

Insights into the phylogenetic relationships within Cixiidae (Hemiptera: Fulgoromorpha): cladistic analysis of a morphological dataset

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Abstract. According to the most recent classifications proposed, the planthopper family Cixiidae comprises three subfamilies, namely Borystheninae, Bothriocerinae and Cixiinae, the latter with 16 tribes. Here we examine morphological characters to present the first phylogenetic reconstructions within Cixiidae derived from a cladistic analysis. We scored 85 characters of the head, thorax, and male and female genitalia for 50 taxa representative of all cixiid subfamilies and tribes and for six outgroup taxa. Analyses were based on maximum parsimony – using both equally weighted and successive weighting procedures – and Bayesian inferences. The monophyly of most currently accepted tribes and subfamilies was investigated through Templeton statistical tests of alternative phylogenetic hypotheses. The cladistic analyses recover the monophyly of Cixiidae, the subfamily Bothriocerinae, and the tribes Pentastirini, Mnemosynini, and Eucarpiini. Successive weighting and Bayesian inference recover the monophyly of the tribe Gelastocephalini, but only Bayesian inference supports the monophyly of Semoniini. The relationships recovered support the groups [Stenophlepsini (Borystheninae + Bothriocerinae)] arising from the tribe Oecleini, and [Andini + Brixidiini + Brixiiini (polyphyletic) + Bennini]. Templeton tests reject the alternative hypothesis of a monophyletic condition for the tribe Pintaliini as presently defined.

Introduction

Cixiidae, one of 21 families of Fulgoromorpha, are composed mainly of phytophagous insects that feed on vascular plant phloem (Bourgoin *et al.*, 2004). Comprising about 160 genera and 2000 species, the family is distributed in all zoogeographical regions (Holzinger *et al.*, 2002), species richness being higher in the tropics. Cixiids are intimately associated with their host plants, which are used for feeding, for mating and oviposition, and as protection against predators (Wilson *et al.*, 1994; Sforza & Bourgoin, 1998). Nymphs develop in humid and dark habitats, usually in the soil, feeding on roots underground, whereas the adults feed and reproduce on the surface, usually on the green parts of the plants (O'Brien & Wilson, 1985; Sforza *et al.*, 1999).

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Some cixiid species are considered pests of economically important crops, acting as vectors of plant pathogens such as viruses, bacterium-like organisms and phytoplasmas. For example, vineyards have been infested by cixiid-transmitted phytoplasmas in Europe (Sforza *et al.*, 1998); and strawberry (Danet *et al.*, 2003) and beet (Sémétey *et al.*, 2007) have been infected by two different bacterium-like organisms of the genus *Phlomobacter*. These bacteria seem to be part of a clade of Y-proteobacteria that include many secondary endosymbionts of Hemiptera, leading Sémétey *et al.* (2007) to suggest that they might have a secondary endosymbiotic function within Cixiidae. Unfortunately, little is known about either the phylogenetic position of Cixiidae within Fulgoromorpha or the relationships among taxa within Cixiidae. A phylogeny of the family would provide a framework with which to understand the evolution of the relationships between Cixiidae and the phytopathogenic organisms they transmit.

The monophyly of Fulgoromorpha and the placement of Cixiidae as one of the earliest branched lineages within the

infraorder are two generally accepted hypotheses (Bourgoin *et al.*, 1997; Yeh & Yang, 1999; Urban & Cryan, 2007). By contrast, the monophyletic condition of Cixiidae is untested and has been questioned several times (Asche, 1988; Bourgoin *et al.*, 1997). Morphologically, the family is characterized by a combination of the following features: the presence of a third ocellus, forewings with tubercles on the veins, apical segment of the rostrum longer than wide, absence of additional transverse veins in the corium and clavus of the forewings, clavus closed, second posterior tarsomere with a row of spines, and piercing ovipositor, among others (Muir, 1930; Kramer, 1983; O'Brien & Wilson, 1985). However, none of these features is exclusive to Cixiidae, nor are they found consistently in its representatives. In a recent study based on data from four molecular loci, Urban & Cryan (2007) also obtained unclear results regarding Cixiidae monophyly, with a parsimony tree suggesting the paraphyly of the family in relation to Delphacidae, but a Bayesian analysis recovering these two families as two monophyletic units forming the sister group of all other Fulgoroidea.

According to Holzinger *et al.* (2002), Cixiidae is currently composed of three subfamilies, namely Bothriocerinae, Borystheninae and Cixiinae (15 tribes), but there is no consensus on the classification of the tribes (Szwedo & Stroinski, 2002). Moreover, seven recently proposed tribes (Emeljanov, 2002) were erected without formal descriptions, i.e. without enumerations of distinguishing features. The tribe Bennarellini, erected by Emeljanov (1989) also failed to receive a formal description. Recently, Szwedo (2004) increased the number of Cixiinae tribes to 16 and elevated Mnemosynina, previously a subtribe of Pentastirini, to the level of tribe.

The only published phylogeny proposed for the family (Emeljanov, 2002) included neither the tribes Bennarellini, Cixiini and Gelastocephalini, nor the subfamily Borystheninae, and suggested that the subfamily Cixiinae was polyphyletic. Supposed synapomorphies were used as a basis for producing this intuitive phylogeny but they are untested by a cladistic analysis.

In this paper, morphological characters of the head, thorax, and male and female genitalia of Cixiidae were used to produce a data matrix, and primary homology hypothesis statements were tested through a cladistic analysis (de Pinna, 1991). We tested for the monophyly of Cixiidae and its subfamilies and tribes and investigated the phylogenetic relationships among these groups.

Material and methods

Taxonomic sampling

The morphological analysis included 50 cixiid species representing 49 genera, and four outgroup species belonging to families derived from basal nodes within Fulgoroidea: Delphacidae, Meenoplidae, Kinnaridae and Achilidae (Muir,

1923, 1930; Asche, 1988; Emeljanov, 1989, 1990, 2002; Bourgoin, 1997; Bourgoin *et al.*, 1997) (Table 1). Because Cixiidae are probably one of the earliest diversified Fulgoromorpha taxa and the inclusion of few derived outgroups could cause polarity problems (Bourgoin *et al.*, 1997; Urban & Cryan, 2007), we included two outgroup species belonging to Cicadomorpha (Table 1), one of the putative Fulgoromorpha sister groups (von Dohlen & Moran, 1995). The other candidate for sister group of Fulgoromorpha, the (Heteroptera + Peloridiidae) group (Sorensen *et al.*, 1995; Ouvrard *et al.*, 2000), is too differentiated morphologically and does not allow reliable comparisons with Cixiidae.

Terminal taxa were chosen based on the classification of Emeljanov (2002), presented by Holzinger *et al.* (2002) in a generic checklist of Cixiidae. The various taxa were also chosen according to their availability for study and the number of genera in each tribe, so that diverse tribes would be better represented in the analysis. Only one representative species of each of the tribes Andini, Bennarellini, Bennini and Stenophlepsini, for which more than one genus is described, was available for this study. It was not possible to examine females of *Phachyntheisa* sp., *Duilius seticulosus*, and *Rhigedanus maculipennis*. Type-species of genera were preferentially included.

Terminology

The terminology of the head characters mostly follows O'Brien & Wilson (1985). Major veins of the forewings are named according to Dworakowska (1988). Every additional branch was named in such a way that the branch closest to the costal margin received a '1', whereas the one closest to the internal margin received a '2'. Other terms for thorax morphology are named according to Emeljanov (1989, 2002). Male abdomen terms mostly follow Bourgoin (1988), whereas female genitalia terms follow Bourgoin (1993).

Phylogeny

All characters were equally weighted, and multistate characters were treated as unordered. In the case of characters for which more than one state was observed for a given taxon, all the states were considered (polymorphic). Characters were reductively coded (Wilkinson, 1995), character states being scored as dashes (–) if not applicable and as question marks (?) if ambiguous or missing. Maximum parsimony (MP) and Bayesian inference (BI) analyses were performed. Under MP, both equally weighted (EW) and successively weighted (SW) analyses were carried out using PAUP* 4.0b10 (Swofford, 2003). Heuristic searches were performed using tree bisection–reconnection (TBR) branch swapping and 500 random-addition replicates. The SW approach (Farris, 1969) was conducted using the maximum value of the rescaled consistency index (RC) (Farris, 1989). Relative support of nodes was assessed with non-parametric

Table 1. Taxa studied and country of origin of the specimens examined. Species in bold are type species of genera.

Subfamily (family for outgroups)	Country
Tribe	
Species	
Borystheninae Emeljanov, 1989	
<i>Borysthenes maculatus</i> Matsumura, 1914	Taiwan
Bothriocerinae Muir, 1923	
<i>Bothrioceretta nigra</i> (Fowler, 1904)	Mexico
<i>Bothriocera signoreti</i> Stål, 1864	Guatemala
Cixiinae Spinola, 1839	
Andini Emeljanov, 2002	
<i>Andes taiensis</i> Van Stalle, 1984	Ivory Coast
Bennarellini Emeljanov, 1989	
Bennarellini gen. sp.	Brazil
Bennini Metcalf, 1938	
Bennini gen. sp.	Papua New Guinea
Brixiidini Emeljanov, 2002	
<i>Brixidia variabilis</i> Synave & Van Stalle, 1984	Ivory Coast
Brixiini Emeljanov, 2002	
<i>Brixia marojelyensis</i> Synave, 1965	Madagascar
<i>Solonaima</i> sp.	Australia
Cajetini Emeljanov, 2002	
<i>Cajeta singularis</i> Stål, 1866	Australia
Cixiini Spinola, 1839	
<i>Achaemenes quinquespinosus</i> Synave, 1960	Reunion Island
<i>Cixiosoma bonaerense</i> Berg, 1883	Argentine and Uruguay
<i>Cixius cucicularius</i> (Linne, 1767)	France
<i>Iolania perkinsi</i> Kirkaldy, 1902	U.S.A. (Hawaii)
<i>Microledrida flava</i> Metcalf, 1923	Mexico
<i>Pachyntheisa</i> sp.	Mexico
<i>Tachycixius pilosus</i> (Olivier, 1791)	France
<i>Trirhacus discrepans</i> Fieber, 1876	France
Duiliini Emeljanov, 2002	
<i>Duilius seticulosus</i> (Lethierry, 1874)	Maroc
Eucarpini Emeljanov, 2002	
<i>Eucarpia taiensis</i> Van Stalle, 1984	Ivory Coast
<i>Kirbyana pagana</i> (Melichar, 1903)	Taiwan
Gelastocephalini Emeljanov, 2000	
<i>Dysoliarus unicornis</i> Fennah, 1949	Australia
<i>Holgus liafredis</i> Löcker & Larivière, 2006	Australia
<i>Rhigedanus maculipennis</i> Emeljanov, 2000	Australia
<i>Wernindia lorda</i> Löcker & Fletcher, 2006	Australia
Mnemosynini Szvedo, 2004	
<i>Mnemosyne camerunensis</i> Distant, 1907	Central African Republic
<i>Mnemosyne cubana</i> Stål, 1866	Cuba
Pentastirini Emeljanov, 1971	
<i>Cyclopoltiarus</i> sp.	Costa Rica
<i>Hyalesthes obsoletus</i> Signoret, 1865	France
<i>Melanoliarus</i> sp.	Guadeloupe
<i>Pentastiridius</i> sp.	France
<i>Reptalus quinquecostatus</i> (Dufour, 1833)	France
Pintaliini Metcalf, 1938	
<i>Aulocorypha punctulata</i> Berg, 1979	Argentina
<i>Cubana cypassis</i> Fennah, 1971	Cayman Islands
<i>Cubanella</i> sp.	Dominican Republic

Table 1. Continued.

<i>Diastrocixius thelius</i> Caldwell, 1945	Panama
<i>Monorachis sordulentus</i> Uhler, 1901	U.S.A.
<i>Notocixius helvolus</i> (Spinola, 1852)	Chile
<i>Pintalia</i> sp.	Brazil
Oecleini Muir, 1922	
<i>Colvanalia taffini</i> (Bonfils, 1983)	Vanuatu
<i>Haplaxius crudus</i> (Van Duzee, 1907)	Colombia
<i>Myndodus adiopodoumensis</i> (Synave, 1962)	Ghana
<i>Mundopa kotoshonis</i> Matsumura, 1914	Taiwan
<i>Nymphocixia unipunctata</i> Van Duzee, 1923	Belize
<i>Oecleus productus</i> Metcalf, 1923	Mexico
<i>Rhamphicixius championi</i> Fowler, 1904	Honduras
Semoniini Emeljanov, 2002	
<i>Betacixius ocellatus</i> Matsumura, 1914	Taiwan
<i>Kuvera tappanella</i> Matsumura, 1914	Taiwan
Stenophlepsini Metcalf, 1938	
<i>Euryphlepsia</i> sp.	Palau Islands
Insertae sedis	
<i>Meenocixius virescens</i> Attié, Bourgoïn & Bonfils, 2002	Reunion Island
Achilidae Stål, 1866	
<i>Catonia picta</i> Van Duzee, 1908	U.S.A.
Delphacidae Leach, 1815	
<i>Prokelisia marginata</i> (Van Duzee, 1897)	U.S.A.
Kinnaridae Muir, 1925	
<i>Nesomicrixia insularis</i> (Synave, 1958)	Reunion Island
Meenoplidae Fieber, 1872	
<i>Nisia atrovonosa</i> (Lethierry, 1888)	Cameroon
Cicadellidae Latreille, 1802	
<i>Agallia consobrina</i> Curtis, 1833	France
Aphrophoridae Amyot & Serville, 1843	
<i>Philaenus</i> sp.	France

bootstrap (Felsenstein, 1985) procedures (1000 pseudo-replicates of 10 random-addition replicates, saving only one tree per replicate) and the Decay index (Bremer, 1988). For the SW analysis, bootstrap values were obtained with the weights assigned in the last iteration. To make sure that the analyses using PAUP recovered the most parsimonious trees, an EW analysis was also run in TNT (Goloboff *et al.*, 2003) with 100 random-addition sequences of random sectorial searches with default options, 40 cycles of drift-accepting suboptimal rearrangements with a maximum fit difference of two, 40 cycles of ratchet, and five rounds of tree-fusing.

BI analysis was run using MRBAYES ver. 3.12 (Ronquist & Huelsenbeck, 2003). In accordance with Lewis (2001), we applied the MkV model for discrete morphological data to our dataset. We ran one analysis assuming equal rates of change among characters, and another with four rate categories of the gamma distribution parameter, thus allowing the rates of character change to be different across characters. In order to find the best-fitting model of the evolution of the data, we compared these two models using Bayes factors (following Kass & Raftery, 1995; Nylander *et al.*, 2004). Values of $2\log_e(B_{10})$ were calculated (the difference in the harmonic means of the log likelihoods of the two models multiplied by two), and values >10 were

considered to be reason to favour one model over the other (Nylander *et al.*, 2004; Wiens *et al.*, 2005). Two independent BI runs were carried out, each with four chains (with incremental heating) of 5×10^6 generations, random starting trees, default priors, and trees sampled every 100 generations. Log-likelihood scores were plotted to determine the number of trees to be discarded (i.e. to determine the length of the burn-in period), and a very conservative burn-in period of 10 000 generations was used.

The Templeton test (Templeton, 1983) was used to evaluate alternative hypotheses of cixiid phylogenetic relationships, in which the tribes Brixiini, Cixiini, Gelastocephalini, Oecleini, Pintaliini, and Semoniini were constrained to be monophyletic. These trees were obtained using PAUP with 10 addition sequences replicates and TBR swapping. As in the unconstrained EW analysis, all constrained analyses yielded several most-parsimonious trees. The topologies of the trees to be compared are taken into account in the Templeton test, so that by chance alone one can obtain false significant or non-significant results when comparing only one pair of most-parsimonious trees among several possible pairs. To avoid such an error, we performed 25 paired comparisons. In each test, one of the most-parsimonious unconstrained trees was compared with one of the most-parsimonious constrained trees, with both trees randomly chosen.

Characters

The comparative analysis resulted in a matrix (Appendix 1) of 85 characters, of which 17 are multistate. The consistency index (CI) of informative characters (maximum value of the variable ones) on the equally weighted parsimony trees is also listed.

Head

1. *Vertex, position in relation to eyes*: (0) in the same plane (Fig. 1A, B, F); (1) in an elevated plane (Fig. 1D, E). CI = 0.50.
2. *Vertex, shape*: (0) laterally compressed; (1) vertically compressed; (2) not compressed vertically or laterally. CI = 0.28.
3. *Median carina of vertex*: (0) absent; (1) present. CI = 0.27.
4. *Posterior margin of vertex, shape*: (0) not tubular; (1) tubular. CI = 1.00.
5. *Frons, shape*: (0) not compressed laterally (Fig. 1B, G, I); (1) laterally compressed (Fig. 1E). CI = 0.50.
6. *Median carina of frons*: (0) present (Fig. 1B, G, I); (1) absent (Fig. 1F). CI = 0.14.
7. *Median carina of frons, aspect*: (0) bifurcate dorsally; (1) linear, dorsally straight. CI = 0.20.
8. *Transversal carina of frons*: (0) present; (1) absent. Emeljanov (2002) refers to this carina as the intermetopal keel. CI = 0.10.
9. *Lateral portion of frons*: (0) without a marked keel; (1) with a marked keel. CI = 1.00.
10. *Lateral margins of frons*: (0) without a basal fold (Fig. 1D, H); (1) with a basal fold (Fig. 1A, C). CI = 1.00.
11. *Lateral margins of frons, aspect*: (0) not forming a keel, (1) forming a keel on the area adjacent to the antennae; (2) forming a prominent keel for its entire length. CI = 0.22.
12. *Median ocellus*: (0) absent; (1) present (Fig. 1B, E, I). CI = 0.27.
13. *Ocellus, position in relation to frontoclypeal suture*: (0) bordering suture (Fig. 1B); (1) outlying suture (Fig. 1E, I). CI = 0.33.
14. *Frontoclypeal suture, contour*: (0) curved upwards (Fig. 1E); (1) curved downwards (Fig. 1B); (2) rectilinear (Fig. 1I); (3) curved outwards. CI = 0.38.
15. *Median carina of clypeus*: (0) present (Fig. 1B, E, I); (1) absent. CI = 0.33.
16. *Median carina of clypeus, extension*: (0) on its entire length; (1) only on ventral portion; (2) only on dorsal portion. CI = 0.29.
17. *Clypeus, apex*: (0) reaching apex of procoxae; (1) reaching middle portion of procoxae; (2) surpassing apex of procoxae. CI = 0.50.
18. *Rostrum, apex*: (0) reaching metacoxae; (1) surpassing metacoxae; (2) not reaching metacoxae. CI = 0.13.
19. *Antennae, position*: (0) between compound eyes; (1) ventral to compound eyes. CI = 1.00.
20. *Antennal pedicel*: (0) without sensilla placoidea; (1) with sensilla placoidea. CI = 1.00.
21. *Antennal pedicel, relative length and width*: (0) about as long as wide (Fig. 1F); (1) longer than wide (Fig. 1D). CI = 0.33.
22. *Antennal pedicel, shape*: (0) reniform (Fig. 1A, C); (1) tubular to circular (Fig. 1D, F). CI = 0.50.
23. *Antennal second projection [after Shih & Yang (1996)], shape*: (0) not spiniform; (1) spiniform. CI = 0.25.
24. *Subantennal carina, lateral view*: (0) absent (Fig. 1D); (1) present (Fig. 1A, F). CI = 0.33.

Thorax

25. *Pronotum, size relative to mesonotum size*: (0) larger; (1) smaller. CI = 1.00.
26. *Pronotum, lateral carinae, position*: (0) following eyes contour (Fig. 1H); (1) not following eyes contour. CI = 0.17.
27. *Pronotum, lateral carina, internal side*: (0) parallel to the external carinae of vertex; (1) not parallel. CI = 0.10.
28. *Pronotum, shape of posterior margin*: (0) angulate (Fig. 1H); (1) rounded. CI = 0.15.
29. *Tegulae*: (0) absent; (1) present. CI = 1.00.
30. *Tegulae, anterior external margin (in ventral view)*: (0) projecting externally; (1) not projecting. CI = 0.09.
31. *Tegulae, shape in lateral view*: (0) angulate; (1) rounded. CI = 0.50.
32. *Mesonotum, apex shape*: (0) pointed (Fig. 1H); (1) rounded. CI = 0.50.

33. *Mesonotum, number of carinae, dorsal view*: (0) three (Fig. 1H); (1) five. CI = 0.20.
34. *Forewings, relative position*: (0) overlapping each other; (1) not overlapping. CI = 0.50.
35. *Forewings, relative position of apexes*: (0) steeply tectiform; (1) slightly tectiform. CI = 0.13.
36. *Forewings, veins*: (0) without tubercles; (1) with tubercles. CI = 0.40.
37. *Forewings, aspect of costal margin*: (0) continuous (Fig. 2A, B); (1) with a basal concavity (Fig. 2C). CI = 0.29.
38. *Forewings, costal margin at base*: (0) prominent; (1) not prominent. CI = 1.00.
39. *Forewings, position of claval apex*: (0) in basal half of forewing (Fig. 2B); (1) in apical half of forewing (Fig. 2A, D). CI = 1.00.
40. *Forewings, divergence of MP from ScP + R + MA*: (0) in basal cell (sensu Emeljanov, 2002) (Fig. 2A); (1) close to basal cell; (2) distant from basal cell (Fig. 2D). CI = 0.03.
41. *Forewings, forking of MP in relation to nodal line*: (0) on nodal line; (1) basad of nodal line; (2) distad of nodal line. CI = 0.15.
42. *Forewings, bifurcation of CuA relative to ScP + R + MA*: (0) CuA bifurcates distad of ScP + R + MA (Fig. 2B, C); (1) CuA bifurcates basad of ScP + R + MA (Fig. 2D); (2) CuA and ScP + R + MA bifurcate at approximately the same level (Fig. 2A). CI = 0.53.
43. *Forewings, bifurcation of CuA in relation to claval sulcus*: (0) median region of claval sulcus; (1) apical one-quarter of claval sulcus. CI = 0.14.
44. *Forewings, intercubital veinlet (sensu Emeljanov 2002)*: (0) connected to claval apex (Fig. 2B); (1) more distal, connected to the internal margin of forewings (Fig. 2C); (2) more proximal, connected to claval sulcus (autapomorphy of Bennarellini). CI = 0.36.
- Emeljanov (2002) interpreted this character in a different way. He split the more distal connection of the intercubital veinlet into two states: one simply more distal, and another in which the intercubital veinlet is elongated and acquires the shape of a third branch of CuA. Here, these attributes are considered as one unique state. Emeljanov (2002) suggested that the elongate intercubital vein could be synapomorphic for Brixidiini, Bennini and Borystheninae, but the species of Bennini and Brixidiini (Fig. 2C) included herein do not exhibit an elongate intercubital vein. In the present analysis, the only species that have elongate intercubital veins are *Brixia variabilis* and *Borysthenes maculatus*. However, whereas this condition seems to be related to the early branching of CuA2 in *Br. variabilis*, it seems to be related to the enlargement of the apical portion of the wing in *Bo. maculatus*.
45. *Forewings, cell between RP + MA1 and RP + MA2*: (0) absent; (1) present (Fig. 2B). CI = 0.09.
46. *Forewings, antepical cell between MP11 and MP12*: (0) absent (Fig. 2C); (1) present (Fig. 2A). CI = 0.13.
47. *Forewings, antepical cell between MP21 and MP22*: (0) absent (Fig. 2A); (1) present (Fig. 2C). CI = 0.5.
48. *Forewings, development of AA in relation to other veins*: (0) more developed; (1) as developed as the other veins. CI = 0.5.
49. *Forewings, transversal vein between claval suture and AA*: (0) absent; (1) present. CI = 0.17.
50. *Forewings, AA, shape at base*: (0) curved; (1) rectilinear. CI = 0.09.
51. *Forewings, jugal vein*: (0) absent; (1) present.
52. *Profemur, ventral subapical portion*: (0) without projection; (1) forming a blunt projection. CI = 0.5.
53. *Metatibia, spines on the external lateral row*: (0) absent; (1) present (Fig. 2E). CI = 0.13.
54. *Metatibia, modified ochre-coloured setae*: (0) absent; (1) present (Fig. 2E). CI = 0.14.
- Emeljanov (2002) considered the ochre-coloured setae and the spines (preceding character) as a single character and suggested that it could be a synapomorphy of Cixiidae. However, some species possess the modified ochre-coloured setae but not spines, leading us to treat the presence or absence of spines and setae as two independent characters.
55. *Metatibia, number of apical spines*: (0) 10–11; (1) 9; (2) 8; (3) 7; (4) 6; (5) 5. CI = 0.75.
56. *Metatibia, apical spines, diastema*: (0) absent (Fig. 2F); (1) present (Fig. 2G). CI = 0.20.
57. *Metatibia, relative size of second and third apical spine of the external group*: (0) approximately equal (Fig. 2F); (1) second longer than third. CI = 0.20.
58. *Metatibia, apical spur*: (0) absent; (1) present.
- The presence of a spur at the apex of the metatibia is an autapomorphy of the family Delphacidae.

Abdomen

59. *Third and fourth abdominal segments, lateral processes*: (0) absent; (1) present.

The presence of lateral appendages on the third and fourth abdominal segments is an autapomorphy of the tribe Bennini. These unusual processes are composed of a cup-like structure filled with a wax cone bearing a marginal seta (Hoch, 1988). The function of these processes is unknown.

60. *Fourth and fifth abdominal segments, lateral expansions*: (0) absent; (1) present.

The lateral expansions of the fourth and fifth abdominal segments are an autapomorphy of the tribe Bennarellini.

Male abdomen.

61. *Sternum 6, number of sclerites, ventral view*: (0) 1; (1) 2. CI = 0.30.
62. *Sternum 7, number of sclerites, ventral view*: (0) 1; (1) 2; (2) 3. CI = 0.22.
63. *Sternum 8, number of sclerites, ventral view*: (0) 1; (1) 2. CI = 0.09.

64. *Medioventral pygofer process*: (0) absent; (1) present (Fig. 2H). CI = 1.00.
65. *Medioventral pygofer process, shape of posterior margin*: (0) angulate; (1) emarginate; (2) rounded; (3) bifid; (4) slightly trilobate. CI = 0.37.
66. *Medioventral pygofer process, maximum width*: (0) at base; (1) along the process. CI = 0.14.
67. *Subgenital plates*: (0) present; (1) absent. CI = 1.00.
68. *Internal margin of styles, aspect*: (0) without spine; (1) with a long spine directed posterolaterally. CI = 0.50.
69. *Tectiform structure, principal axis of anterior expansions*: (0) longitudinal; (1) transversal (Fig. 2J). CI = 1.00.
70. *Periandrium, symmetry*: (0) symmetric; (1) asymmetric (Fig. 2I, J). CI = 1.00.
71. *Aedeagus (or flagellum), shape*: (0) not flagelliform; (1) flagelliform (Fig. 2I). CI = 1.00.

The aedeagus of Cixiidae is generally named the flagellum because of its shape and difficulties in homology assessment of the various parts of the phallic complex of Fulgoromorpha families. The flagellum, the name commonly adopted by authors working on cixiids (Kramer, 1983; Hoch, 2005), is here tentatively interpreted as the aedeagus, as suggested by Bourgoïn (1988) and Bourgoïn & Huang (1990). Some authors divide the aedeagus into the periandrium and flagellum (for example Van Stalle, 1987), in that case the aedeagus is taken as the whole phallic complex. However, in the insect basal plan the aedeagus and the periandrium are different parts of the phallic complex that could be lost subsequently in several groups (Snodgrass, 1935).

72. *Anal tube, symmetry*: (0) symmetric; (1) asymmetric. CI = 0.14.
73. *Anal tube, shape of apical margin*: (0) rounded; (1) angulate; (2) irregular; (3) rectilinear; (4) interrupted medially; (5) concave; (6) wavy. CI = 0.30.
74. *Anal style, size relative to sternite XI*: (0) longer; (1) shorter; (2) approximately the same size. CI = 0.22.

Female abdomen.

75. *Ovipositor, degree of development*: (0) reduced (Fig. 2K), (1) well developed (Fig. 2L). CI = 0.50.

The size differences of the gonapophyses illustrate the reduced and the developed ovipositor types (Fig. 2K, L). Reductions and enlargements of the ovipositor complex are well known in cixiid taxa, as well as in other Fulgoroidea families (Bourgoïn, 1993; Wilson *et al.*, 1994; Holzinger *et al.*, 2002). The reduced type is usually associated with eggs being deposited on plant tissue and covered with wax and exogenous material, whereas the enlarged ovipositor complex seems to be used to insert the eggs into the plant tissue (Bourgoïn, 1993; Wilson *et al.*, 1994).

76. *Gonapophysis 8, row of setae below fibulae*: (0) absent; (1) present. CI = 0.10.
77. *Gonapophysis 8, setae below fibulae*: (0) conspicuous; (1) reduced. CI = 0.20.

78. *Gonapophysis 8, basal lobe*: (0) absent; (1) present. CI = 1.00.
79. *Gonapophysis 9, contour of dorsal margin on apical portion*: (0) smooth; (1) forming teeth. CI = 0.10.
80. *Gonapophysis 9, connection of dorsal, or internal, margin*: (0) fused; (1) separate. CI = 0.50.
81. *Gonoplates, shape of apex*: (0) rounded; (1) angulate; (2) concave. CI = 0.14.
82. *Segment 9, position of gonapophysis*: (0) central; (1) displaced ventrally. CI = 1.00.
83. *Segment 9, shape*: (0) not truncate (Fig. 2L); (1) truncate (Fig. 2K). CI = 0.14.
84. *Segment 9, multi-pointed setae, posterior view*: (0) absent; (1) present. CI = 1.00.
85. *Anal tube, shape*: (0) not diamond-shaped; (1) diamond-shaped. CI = 1.00.

Results

The EW analysis yielded 106 most-parsimonious trees of 475 steps [CI = 0.27, and retention index (RI) = 0.54 excluding uninformative characters]. The analyses with PAUP and TNT yielded the same strict consensus tree (Fig. 3). The monophyly of Cixiidae was recovered in all most-parsimonious trees. The subfamily Bothriocerinae and the Cixiinae tribes Pentastirini, Mnemosynini, and Eucarpiini came out as monophyletic. The monophyly of the tribes Brixini, Cixiini, Gelastocephalini, Oecleini, Pintaliini, and Semoniini was not recovered. However, based on all 25 pairwise comparisons of constrained and unconstrained trees (Templeton tests, see Methods), the hypothesis of monophyly could not be rejected for the tribes Brixini ($0.25 \geq P \geq 0.16$), Cixiini ($0.17 \geq P \geq 0.06$), Gelastocephalini ($0.90 \geq P \geq 0.82$), Oecleini ($0.74 \geq P \geq 0.66$), and Semoniini ($0.88 \geq P \geq 0.78$). Monophyly was rejected for Pintaliini only ($0.04 \geq P \geq 0.01$).

The SW analysis stabilized after two iterations, retaining one most-parsimonious tree of 61 335 weighted steps (CI = 0.58 and RI = 0.76) (Fig. 4). The synapomorphies of the clades recovered in this tree are presented in Appendix 2. In addition to the clades recovered in the EW analysis, the Gelastocephalini were monophyletic in the SW tree (Fig. 4). The tribes Mnemosynini and Pentastirini clustered together (Fig. 4). In general, clades found to be monophyletic in both MP analyses have low bootstrap and Bremer support values (Figs 3, 4).

In the BI analyses, the total harmonic means (obtained with the *sump* command in MRBAYES) were -1979.78 and -1867.84, and the resulting $2\log_e(B_{10})$ of the Mk_v/Mk_v + Γ was 111.94, leading us to conclude that the implementation of a gamma shape parameter is a better choice for the present dataset. This BI analysis recovered the monophyly of Cixiidae, as well as that of the tribes Eucarpiini, Gelastocephalini, Mnemosynini, Pentastirini, and Semoniini (Fig. 5). However, most of the tribes found to be monophyletic have

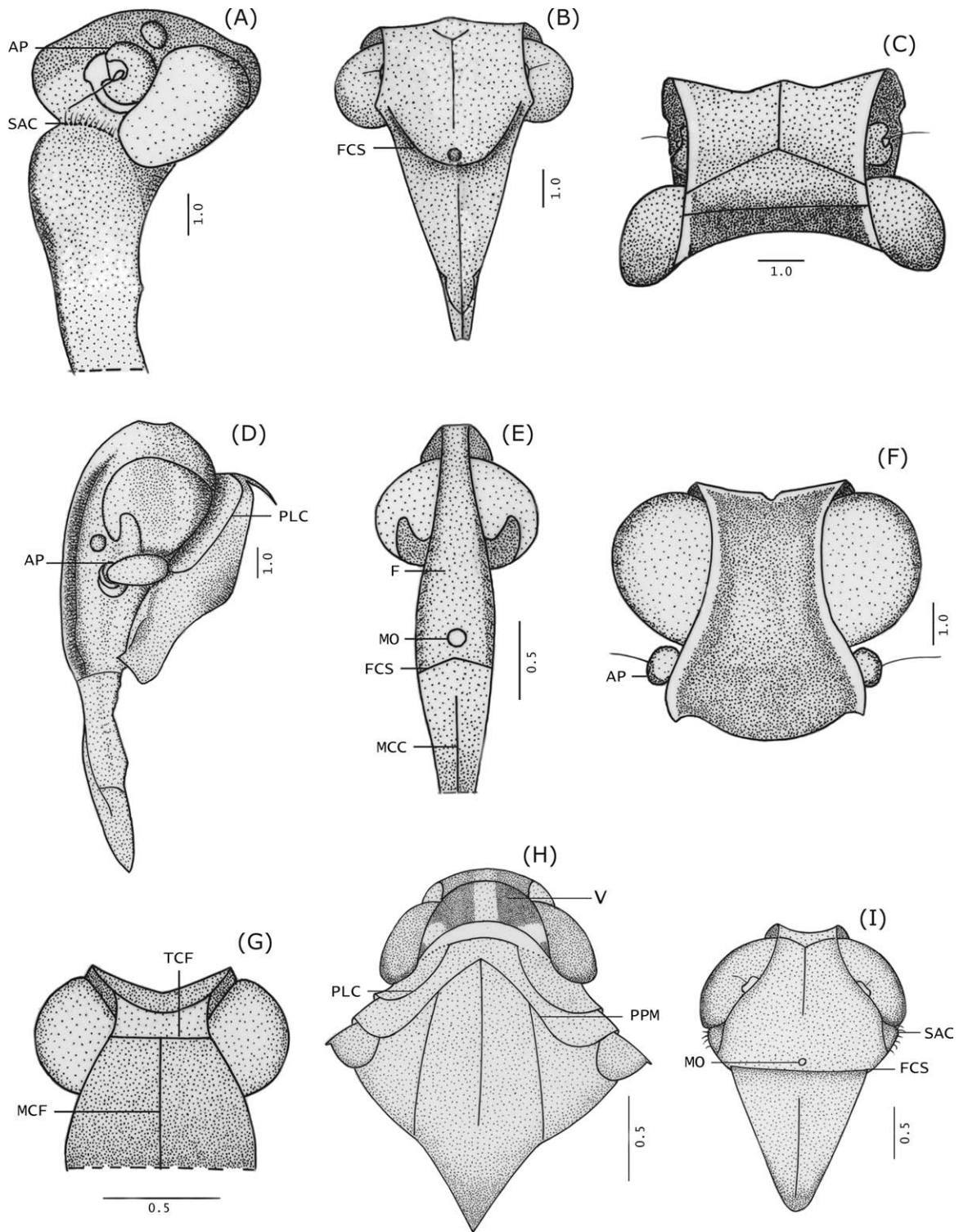


Fig. 1. Head and thorax characters. A–C, *Bothrioceretta nigra*, head: (A) lateral view, (B) frontal view, (C) dorsal view; D–E, *Brixia marojelyensis*: (D) head and pronotum, lateral view, (E) head, frontal view; (F) *Hyalesthes obsoletus*, head, frontal view; G–H, *Tachycixius pilosus*: (G) head, frontodorsal view, (H) head, pronotum, and mesonotum, dorsal view; (I) *Euryphlepsia* sp., head, lateral view. AP, antennal pedicel; F, frons; FCS, frontoclypeal suture; MCC, median carina of clypeus; MCF, median carina of frons; MO, median ocellus; PLC, pronotum lateral carina; PPM, pronotum posterior margin; SAC, subantennal carina; TCF, transversal carina of frons; V, vertex. Scales are in millimetres.

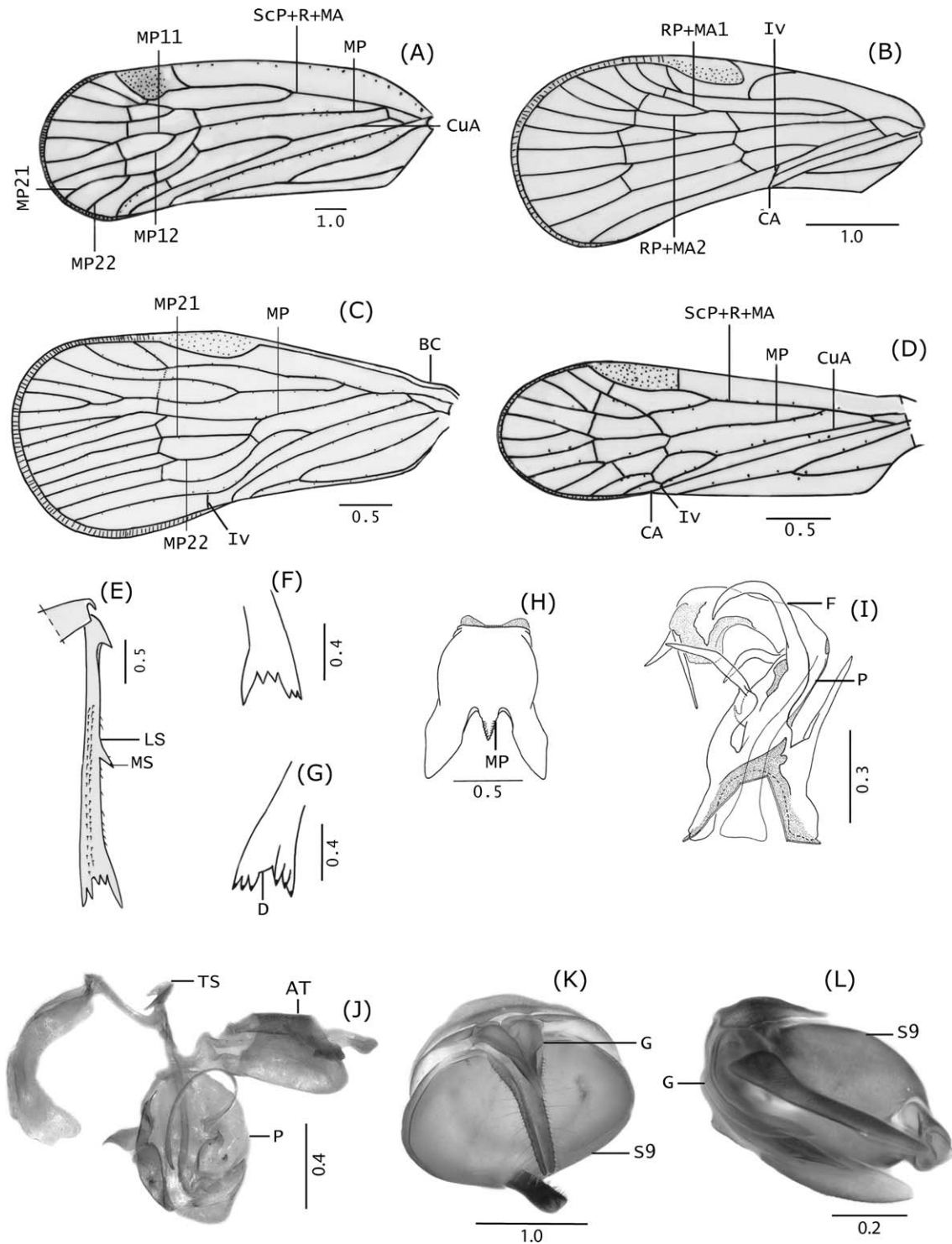


Fig. 2. Forewings, metatibiae, male and female genitalia characters. A–D, forewings: (A) *Cixius cunicularius*, (B) *Bothrioceretta nigra*, (C) *Brixia marojelyensis*, (D) *Haplaxius crudus*; (E) *Mnemosyne* sp., metatibia; F–G, metatibial apical spines: (F) *Mnemosyne* sp., (G) *Haplaxius crudus*; H–J, male genitalia: (H) *Melanoliarius orizicola*, pygofer, ventral view, (I) *Melanoliarius kindli*, phallic complex, ventral view, (J) *Andes* sp., styles, tectiform structure, phallic complex and anal tube, lateral view; K–L, female genitalia, segment nine and ovipositor: (K) *Mnemosyne* sp., posterior view, (L) *Bothrioceretta nigra*, posterior view. AT, anal tube; BC, basal concavity; CA, claval apex; D, diastema; F, flagellum; G, gonapophyses of ovipositor; Iv, intercubital veinlet; LS, lateral spine; MP, median process of pygofer; MS, modified ochre-coloured seta; P, perianthrium; S9, segment nine; TS, tectiform structure. Other abbreviations of forewing veins are as found in the text. Scales are in millimetres.

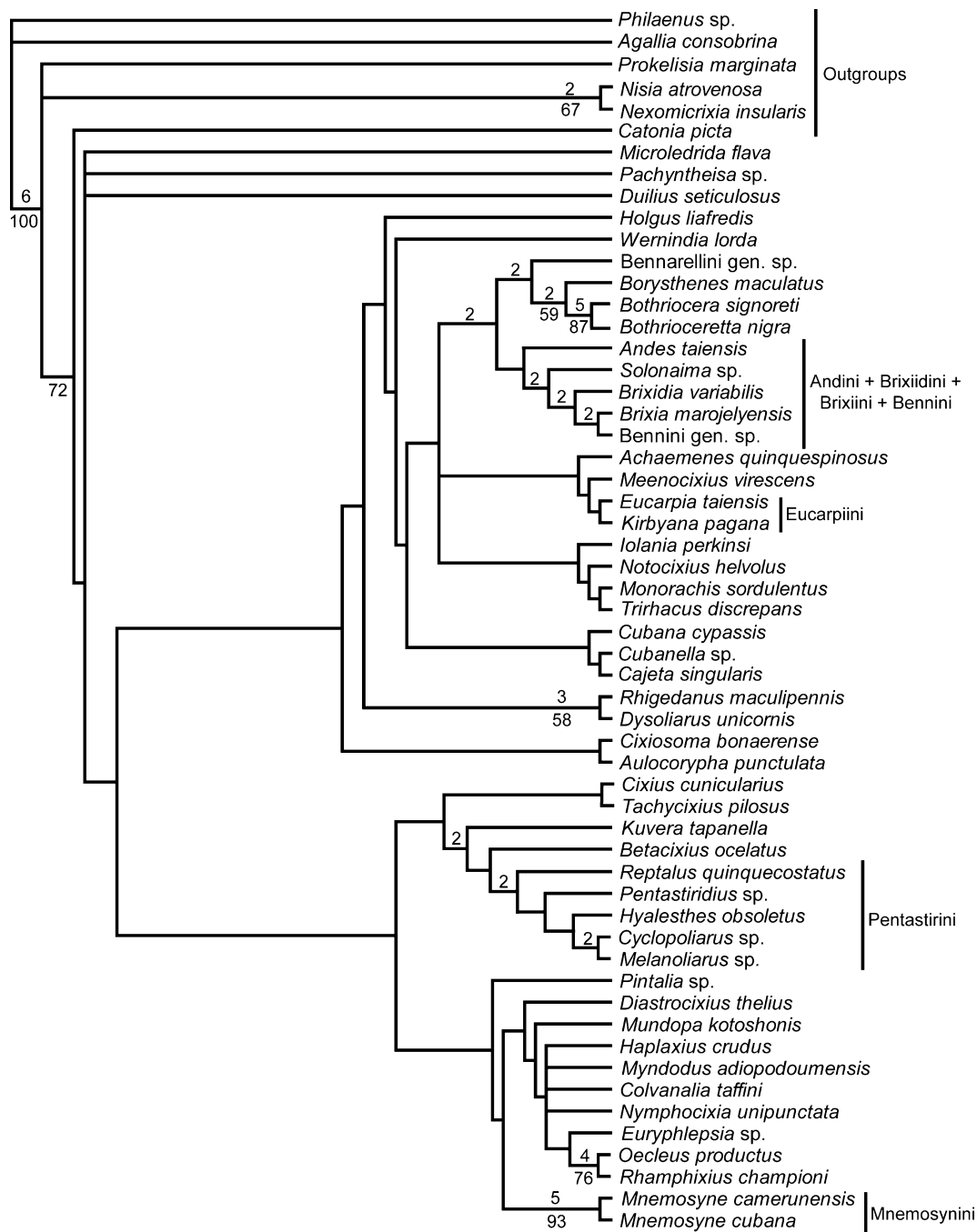


Fig. 3. Strict consensus of the 106 most-parsimonious cladograms from the equally weighted analysis. The numbers below branches are bootstrap values (when >50%) and those above are Decay values (when >1).

low clade posterior probabilities (Fig. 5). As in the MP trees, the tribes Cixiini, Pintaliini, and Oecleini were non-monophyletic.

Concerning the relationships among the various subfamilies and tribes, the three analyses recovered a clade formed by (Andini + Brixini + Brixidiini + Bennini) (Figs 3–5). The subfamily Borystheninae was also consis-

tently placed as a sister taxon of Bothriocerinae (Fig. 3). Both the SW and the BI topologies placed Stenophlepsini as the sister taxon of (Borystheninae + Bothriocerini), and the clade [Stenophlepsini + (Borystheninae + Bothriocerini)] is placed as arising from the tribe Oecleini (Figs 4, 5). Synapomorphies of the clades recovered in the SW tree are listed in Appendix 2.

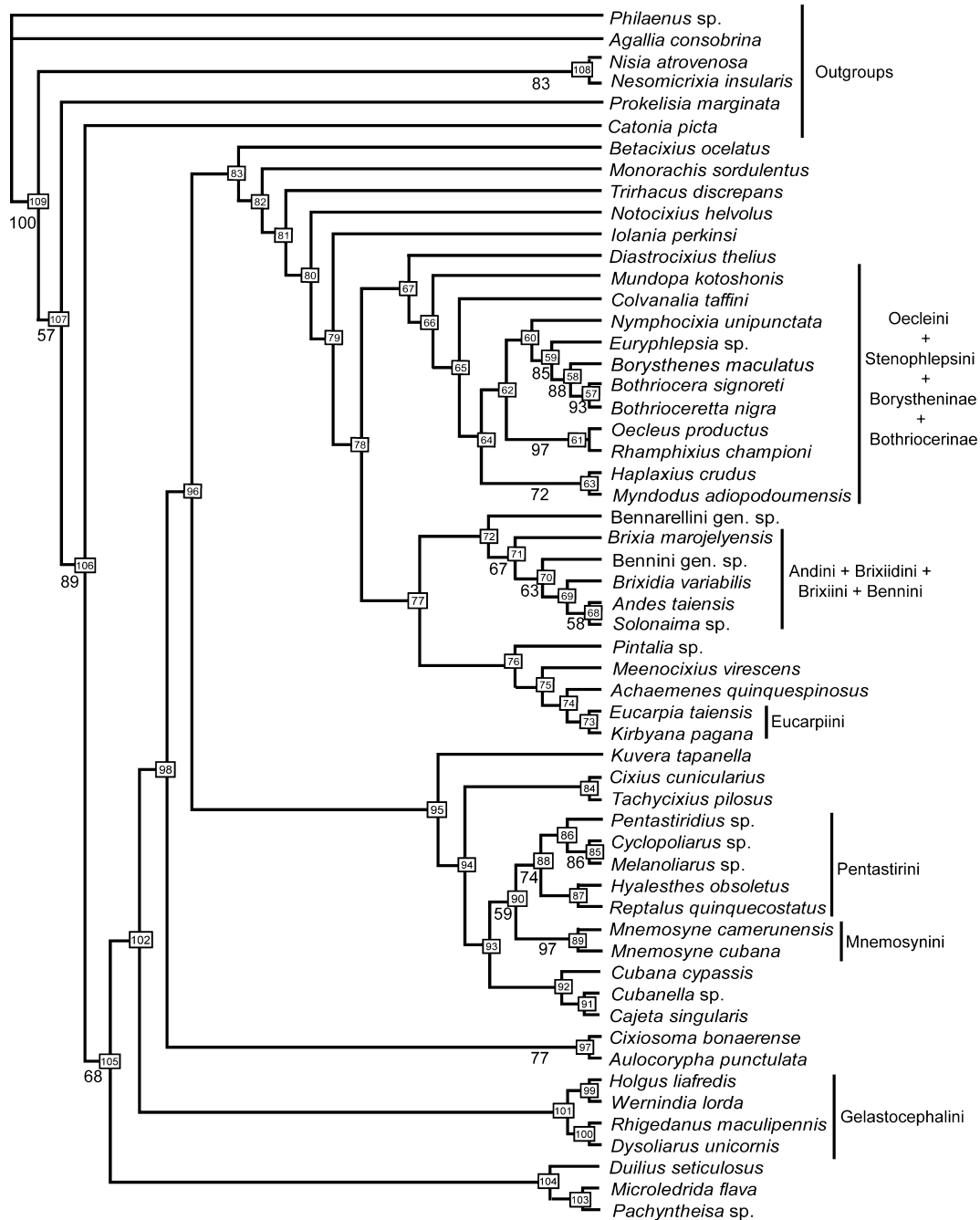


Fig. 4. The most-parsimonious cladogram derived under the successive weighting procedure. The numbers below branches are bootstrap values (when >50%). Node numbers are framed.

Discussion

This study represents the first attempt to use a cladistic analysis to elucidate the relationships within Cixiidae. Although the high level of homoplasy observed in our dataset led to a loss of resolution of the reconstructed phylogenies (support indices shown in Figs 3–5), some clear patterns emerged. First, the monophyly of Cixiidae

was recovered using both parsimony and Bayesian inference. Second, both kinds of analyses also recovered the clade formed by [Andini + Brixidiini (polyphyletic) + Brixidiini + Bennini] and another by (Borystheninae + Bothriocerinae). Third, in the BI and in the SW MP topologies, Stenophlepsini manifested as sister to (Bothriocerinae + Borystheninae), this clade arising from Oecleini.

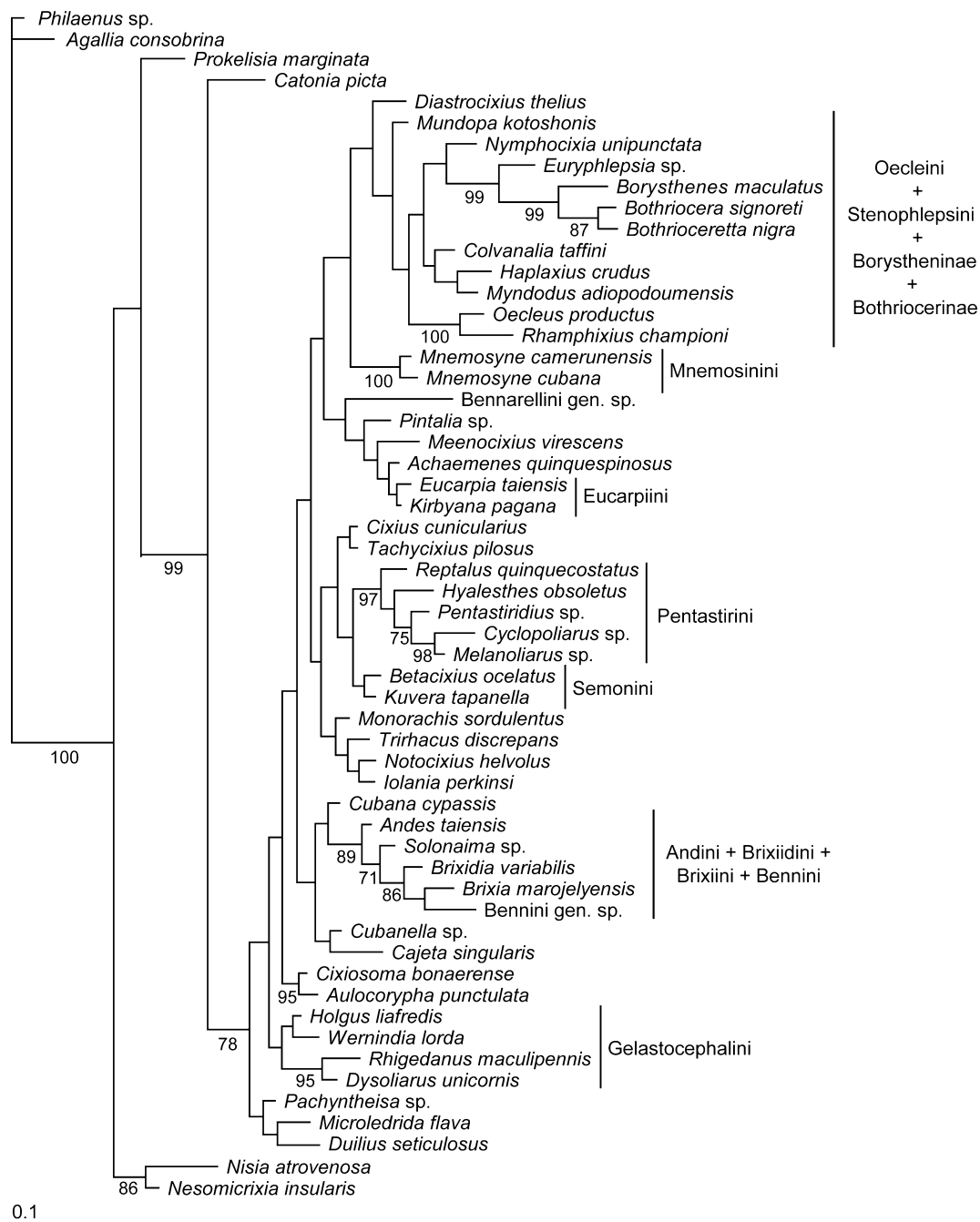


Fig. 5. Bayesian inference topology of cixiid morphological characters derived with Mk_v + Γ. Numbers below branches are posterior probability values (when >70%).

Monophyly of Cixiidae

The monophyly of Cixiidae has been contested primarily because cixiids are distinguished by a combination of features, but no unique characters have been recognized for the family. Muir (1923) suggested that most of the Fulgoromorpha families might have evolved from cixiids, and Ashe (1988) pointed out the absence of synapomorphic

traits for the family. Urban & Cryan (2007) obtained inconclusive results regarding Cixiidae monophyly. Whereas their Bayesian trees supported the monophyly of the family, parsimony analyses pointed to a paraphyletic Cixiidae, with Delphacidae arising from within the Cixiidae. Although cixiid monophyly is a recurring uncertain issue, the hypothesis that Cixiidae and Delphacidae form a monophyletic group is a consensus in the literature on Fulgoroidea

phylogeny (Asche, 1988; Muir, 1923, 1930; Bourgoïn *et al.*, 1997; Emeljanov, 1990; Urban & Cryan, 2007). In the present study, the monophyletic status of Cixiidae was recovered using all types of analyses, but with low support indices. Surprisingly, *Catonina picta* (Achilidae) appeared as sister group of Cixiidae in the topologies produced by MP and BI analyses (Figs 3–5). However, several studies have documented a clear separation between the more anciently diversified Fulgoroidea (Cixiidae and Delphacidae) and the remaining families, be it on the basis of differing ovipositor morphology or of molecular data (Asche, 1988; Bourgoïn, 1993; Bourgoïn *et al.*, 1997; Urban & Cryan, 2007).

Among the features supporting the Cixiidae clade, the only non-homoplastic synapomorphy (EW and SW analyses) is the flagelliform aedeagus. However, in insects of the subfamily Asiracinae, the most anciently diversified subfamily within Delphacidae, the aedeagus is also flagelliform (Asche, 1990). Further investigations are needed to determine whether or not the flagelliform aedeagus was acquired independently in Cixiidae and some Delphacidae.

The other characters supporting Cixiidae monophyly in the SW tree are the following homoplastic synapomorphies: the sixth and seventh abdominal sternites formed by two sclerites, six spines on the hind-tibiae apex, and the presence of tubercles on the forewing veins. Among these, the character typically invoked to define the family is the tubercles on the forewing veins (O'Brien & Wilson, 1985; Wilson, 2005). Among the other features often used to separate Cixiidae from the other Fulgoroidea is the presence of a third ocellus (Kramer, 1983). However, this character goes through a number of reversions and gains in the present analyses, proving less characteristic of Cixiidae than was previously thought. Emeljanov (2002) cited two synapomorphies for Cixiidae in his intuitive phylogeny, one of which is a nymphal character. Considering the very small number of species for which nymphal stages are known (Emeljanov, 2002), it is difficult to verify whether this nymphal character could be extrapolated to all representative taxa of the family. The second character mentioned by Emeljanov (2002) as a synapomorphy is the lateral spines of the metatibiae with a short thick seta. This character is interpreted as two independent ones here (53 and 54 – see character description for justification). Our results show that neither comes out as synapomorphies for Cixiidae. Other characters historically mentioned as distinguishing of Cixiidae are related to the piercing ovipositor present in most cixiids and all delphacids (Muir, 1923). The latter are easily distinguished by the presence of a spur on the metatibiae. In fact, most of the characters used to determine Cixiidae are present also in Delphacidae, the distinction between these two families being the apomorphic metatibial spur of delphacids.

Subfamily and tribal relationships

With regard to relationships among currently recognized cixiid groups, the subfamilies Bothriocerinae and Borysthe-

ninae clustered together in all analyses. Representatives of Bothriocerinae are found in the New World, whereas Borystheninae occur in Ethiopian and Oriental regions. Although this recent distribution pattern is not in agreement with the suggestion of the close relationship proposed here, Szewo (2002) recently described a new Bothriocerinae fossil genus, *Bothriobaltia*, from Baltic Amber (Scandinavian Peninsula). The presence of *Bothriobaltia* in the Palearctic region may indicate a broader ancient distribution of Bothriocerinae and reconcile the apparent conflict between actual biogeographical data and the (Borystheninae + Bothriocerinae) clade.

The (Bothriocerinae + Borystheninae) clade joined the Stenophlepsiini (arising from within the Oecleini) in both the SW and the BI analyses (Figs 4, 5). These groups have frequently been considered related. When he described the genus *Euryphlepsia*, Muir (1922) put it into the Oecleini. On the basis of the presence of a subantennal carina, he then changed this classification and erected the Bothriocerinae, including the genera *Bothriocera*, *Euryphlepsia*, *Stenophlepsia*, and *Borysthenes* (Muir, 1923, 1925). On the basis of the presence of a diastema on the apical spines of metatibiae, Emeljanov (2002) suggested that Bothriocerinae and Oecleini formed a monophyletic group, with Stenophlepsiini as sister group. However, Emeljanov (1989) separated *Borysthenes* from Bothriocerinae, arguing that the subantennal carinae and the wings similarities between *Bothriocera* and *Borysthenes* were not homologous. However, the possibility that all these tribes and subfamilies may form one unique clade, as suggested by both the SW and BI analyses, had never been raised. If Oecleini are paraphyletic, they might be either sunk or raised to subfamily status, as the tribe could not comprise the subfamilies Borystheninae and Bothriocerinae.

At the tribal level, Andini, Bennini, Brixiidini and Brixiini (polyphyletic) clustered in the MP and BI analyses (Figs 3–5), although with some differences in the relationships among them. Emeljanov (2002) proposed that these tribes form a monophyletic unit, based on the steeply tectiform forewings, which is not a synapomorphy here but is indeed the most frequent condition of this character in species of these tribes. The fact that the monophyly of Brixiini was not recovered in MP and BI analyses suggests that it might be justifiable to consider Andini + Bennini + Brixiidini + Brixiini as a unique tribe. However, a more comprehensive analysis of this group of tribes is necessary to confirm its monophyly.

Conclusions

In conclusion, the cladograms obtained in this study corroborate the monophyly of the family Cixiidae and some of its currently recognized subfamilies and tribes. However, the performed Templeton tests indicate that the monophyly of most of the tribes cannot be rejected based on the present data. Further studies are needed to assess the groups

recovered here and to examine some of the patterns recovered by the analyses. A study based on molecular data using genes with different evolutionary rates would further resolve the relationships of these taxa, which seem to be obscured by the significant level of homoplasy in cixiid morphological characters.

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Appendix 1. Morphological data matrix. The first six taxa are outgroups. Polymorphisms are indicated by letters: a = 0, 1; b = 0, 2; c = 1, 2; d = 3, 4; e = 3, 4; codes for inapplicable data: '?', for unavailable or doubtful data.

	1	2	3	4	5	6	7	8
<i>Philaenus</i> sp.	020001-10--0-31-020001-00--00--0-110011--00--0-101001020-0000000--00--006010-000001000							
<i>Agallia consobrina</i>	010001-10--0-31-000001-00--20--0-110011--00--0-100010??-0000000--00--00??10-00001100?							
<i>Prokelisia marginata</i>	02100011000-20101111001011100010010211-00010000105011000000--1010000010-01001000							
<i>Catonia picta</i>	0210001010-0000011000101010000100110c01??101001130000000011010-?000010-10120--0							
<i>Nisia atrovirens</i>	010001-11020-20021110100101110-1001110-21-00000000010-0000000--10000040??????????							
<i>Nesomicricta insularis</i>	020001-11000-200211101101011000010011021000101000020-0000000--10000000??0??0??0							
<i>Borysiethes maculatus</i>	01000011021120101100011001110100100100010111010001401000110120101112011100001000							
<i>Bothriocera signoreti</i>	021000111211100111100?1111101001001002100110001014110001101001011100010-01001000							
<i>Bothriocera nigra</i>	0100011121110011100011111010010010021000101000101411000111121101110011001011000							
<i>Andes taiensis</i>	120010-01021120001101100011000110110210111010000140000011100101100110?01001000							
<i>Brixidia variabilis</i>	120011-0102110000111110100100101010010011000014000001101001011101010?01001000							
<i>Brixia maroelyensis</i>	020011-11021100001111101001100010111101010110100001400000100100101112110?00001000							
<i>Solonaima</i> sp.	120010102112000111110100110001101101c01110100000140000011010010111020110?01000							
<i>Achaemenes quinquespinosus</i>	02000101010-000001101101000110001011110010001010000004000001?01001011100010-010001000							
<i>Cixius cunicularius</i>	02?00010101100000?1101101000100001110100c011101010114000001?010010111030110000001100							
<i>Cixiosoma bonaerense</i>	02100011010-00000110110100010000111001110111010001140000011010010111000111000001000							
<i>Microletrida flava</i>	02100011010-20102110010110000110110100?01010011400000110120101103011000001000							
<i>Pachyntheisa</i> sp.	021001-11010-0000?1101?01??1?000111011010-10010011400000??1?01110000??0??0??0??							
<i>Tachycixius pilosus</i>	021000101000000101010101000011011010101101010101000001101001011030110000011100							
<i>Duilius setulosus</i>	02100011010-01-121101001000100011011010000111000040000111101011000??0??0??0??							
<i>Eucarpia taiensis</i>	02100011010-00001101100011000101110010101010000040000000100101100010-01011000							
<i>Haplaxius crudus</i>	0200011010020010101?01111000011101120000001000000410000??1211011100210-01011000							
<i>Mundopa kotoshonis</i>	02000011011000012110110110100101101001011010010001410000111001011100010-01001000							
<i>Myndodus adiopodoumensis</i>	021000110110200121101101101000?11011201001101000?00410000?2?121101112010-01011000							
<i>Colvanalia taffini</i>	0200001101102001011011010001101100100100100000041000011100101112010-000001000							
<i>Nymphocixia unipunctata</i>	00100011021020012110111101000011111000-10010000041000011010010111010100001000							
<i>Oecleus productus</i>	000110?110011200111101?011110001?10112011-000100000141000011100101100010001011100							
<i>Rhamphixius championi</i>	000111-010211200121101101-011000111011202100001010001410000101141101112011101001100							
<i>Cyclopolarus</i> sp.	0210000101000000110110110100001101101000-100100000410000110100101110101010111							
<i>Hyalesthes obsoletus</i>	02a00011010-01-00110101010001100110001000100010010011400000101211011100000-001010110							
<i>Melanoliarus</i> sp.	02100000101a0000001101101010011011012001010101001140000010010110110000-101-11111							
<i>Pentastiridius</i> sp.	0200000101a0000011011010100111011000010100001140000010010101105001000011110							
<i>Mnemosyne camerounensis</i>	02000011011000001101101101101101101101101101101101101101101101101101101100000011100							
<i>Mnemosyne cubana</i>	02000011011000010110110110110-1110110110011011001?41000011012110111000011000011100							
<i>Aulocorypha punctulata</i>	02100011010-00000110110101000110011101101010011400000?010010111042111000001000							
<i>Cubana cypassis</i>	02000010102000001101101010001011001010100114000001110010111001101001010001000							
<i>Cubanella</i> sp.	020000101110000011011010010010100100							
<i>Meenocixius virescens</i>	020000110110200000101101000101111011-0000010004100001?1001011105010-000001000							
<i>Monorachis sordidulus</i>	0210001101100000110110100010101001010001140000011100101110001110000100001000							
<i>Diastrocixius thelius</i>	020001-1102100000110110111010000110110011011010001410000??1001011105011001001100							

Appendix 1. Continued.

	1	2	3	4	5	6	7	8
<i>Notocixius hevolus</i>	021000101010-0000011101100001000011101100201000100000004000000111001011103010-01001000							
<i>Pintalia</i> sp.	0200001110110000001101101100010001011100101110010110101000140000011?120101100011000011000							
<i>Betacixius ocelatus</i>	021001-1101000000110110101100001110110000101000011400000110010111000110001011000							
<i>Kuvera tapanella</i>	02100011101100000011011010001000011101101b0101010001400000110100101100011000011100							
<i>Reptalus quinquecostatus</i>	02?000101011000000110110111000011101100001010000114000001201011111120010000111110							
<i>Iolania perkinsi</i>	020000101010-000011101101000100011110110010101000001400000111001011105010-000001000							
<i>Bennarellini</i> gen. sp.	010000111021120101111101000100001001110111211011000004010011101211011100210-01011000							
<i>Trirhachus discrepans</i>	02100010101100020111011010001100011101102a0100010000114000001101001011103010-000001000							
<i>Euryphlepsia</i> sp.	02000001102112001211000111101100011001120011100100010000-00011010010111?0110001011000							
<i>Holgus liafredis</i>	021000-11010-0000011011010001100011101110?0a0?0101001140000011100101100010-010001000							
<i>Wernindia lorda</i>	020000-11010-0000111011000011000101011101000101001140000011010111011011011011000							
<i>Kirbyana pagana</i>	021000111010-0000011011010001100010111001010100000040000??1001011100010-010001000							
<i>Bennini</i> gen. sp.	12a011-0102110010111101000110001011100111101000011401010111001011121110000011100							
<i>Cajeta singularis</i>	010000101010-00002110110111110001010110a01?101100001200000111001011100210-01011100							
<i>Rhigedanus maculipennis</i>	021001-11020-000021101101111100001110111200?1011100115110001?010010111002???????????							
<i>Dysolotarus unicoloris</i>	021001-11010-0000?110110101010001110111100111?1110011411100000010010?11?0110-010001000							

Appendix 2. List of apomorphies for the nodes in the most-parsimonious tree in Fig. 4 (SW tree) with fast optimization. Non-homoplastic synapomorphies are in bold type.

Node	Apomorphies
109	9 (1) , 14 (2), 15 (0), 18 (1), 19 (1) , 20 (1) , 25 (1) , 29 (1) , 43 (1), 66 (1)
107	3 (1), 6 (0), 55 (3), 69 (1)
106	11 (1), 14 (0), 18 (0), 40 (1), 43 (0), 50 (1), 54 (1), 64 (1) , 70 (1)
105	36 (1), 55 (4), 61 (1), 62 (1), 71 (1) , 76 (1)
102	23 (1), 46 (1), 65 (0)
98	30 (0), 44(1)
96	40 (0), 81 (1)
83	50 (0)
82	12 (1), 18 (1), 27 (0), 46 (0), 63 (1), 81 (0)
81	8 (0), 73 (3)
80	12 (0), 53 (0)
79	3 (0), 46 (1), 73 (0)
78	8 (1), 12 (1), 45 (1)
67	26 (1), 27 (1), 44 (0), 56 (1), 79 (1)
66	17 (1), 18 (2), 46 (0)
65	14 (2), 30 (1), 54 (0), 72 (1), 73 (2)
64	40 (2)
62	2 (0), 11 (2), 13 (1), 76 (1)
60	24 (1) , 42 (0), 44 (1), 63 (0)
59	2 (1), 7 (0), 22 (0) , 23 (0), 36 (0), 52 (1), 56 (0), 57 (1)
58	18 (1), 28 (1), 32 (1), 34 (0), 39 (0) , 40 (0), 42 (1), 46 (1), 54 (1), 65 (2)
57	7 (1), 10 (1) , 14 (1), 41 (2), 44 (0), 56 (1), 73 (0)
61	4 (1) , 5 (1), 43 (1), 45 (0), 54 (1), 83 (1)
63	62 (2), 65 (2), 67 (1), 81 (1)
77	30 (1), 35 (0), 37 (1), 63 (0)
72	11 (2), 13 (1), 21 (1), 28 (1), 74 (1)

Appendix 2. Continued.

Node	Apomorphies
71	5 (1), 6 (1), 72 (1), 73 (2)
70	1 (1) , 8 (0), 28 (0)
69	37 (0), 72 (0), 74 (0), 77 (1), 79 (1)
68	6 (0), 14 (2), 35 (1), 41 (1)
76	18 (0)
75	45 (0), 54 (0)
74	12 (0), 62 (0), 79 (1)
73	3 (1), 61 (0)
95	83 (1)
94	8 (0)
84	45 (1), 73 (3)
93	3 (0), 18 (1), 30 (1)
90	33 (1), 67 (1), 75 (0)
88	42 (0), 80 (1), 84 (1)
86	7 (0), 62 (0)
85	3 (1), 41 (1), 76 (0), 78 (1), 81 (0), 85 (1)
87	18 (0), 30 (0)
89	8 (1), 12 (1), 26 (1), 31 (1), 41 (1), 44 (0), 49 (1), 56 (1), 65 (2)
92	63 (1), 74 (1), 76 (0), 79 (1)
91	26 (1), 35 (0)
97	38 (0) , 41 (1), 45 (1), 77 (1)
101	74 (1), 76 (0), 79 (1)
99	27 (0)
100	6 (1), 18 (2), 41 (1), 42 (0), 45 (1), 49 (1), 56 (1), 57 (1), 62 (0)
104	18 (2)
103	28 (1), 30 (0), 45 (1), 65 (2)
108	17 (2), 42 (2), 53 (0)