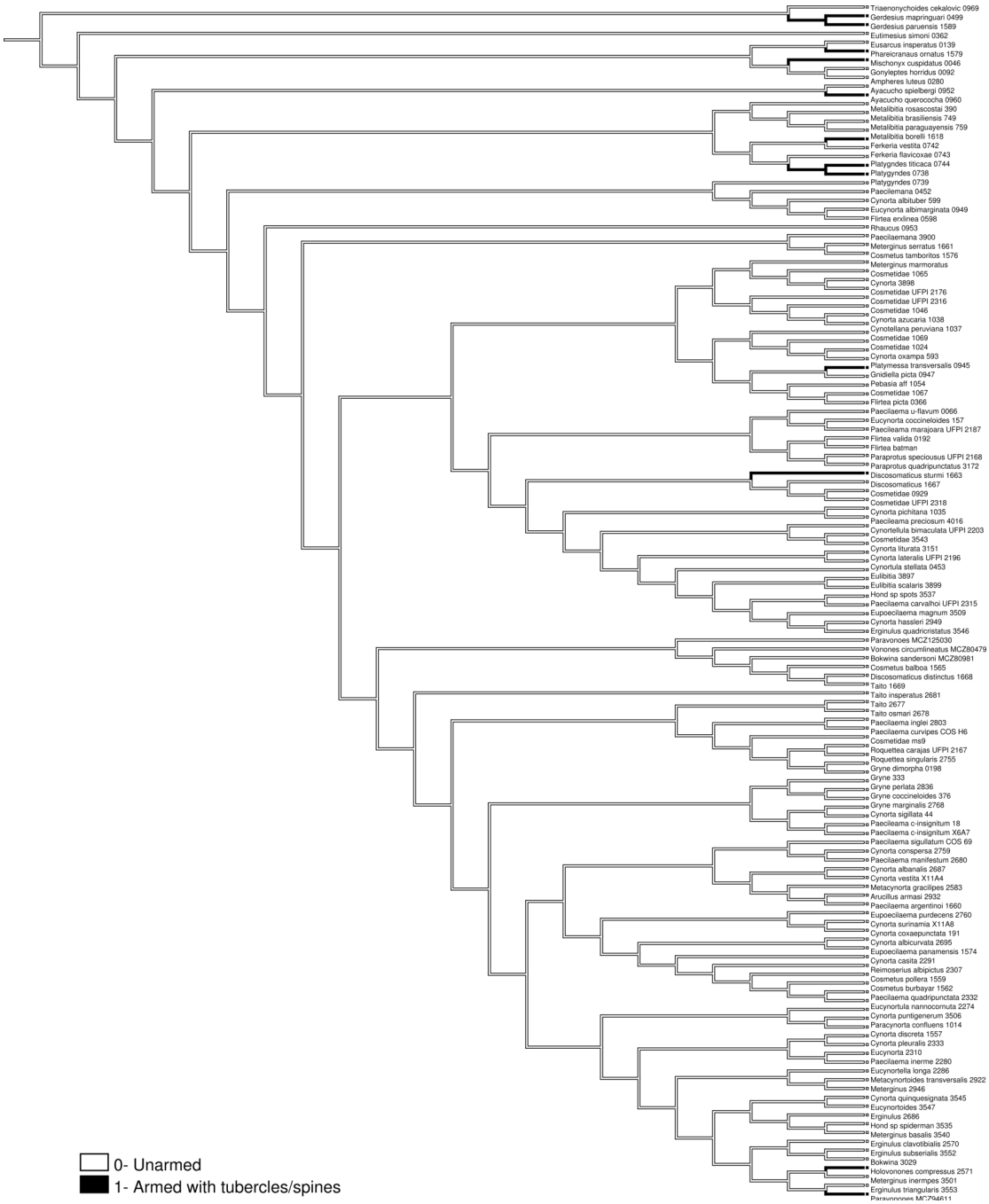


Figure 24: Character 18. DS. Opithosoma. Free tergite I armature (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.10; RI= 0.182): Parsimony reconstruction (Unordered) [Steps: 10]

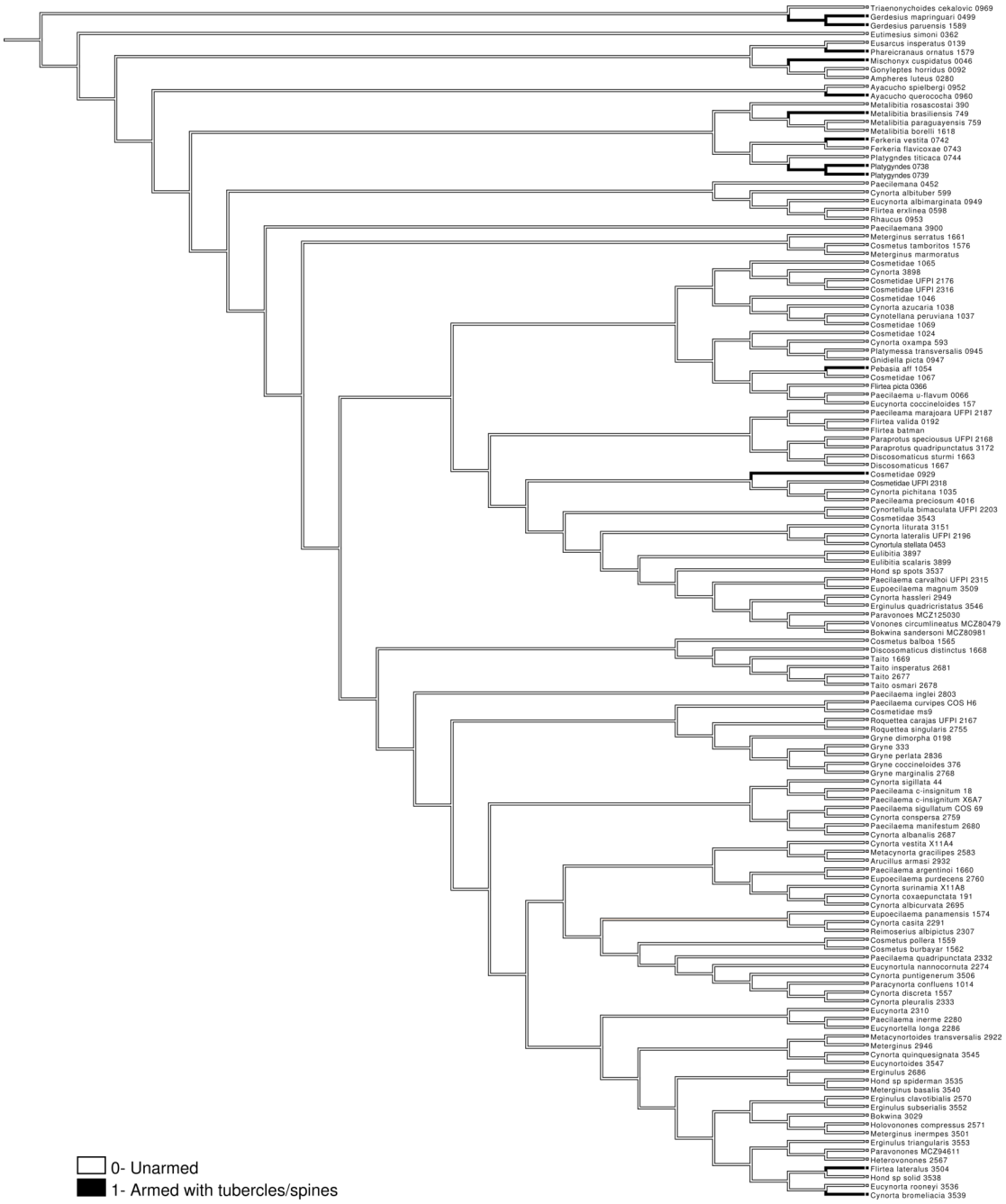


Figure 25: Character 19. DS. Opisthosoma. Free tergite II armature (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.091; RI= 0.167): Parsimony reconstruction (Unordered) [Steps: 11]

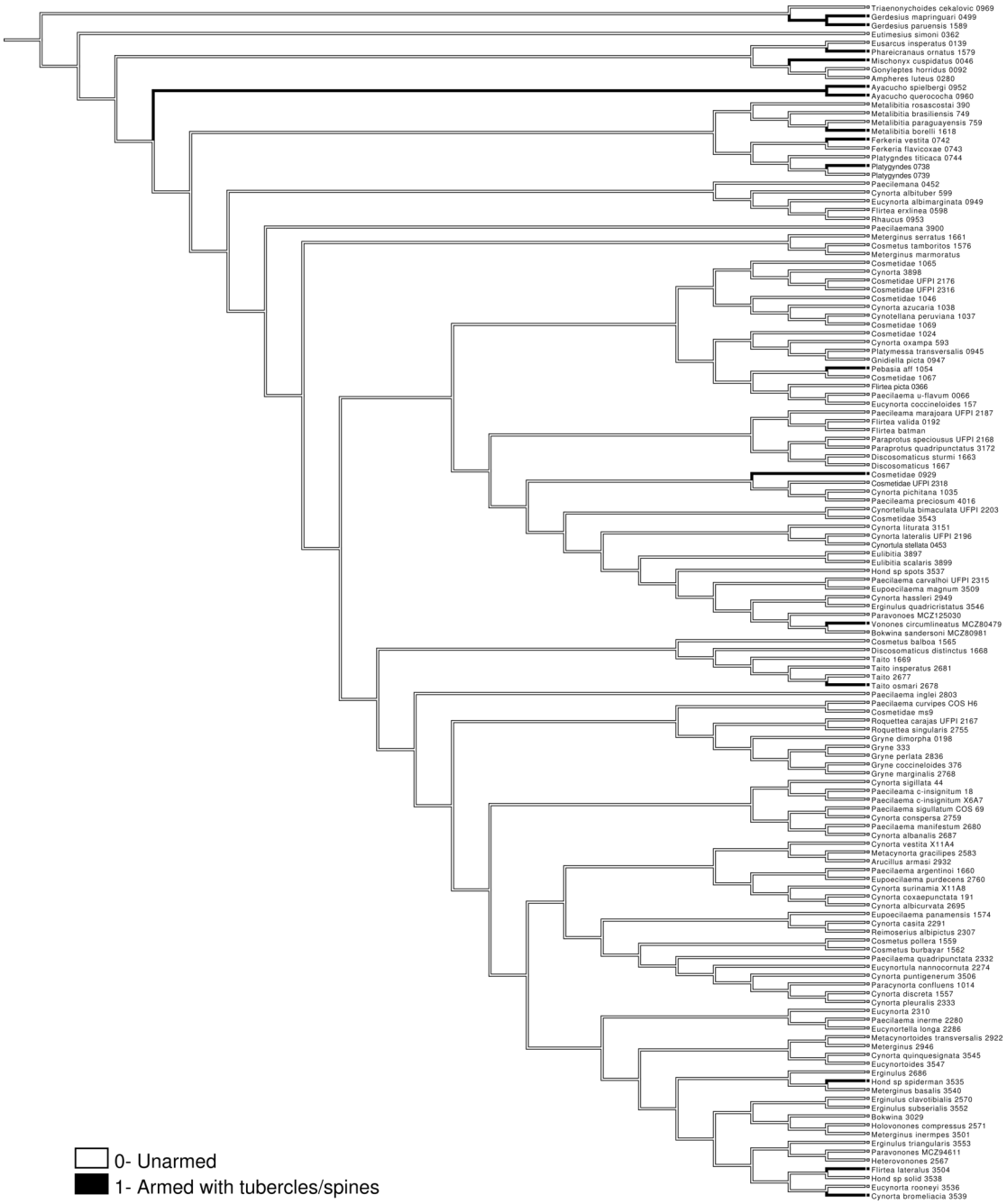


Figure 26: Character 20. DS. Opisthosoma. Free tergite III armature (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.071; RI= 0.133): Parsimony reconstruction (Unordered) [Steps: 14]

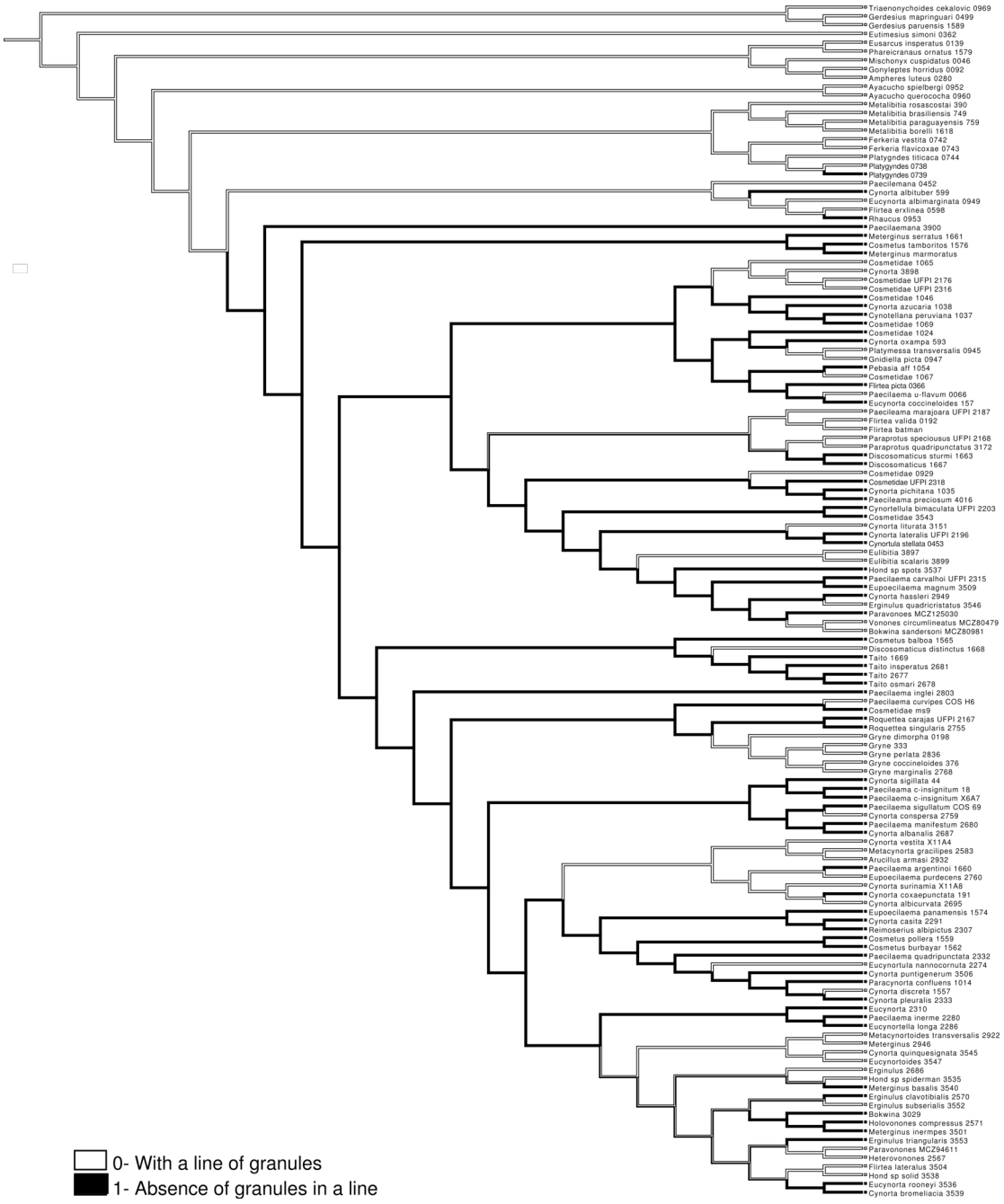


Figure 27: Character 22. DS. Opithosoma. Posterior margin (CI= 0.033; RI= 0.561): Parsimony reconstruction (Unordered) [Steps: 30]

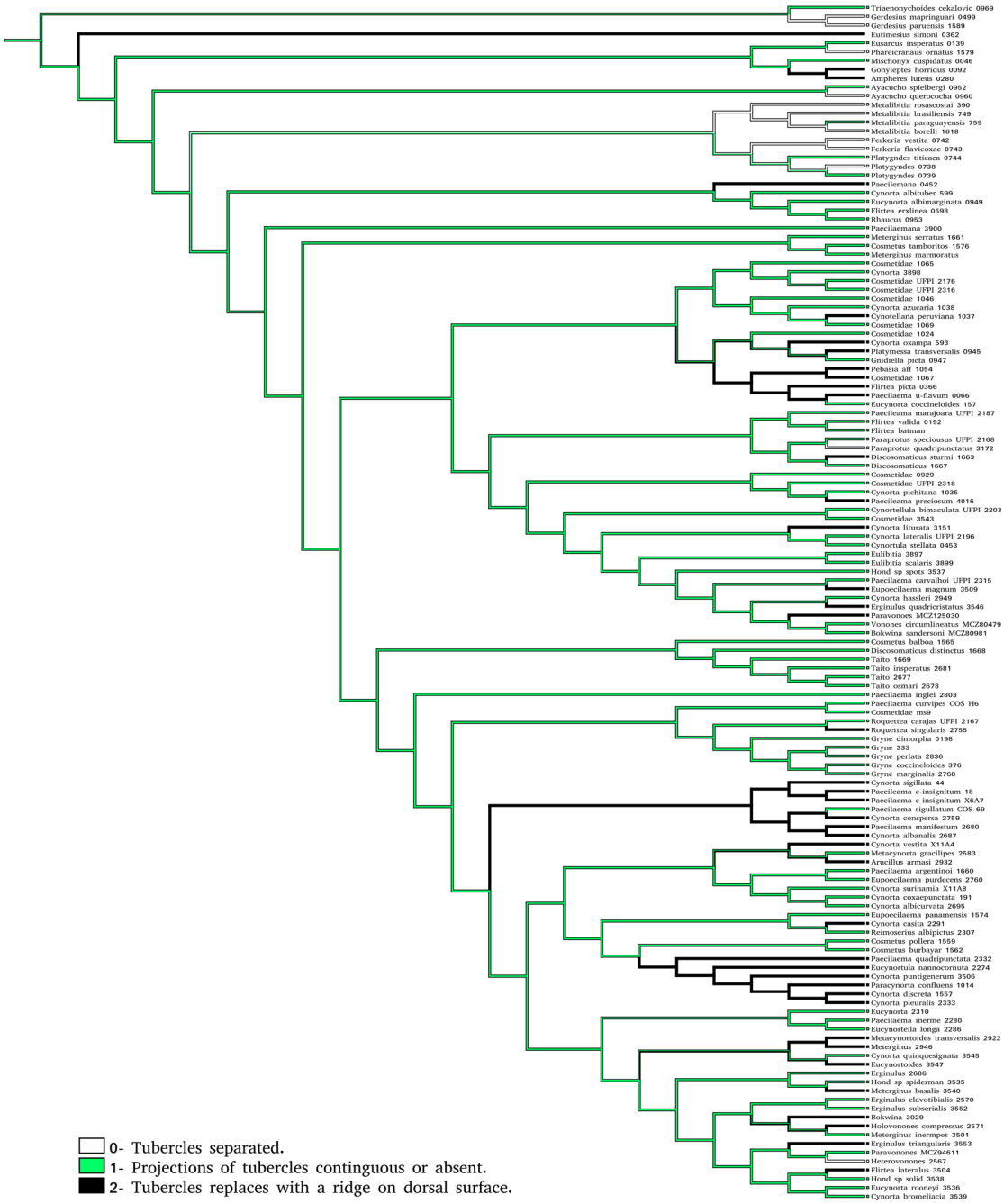


Figure 28: Character 25. Pedipalp. Femur. Arrangement of dorsal tubercles (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.057; RI= 0.313): Parsimony reconstruction (Unordered) [Steps: 35]

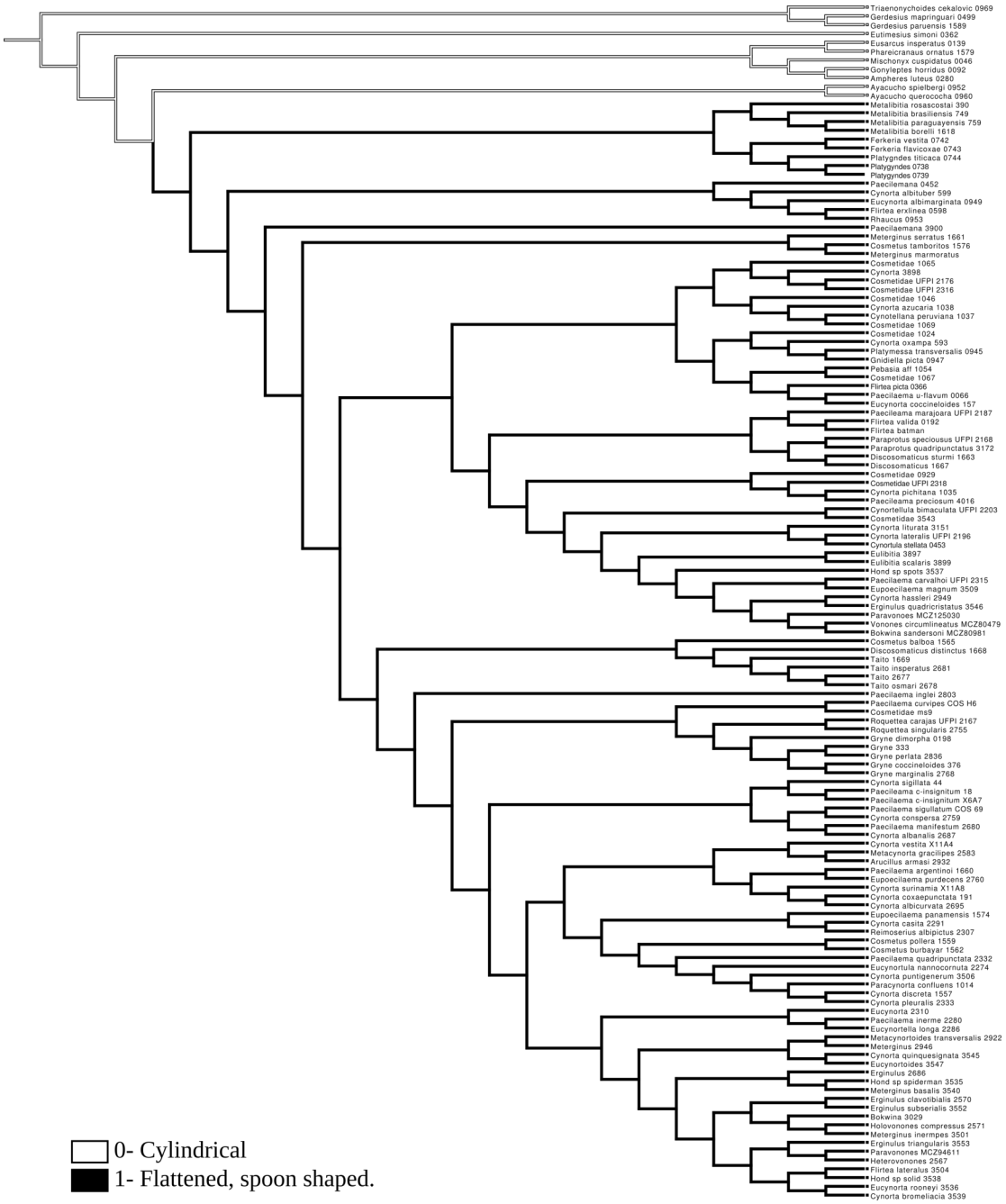


Figure 29: Character 26. Pedipalp. Tibia. Dorsal-ventral shape (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 1.00; RI= 1.00): Parsimony reconstruction (Unorderd) [Steps: 1]

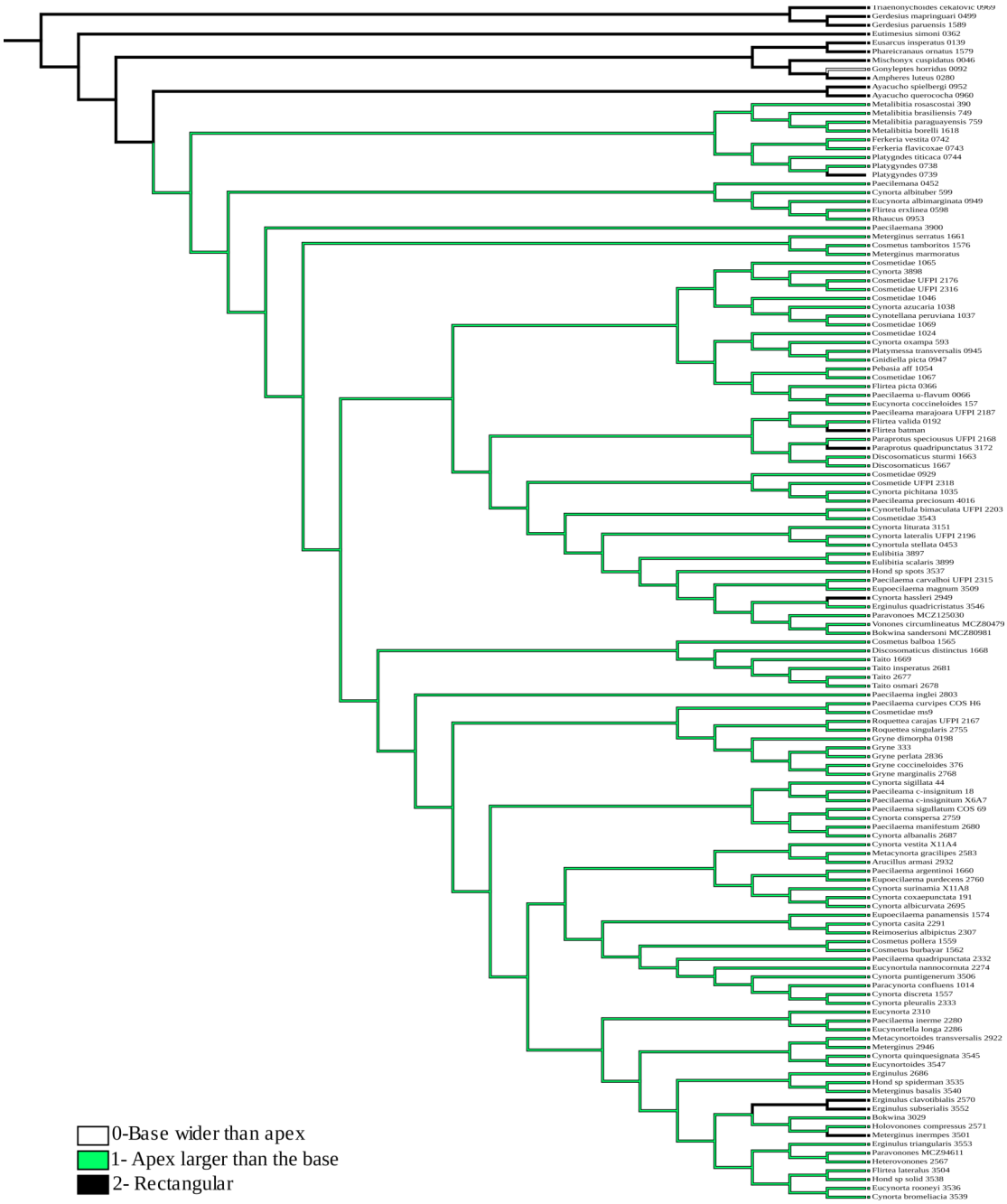


Figure 30: Pedipalp. Tibia. Shape in lateral view (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017): Parsimony reconstruction (Unordered) [Steps: 7]

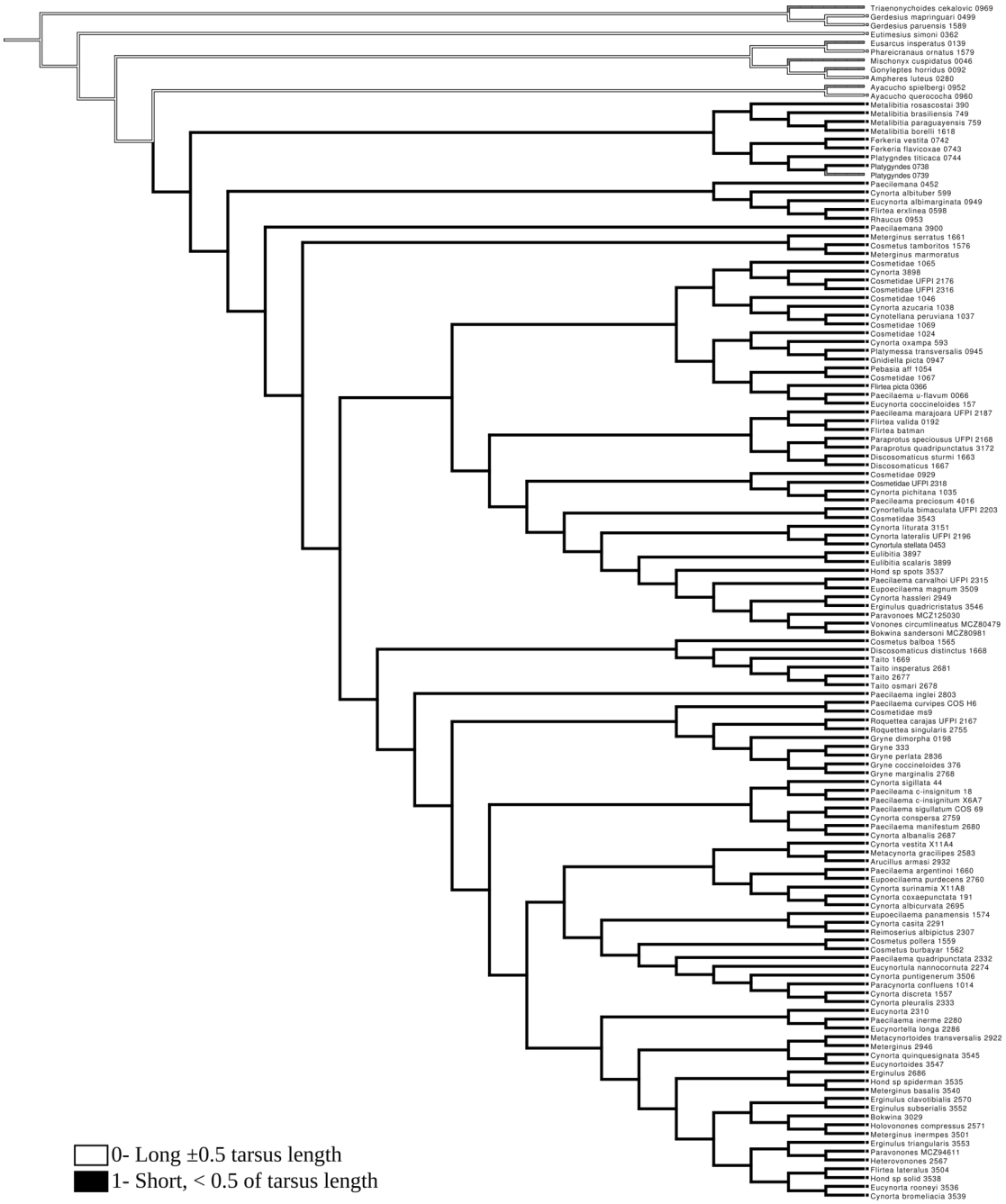


Figure 31: Character 29. Pedipalp. Tarsus. Size of setae (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 1.00; RI= 1.00): Parsimony reconstruction (Unordered) [Steps: 1]

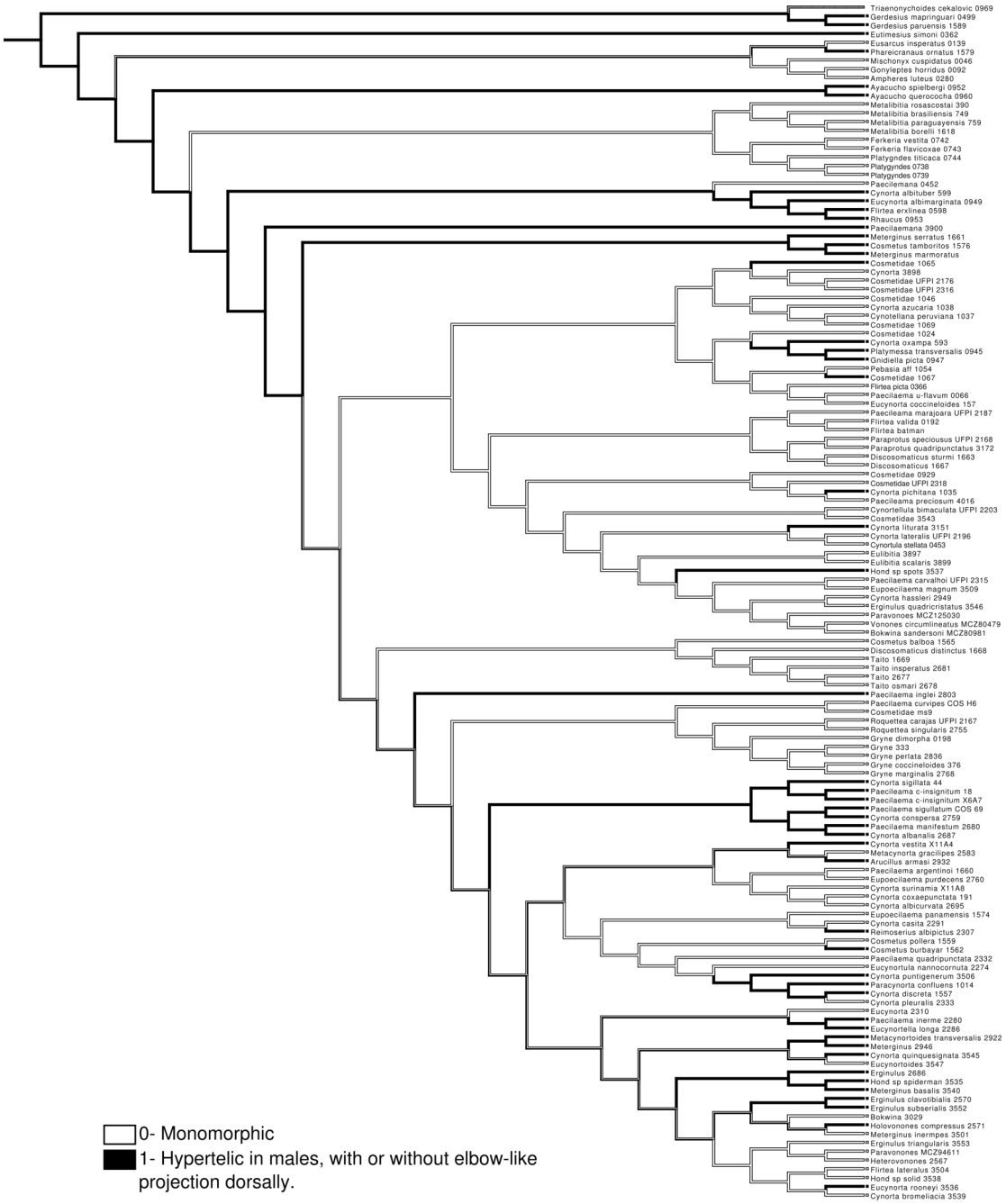


Figure 32: Character 30. Cheliceral hand (Segment II) shape (CI= 0.037; RI= 0.447): Parsimony reconstruction (Unordered) [Steps: 26]

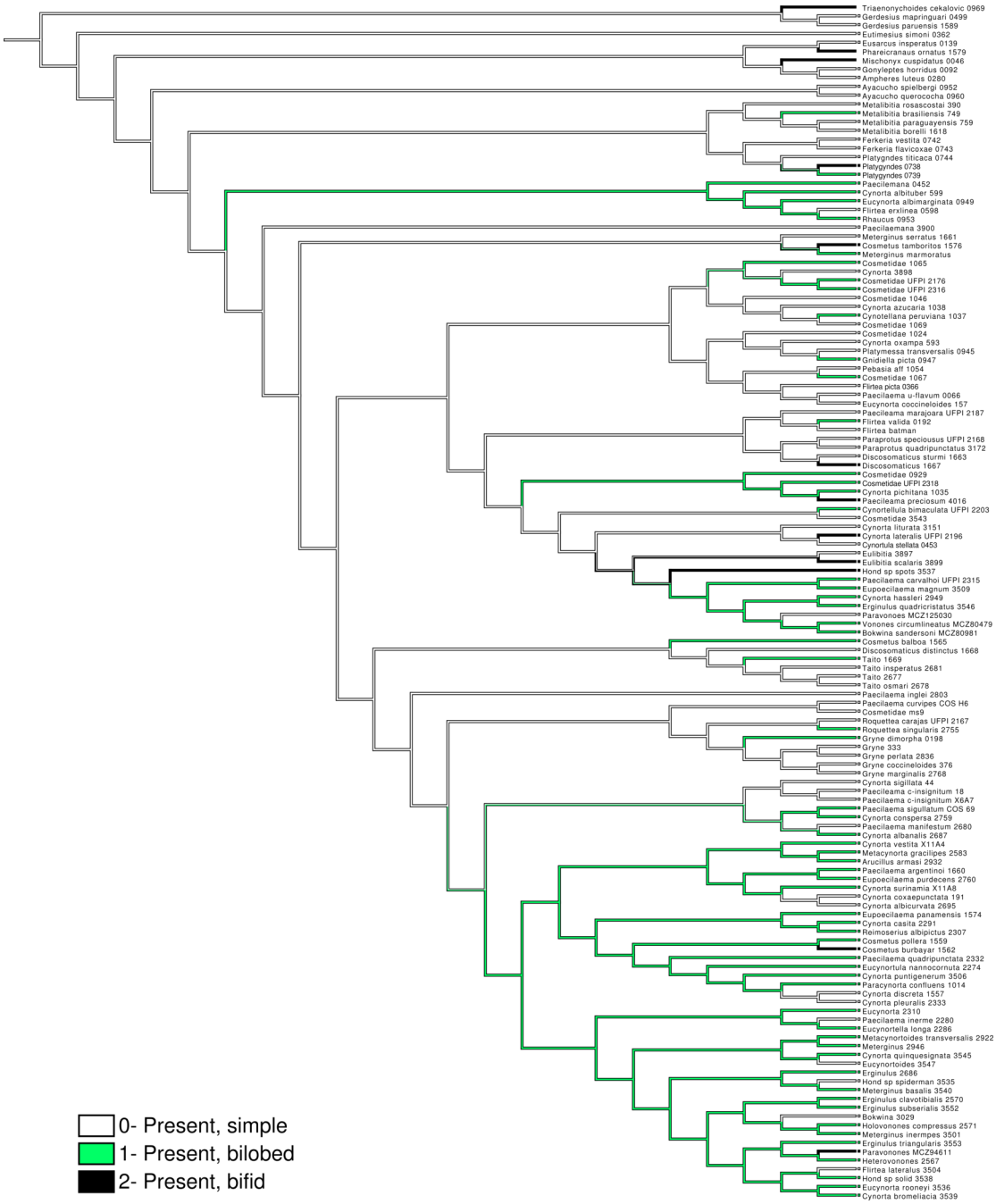


Figure 33: Character 35. Legs. Coxa II. Prolateral dorsal apophysis (modified from Ferreira thesis, 2006, and Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.053; RI= 0.471): Parsimony reconstruction (Unordered) [Steps: 38]

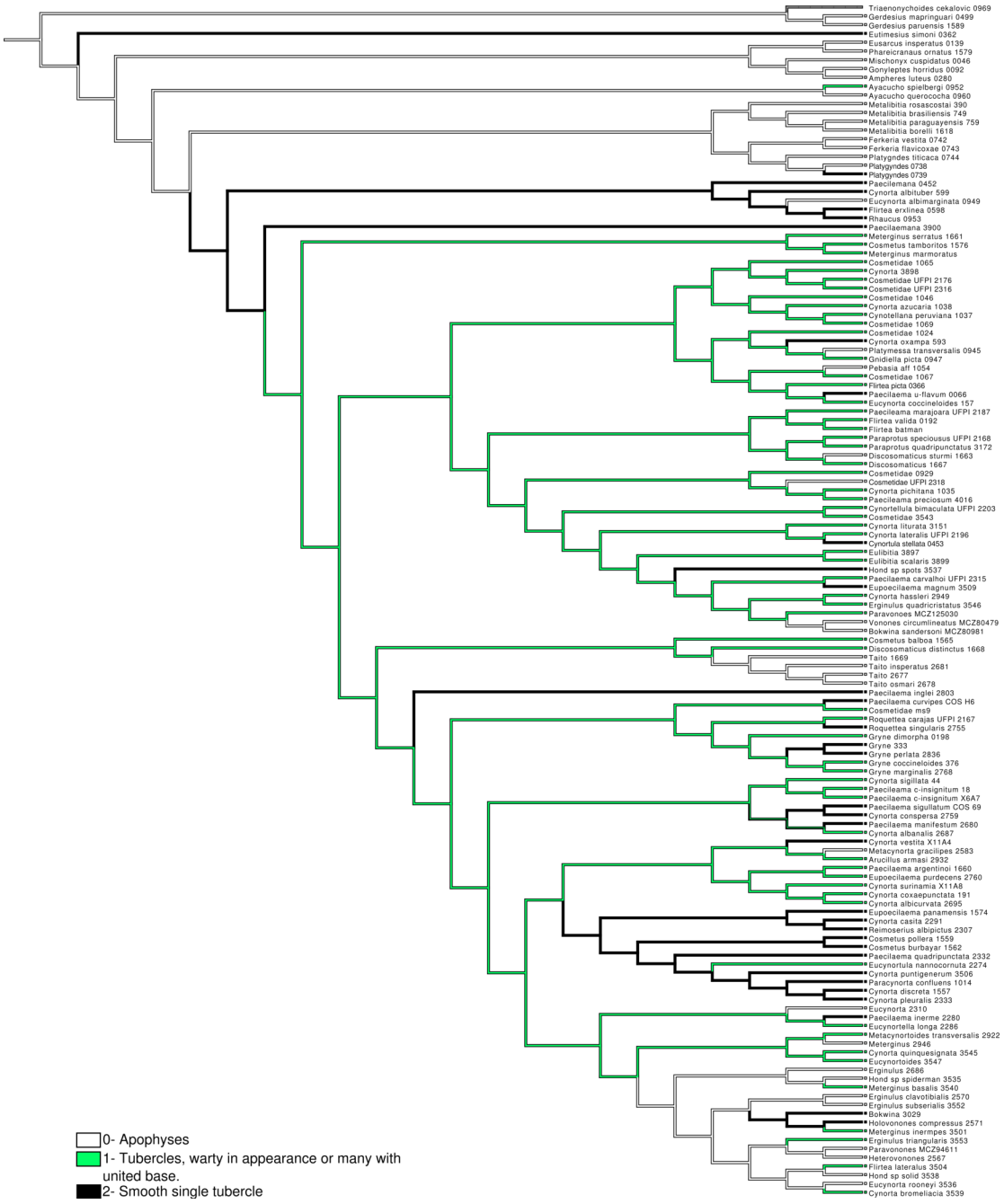


Figure 34: Character 40. Legs. Coxa IV. Apical Prolateral armature (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.054; RI= 0.500):Parsimony reconstruction (Unordered) [Steps: 37]

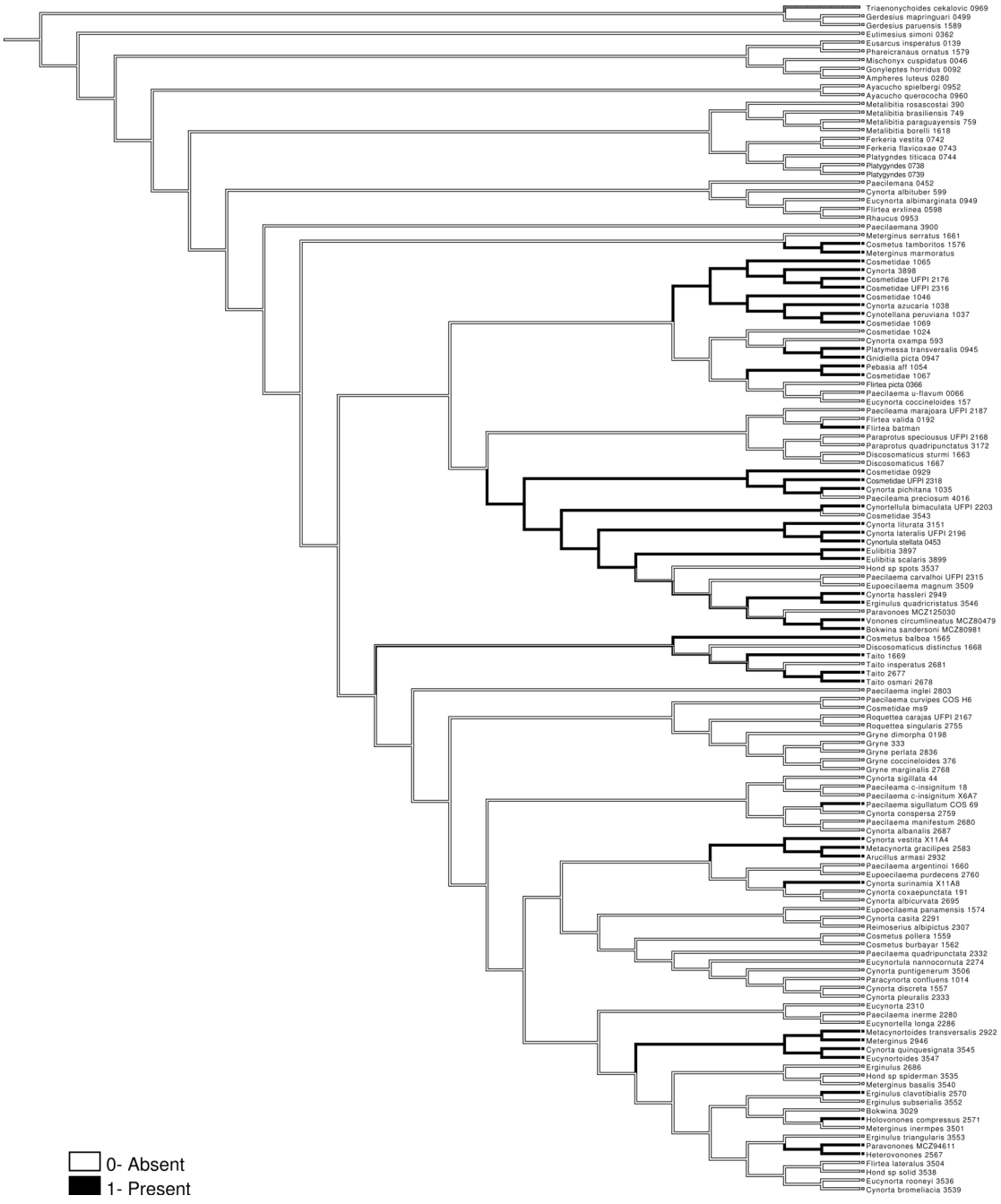


Figure 35: Character 42. Legs. Coxae IV. Clavi Ingiunes (CI= 0.048; RI= 0.545): Parsimony reconstruction (Unordered) [Steps: 21]

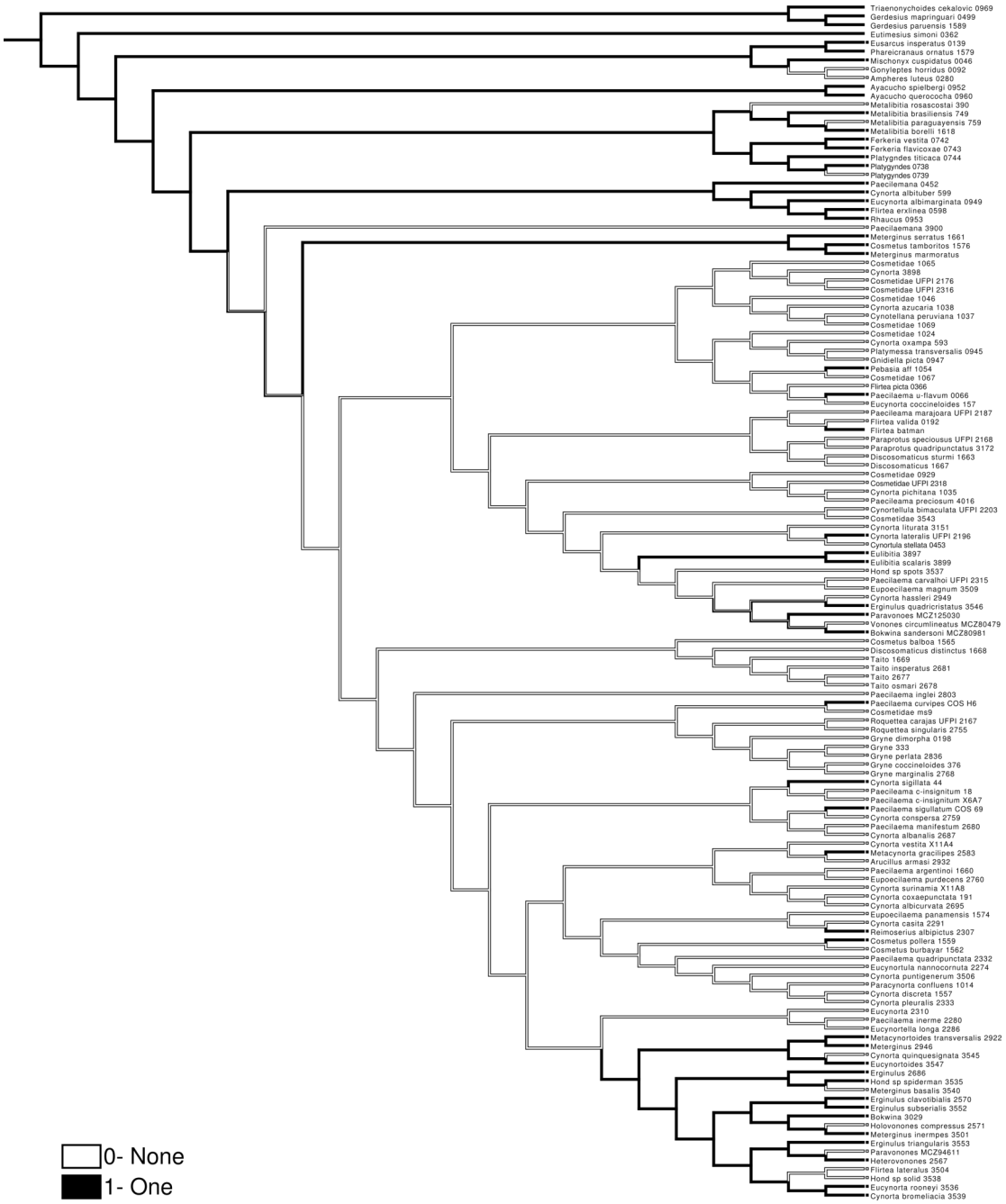


Figure 36: Character 51. Femur III. Armature (CI= 0.04; RI= 0.429): Parsimony reconstruction (Unordered) [Steps: 25]

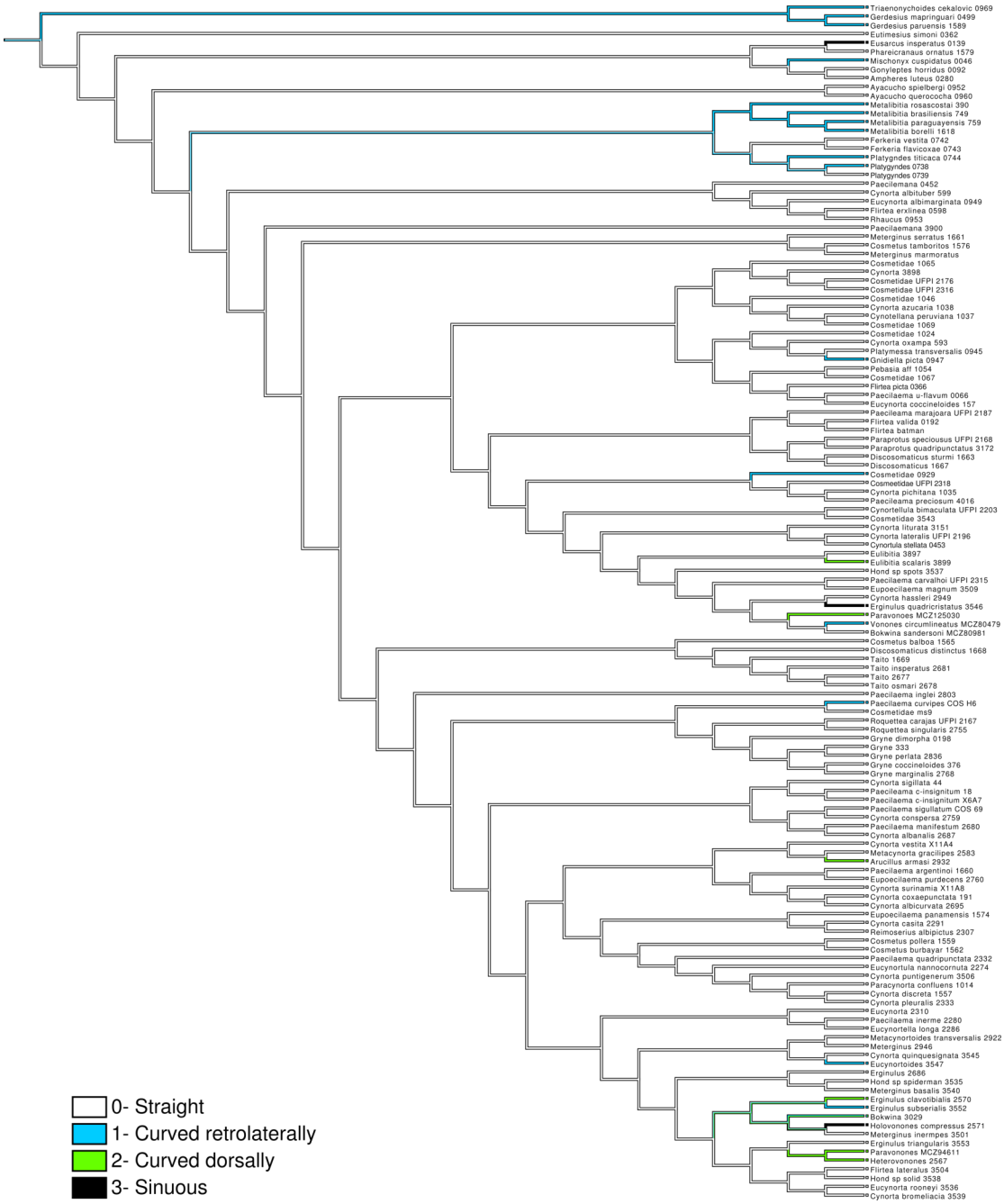


Figure 37: Character 52. Legs. Femur IV. Shape (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.190; RI= 0.261): Parsimony reconstruction (Unordered) [Steps: 21]

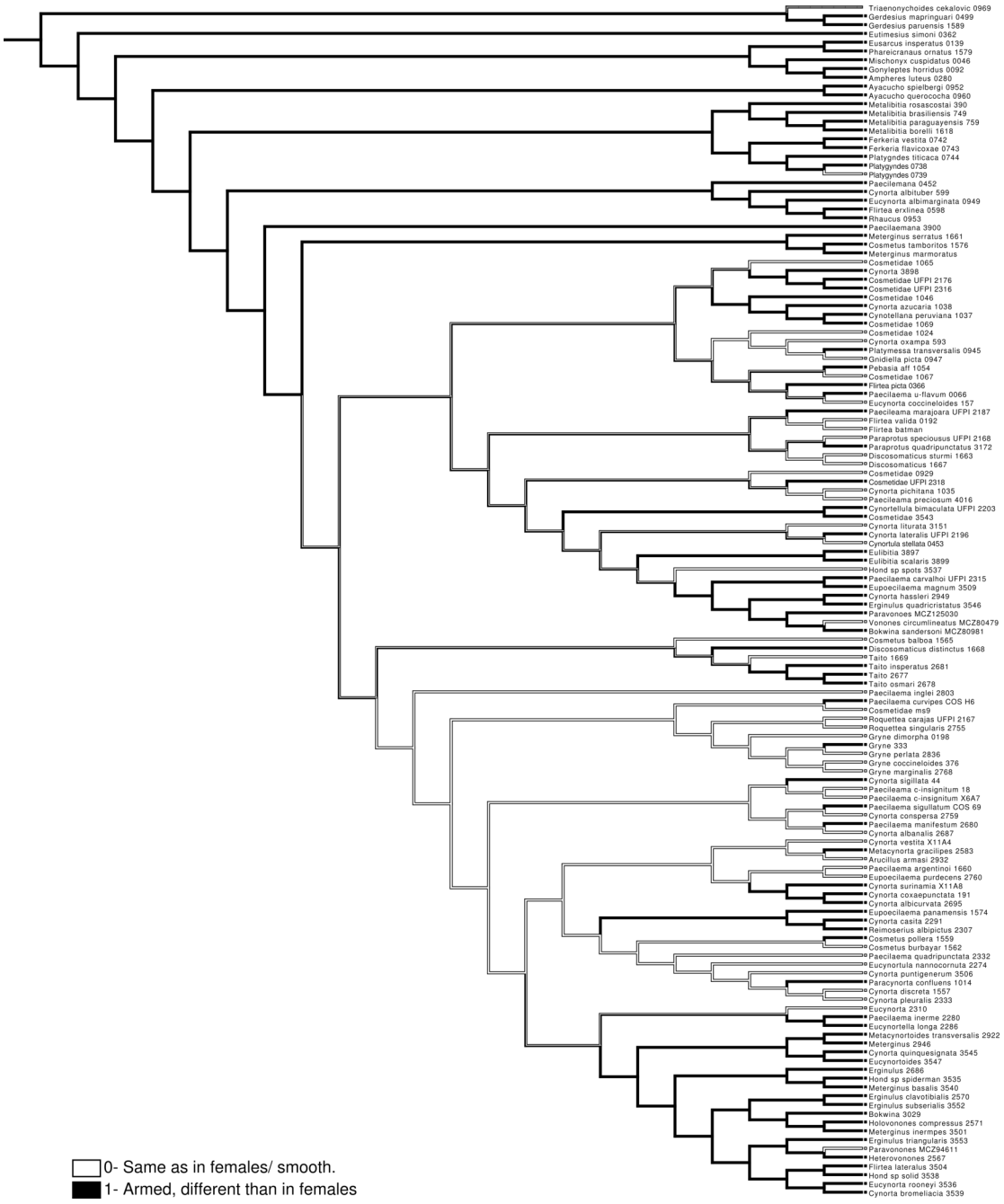


Figure 38: Character 53. Legs. Femur IV. Retrolateral Armature (CI= 0.032; RI= 0.318). Parsimony reconstruction (Unordered) [Steps: 31]

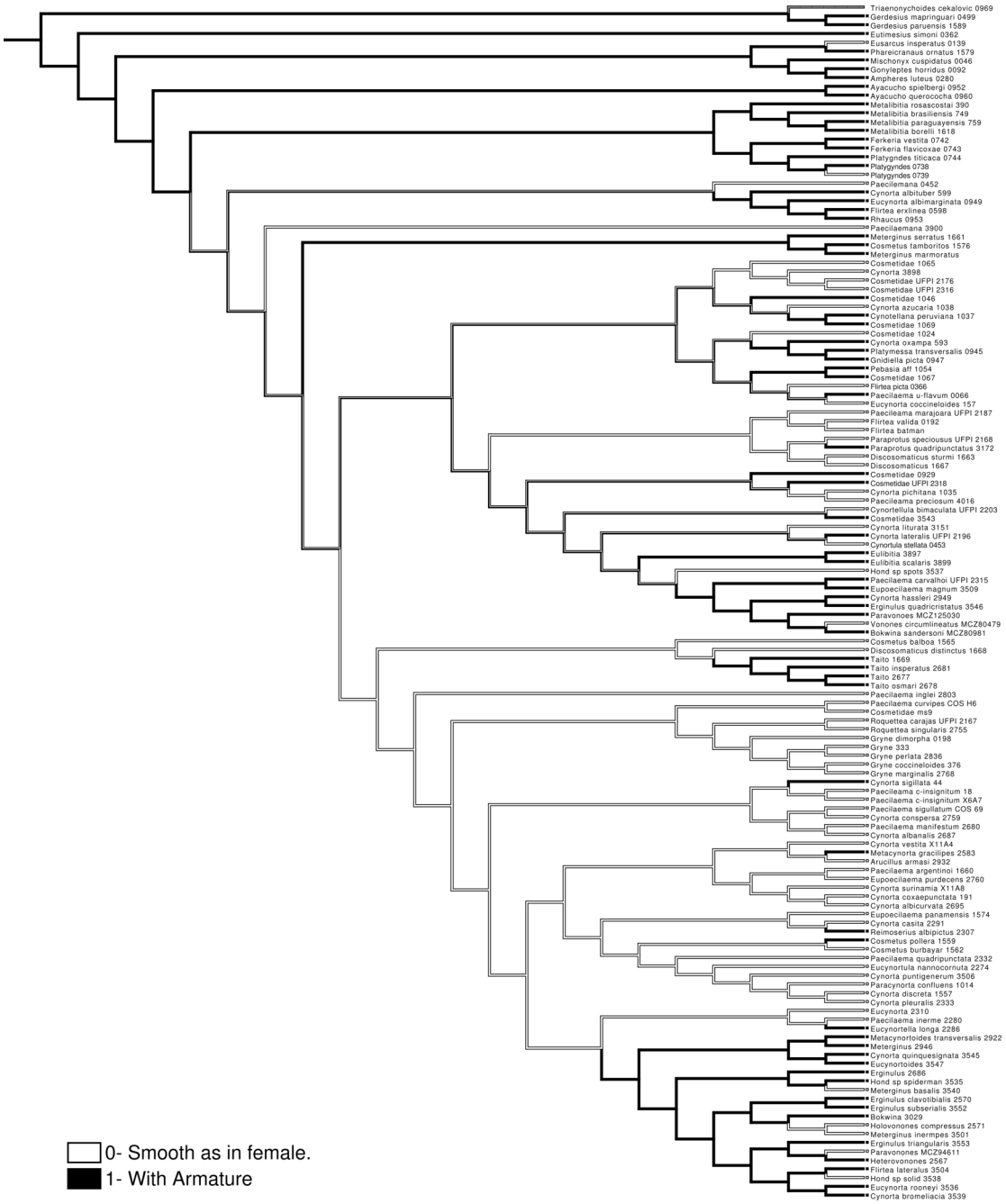


Figure 39: Character 54. Legs. Femur IV. Prolateral Armature. Male (CI= 0.034; RI= 0.569): Parsimony reconstruction (Unordered) [Steps: 29]

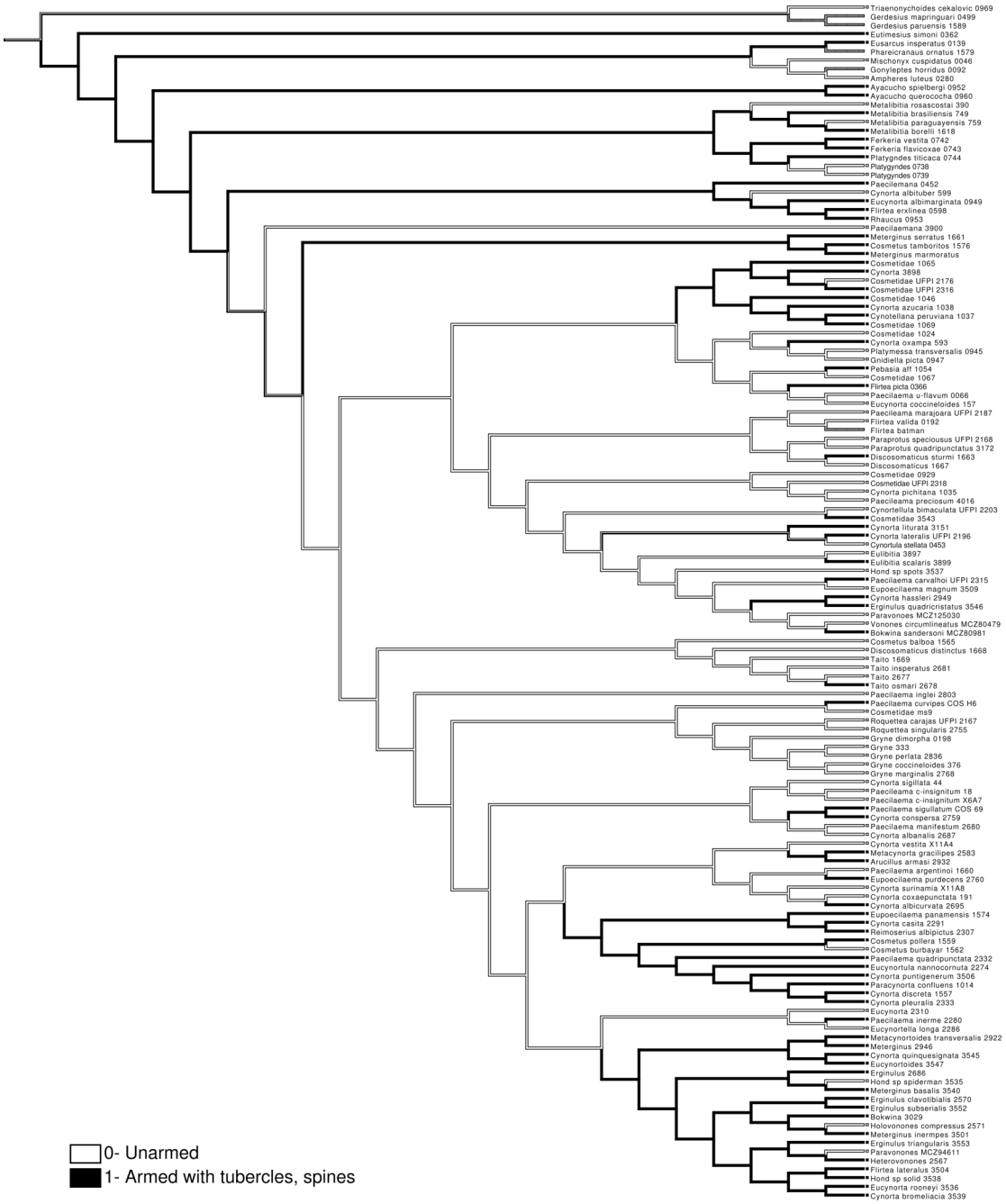


Figure 40: Character 56. Legs. Patella IV. Armature (CI= 0.029; RI= 0.45): Parsimony reconstruction (Unordered) [Steps: 34]

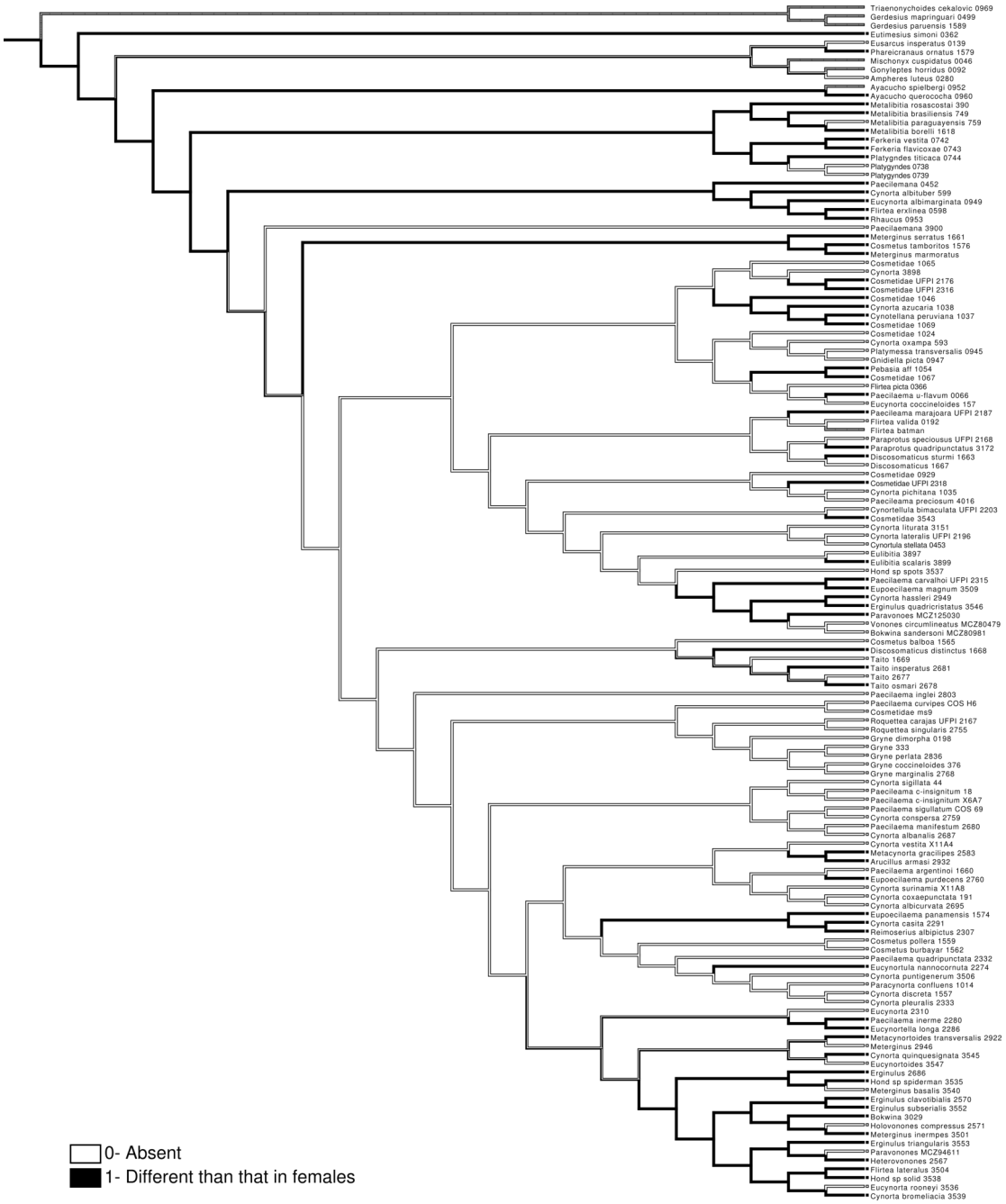


Figure 41: Character 57. Legs. Tibia IV, sexual dimorphism (CI= 0.030; RI= 0.475): Parsimony reconstruction (Unordered) [Steps: 33]

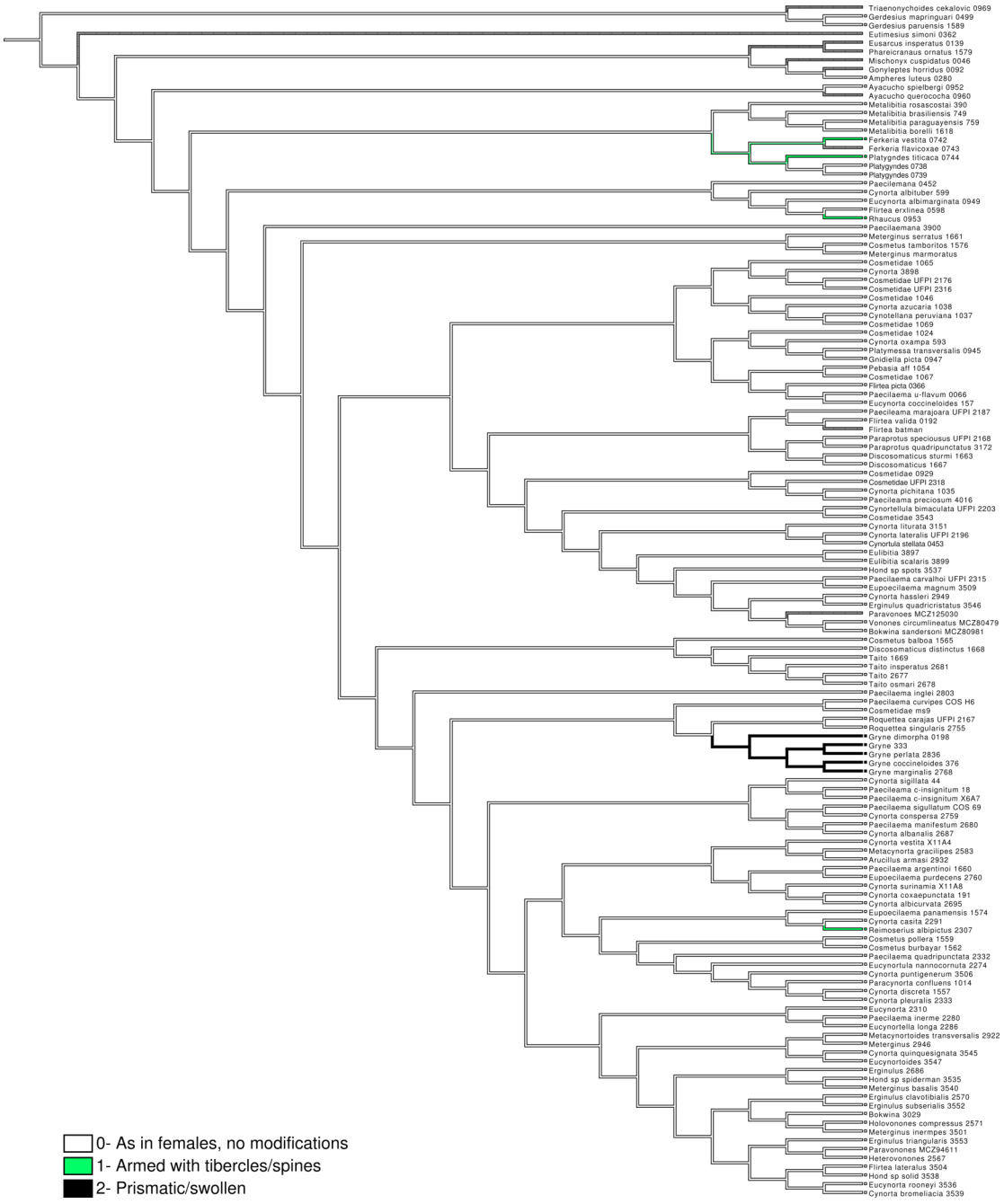


Figure 42: Character 59. Legs. Metatarsus IV. Sexual dimorphism (CI= 0.40; RI= 0.571): Parsimony reconstruct (Unordered) [Steps: 5]

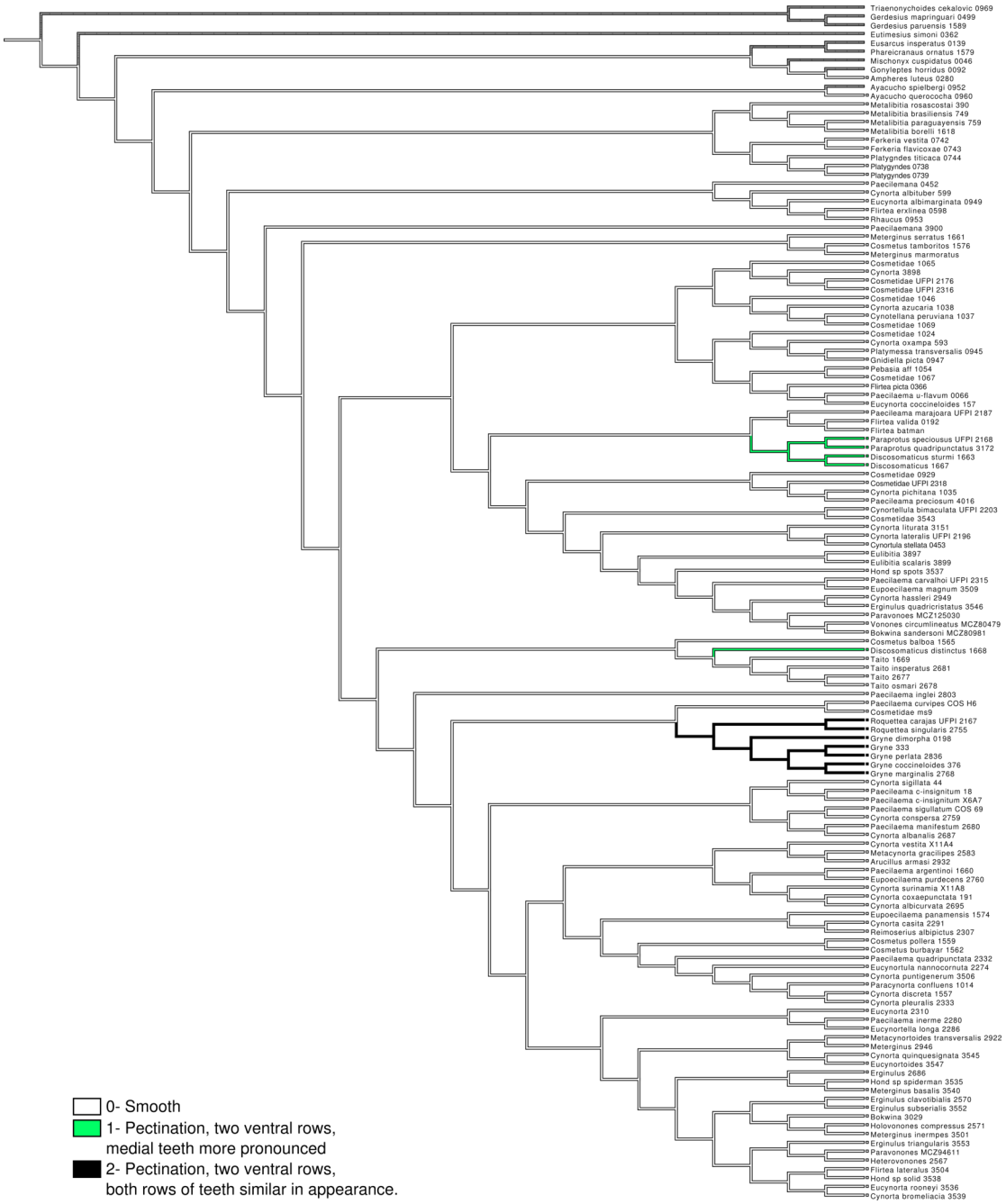


Figure 43: Character 60. Legs. Tarsus. Claws (CI= 0.667; RI= 0.90): Parsimony reconstruction (Unordered) [Steps: 3]

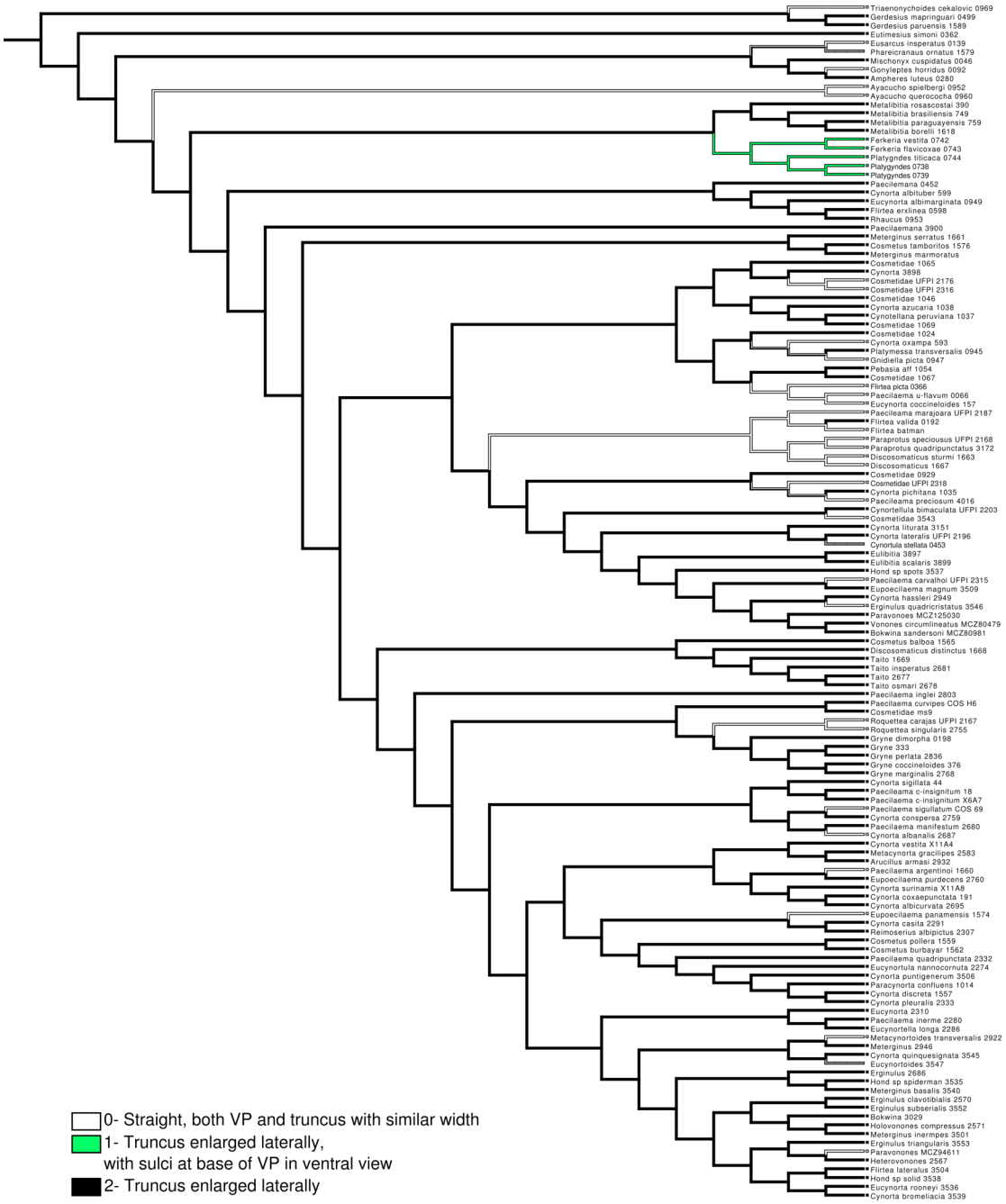


Figure 44: Character 61. Penis. Truncus. In relation to ventral plate (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.087; RI= 0.382): Parsimony reconstruction (Unordered) [Steps: 23]

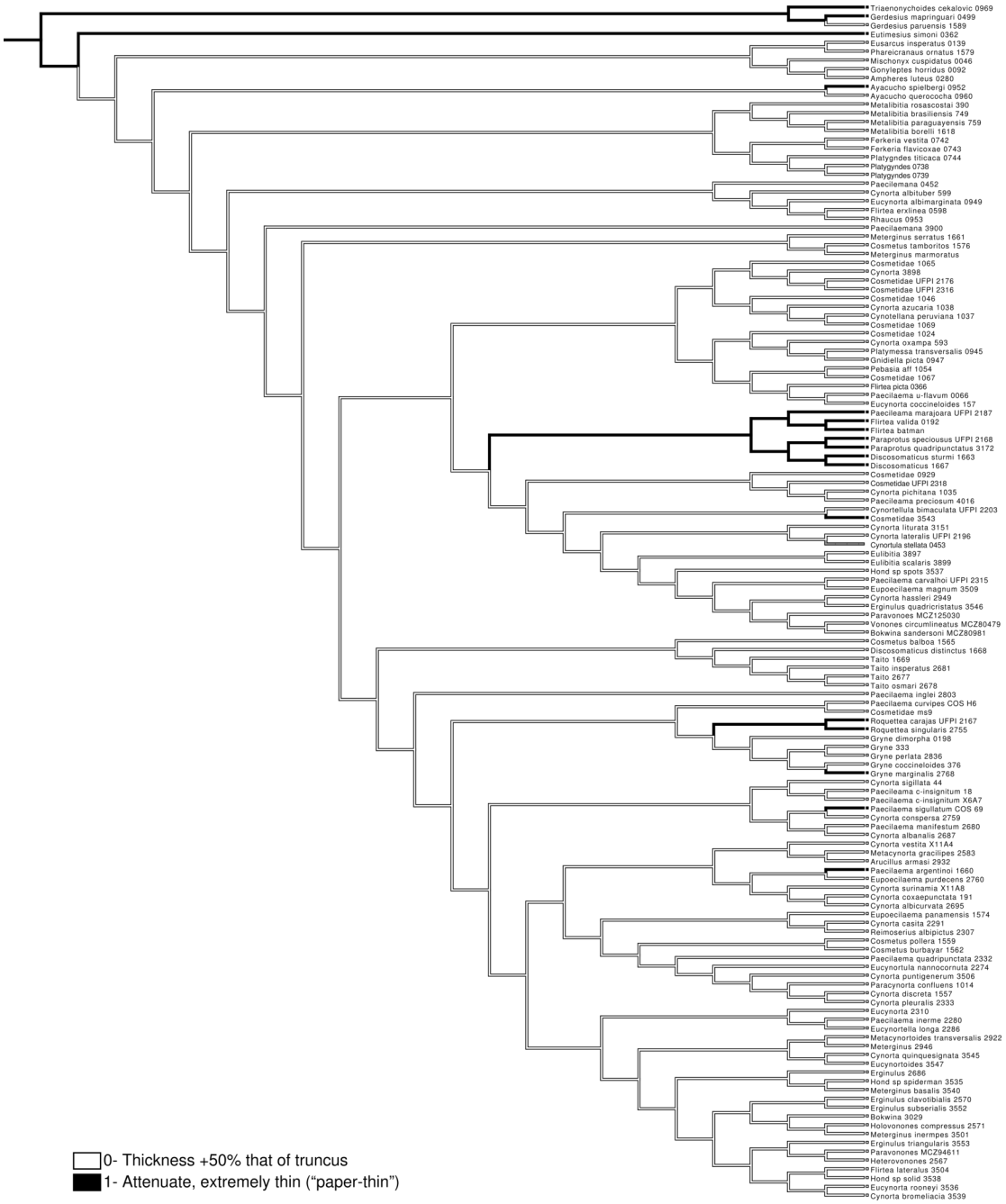


Figure 45: Character 63. Penis. VP. Thickness in lateral view (CI= 0.111; RI= 0.500): Parsimony reconstruction (Unordered) [Steps: 9]

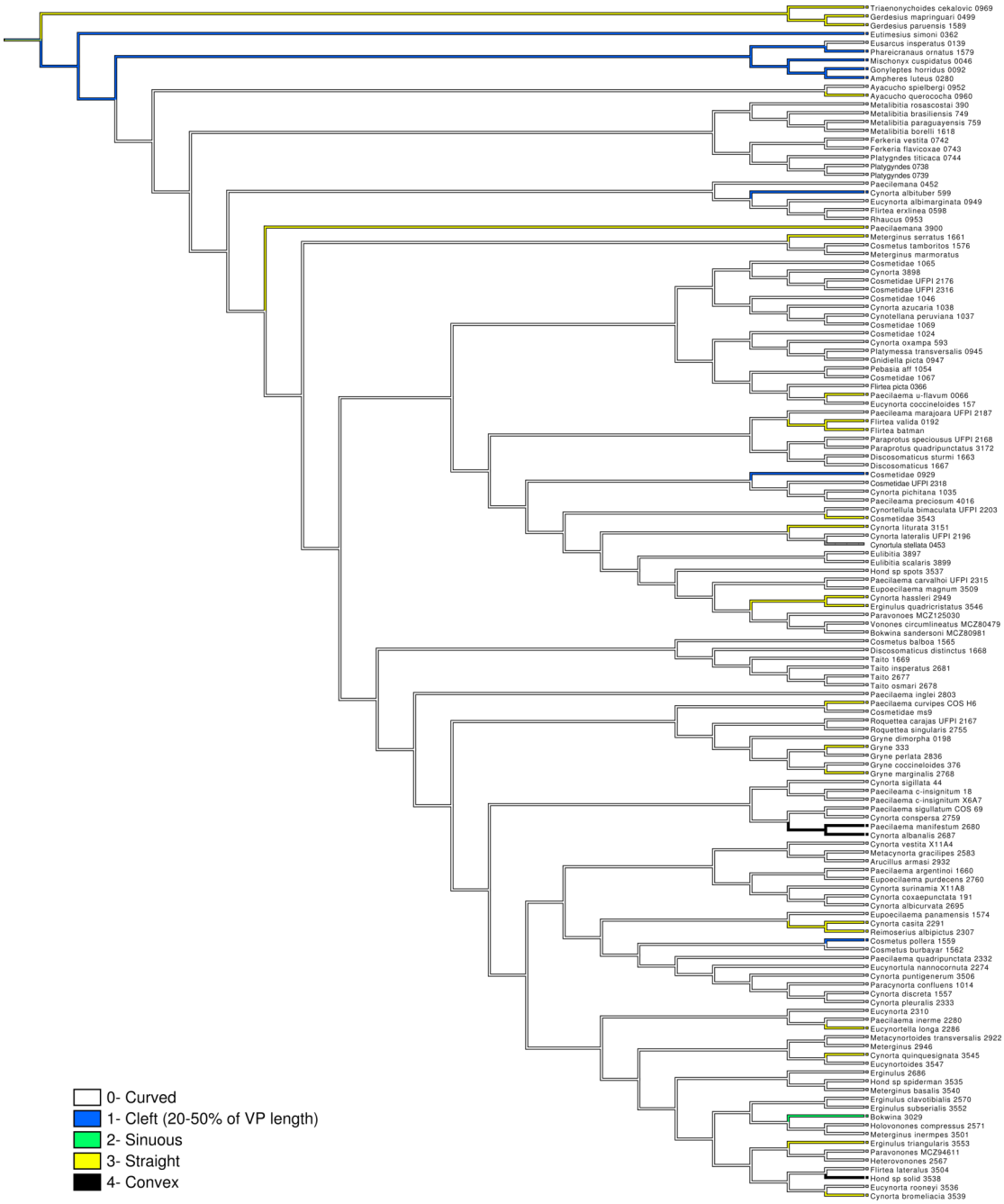


Figure 46: Character 64. Penis. VP. Shape of apical margin (CI= 0.16; RI= 0.30): Parsimony reconstruction (Unordered) [Steps: 25]

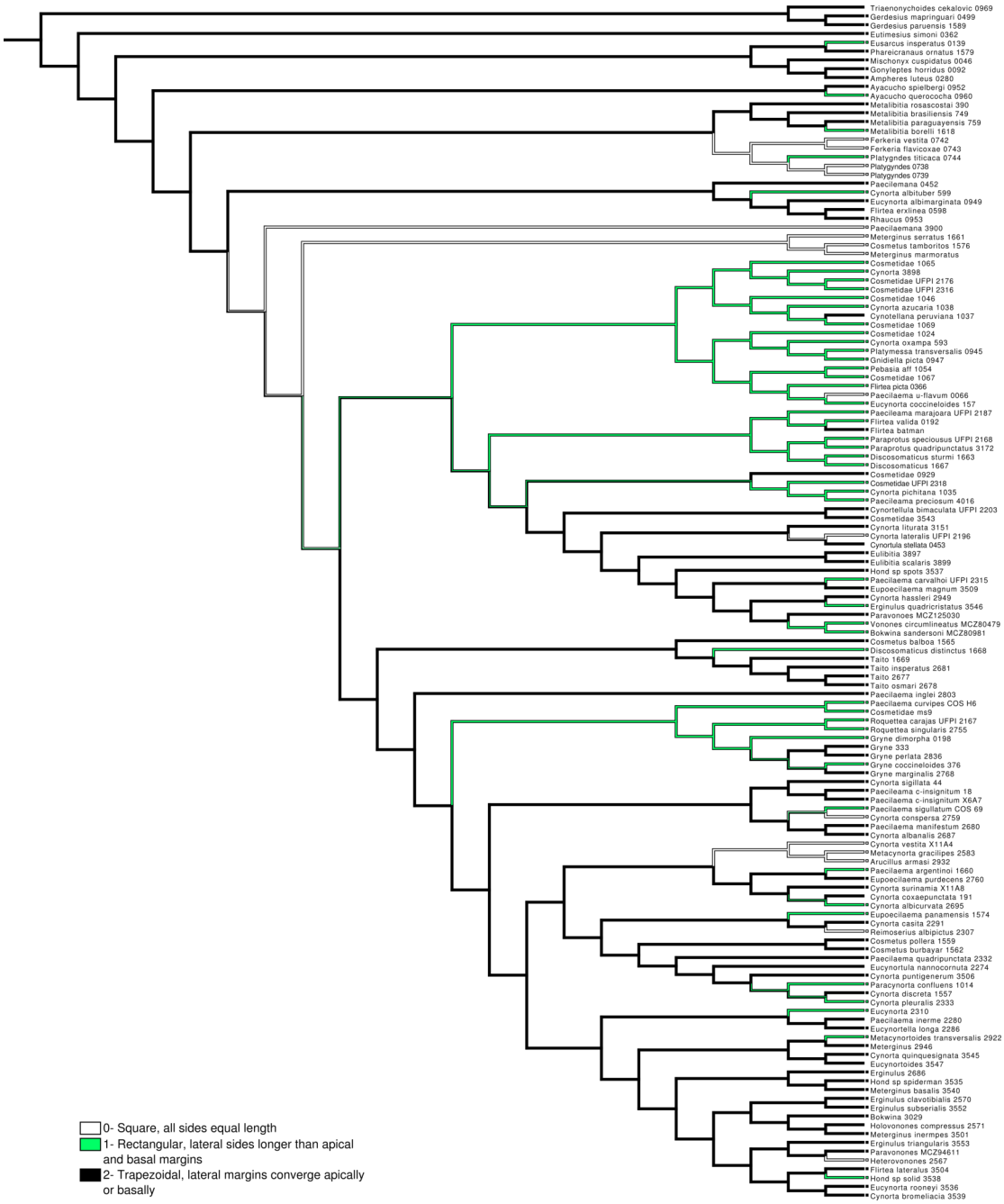


Figure 47: Character 65. Penis. VP. General shape of VP (CI= 0.059; RI= 0.492): Parsimony reconstruction (Unordered) [Steps: 34]

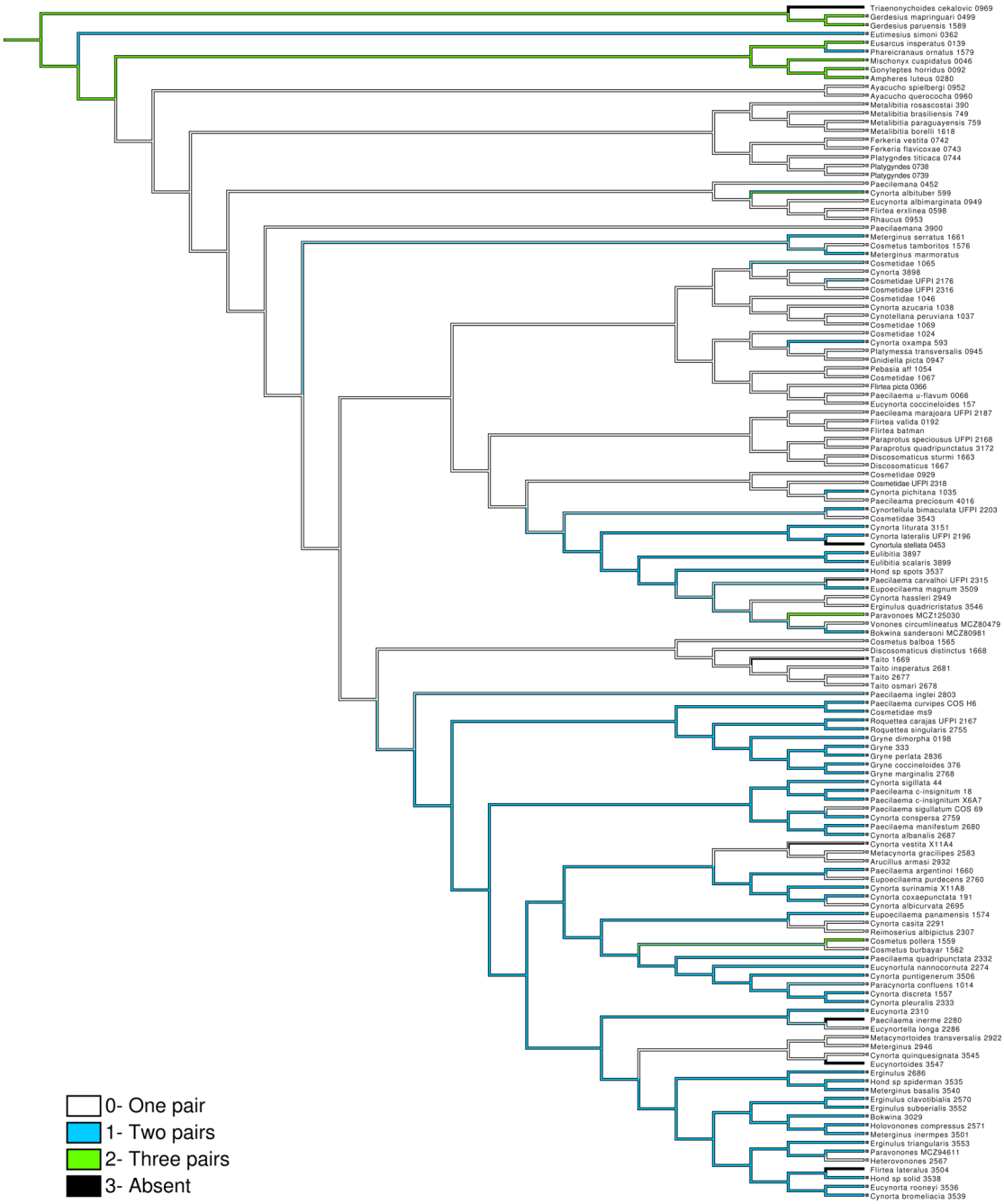


Figure 48: Character 66. Penis. VP. Macrosetae (MS) A (modified from Kury & Villarreal 2015) (CI= 0.303; RI= 0.610): Parsimony reconstruction (Unordered) [Steps: 33]

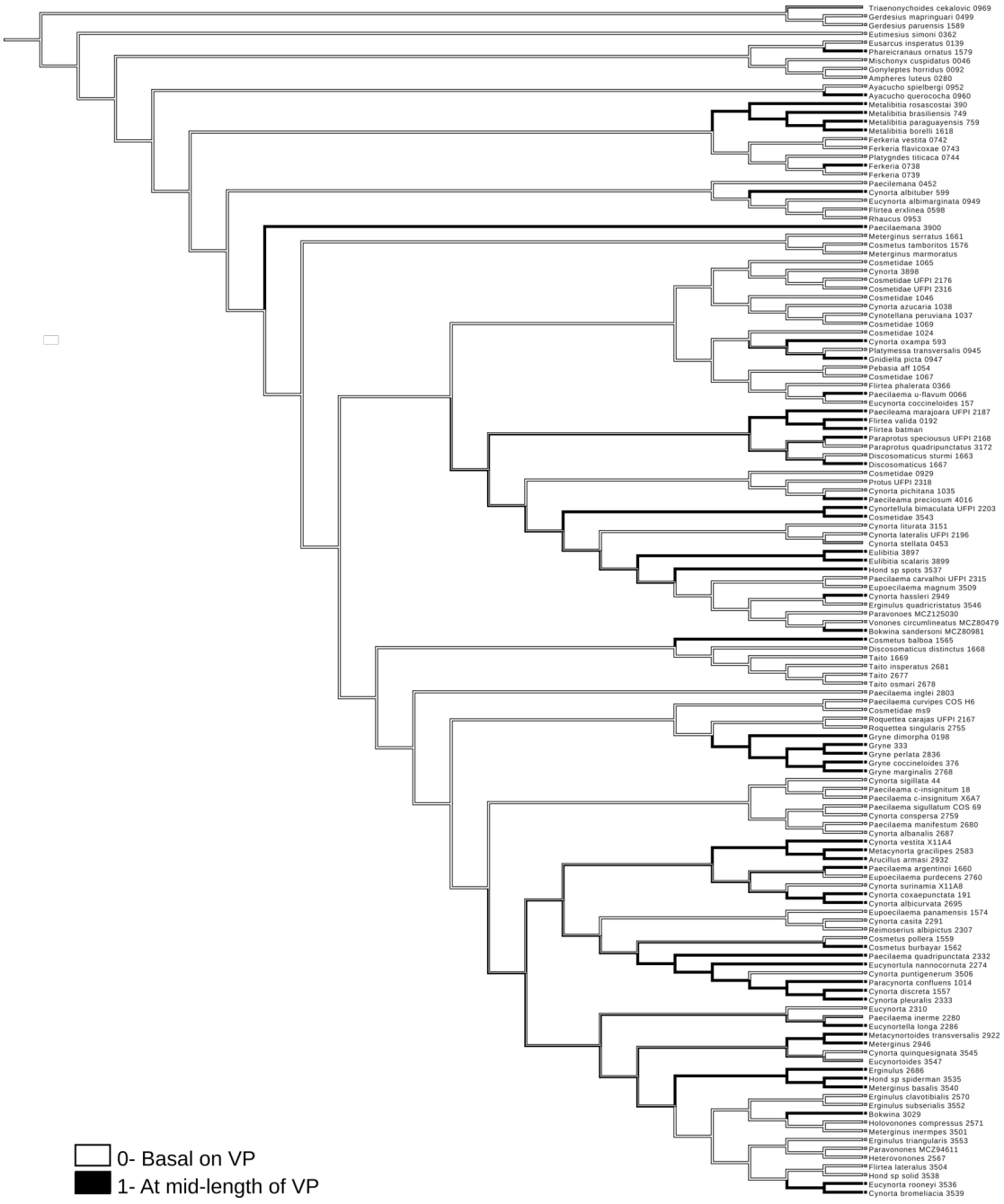


Figure 49: Character 69. Penis, VP, MS-A, most apical pair position (CI= 0.323; RI= 0.412): Parsimony reconstruction (Unordered) [Steps: 31]

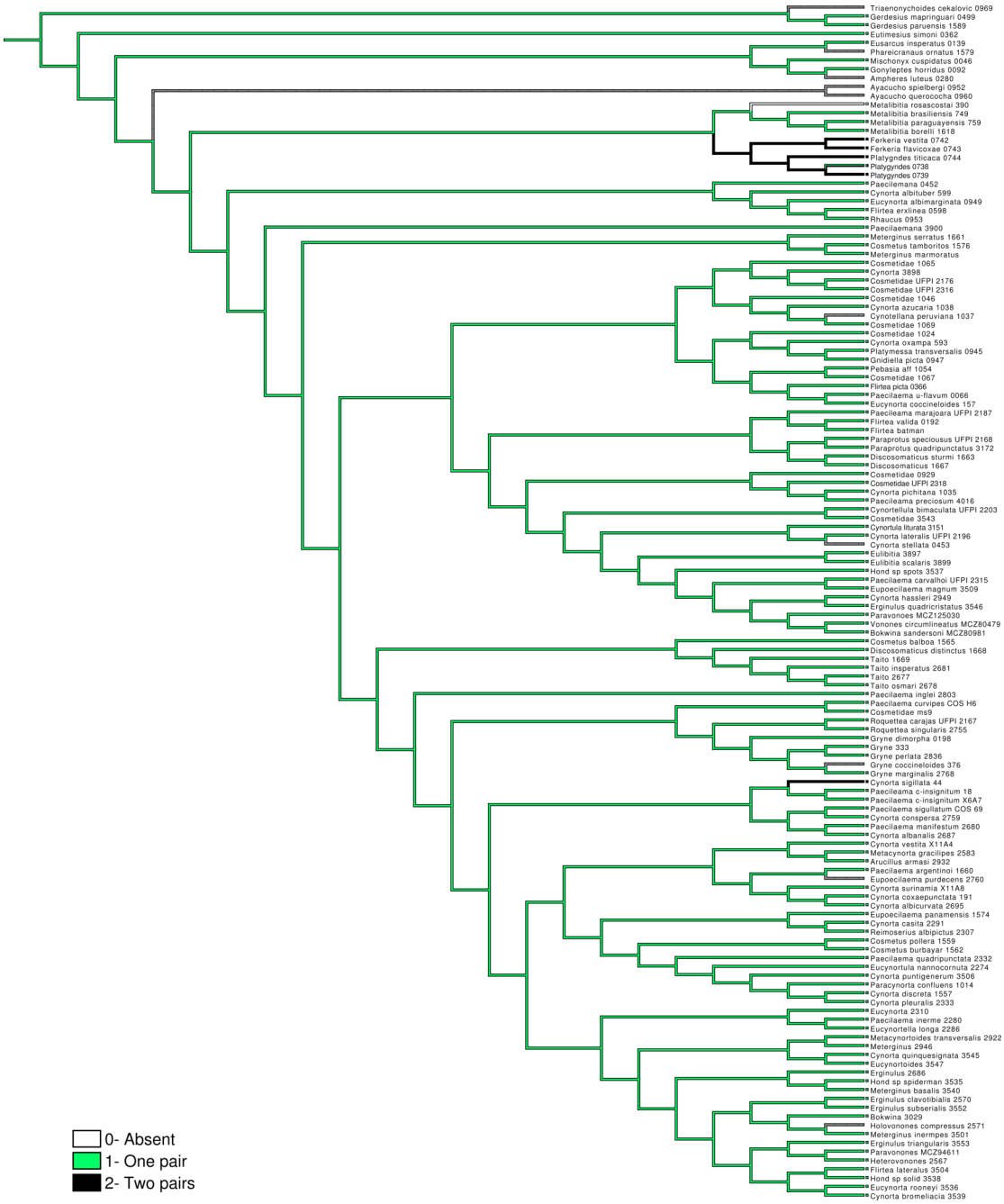


Figure 50: Character 70. Penis. VP. MS-B. (only visible in ventral view SEM) (modified from Kury & Villarreal 2015; Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.75; RI= 0.75): Parsimony reconstruction (Unordered) [Steps: 4]

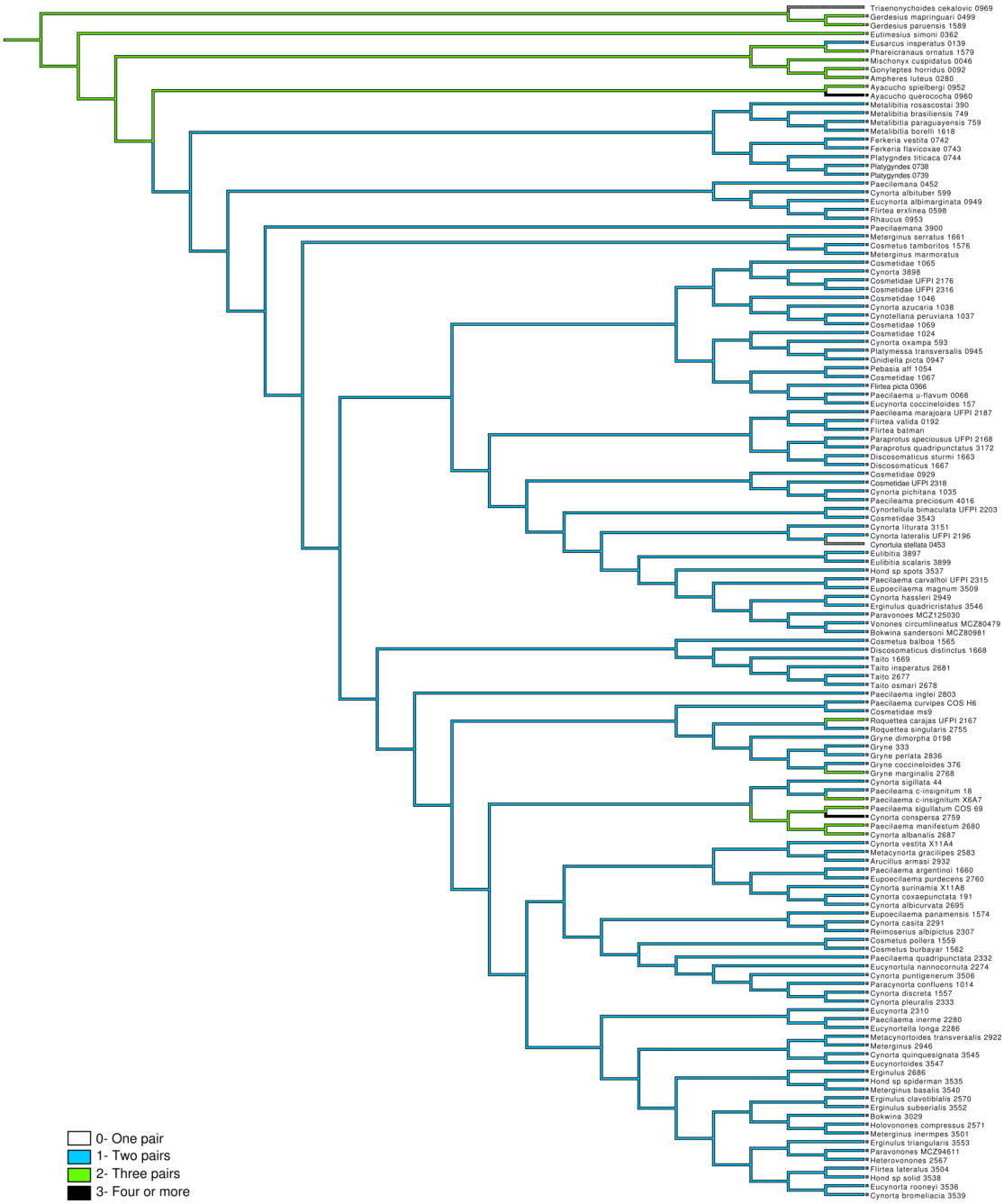


Figure 51: Character 72. Penis. VP. MS-C. (modified from Kury & Villarreal 2015; Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.25; RI= 0.571): Parsimony reconstruction (Unordered) [Steps: 8]

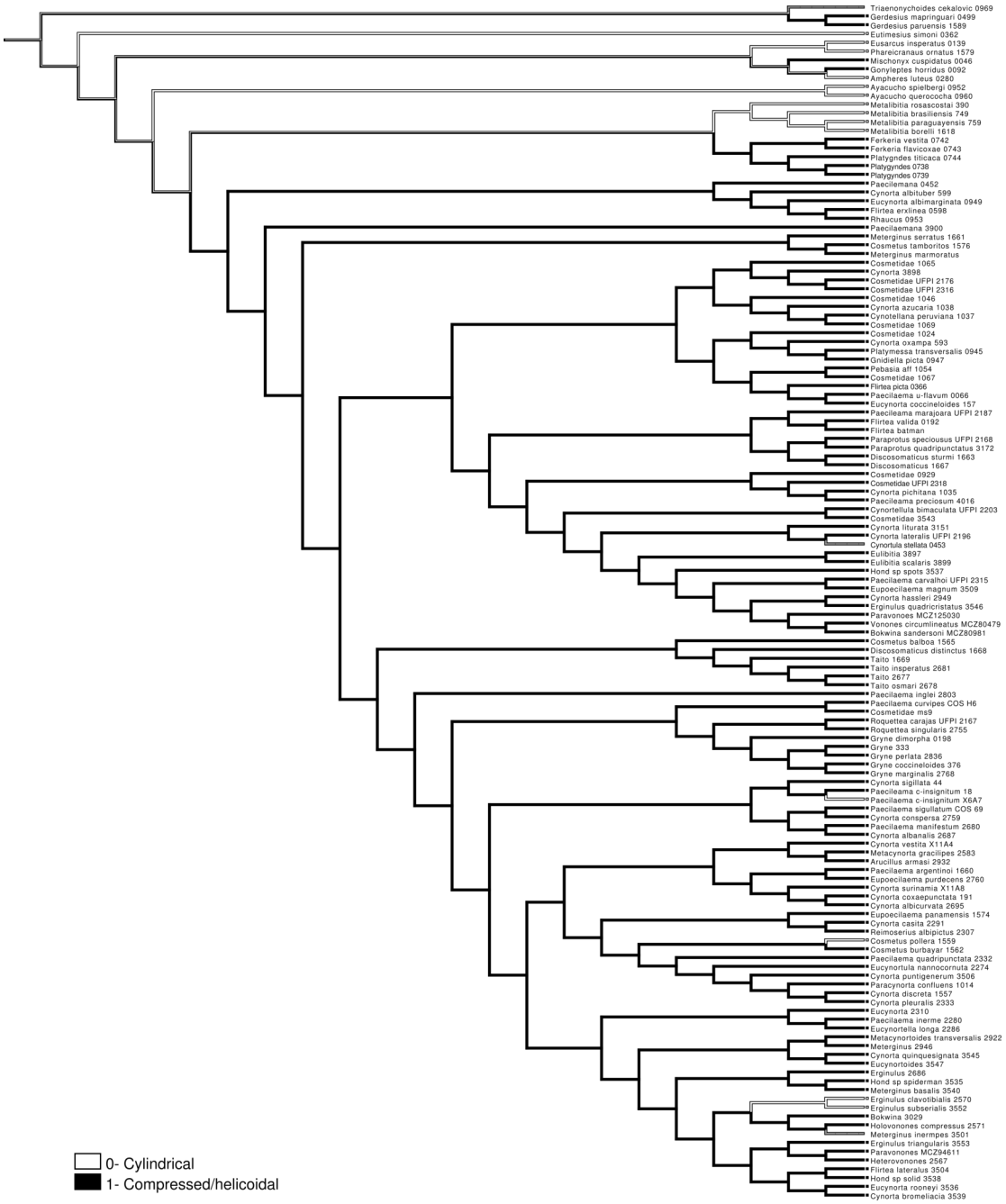


Figure 52: Character 73. Penis. VP. MS-C. Shape (CI= 0.125; RI= 0.462): Parsimony reconstruction (Unordered) [Steps: 8]

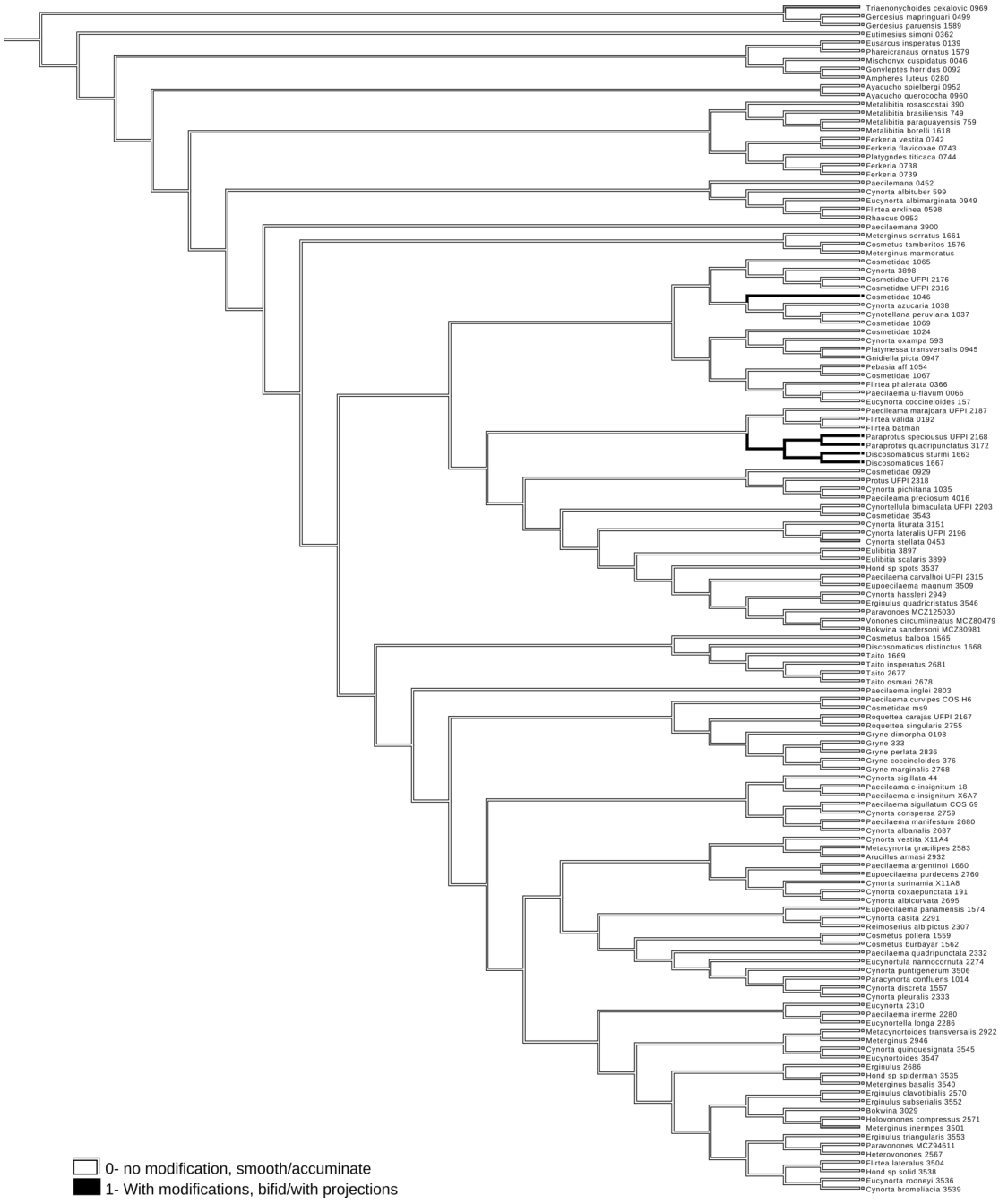


Figure 53: Character 74. Penis. VP. MS-C. Apical tip (CI= 0.50; RI= 0.75): Parsimony reconstruction (Unordered) [Steps: 2]

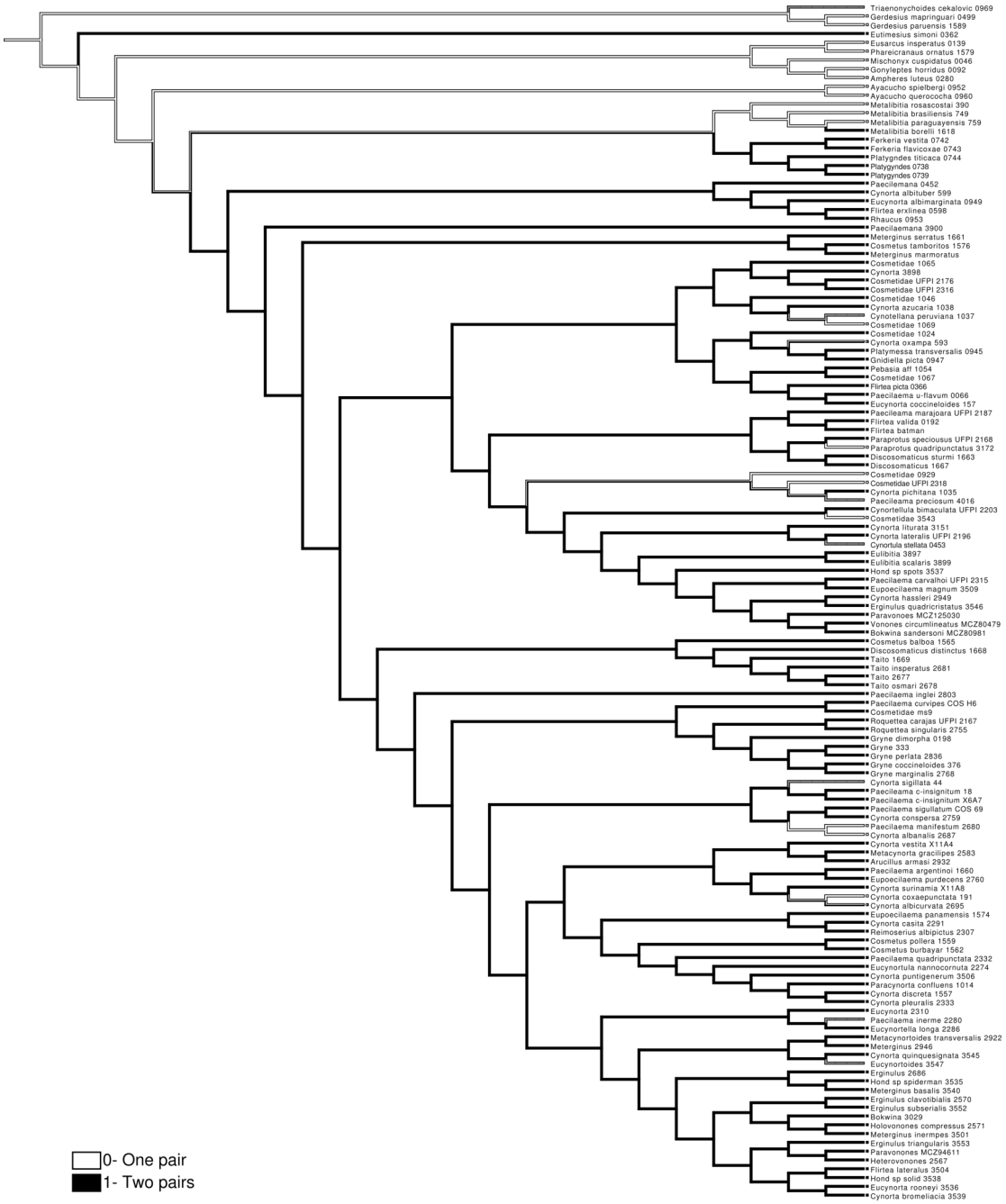


Figure 54: Character 76. Penis. VP. MS-D. (modified from Kury & Villarreal 2015; Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.231; RI= 0.474): Parsimony reconstruction (Unordered) [Steps: 13]

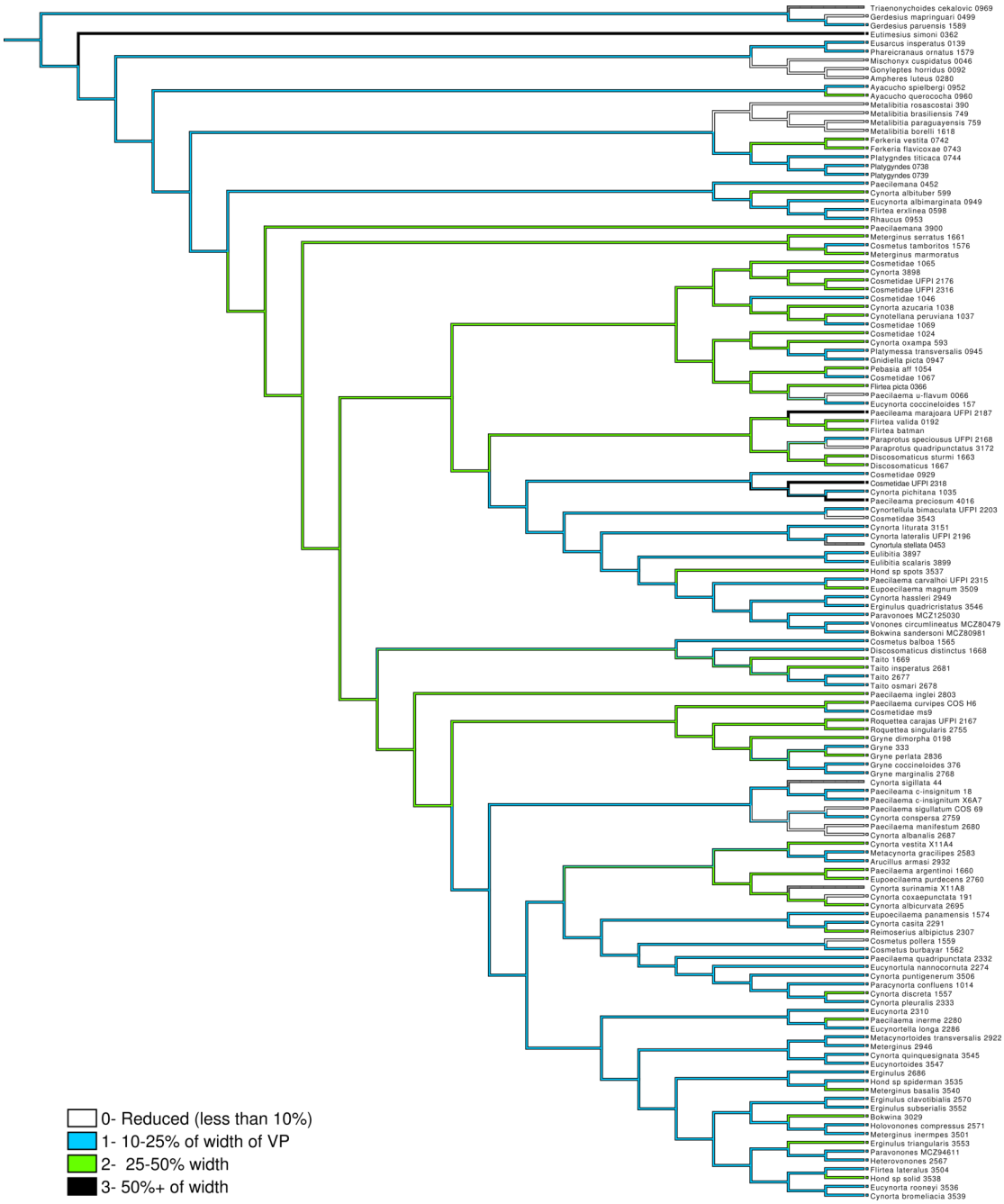


Figure 55: Character 77. Penis. VP. MS. Length of MSD1 (compare to the widest portion of VP) (CI= 0.068; RI= 0.305): Parsimony reconstruction (Unordered) [Steps: 44]

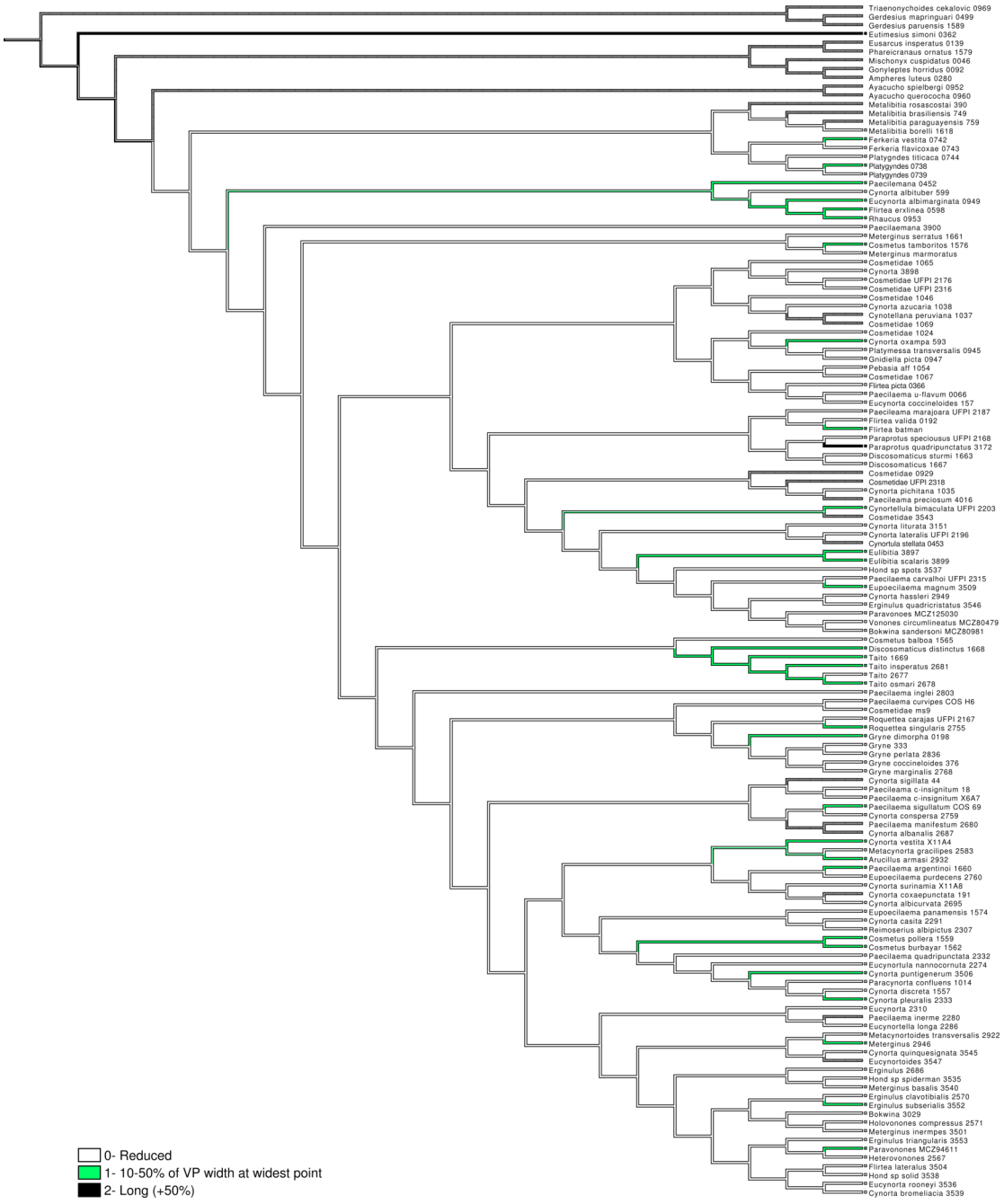


Figure 56: Character 78. Penis. VP. MS. Length of MSD2 (CI= 0.077; RI= 0.20): Parsimony reconstruction (Unordered) [Steps: 26]

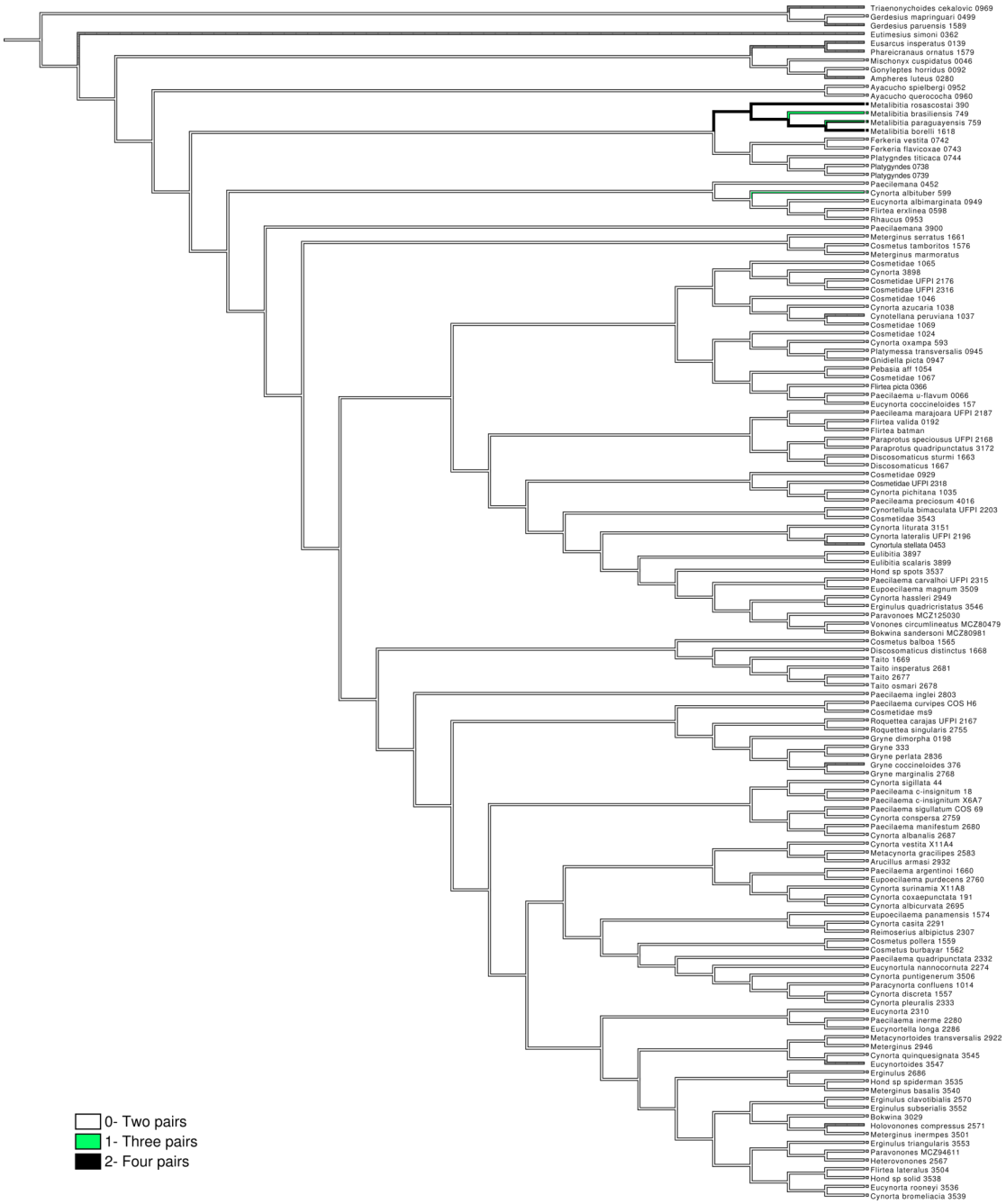


Figure 57: Character 79. Penis. VP. MS Number of MSE (modified from Kury & Villarreal 2015; Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 1.00; RI= 1.00): Parsimony reconstruction (Unordered) [Steps: 4]

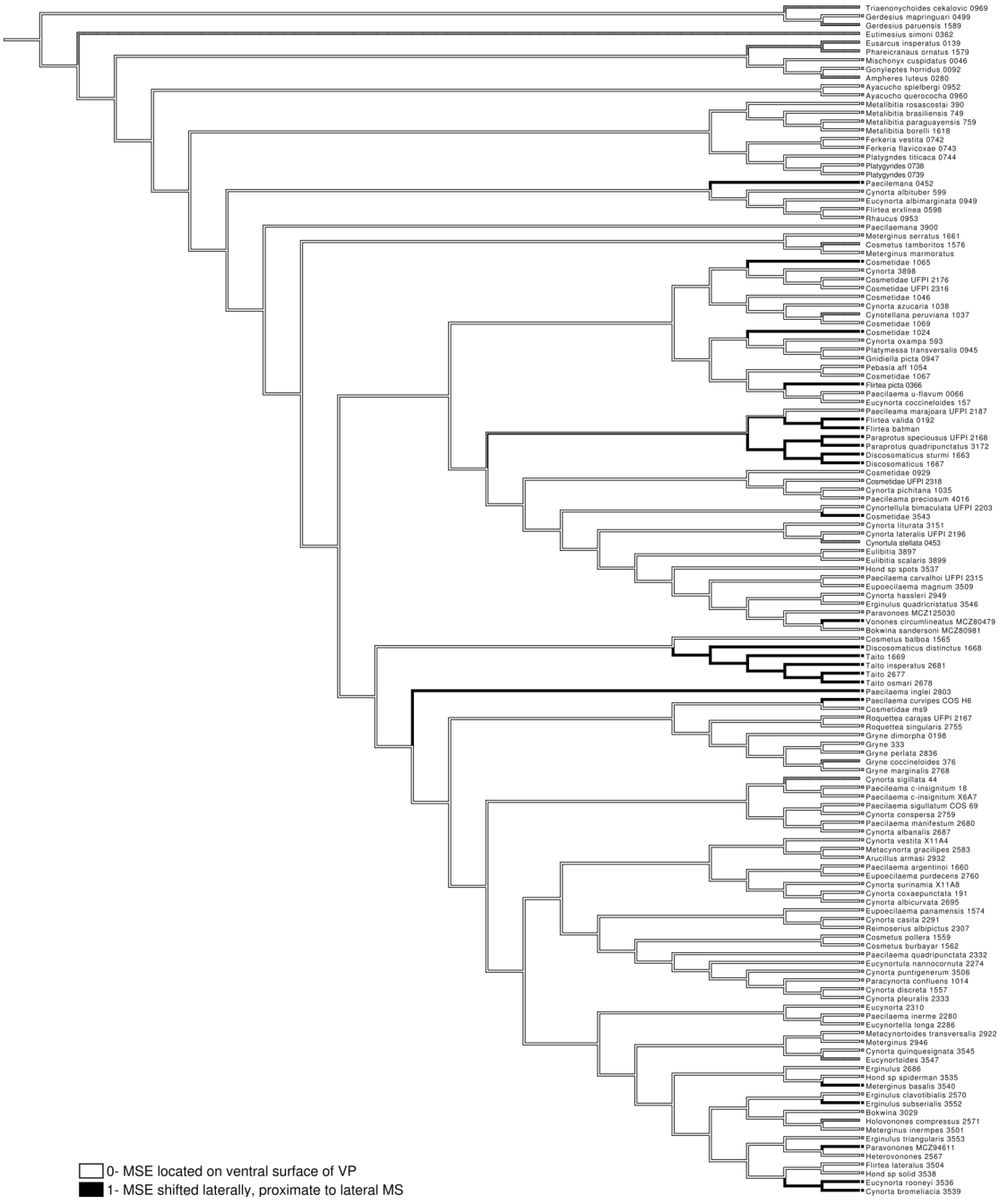


Figure 58: Character 80. Penis. VP. MS-E. (CI= 0.067; RI= 0.391): Parsimony reconstruction (Unordered) [Steps: 15]

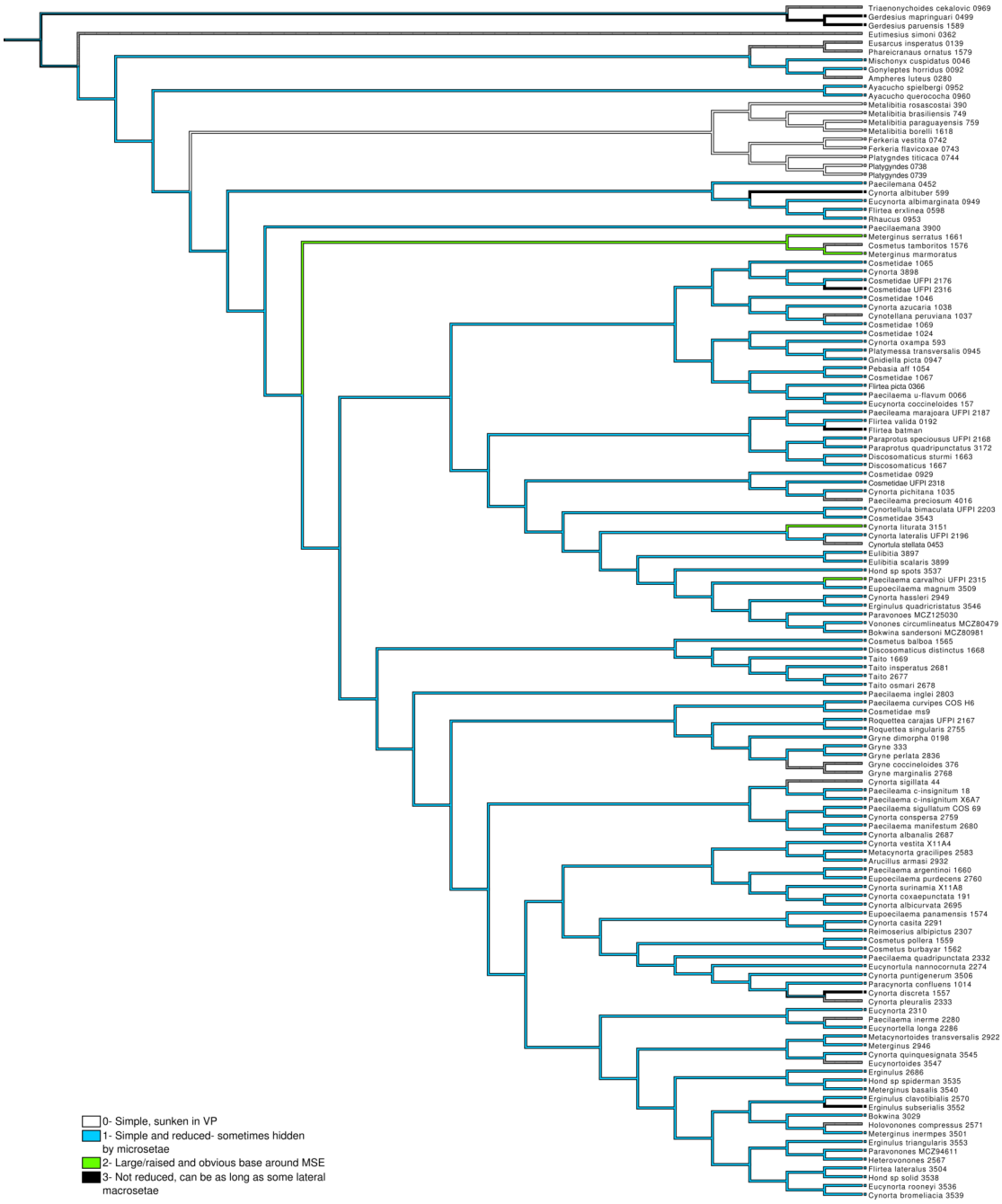


Figure 59: Character 81. Penis. VP. MS-E. Appearance (CI= 0.30; RI= 0.588): Parsimony reconstruction (Unordered) [Steps: 10]

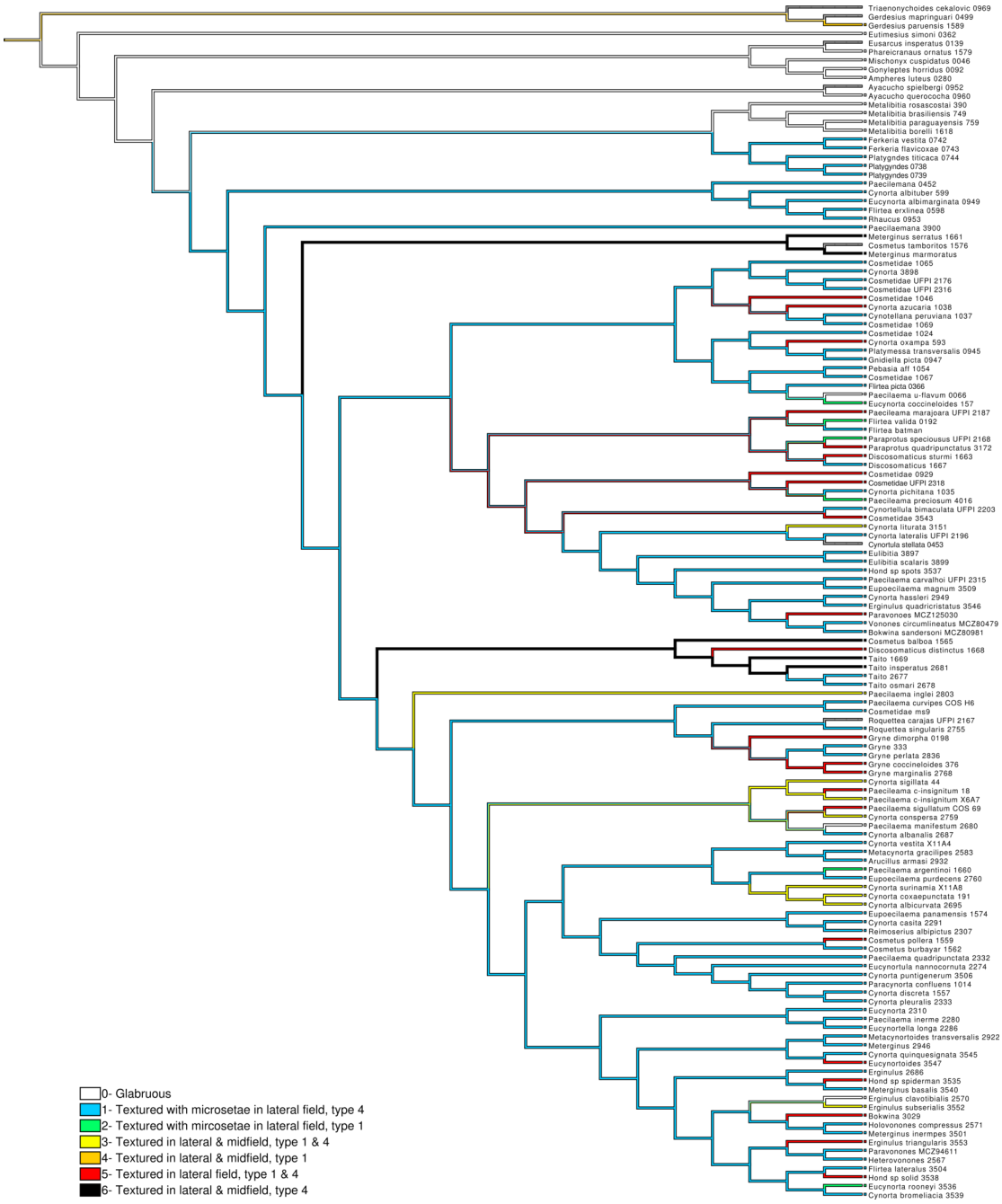


Figure 60: Character 83. Penis. VP. Texture and microsetae type on ventral surface (modified from Kury 2016, and Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.143; RI= 0.28): Parsimony reconstruction (Unordered) [Steps: 42]

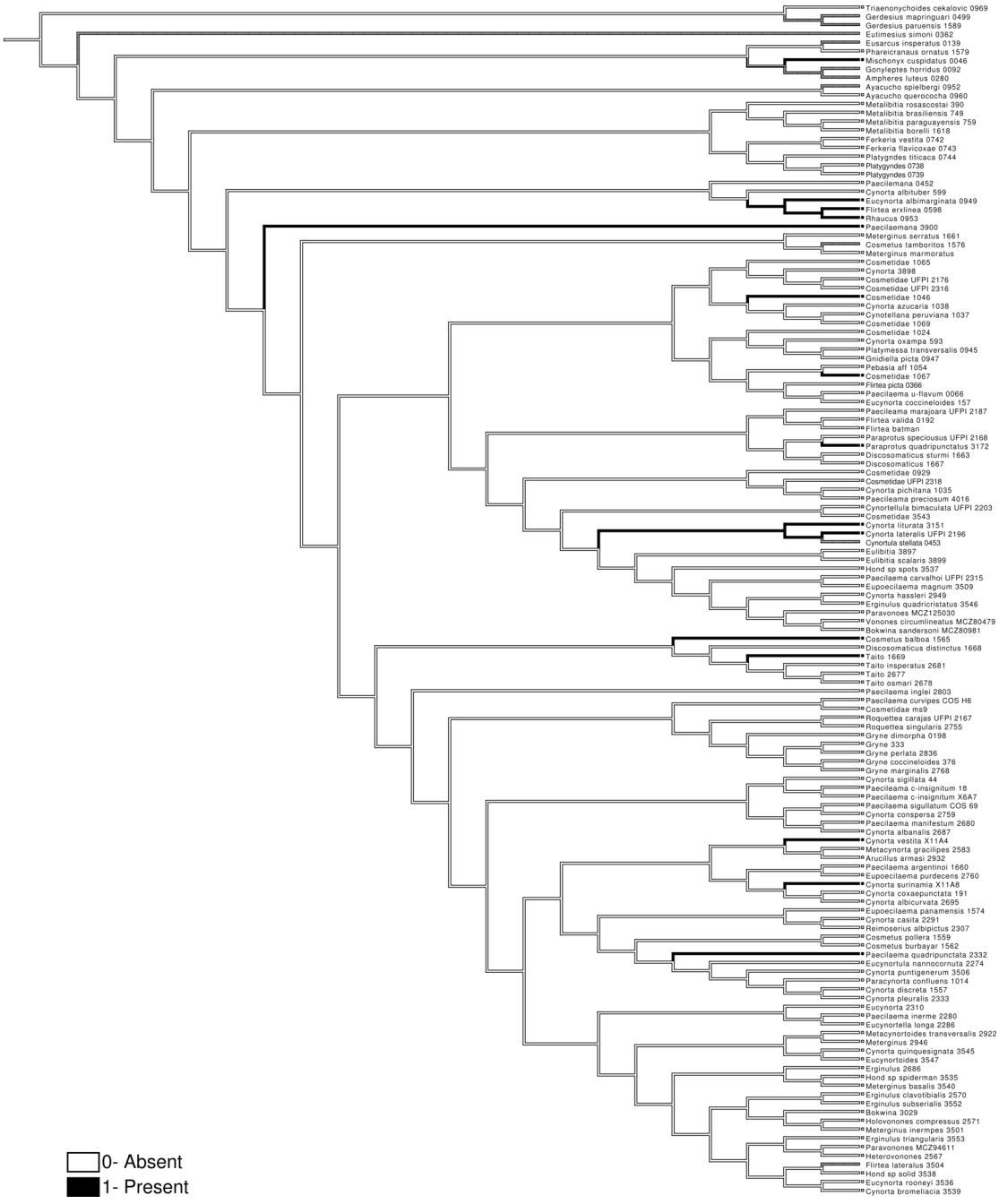


Figure 61: Character 84. Penis. VP. Microsetae (type 4) on dorsal surface (CI= 0.083; RI= 0.214): Parsimony reconstruction (Unordered) [Steps: 12]

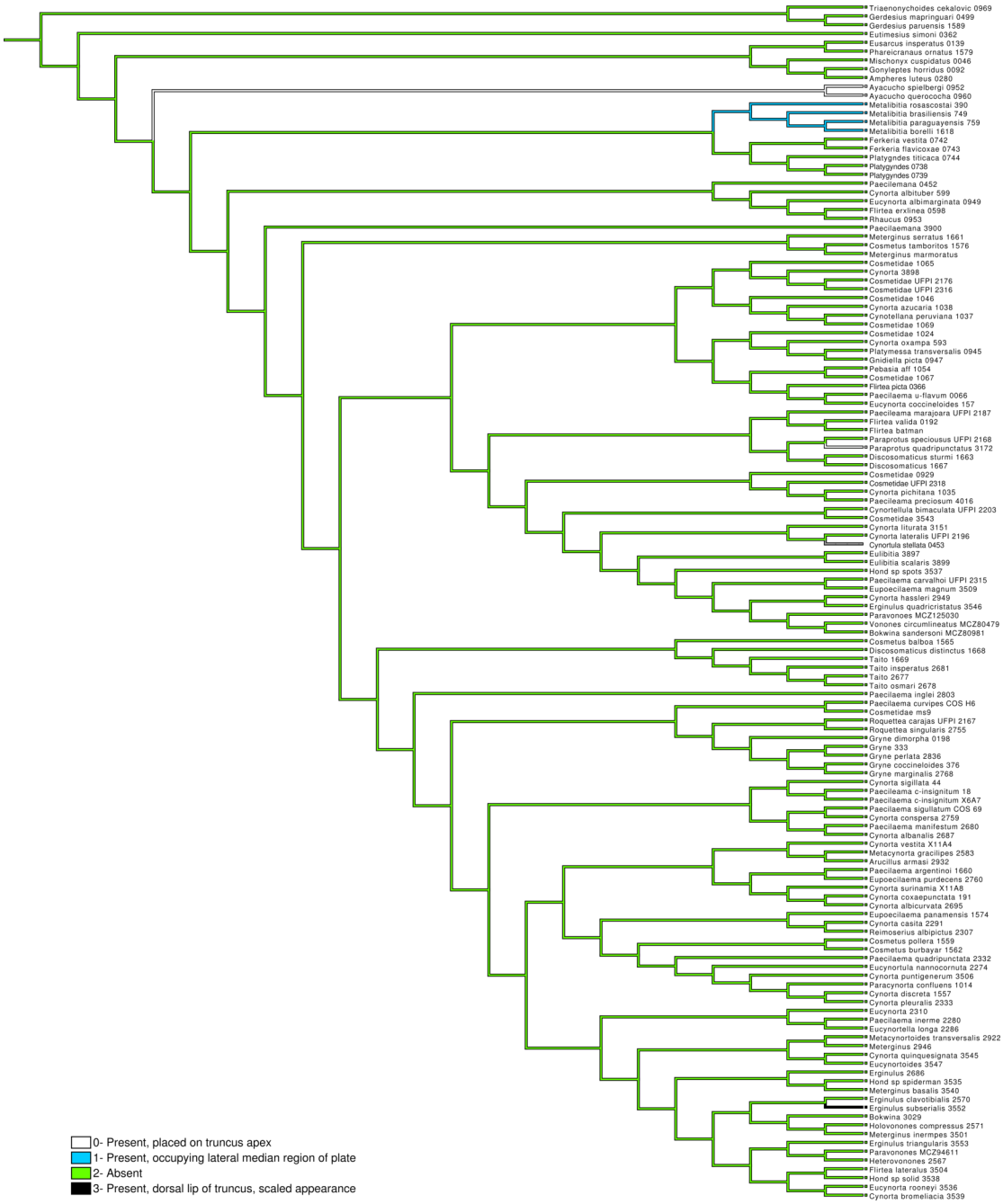


Figure 62: Character 85. Penis. Membranous extension of glans (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.75; RI= 0.80):Parsimony reconstruction (Unordered) [Steps: 4]

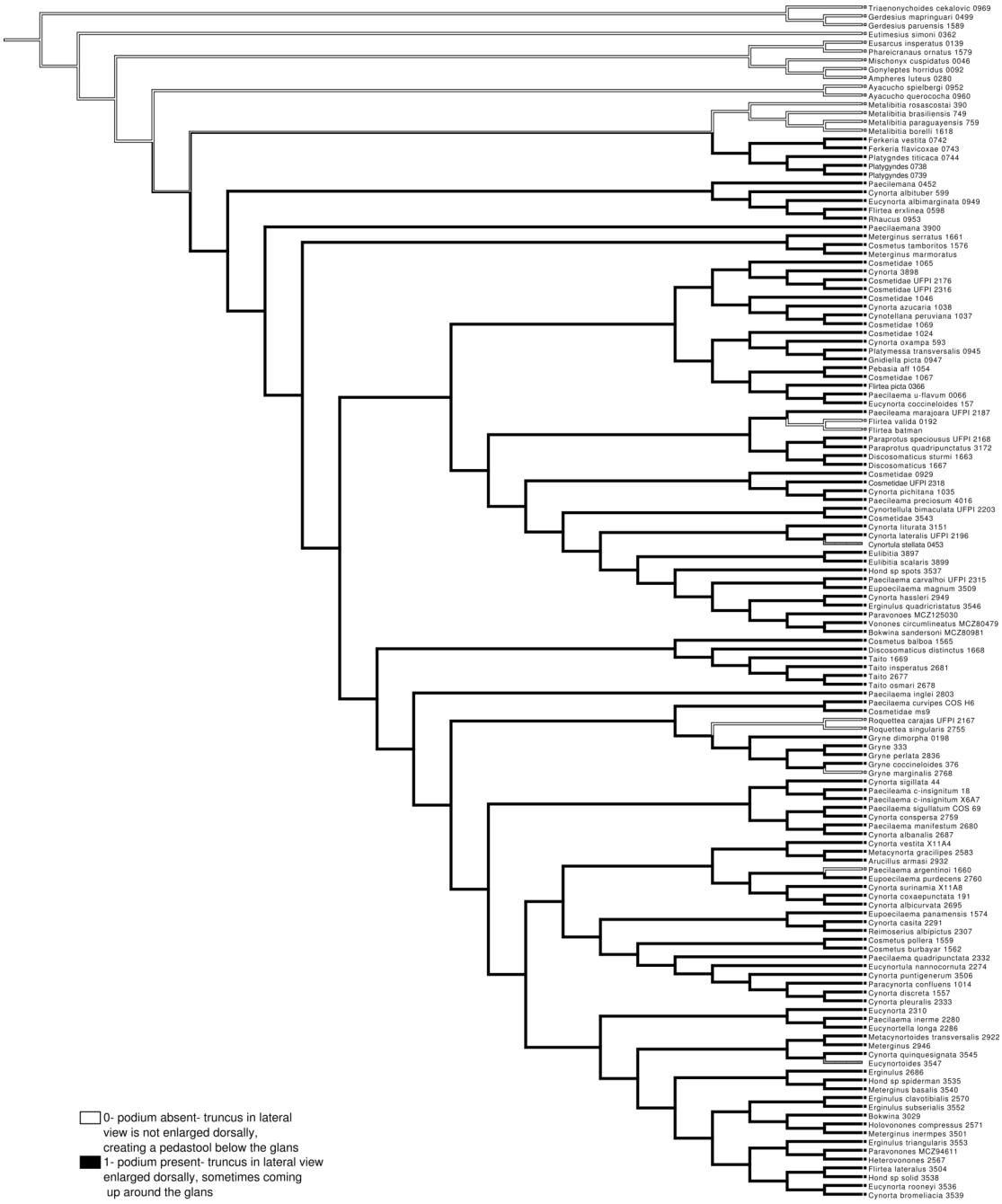


Figure 63: Character 86. Penis. VP. Glans. (modified from Medrano & Kury 2018) (CI= 0.167; RI= 0.75): Parsimony reconstruction (Unordered) [Steps: 6]

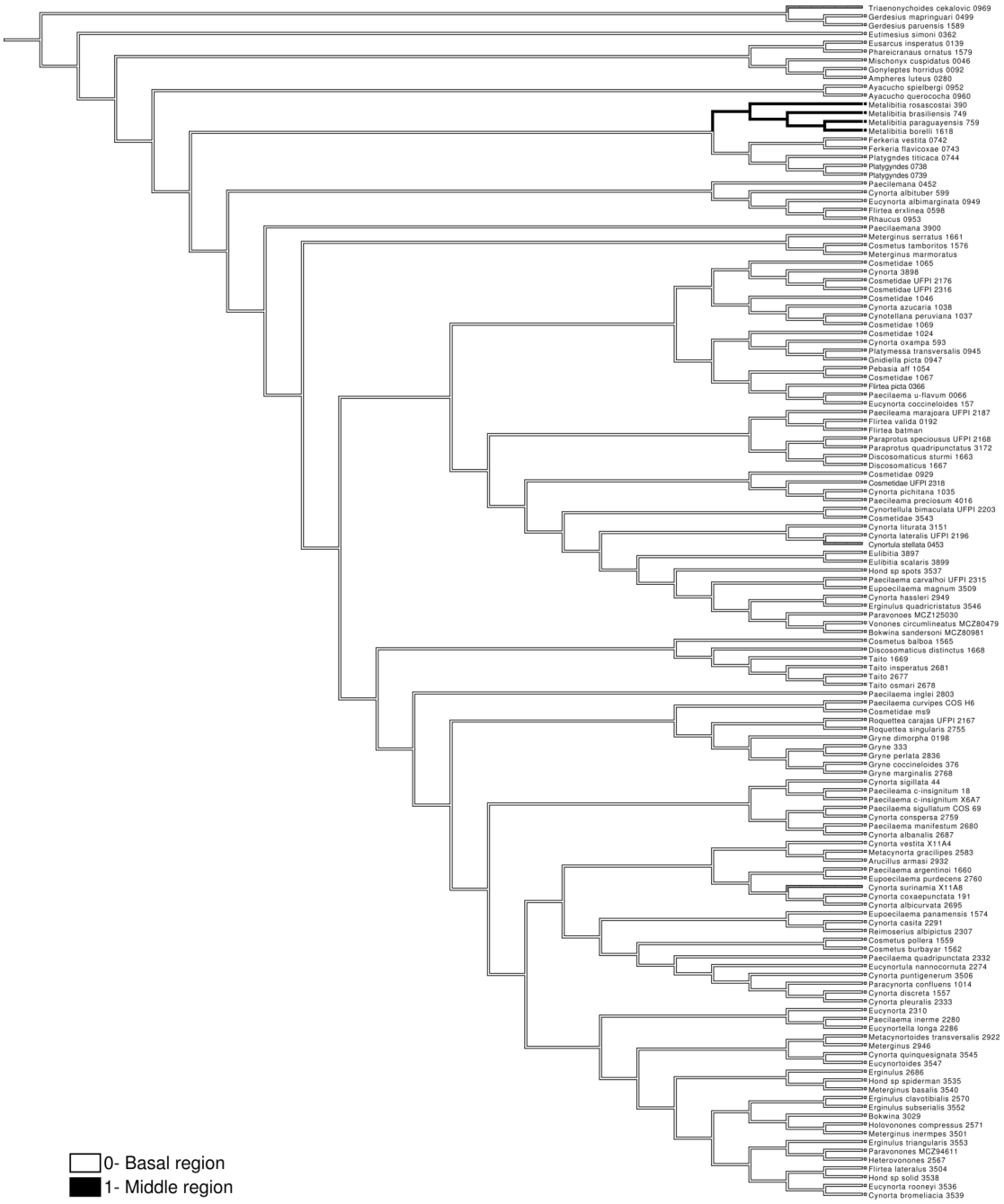


Figure 64: Character 88. Penis. Glans. Position of base of glans on ventral plate (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 1.00; RI= 1.00): Parsimony reconstruction (Unordered) [Steps: 1]

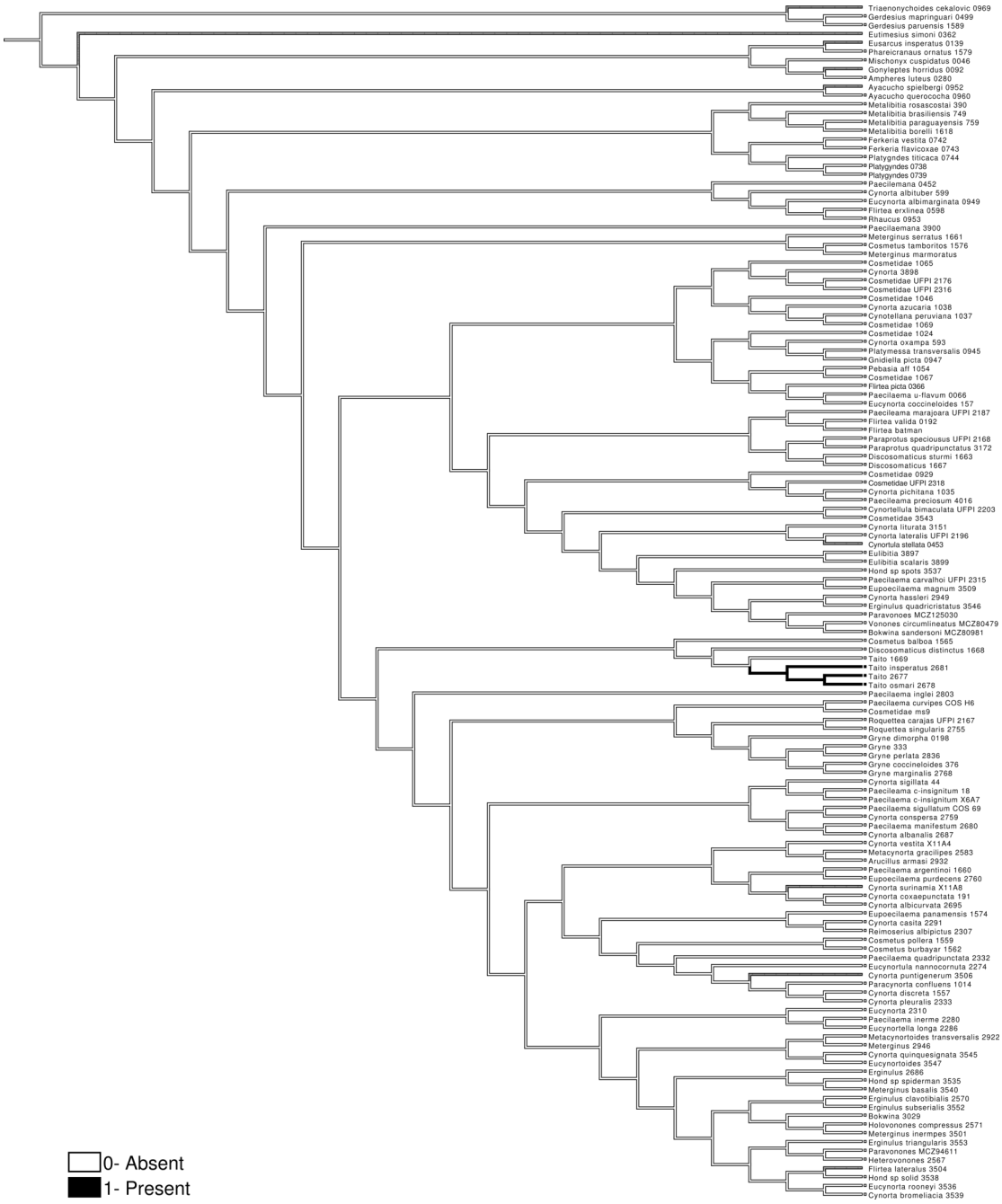


Figure 65: Character 89. Penis. Glans. Bump scales (see Kury & Baros 2014) (CI= 1.00; RI= 1.00): Parsimony reconstruction (Unordered) [Steps: 1]

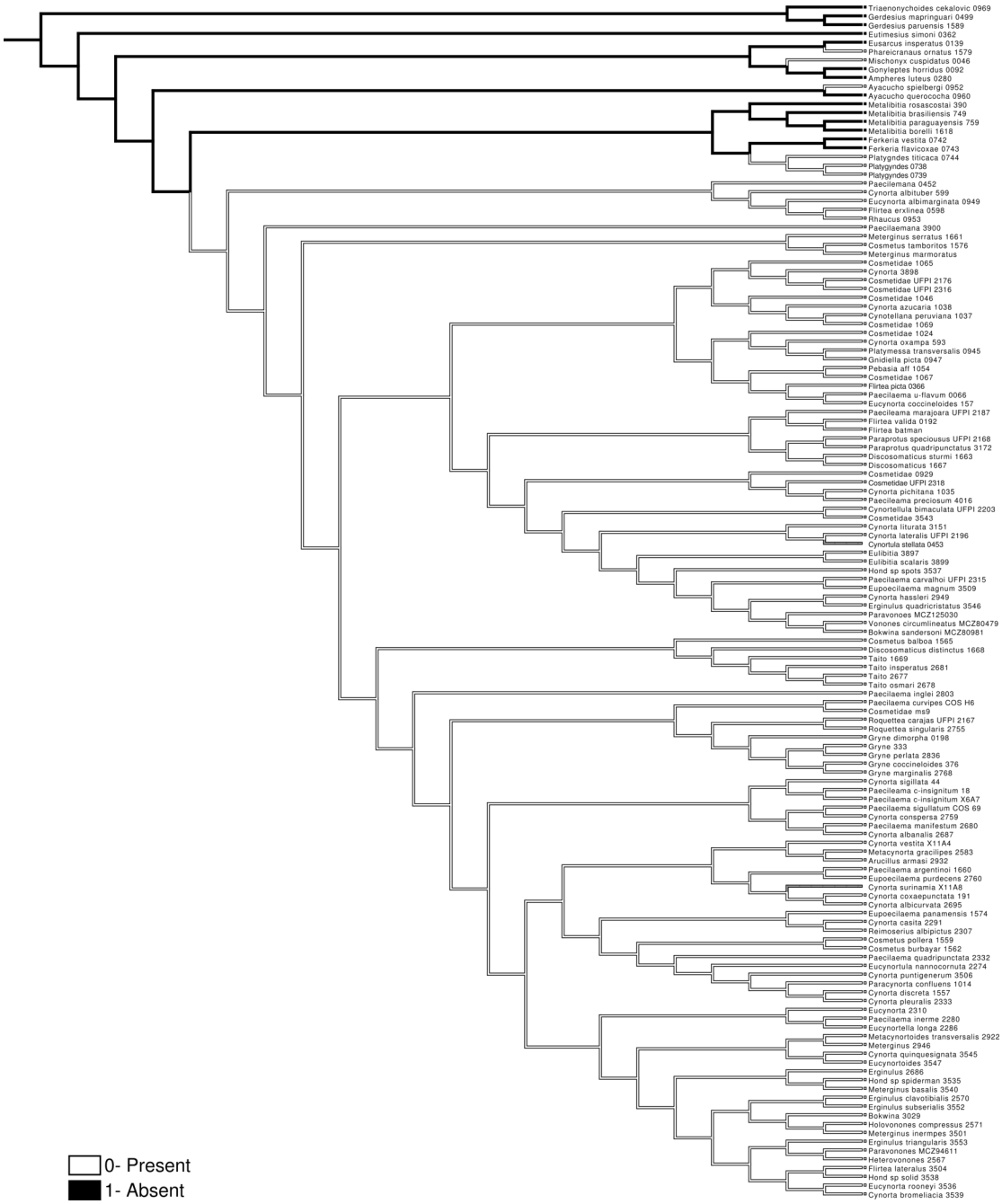


Figure 66: Character 90. Penis. Dorsal process of glans (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.20; RI= 0.692): Parsimony reconstruction (Unordered) [Steps: 5]

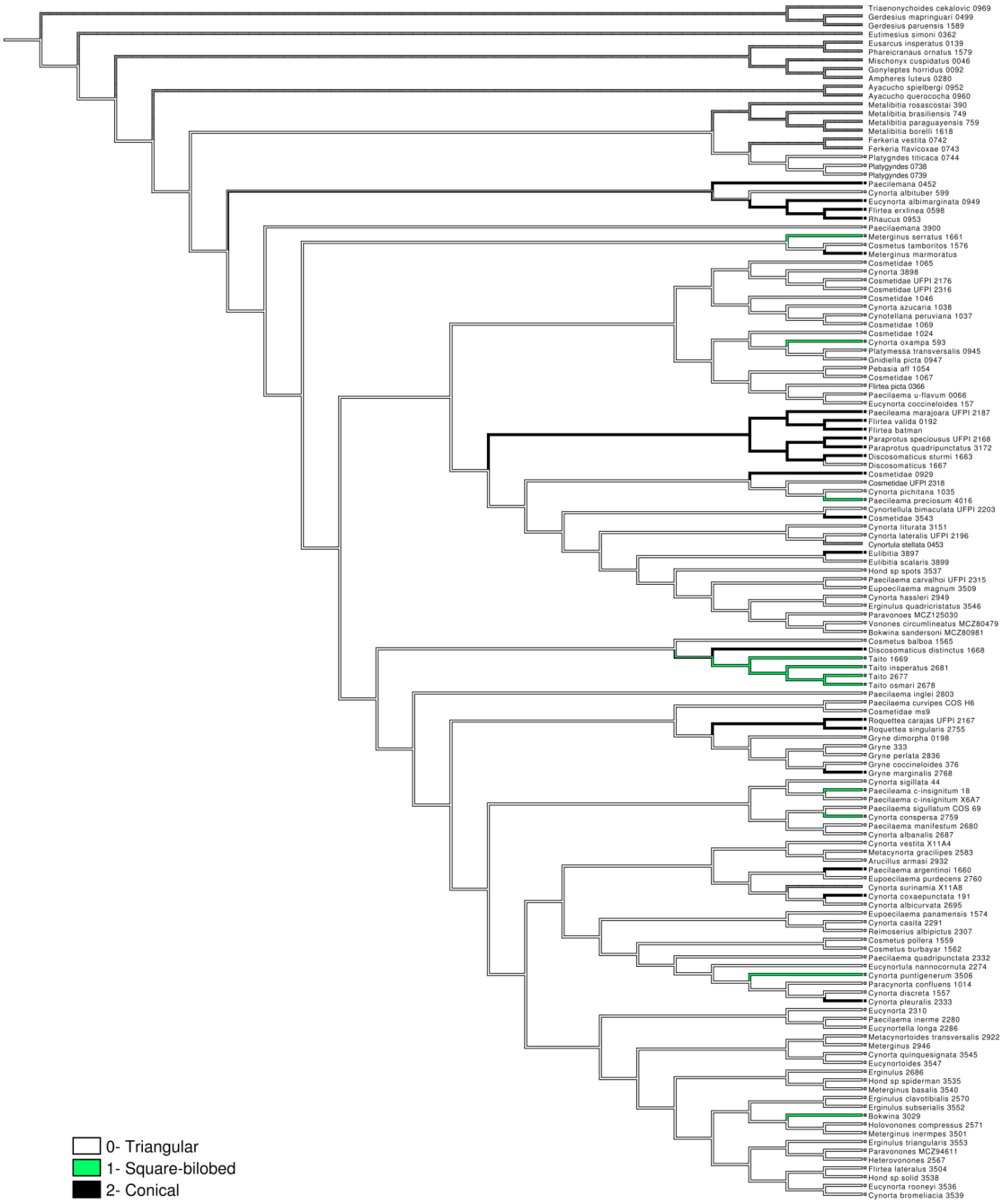


Figure 67: Character 91. Penis. Dorsal process. Shape (modified from Kury & Barros, 2014; Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.091; RI= 0.333): Parsimony reconstruction (Unordered) [Steps: 22]

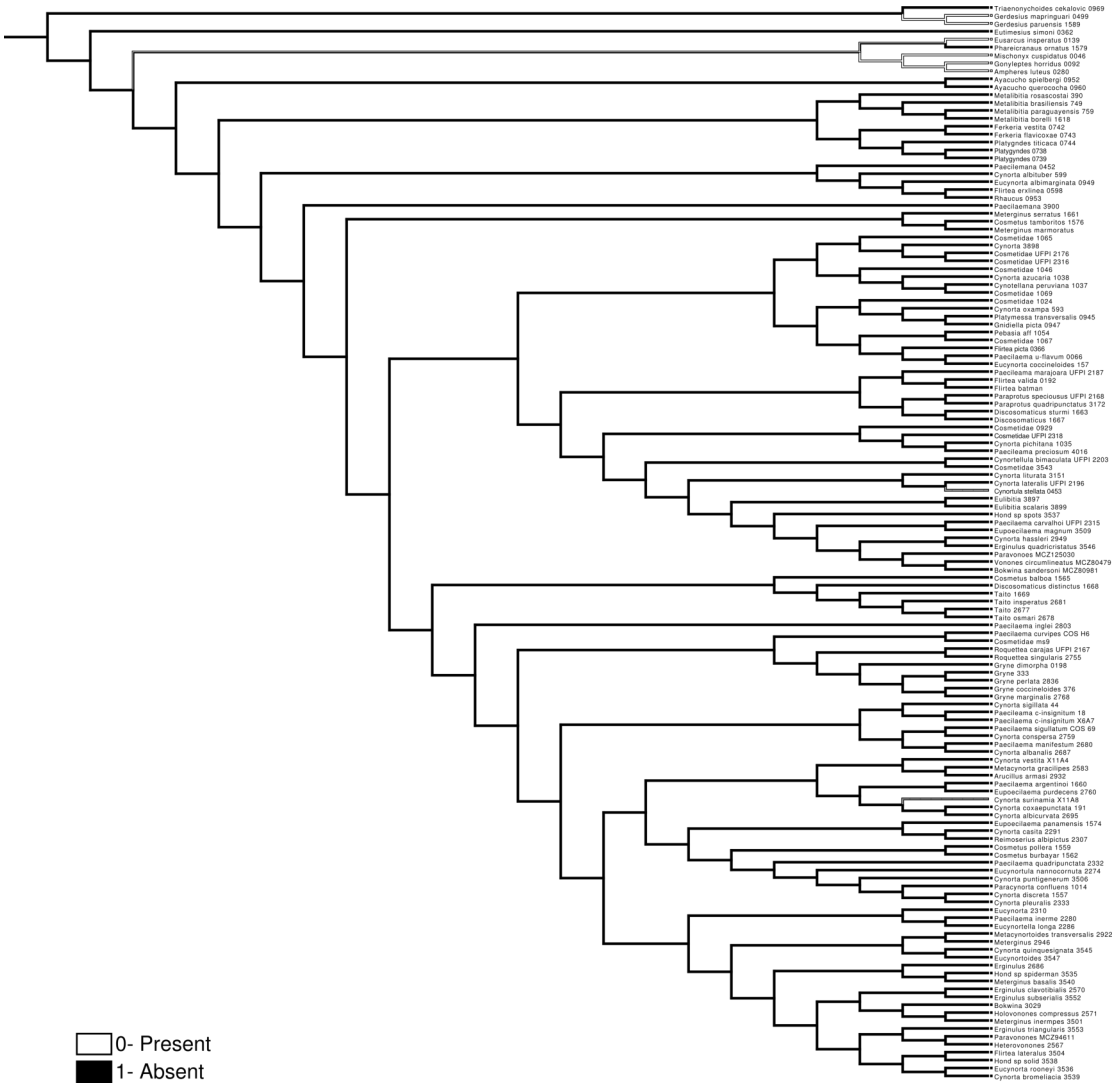


Figure 68: Character 92. Penis. Ventral process of stylus (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017) (CI= 0.333; RI= 0.60): Parsimony reconstruction (Unordered) [Steps: 3]

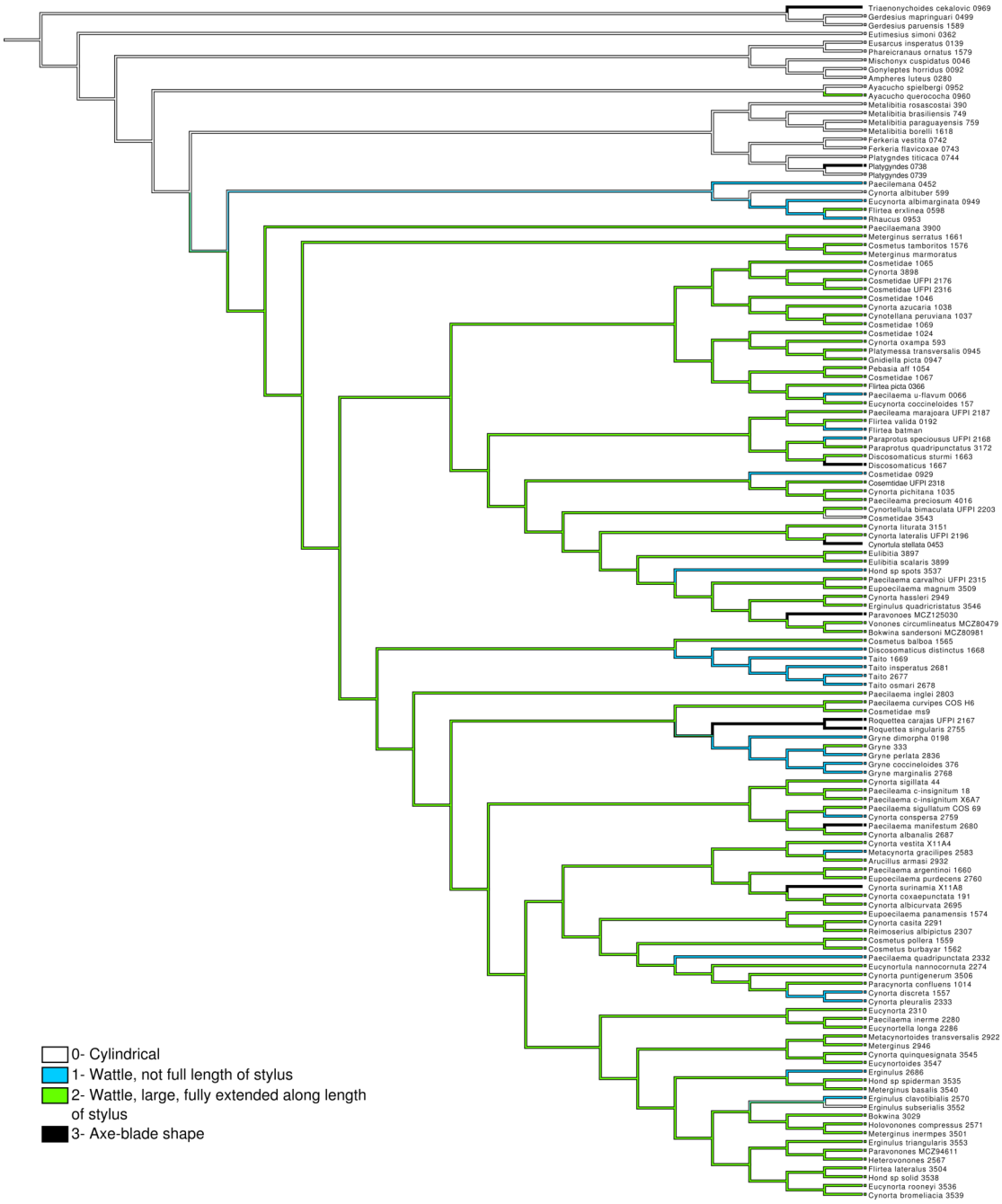


Figure 69: Character 94. Penis. Stylus. Apex Shape (CI= 0.115; RI= 0.511): Parsimony reconstruction (Unordered) [Steps: 26]

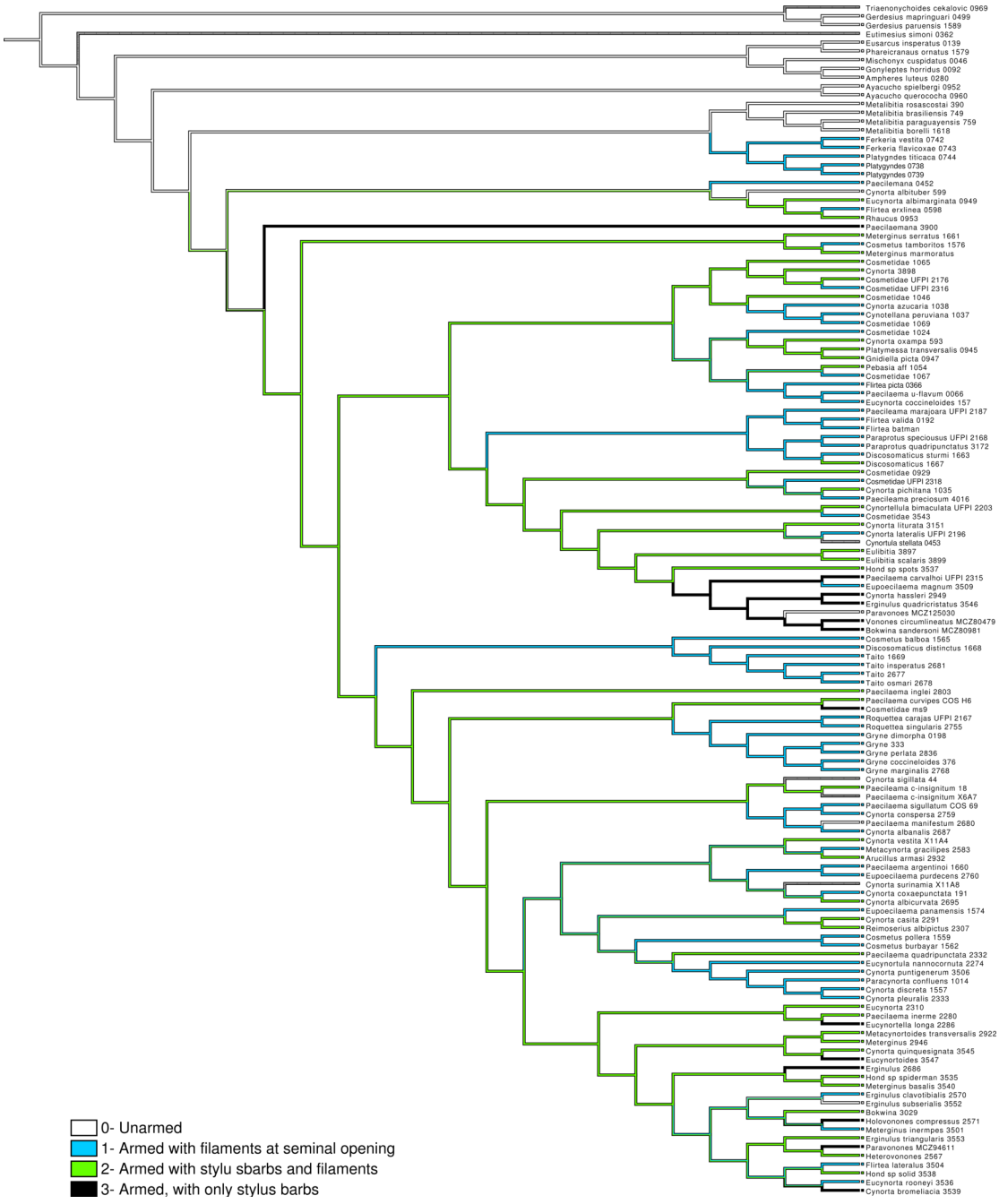


Figure 70: Character 95. Penis. Stylus. Apex ornamentation (modified from Coronato-Ribeiro & Pinto-da-Rocha 2017 and Medrano Kury 2018) (CI= 0.0698; RI= 0.403): Parsimony reconstruction (Unordered) [Steps: 43]

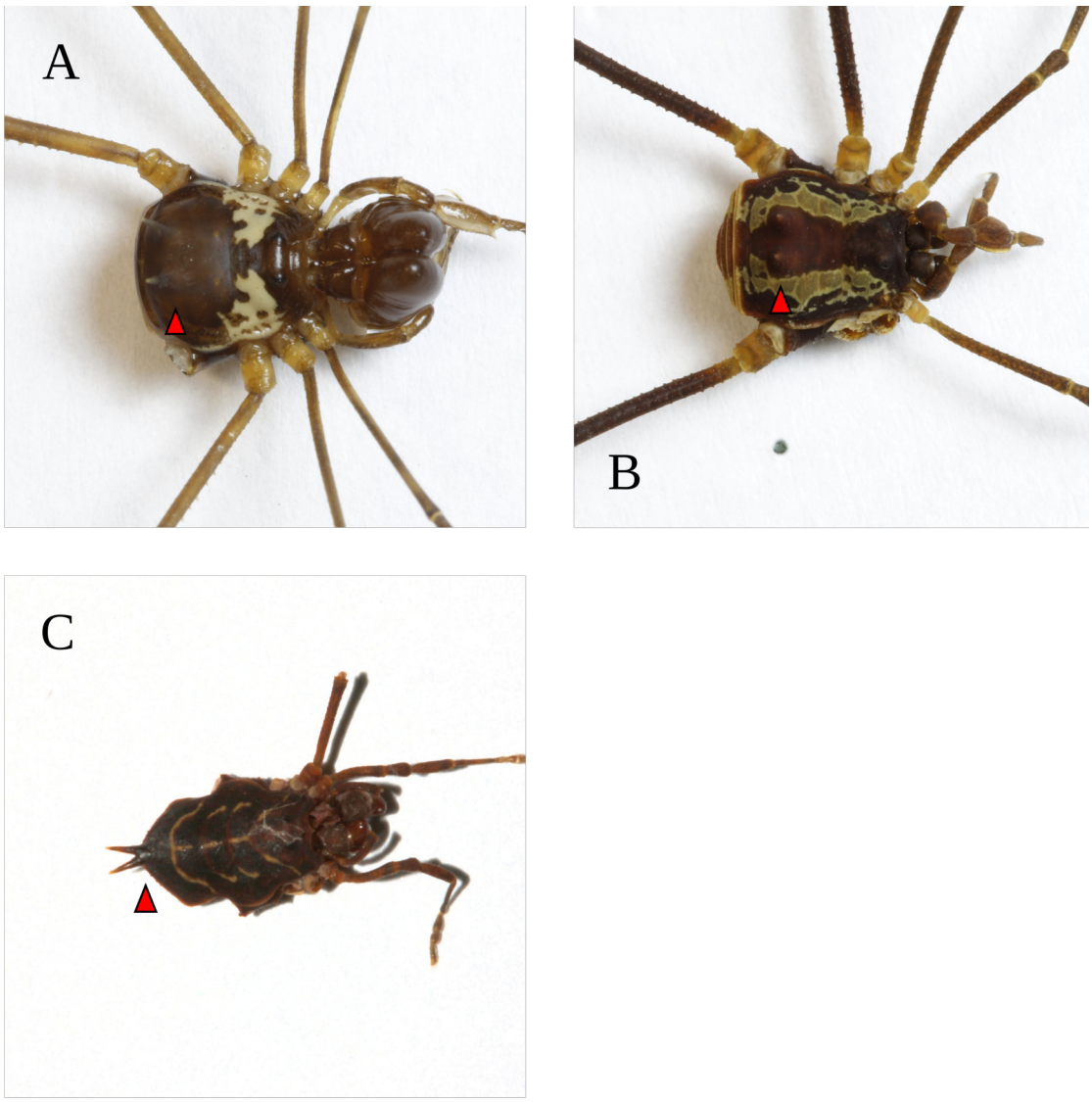


Figure 71: Dorsal images demonstrating DS armature: **A.** *Meterginus serratus*, red arrow indicates smooth base of Area III spines; **B.** *M. marmoratus*, red arrow indicates granulated base of Area III spines; **C.** *Arucillus armasi*, red arrow indicates Area IV spines.

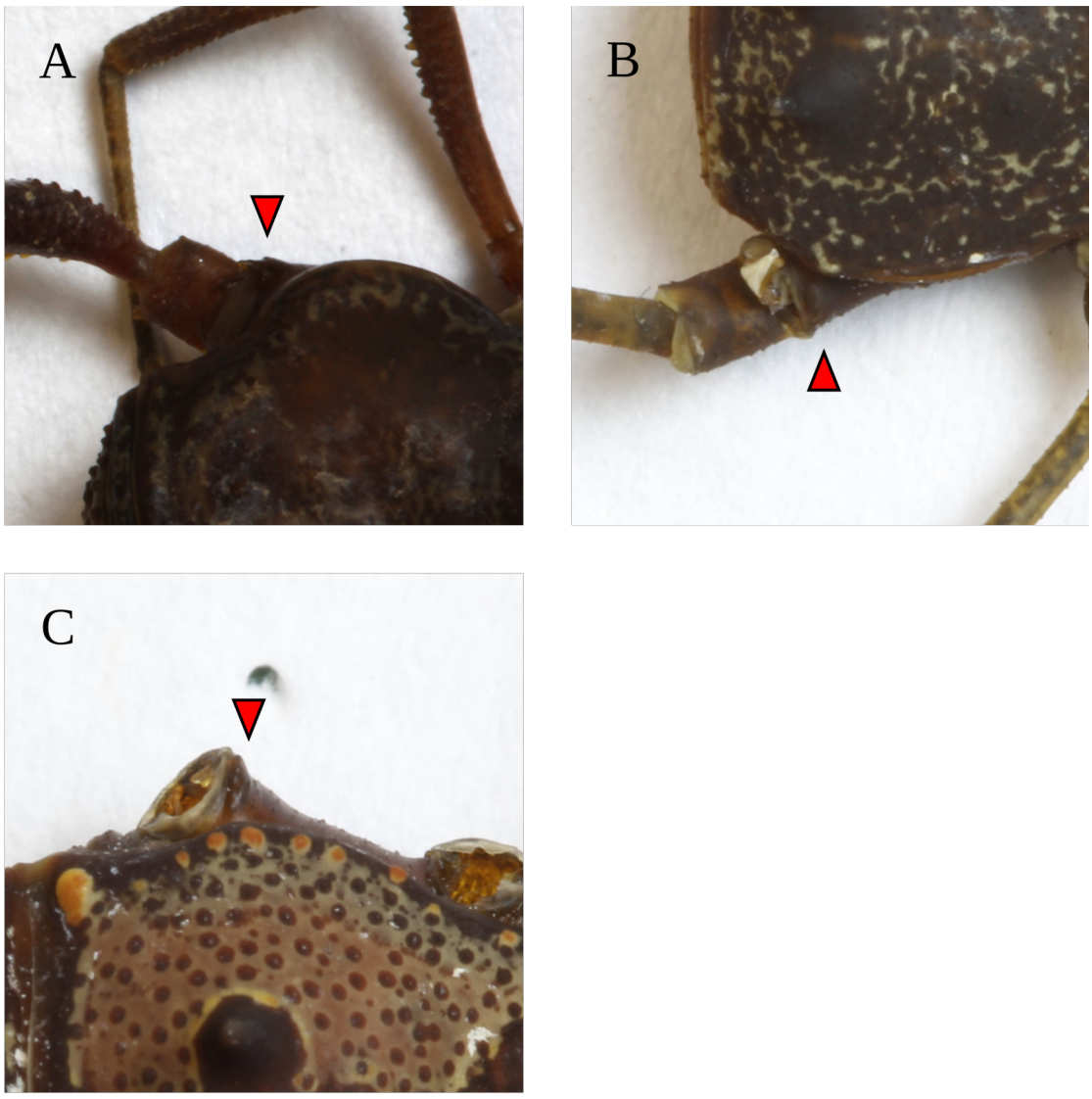


Figure 72: Dorsal images of coxae IV demonstrating forms of prolateral dorsal armature. Red arrows point to armature: **A.** *Erginulus subserialis*; **B.** *Gryne coccineloides*; **C.** *G. dimorpha*.

Figure 73: Leg IV: **A.** *Holovonones compressus*, ventral view; **B.** *Heterovonones* sp. 2567, ventral view; **C.** *Rhaucus* sp. 0953, retrolateral view.

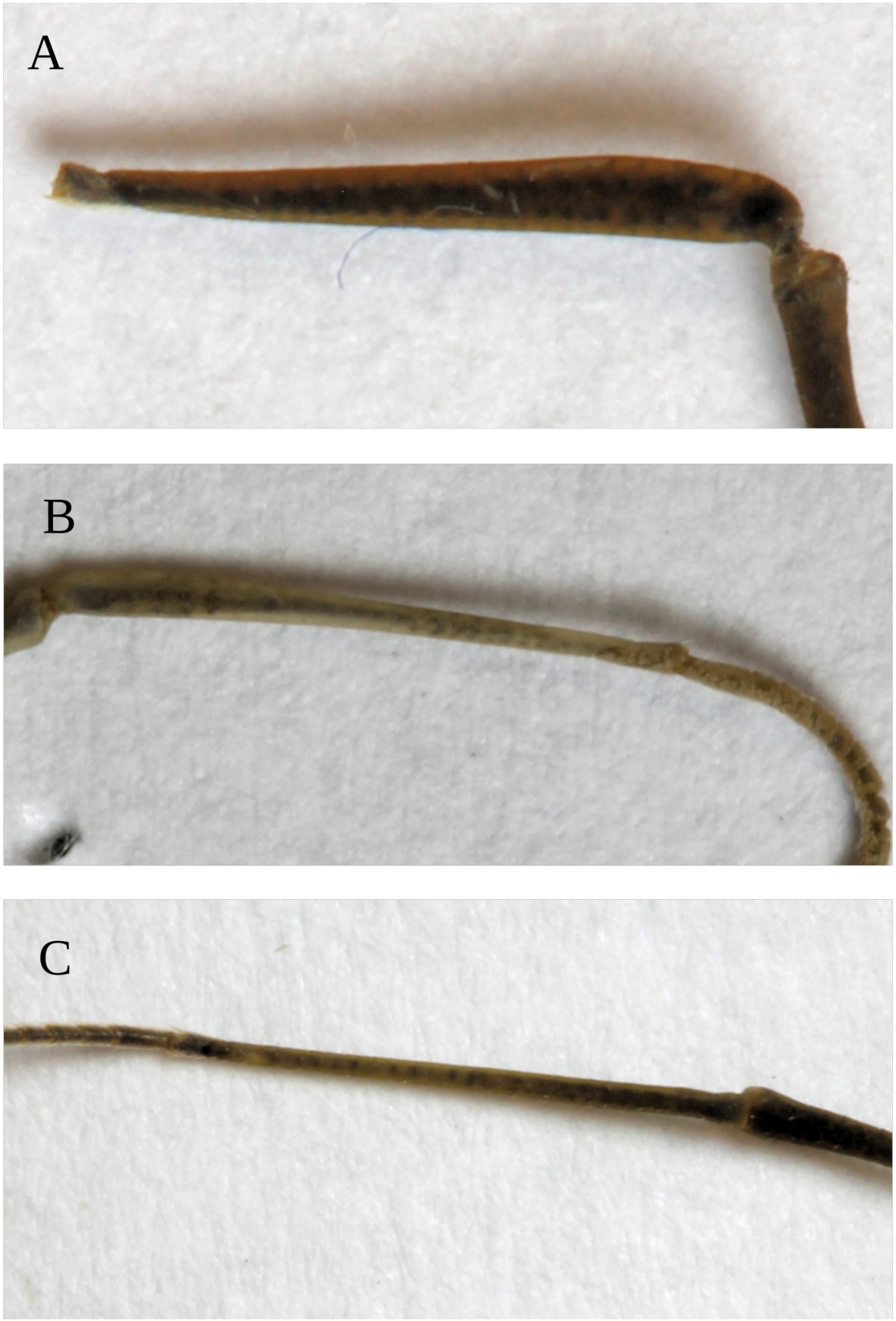


Figure 74: Lateral view of metatarsi of *Gryne* species: **A.** *Gryne coccineloides*; **B.** *G. dimorpha*; **C.** *G. perlata*.

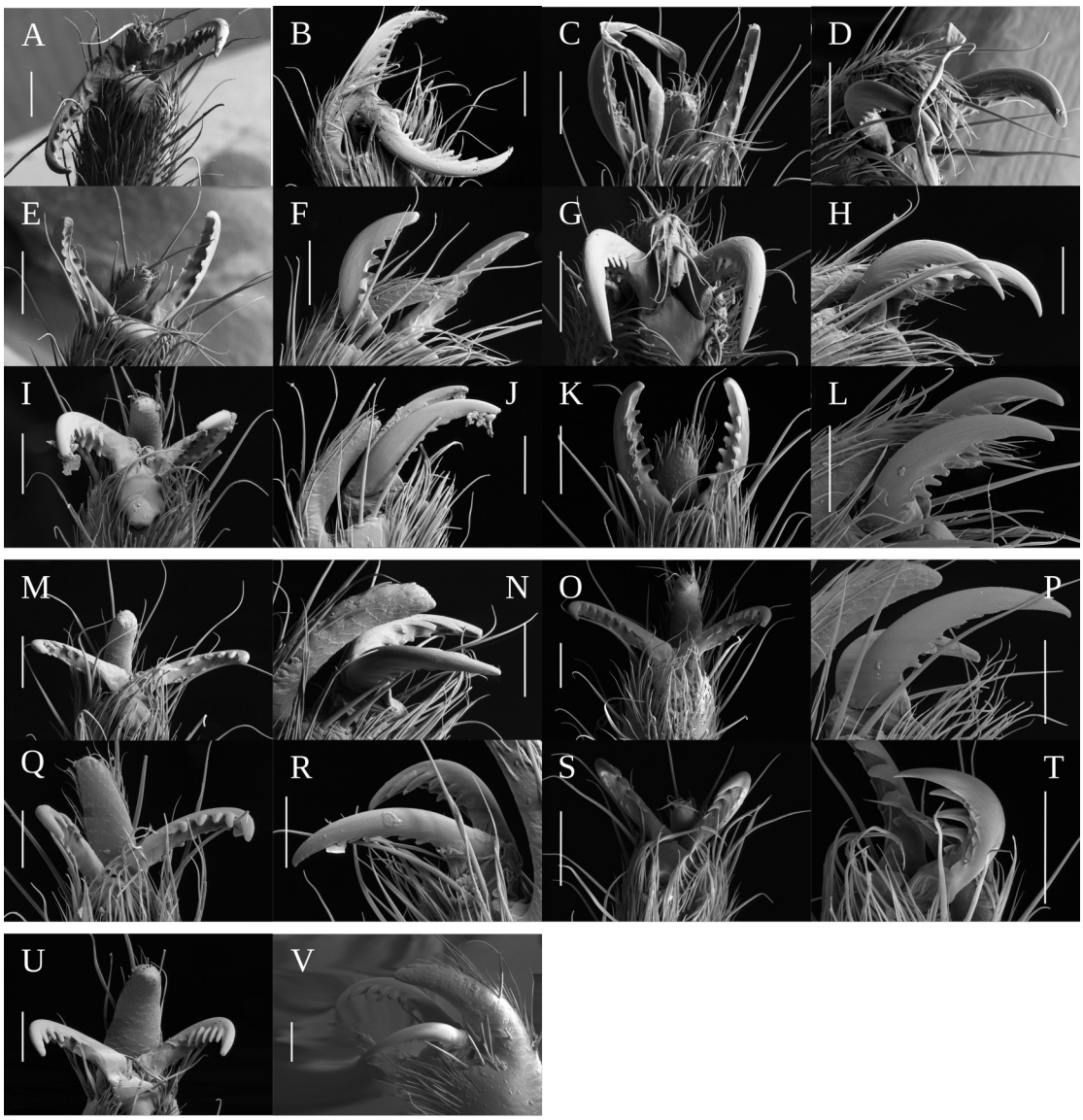


Figure 75: Leg IV tarsal claws of Discsomaticinae species (A-L, *Gryne*/*Roquettea* group, M-T, *Paraprotus*/*Discosomaticus* group, all scale bars 100 m): **A-B.** *Gryne marginalis*; **C-D.** *G. coccineloides*; **E-F.** *G. perlata*; **G-H.** *G. dimorpha*; **I-J.** *Roquettea singularis*; **K-L.** *R. carajas*; **M-N.** *Paraprotus quadripunctatus*; **O-P.** *P. speciousus*; **Q-R.** *Discosomaticus* sp. 1667; **S-T.** *D. sturmi*; **U-V.** *D. distinctus*.

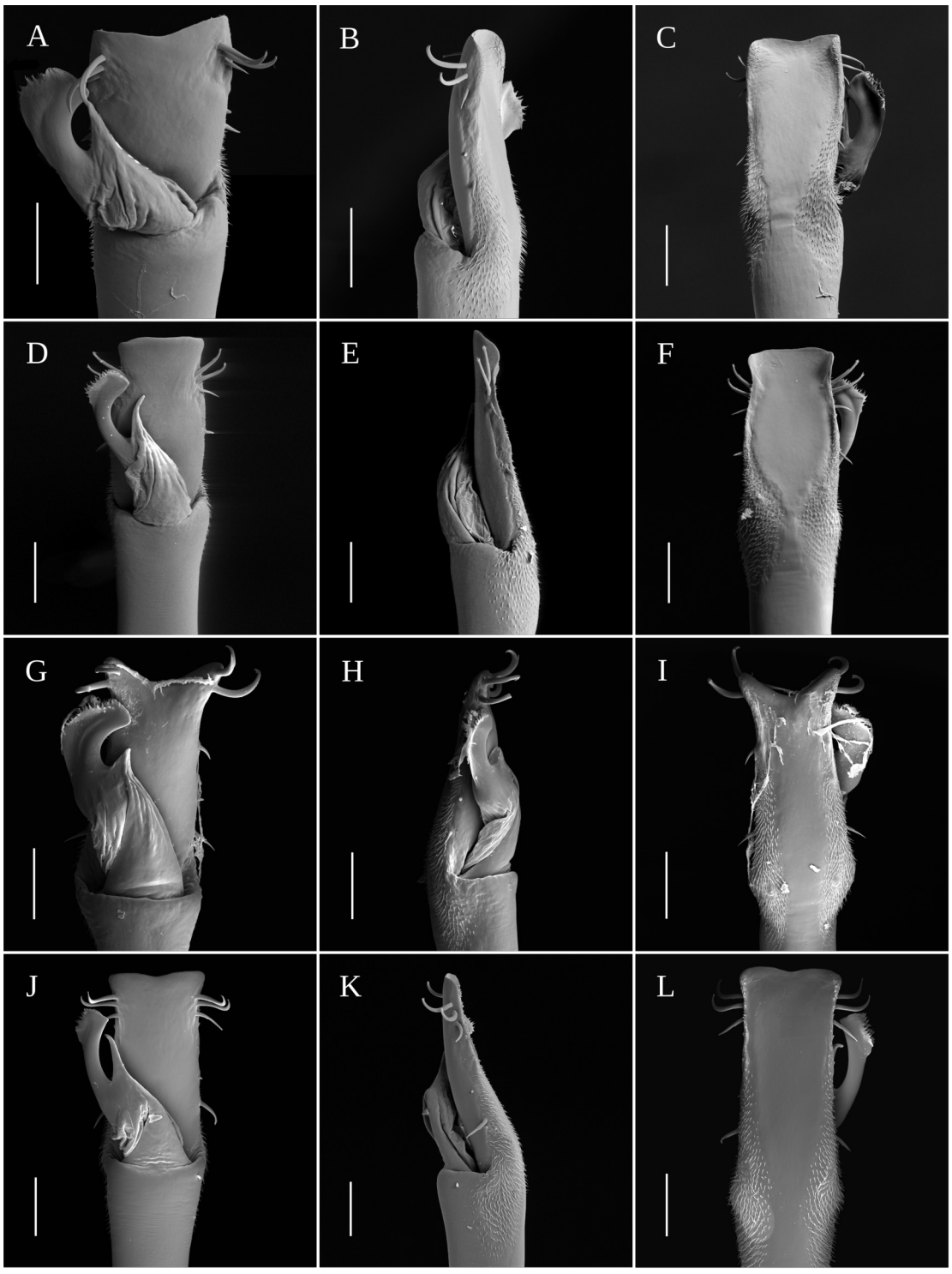


Figure 76: (clade 6, FIGS 1-5, all scale bars = 100 m): **A-C.** *Paraprotus quadripuntatus*, **A.** dorsal, **B.** lateral, **C.** ventral; **D-F.** *P. speciousus*, **D.** dorsal, **E.** lateral, **F.** ventral; **G-I.** *Discosomaticus sturmi*, **G.** dorsal, **H.** lateral, **I.** ventral; **J-L.** *Discosomaticus* sp. 1667, **J.** dorsal, **K.** lateral, **L.** ventral.

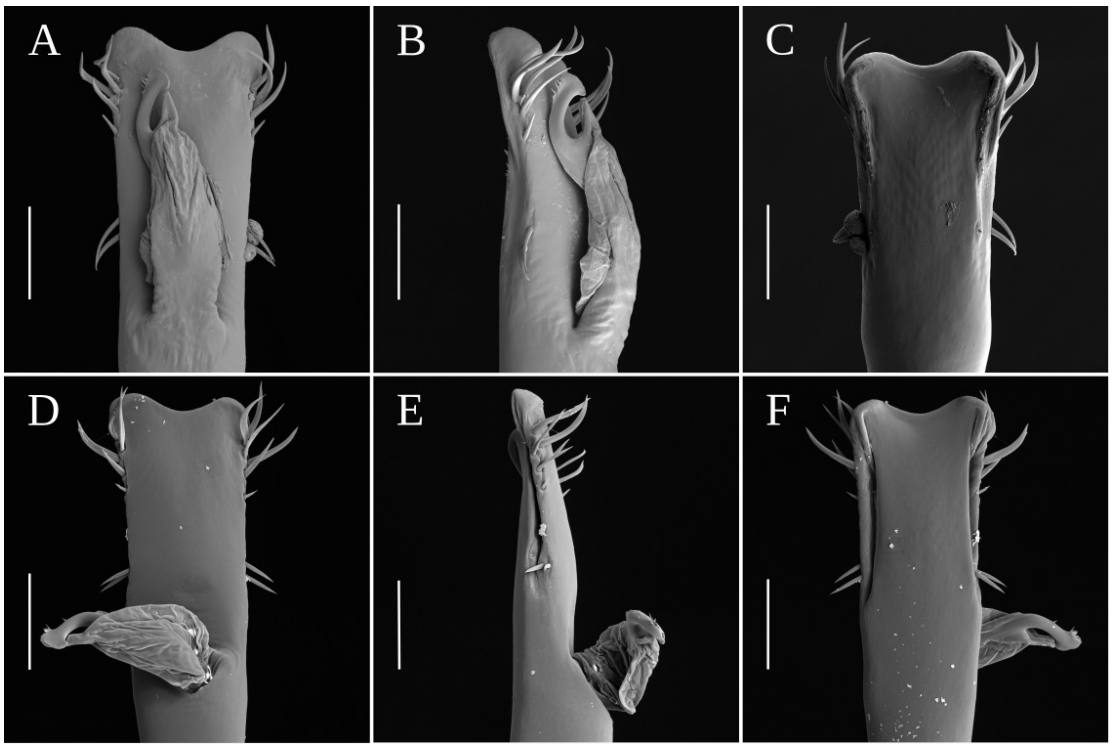


Figure 77: (clade 10, FIGS 1-5, all scale bars = 100 m): **A-C.** *Roquettea singularis*, **A.** dorsal, **B.** lateral, **C.** ventral; **D-F.** *R. carajas*, **D.** dorsal, **E.** lateral, **F.** ventral.

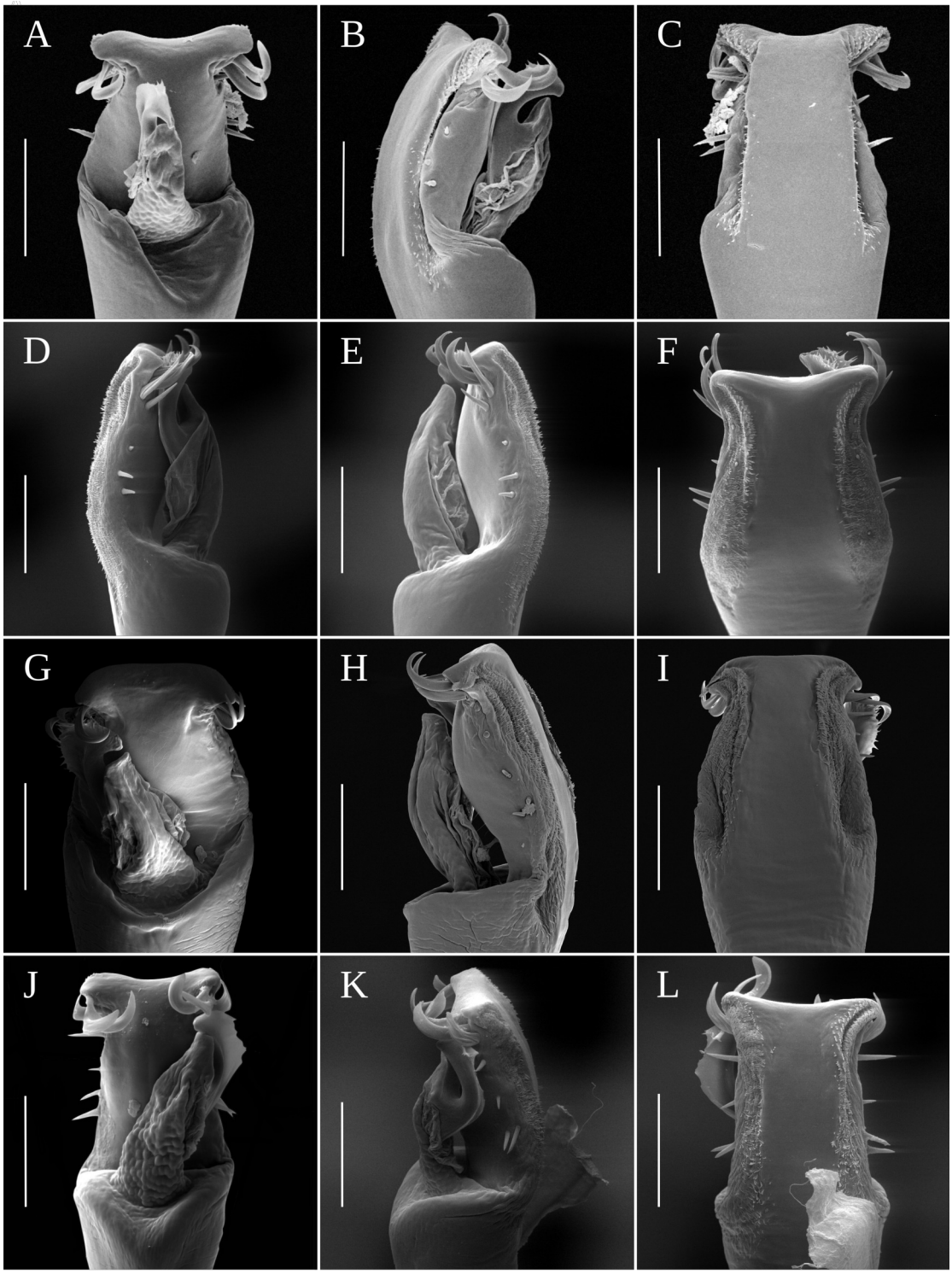


Figure 78: (clade 10, FIGS 1-5, all scale bars = 100 m): **A-C.** *Gryne coccineloides*, **A.** dorsal, **B.** lateral, **C.** ventral; **D-F.** *G. perlata*, **D.** lateral, **E.** lateral 2, **F.** ventral (dorsal view missing); **G-I.** *Gryne* sp. 0333, **G.** dorsal, **H.** lateral, **I.** ventral; **J-L.** *G. dimorpha*, **J.** dorsal, **K.** lateral, **L.** ventral.

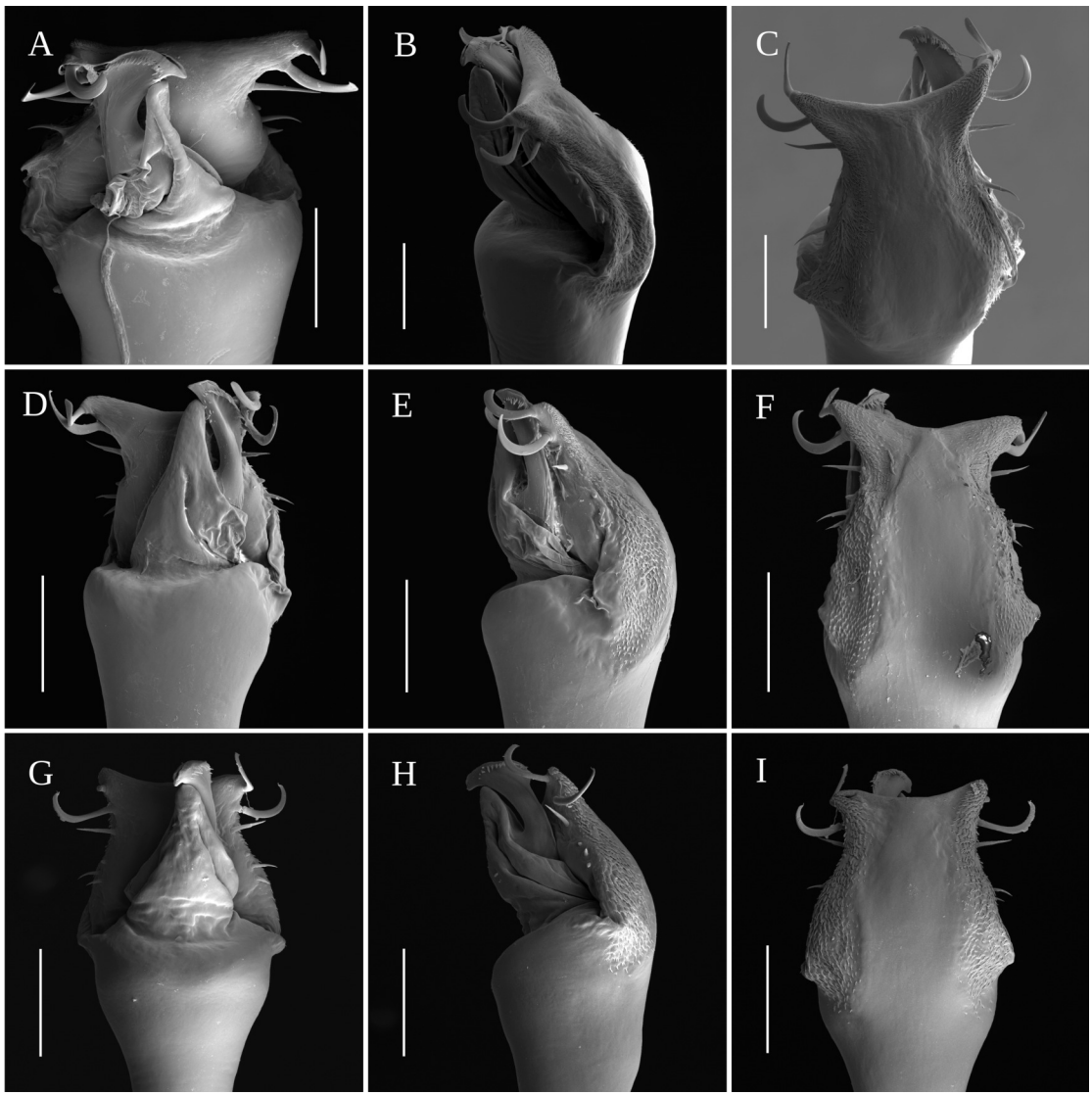


Figure 79: (clade 12, FIGS 1-5, all scale bars = 100 m): **A-C.** *Meterginus basalis*, **A.** dorsal, **B.** lateral, **C.** ventral; **D-F.** Honduran sp. 3535, **D.** dorsal, **E.** lateral, **F.** ventral; **G-I.** *Erginulus* sp 2686, **G.** dorsal, **H.** lateral, **I.** ventral.

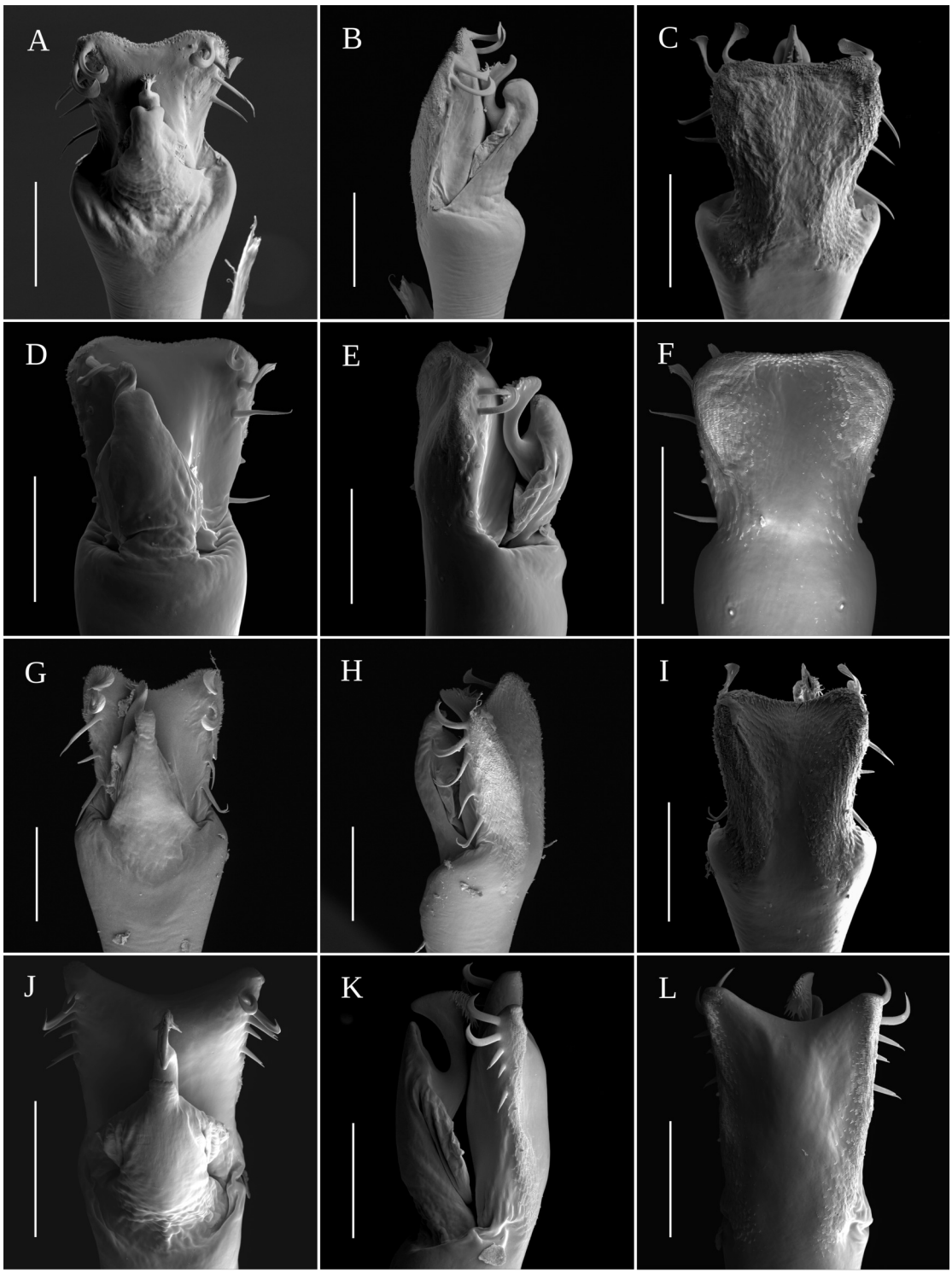


Figure 80: (clade 9, FIGS 1-5, all scale bars = 100 m): **A-C.** *Taito* sp. 1669, **A.** dorsal, **B.** lateral, **C.** ventral; **D-F.** *Taito* sp. 2677, **D.** dorsal, **E.** lateral, **F.** ventral; **G-I.** *Taito insperatus*, **G.** dorsal, **H.** lateral. **I.** ventral. **J-L.** *Discosomaticus distinctus*, **J.** dorsal, **K.** lateral, **I.** ventral.

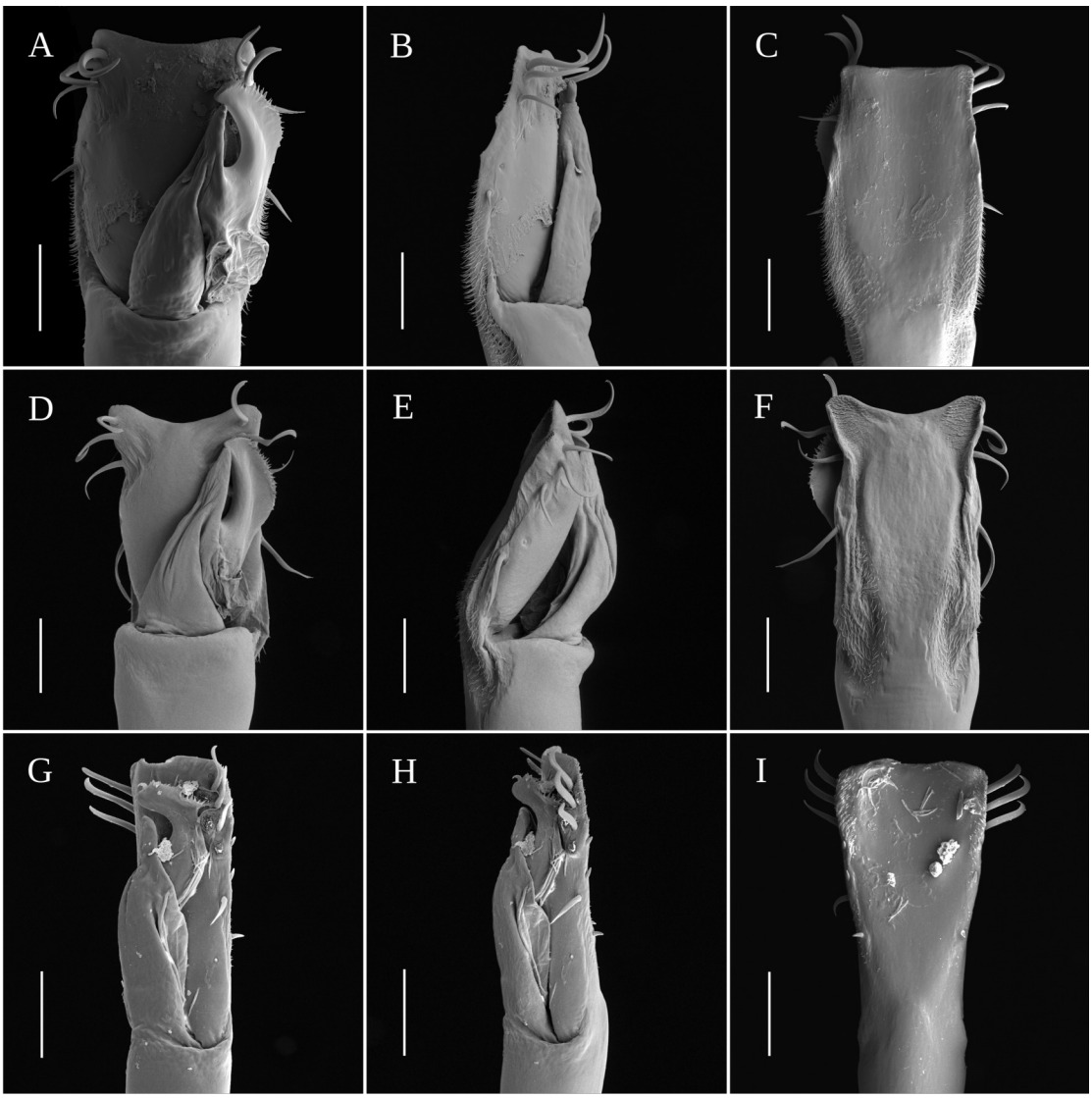


Figure 81: (clade 6, FIGS 1-5, all scale bars = 100 m): **A-C.** *Flirtea valida*, **A.** dorsal, **B.** lateral, **C.** ventral; **D-F.** *Paecileama marajoara*, **D.** dorsal, **E.** lateral, **F.** ventral; **G-I.** *F. batman*, **G.** dorsal, **H.** lateral, **I.** ventral.

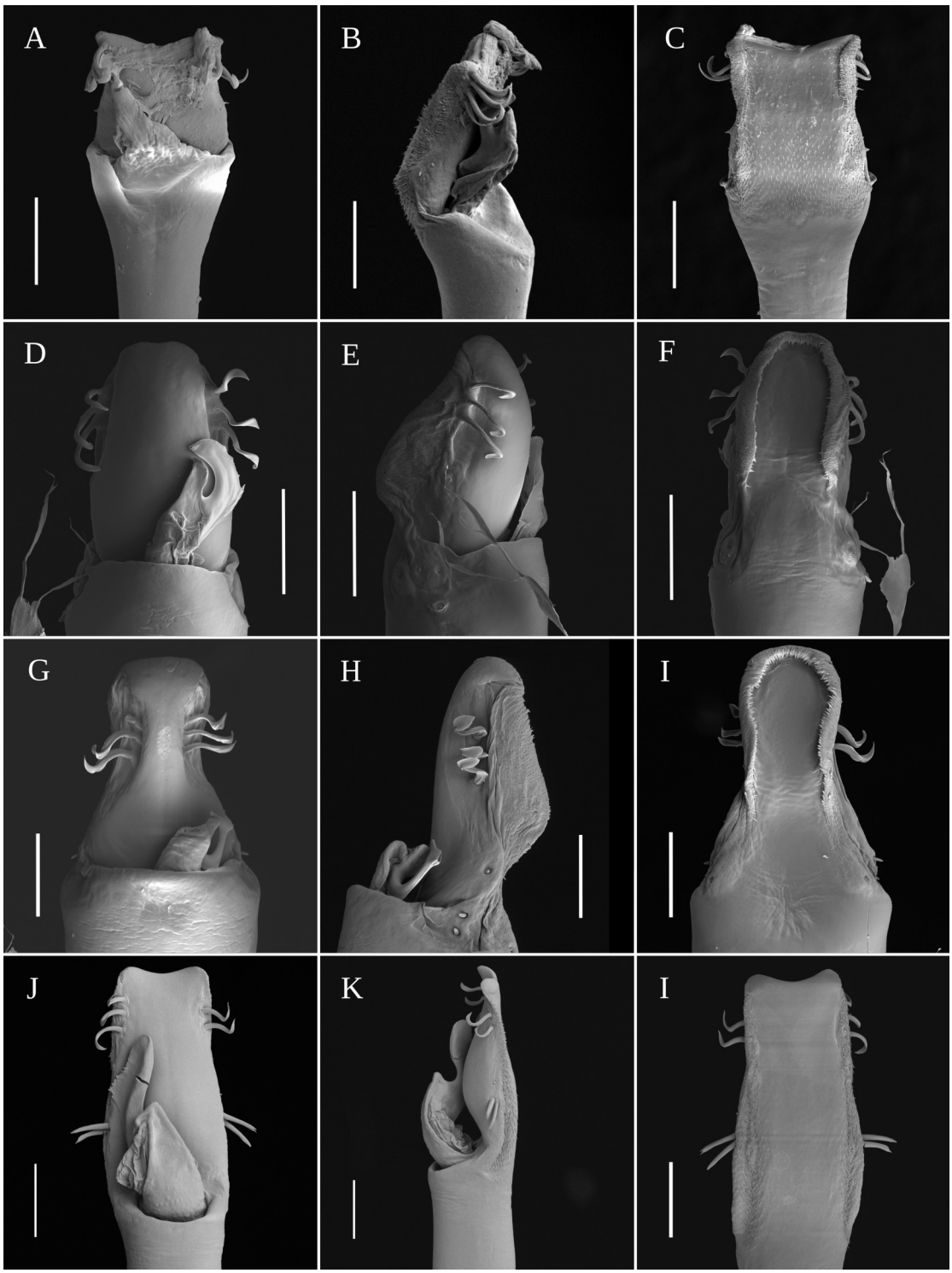


Figure 82: (clade 11, FIGS 1-5, all scale bars = 100 m): **A-C.** C*Cynorta conspersa*, **A.** dorsal, **B.** lateral, **C.** ventral; **D-F.** *C. albanis*, **D.** dorsal, **E.** lateral, **F.** venrtral; **G-I.** *Paecileama manifestum*, **G.** dorsal, **H.** lateral, **I.** ventral; **J-L.** *P. sigillatum*, **J.** dorsal, **K.** lateral, **L.** ventral.

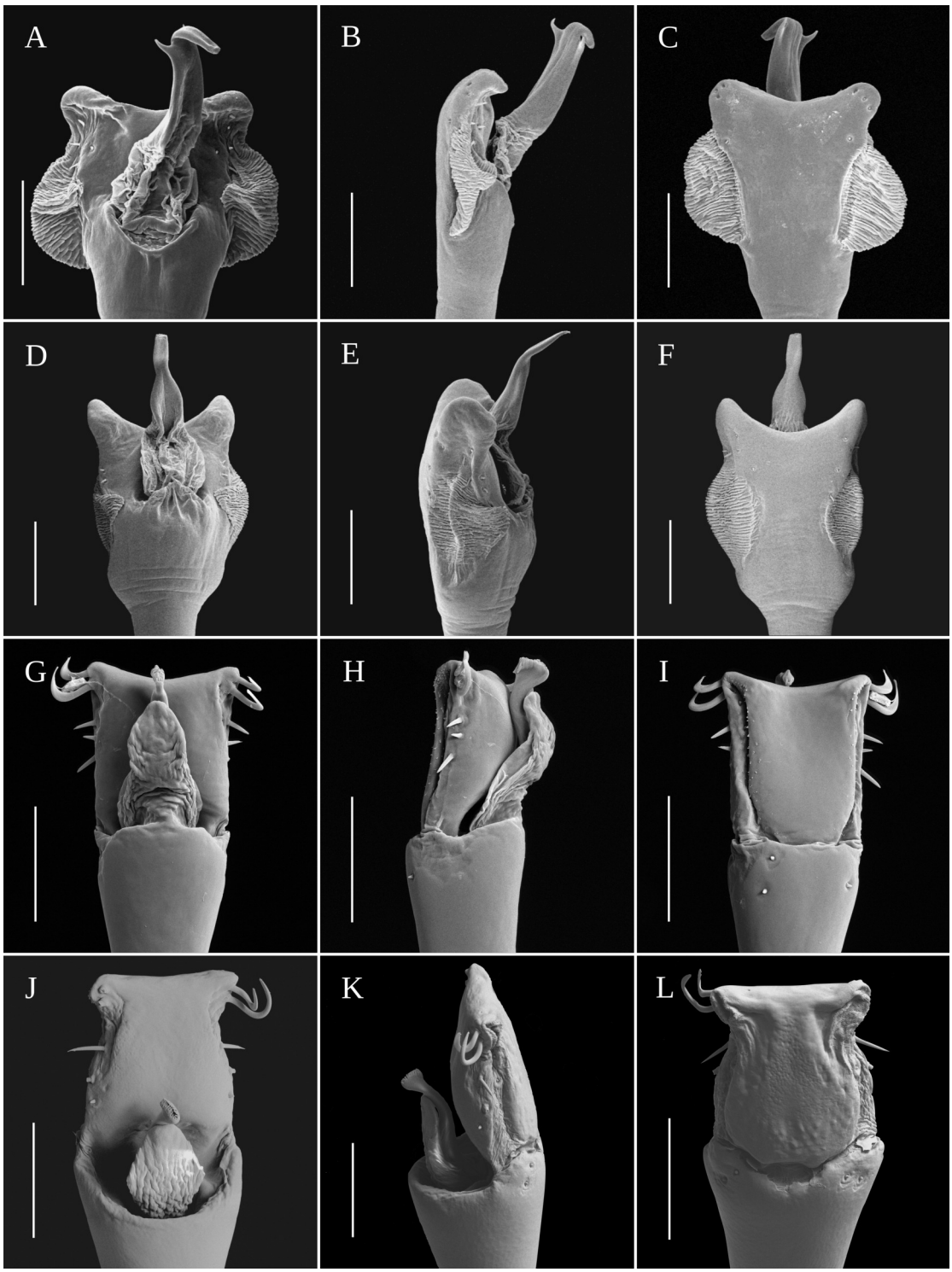


Figure 83: (clade 1, FIGS 1-5, all scale bars = 100 m): **A-C.** *Metalibitia paraguayensis*, **A.** dorsal, **B.** lateral, **C.** vetnral; **D-F.** *M. borelli*, **D.** dorsal, **E.** lateral, **F.** ventral; **G-I.** *Platygyndes* sp. 0738, **G.** dorsal, **H.** lateral, **I.** ventral;**J-L.** *Platygyndes* sp. 0739, **J.** dorsal, **K.** lateral, **L.** ventral.

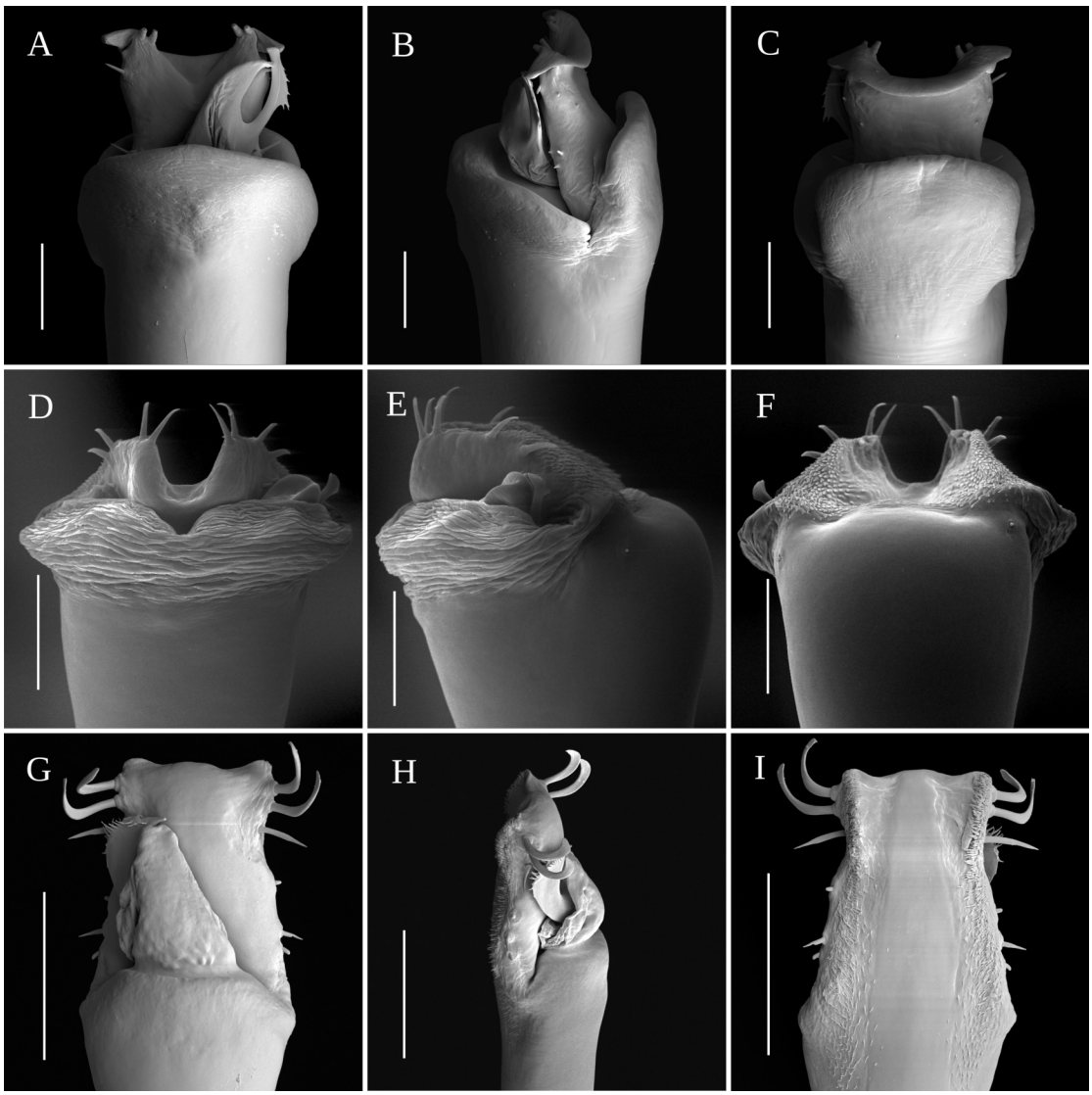


Figure 84: (clade 13, FIGS 1-5, all scale bars = 100 m): **A-C.** *Erginulus clavotibialis*, **A.** dorsal, **B.** lateral, **C.** ventral; **D-F.** *E. subserialis*, **D.** dorsal, **E.** lateral, **F.** ventral; **G-I.** *Bokwina* sp. 3029, **G.** dorsal, **H.** lateral, **I.** ventral.

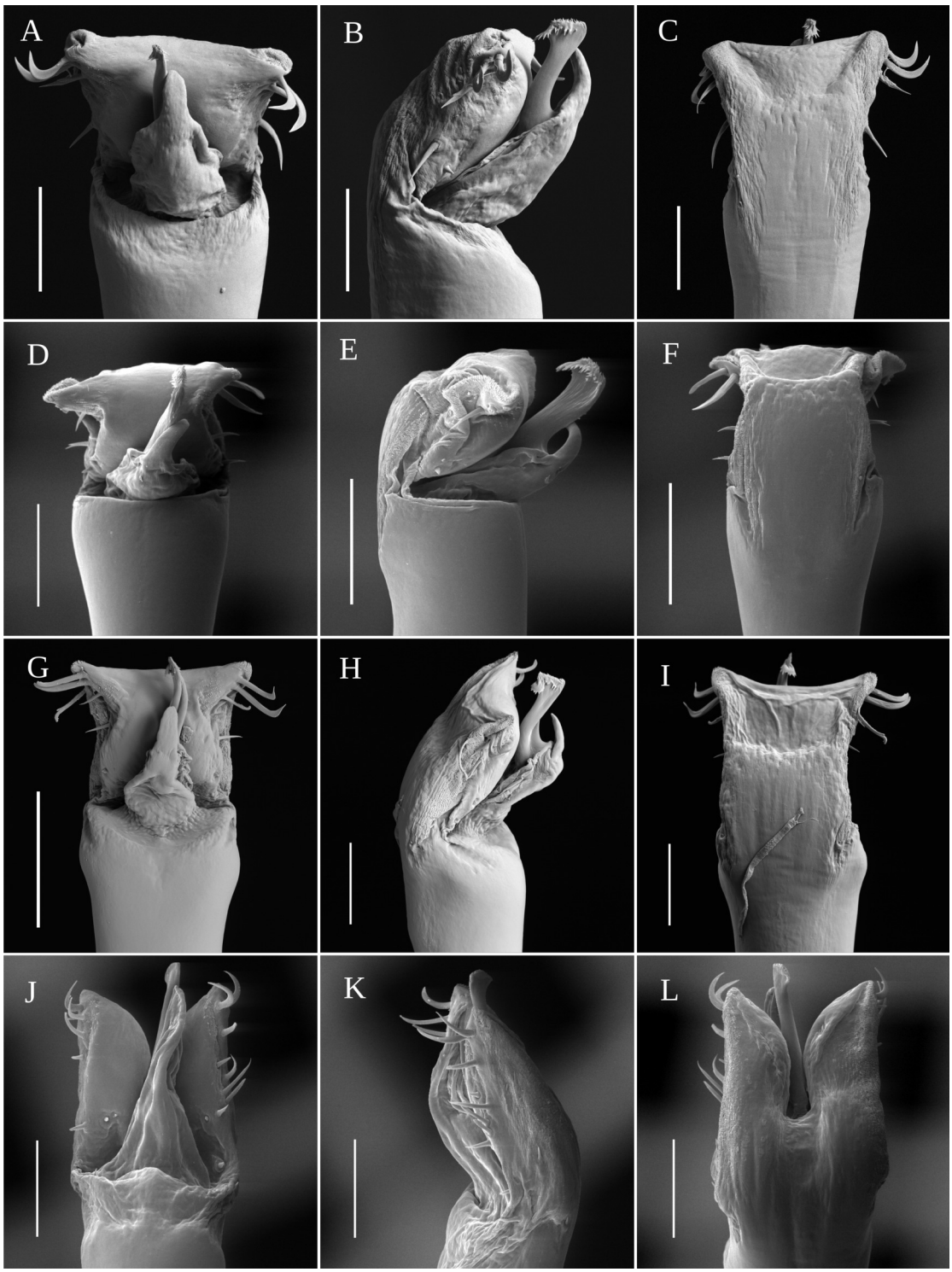


Figure 85: (clade 2, FIGS 1-5, all scale bars = 100 m): **A-C.** *Rhaucus* aff. 0953, **A.** dorsal, **B.** lateral, **C.** ventral; **D-F.** *Flirtea erxlinea*, **D.** dorsal, **E.** lateral, **F.** ventral; **G-I.** *Eucynorta albimarginata*, **G.** dorsal, **H.** lateral, **I.** ventral; **J-L.** *Cynorta albituber*, **J.** dorsal, **K.** lateral, **L.** ventral.

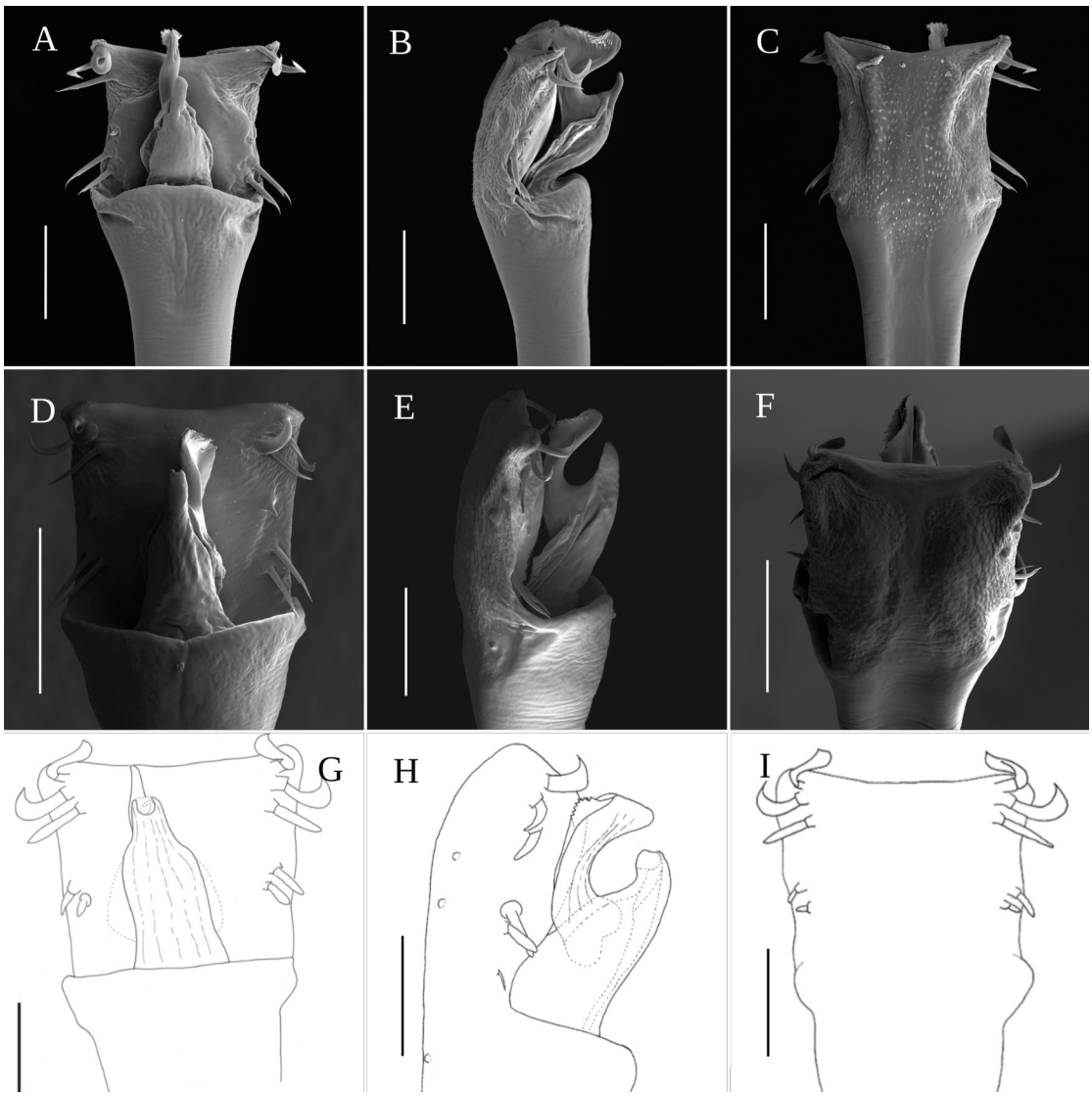


Figure 86: (clade 3, FIGS 1-5, all scale bars = 100 m): **A-C.** *Meterginus serratus*, **A.** dorsal, **B.** lateral, **C.** ventral; **D-E.** *M. marmoratus*, **D.** dorsal, **E.** lateral, **F.** ventral; G-I *Cosmetus tamboritos* (images from Coronato-Ribeiro & Pinto-da-Rocha, 2015), **G.** dorsal, **H.** lateral, **I.** ventral.

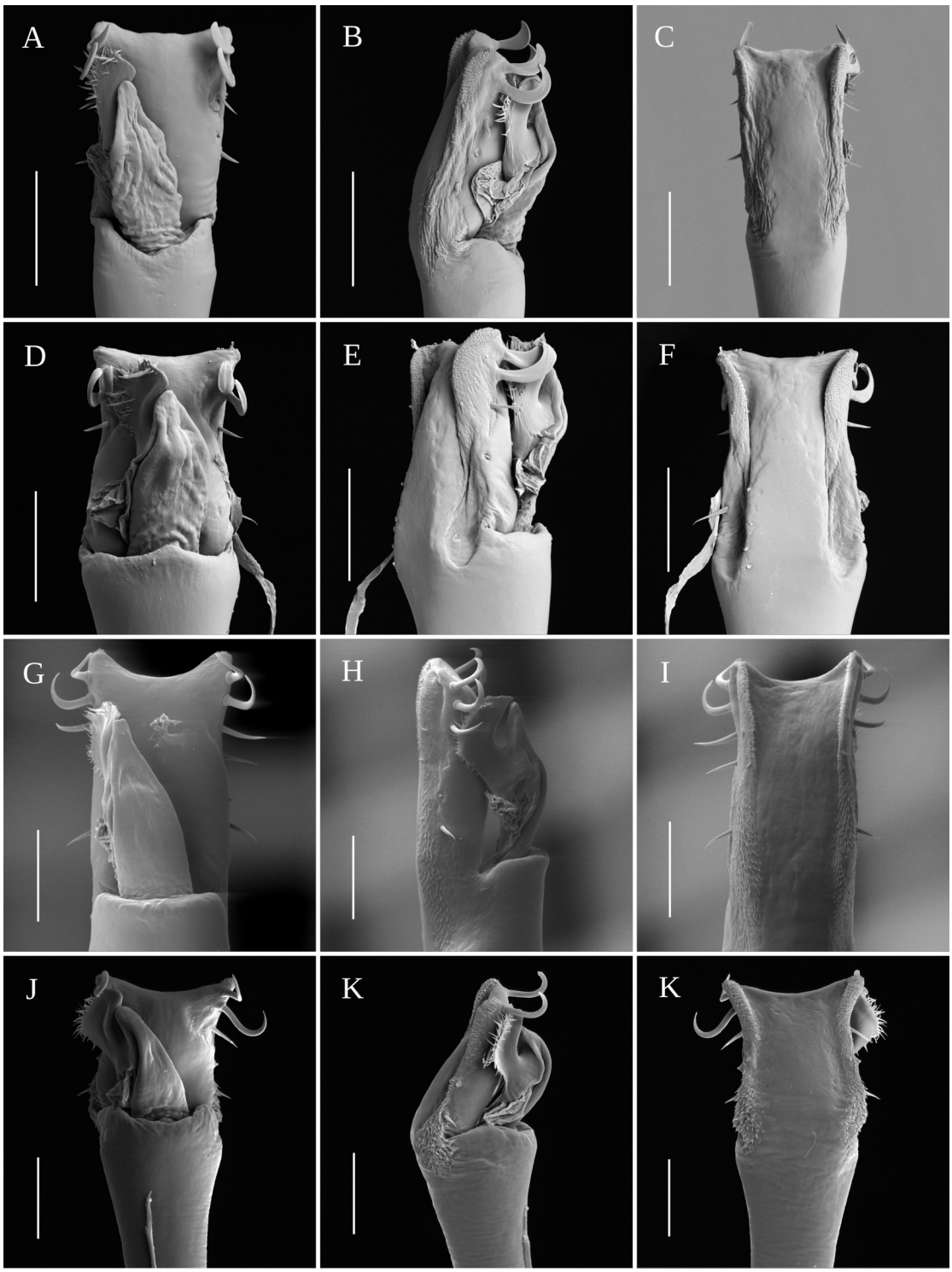


Figure 87: (clade 5, FIGS 1-5, all scale bars = 100 m): **A-C.** *Gnidiella picta*, **A.** dorsal, **B.** lateral, **C.** ventral; **D-F.** *Platymessa transversalis*, **D.** dorsal, **E.** lateral, **F.** ventral; **G-I.** *Cynorta oxampa*, **G.** dorsal, **H.** lateral, **I.** ventral; **J-L.** Cosmetidae sp. 1024, **J.** dorsal, **K.** lateral, **L.** ventral.

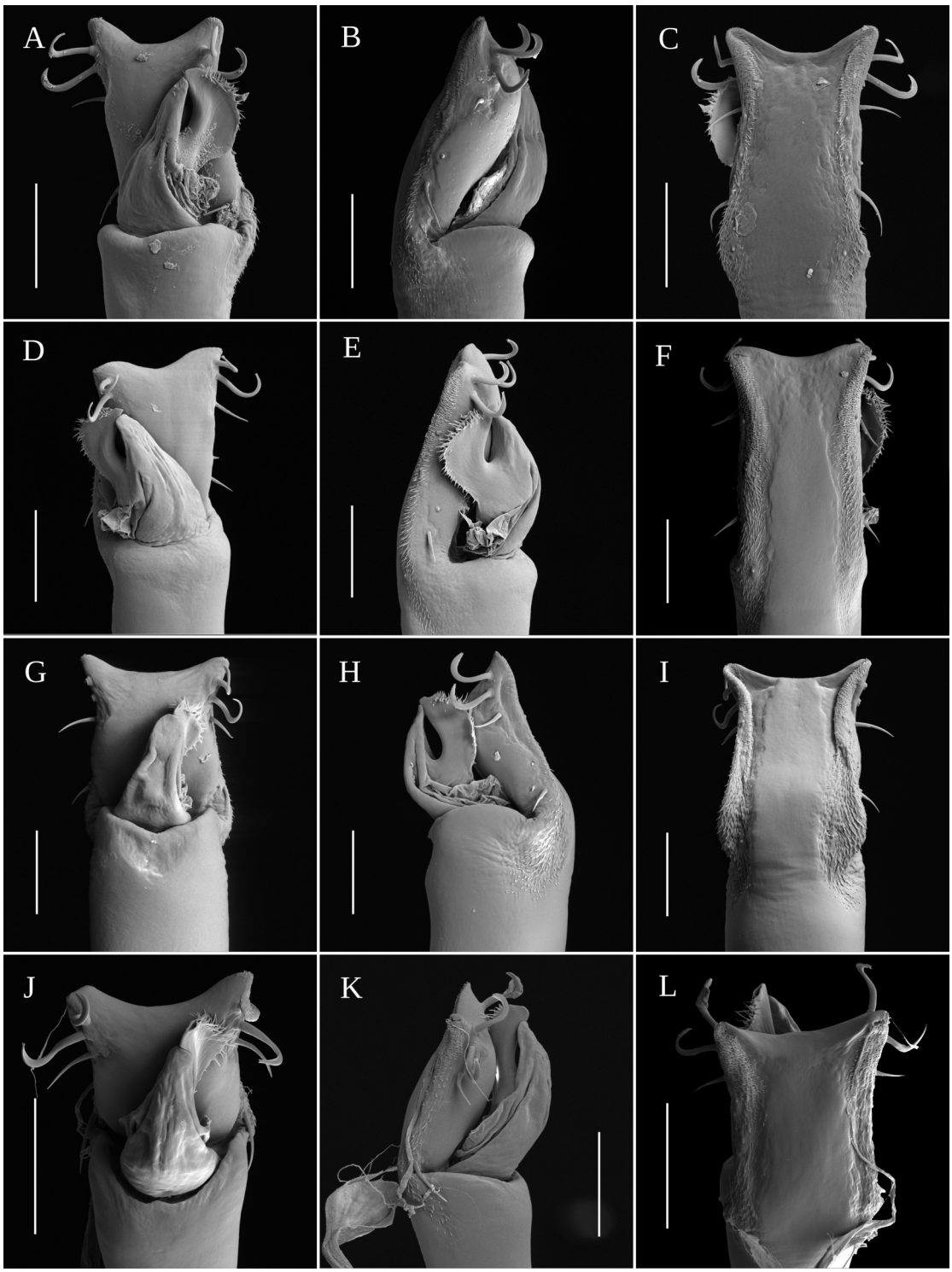


Figure 88: (all scale bars = 100 m): **A-C.** *Cynorta azucaria*, **A.** dorsal, **B.** lateral, **C.** ventral; **D-F.** Cosmetidae sp. 1046, **D.** dorsal, **E.** lateral, **F.** ventral; **G-I.** *Cynorta pichitana*, **G.** dorsal, **H.** lateral, **I.** ventral; **J-L.** Cosmetidae sp. 1069, **J.** dorsal, **K.** lateral, **L.** ventral.

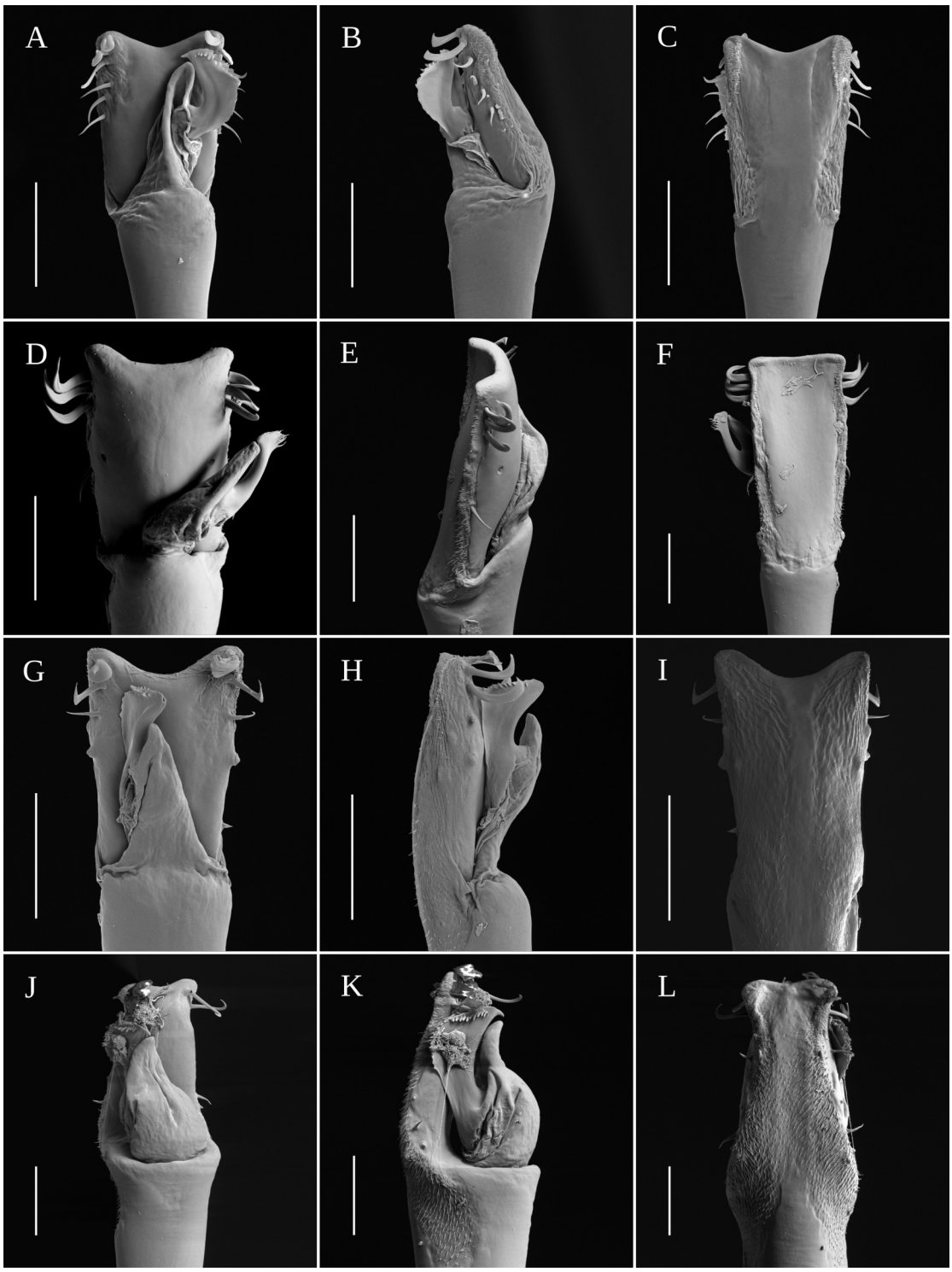


Figure 89: (all scale bars = 100 m: **A-C.** *Cynortellula bimaculata*, **A.** dorsal, **B.** lateral, **C.** ventral; **D-F.** Cosmetidae sp. 3543, **D.** dorsal, **E.** lateral, **F.** ventral; **G-I.** *Paecileama carvalhoi*, **G.** dorsal, **H.** lateral, **I.** ventral; **J-L.** *Cynorta liturata*, **J.** dorsal, **K.** lateral, **L.** ventral.

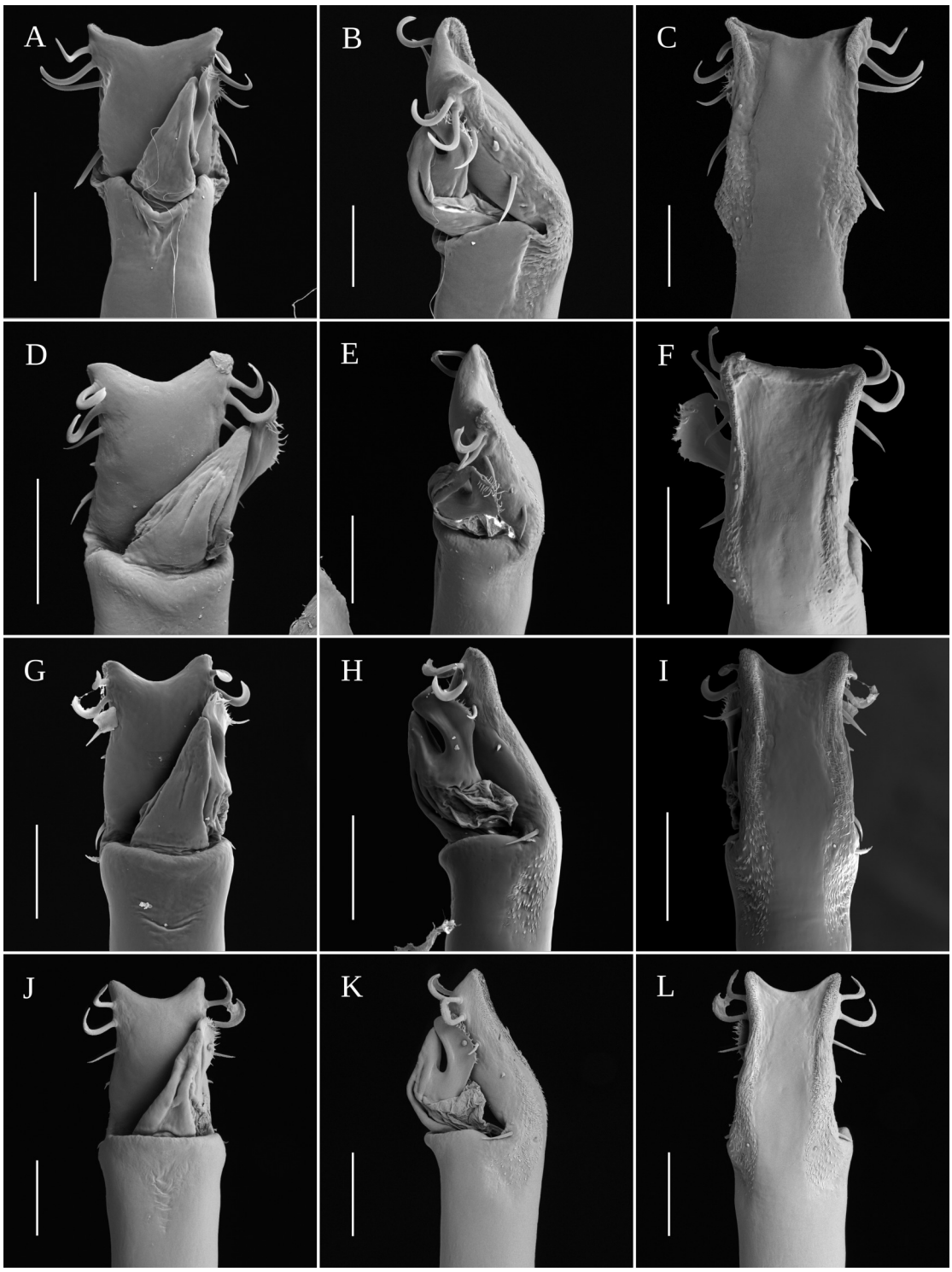


Figure 90: (all scale bars = 100 m): **A-C.** Cosmetidae sp. 1065, **A.** dorsal, **B.** lateral, **C.** ventral; **D-F.** *Cynorta* sp. 3898, **D.** dorsal, **E.** lateral, **F.** ventral; **G-I.** Cosmetidae sp. UFPI2176, **G.** dorsal, **H.** lateral, **I.** ventral; **J-L.** Cosmetidae sp. UFPI2316, **J.** dorsal, **K.** lateral, **L.** ventral.

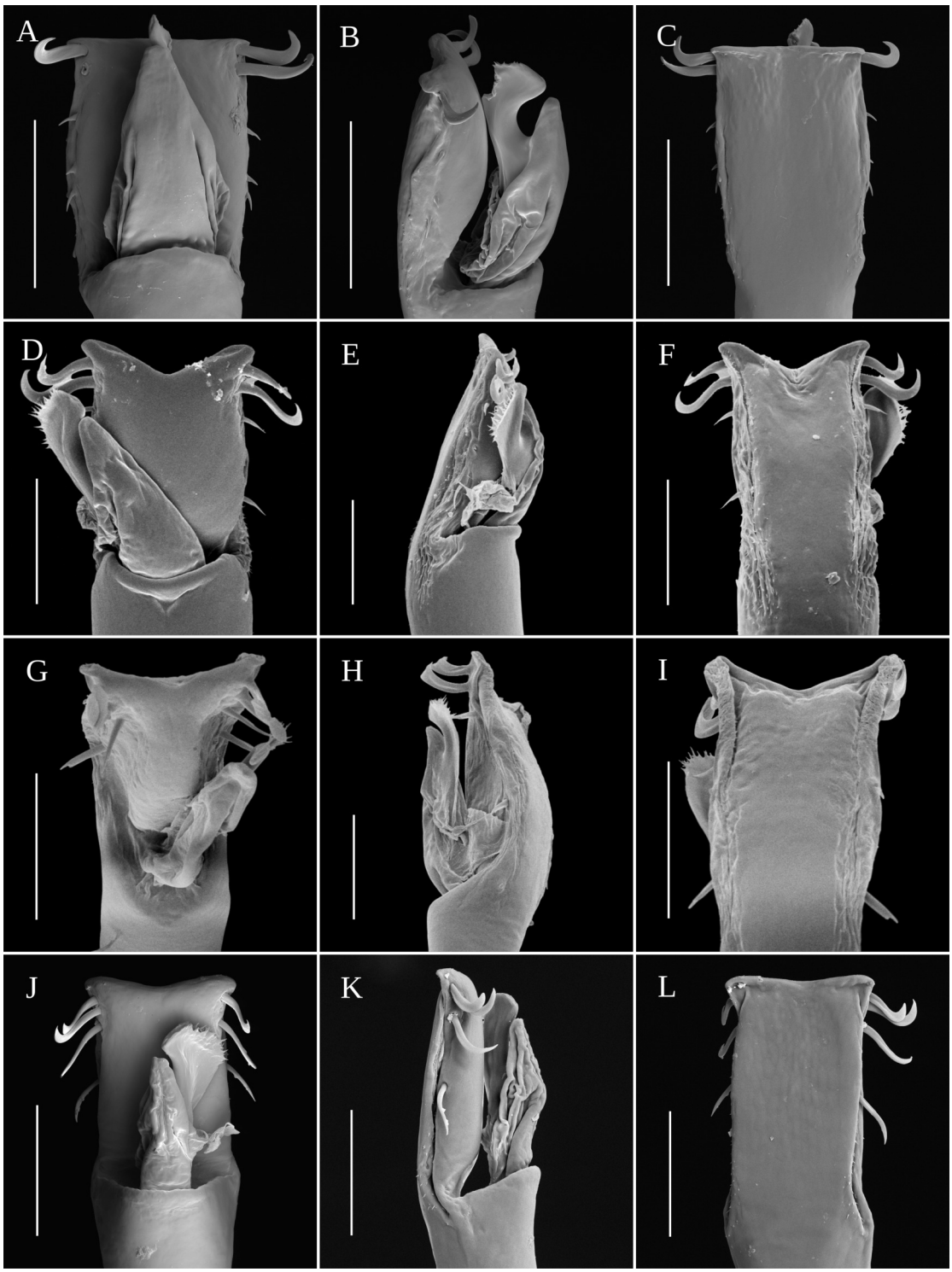


Figure 91: (all scale bars = 100 m): **A-C.** *Paecileama u-flavum*, **A.** dorsal, **B.** lateral, **C.** ventral; **D-F.** *Eucynorta coccineloides*, **D.** dorsal, **E.** lateral, **F.** ventral; **G-I.** *Flirtea picta*, **G.** dorsal, **H.** lateral, **I.** ventral; **J-L.** *P. preciosum*, **J.** dorsal, **K.** lateral, **L.** ventral.

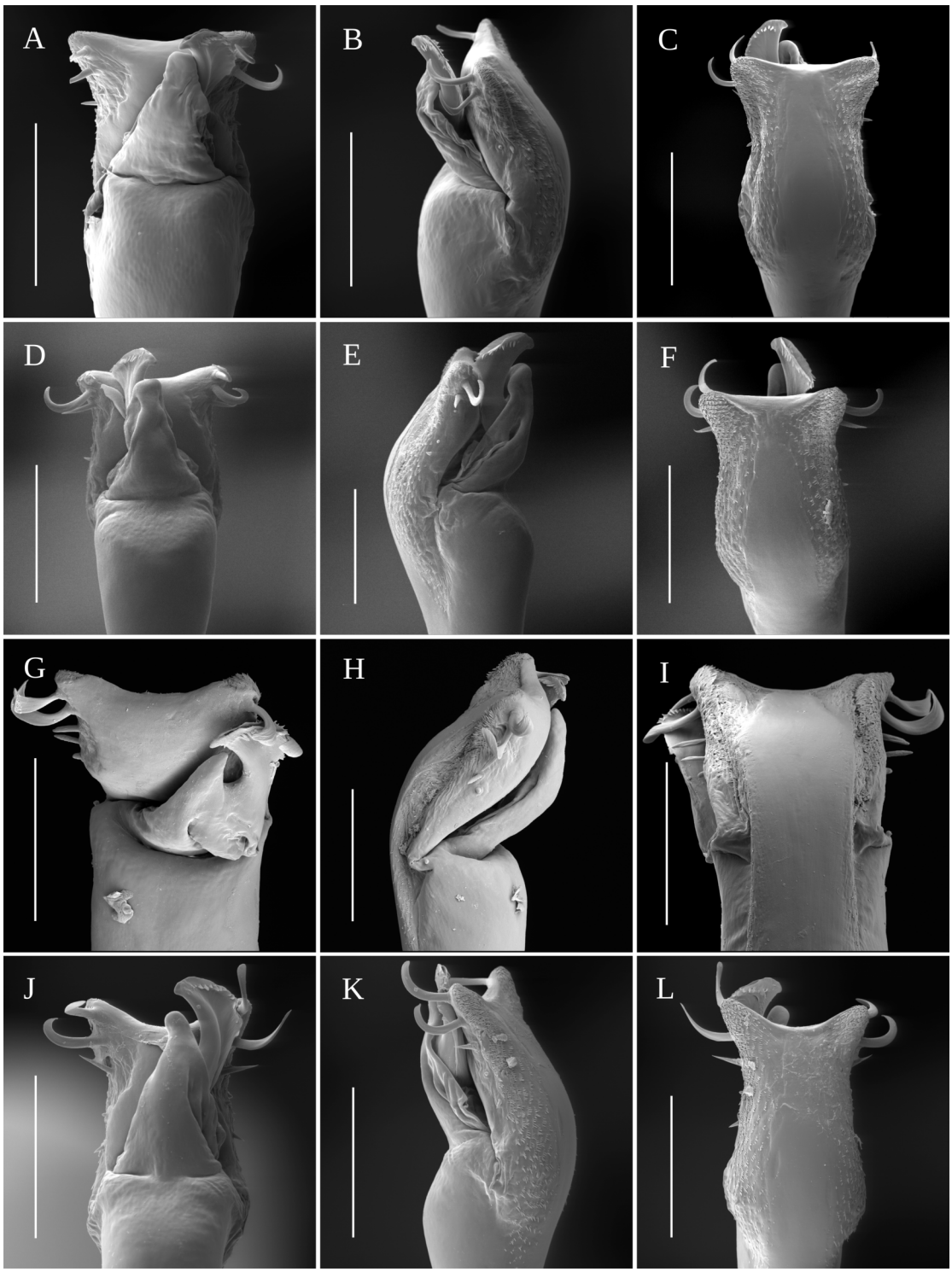


Figure 92: (all scale bars = 100 m): **A-C.** *Bokwina sandersoni*, **A.** dorsal, **B.** lateral, **C.** ventral; **D-F.** *Metacynorta gracilipes*, **D.** dorsal, **E.** lateral, **F.** ventral; **G-I.** *Arucillus armasi*, **G.** dorsal, **H.** lateral, **I.** ventral; **J-L.** *Vonones circumlineatus*, **J.** dorsal, **K.** lateral, **L.** ventral.

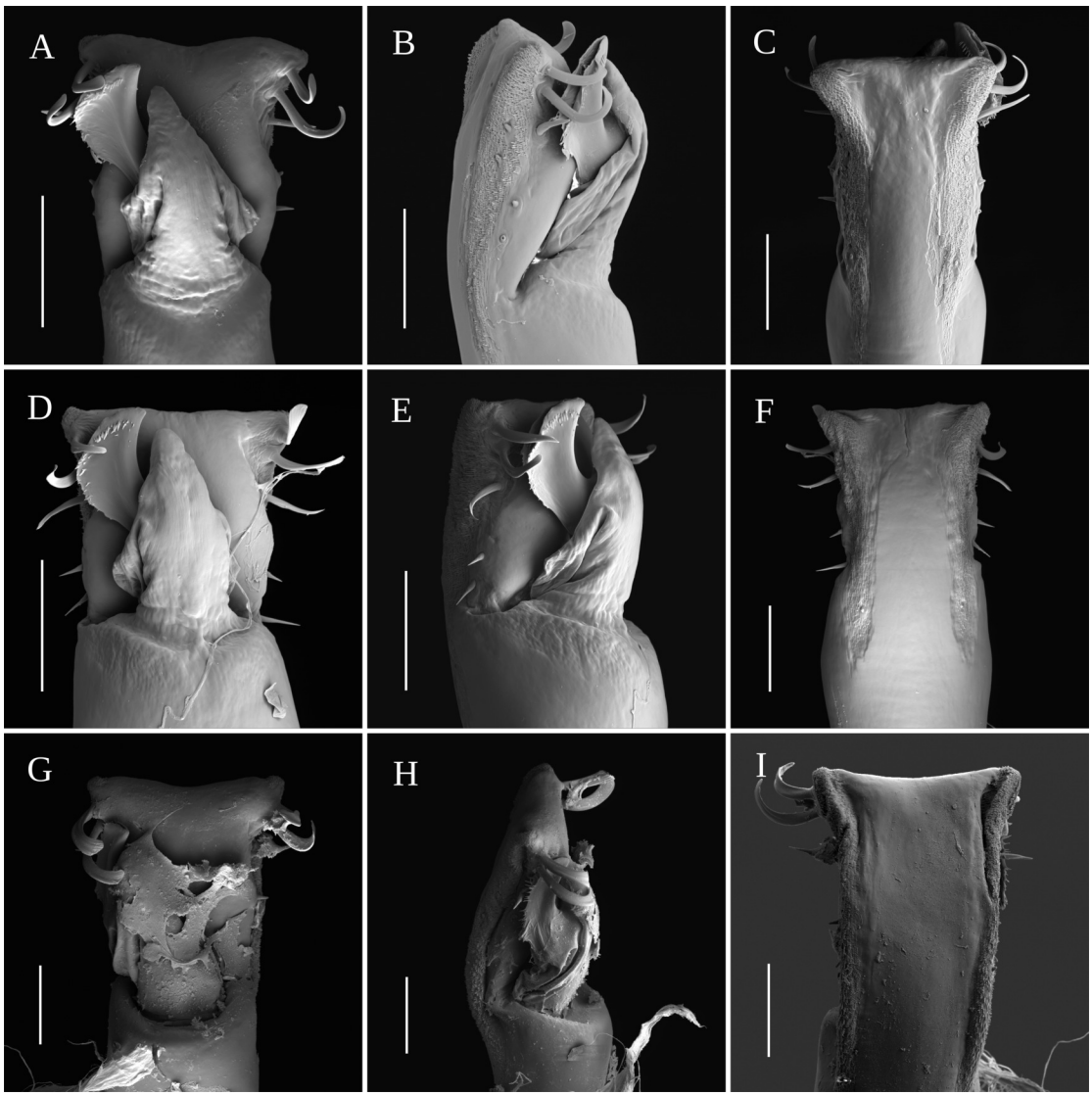


Figure 93: (all scale bars = 100 m): **A-C.** *Cynorta casita*, **A.** dorsal, **B.** lateral, **C.** ventral; **D-F.** *Reimoserious albipictus*, **D.** dorsal, **E.** lateral, **F.** ventral; **G-I.** *Eupoecileama panamensis*, **G.** dorsal, **H.** lateral, **I.** ventral.

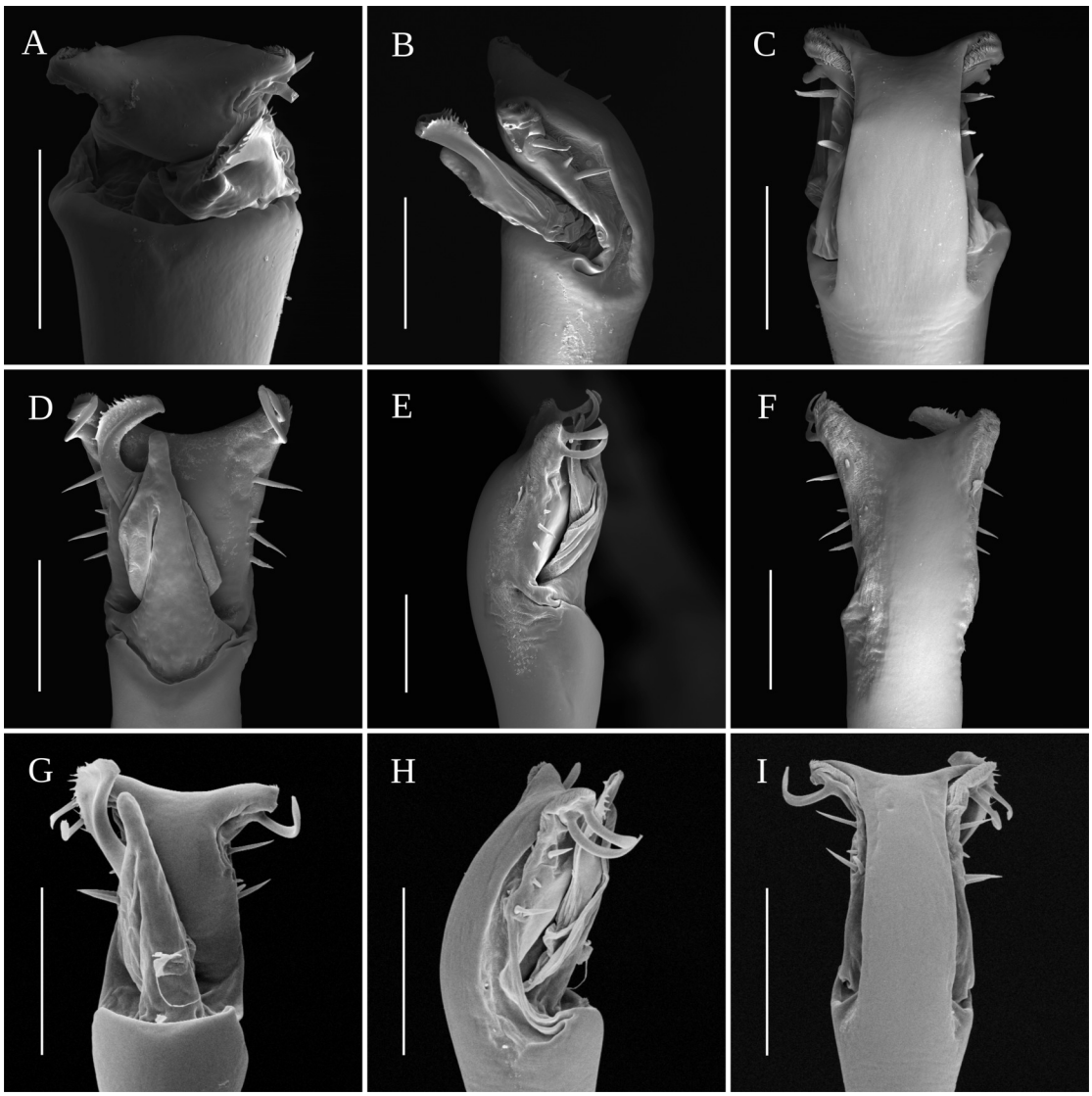


Figure 94: (all scale bars = 100 m): **A-C.** *Paracynorta confluens*, **A.** dorsal, **B.** lateral, **C.** ventral; **D-F.** *Cynorta discreta*, **D.** dorsal, **E.** lateral, **F.** ventral; **G-I.** *C. pleuralis*, **G.** dorsal, **H.** lateral, **I.** ventral.

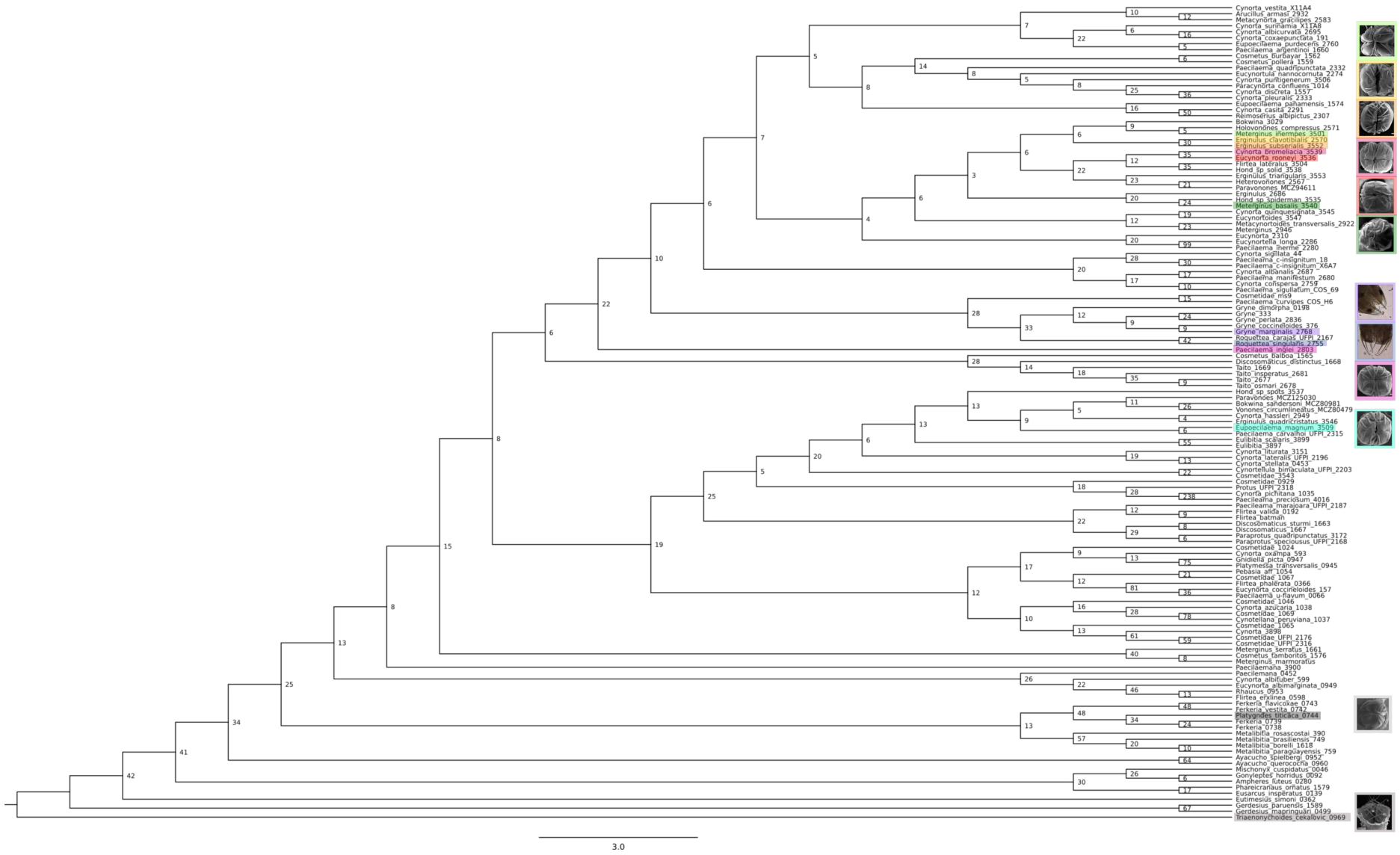


Figure 95: POY ATMD phylogeny with example images of various ovipositors from Cosmetidae, as well as the root species (Some images used here for demonstrative purposes can be found in Walker et al., 2014 and Townsend et al., 2015).