



A cladistic insight into the higher level classification of Baetidae (Insecta: Ephemeroptera)

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Abstract. Baetidae was one of the first families established for mayflies (Ephemeroptera). After more than 200 years of progressive research, Baetidae is now known as the most species-rich family in the order. Two competing proposals of family division were proposed: Cloeoninae and Baetinae, or Protopatellata and Anteropatellata. Both classifications were established without cladistic support. The purpose of this paper is to investigate the phylogenetic relationships of the family Baetidae using morphological evidence and evaluate these classification schemes. The matrix included 245 morphological characters derived from larval and adult stages across 164 species in 98 genera. The resulting tree recovered Anteropatellata, Protopatellata and Cloeoninae as not monophyletic; Baetinae and Baetovectata were recovered as monophyletic with the same circumscription. Subimaginal and imaginal characters presented the lowest homoplastic values and were the best to understand the higher relationships.

Introduction

Baetidae Leach was one of the first families of mayflies established (1815), initially with only two genera: *Baetis* Leach, which encompassed the species with two pairs of wings, and *Cloeon* Leach, the species with a single pair of wings. Although the fossil record of Ephemeroptera dates back to the Carboniferous (Salles *et al.*, 2015), it is not until the Early Cretaceous where the first species of Baetidae appear in the fossil record (Poinar, 2011).

After more than 200 years of research, Baetidae is now the most taxon-rich family in the order, with approximately

110 genera and 1100 species constituting a quarter of the world's mayfly diversity (Gattolliat & Nieto, 2009; Sartori & Brittain, 2015). The family is present on almost all land-masses except Antarctica, New Zealand and some oceanic islands (Edmunds *et al.*, 1976). Baetids inhabit diverse freshwater habitats, both lentic and lotic. The worldwide distribution of Baetidae and its considerable diversity are reflected in its long history of taxonomic review and conflicting concepts of the suprageneric classification based on inferences from morphology (Kazlauskas, 1972; Riek, 1973; McCafferty & Waltz, 1990; Gillies, 1991; Kluge, 1997, 2004).

Baetidae was placed in the suborder Pisciforma, superfamily Baetoidea, by McCafferty (1991), or in the Tetramerotarsata and Tridentiseta by Kluge (2004), but always as sister to Siphlaenigmatidae (Staniczek, 1997). Later, Ogden & Whiting (2005) and Ogden *et al.* (2009, 2019) did not recover any of these clades as monophyletic. Instead, they recovered Baetidae close to the

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Fig. 1. Baetidae, live specimens: (A) female imago of *Similicloeon simile* (Eaton); (B) male imago of *Centroptilum luteolum* (Müller); (C) male imago of *Cloeodespenai* (Moriyama & Edmunds); (D) female imago of a new species of *Callibaetis* Eaton; (E) larva of *Alaimites muticus* (Linnaeus); (F) larva of *Americabaetis longetron* Lugo-Ortiz & McCafferty; (G) larva of *Guajirolus ektrapelogglossa* Flowers; (H) larva of *Nanomis galera* Lugo-Ortiz & McCafferty. All photographs are by FFS. [Colour figure can be viewed at wileyonlinelibrary.com].

base of Ephemeroptera, with Siphuriscidae as a sister group of all other mayfly clades (Ogden *et al.*, 2019).

The best-known concept for division of the family was proposed by Kazlauskas (1972), recognizing Cloeoninae Kazlauskas for those baetids with a single marginal intercalary

vein on the forewing (Figs 1A, B) and Baetinae Leach for those with two marginal intercalary veins on the forewing (Fig. 1C). This division was later supported by Landa & Soldan (1985) and especially by Gillies (1991), adding two characteristics to distinguish the two subfamilies. The first is the setal tuft at the

base of the prosthema of the mandible (first correlated to a high degree with the venational differences by Demoulin, 1965), which is present in Cloeoninae and absent in Baetinae, and the second is the double row of denticles on the tarsal claws in Cloeoninae, which is reduced to one in Baetinae (Landa & Soldan, 1985). In order to corroborate the combination of these three characteristics (intercalary veins, tufts of setae on the mandible, and the number of rows of denticles in the claw) for the diagnosis of the subfamilies, Gillies (1991) quantified the number of known African genera that present these characteristics; Gillies (1991) also made comparisons with some Neotropical, Palearctic, Nearctic and Oriental genera. However, combinations of characteristics are not evidence of an evolutionary relationship, and, in any case, the increasing number of species and genera being described has progressively decreased the number of matches. While Gillies (1991) proposed these combinations, he commented that the analysis of suprageneric relationships in only two global lineages supported by three characteristics is a simplistic approach that causes a number of potential problems.

Gillies (1991) also recognized a third subfamily, Callibaetinae Riek. This subfamily was proposed by Riek (1973) and was synonymized with Cloeoninae by Landa & Soldan (1985). Callibaetinae only contains *Callibaetis* Eaton (Fig. 1D), a Pan-American genus that is characterized by the presence of both types of intercalary veins (single and double) and by an ornamented cuticle.

Two new classifications of the generic and suprageneric diversity of Baetidae were proposed since the work of Gillies (1991). The first was the establishment of genus complexes, such as the *Baetodes* complex (Lugo-Ortiz & McCafferty, 1996; Nieto, 2015), *Baetis* complex (Lugo-Ortiz & McCafferty, 1998a), *Centroptiloides* complex (Lugo-Ortiz & McCafferty, 1998c) and the *Cloeodes* complex (Lugo-Ortiz & McCafferty, 1998b). These arrangements were intended to delineate monophyletic lineages within the family, irrespective of the existing classification of subfamilies. These establishments of complexes implied the erection of several of new genera as well as numerous new combinations or reassignments (Waltz & McCafferty, 1987; McCafferty & Waltz, 1990; Lugo-Ortiz & McCafferty, 1998c). As no formal global reconstruction was undertaken, many genera were not assigned to a complex (Gattolliat *et al.*, 2008).

The second classification, proposed by Kluge (1997, 2004) and Kluge & Novikova (2011), is based on a non-Linnaean approach – the Rank-free System. In this system, the family Baetidae is treated as Liberevenata Kluge, or Baetis/fg3, which is divided into two groups, the extinct plesiomorphon *Palaeocloeon*/fg1 Kluge and Turbanoculata Kluge or Baetis/fg4. Turbanoculata is divided into the plesiomorphon Protopatellata Kluge & Novikova and Anteropatellata Kluge or Baetis/fg5. Protopatellata is diagnosed primarily through the patella-tibial suture present on the middle and hind legs of the larva only, whereas in Anteropatellata, this suture is present on all of the legs of the larva. In Anteropatellata, the first major group is Baetovectata Kluge & Novikova or Baetis/fg6, which is characterized mainly by the

double marginal intercalary veins and the arched shape of the gonovectes.

Few phylogenetic reconstructions have been conducted since the beginning of the 21st century (Monaghan *et al.*, 2005; Gattolliat *et al.*, 2008; Nieto, 2010; Salles *et al.*, 2015). Monaghan *et al.* (2005) corroborated the monophyly of Baetinae and Cloeoninae mainly based on specimens from continental Africa and Madagascar. They also included Asian and European taxa in analyses, which indicated that the two proposed subfamilies may represent a deep subdivision of Baetidae globally. Gattolliat *et al.* (2008) corroborated the monophyly of Baetinae for African members of the family, with inconclusive results related to Cloeoninae. However, Nieto (2010) recovered Baetinae and Cloeoninae as paraphyletic in a review of the relationships among the South American baetids, although the findings of this study also emphasized the need for a further and more detailed analysis of the problem to better define these relationships. Clearly, analyses that include genera from other biogeographical regions of the world will be necessary before confident conclusions can be made on the internal relationships within Baetidae.

Considering the existence of independent proposals for the higher-level classification of the family, and the absence of a comprehensive cladistic analysis to support the proposed groups, two fundamental questions remain. Firstly, do the proposed classifications reflect natural groups? Second, are the current morphological characteristics attributed to subdivisions useful for recognizing phylogenetic relationships? The purpose of this paper, therefore, is to provide the first quantitative investigation of phylogenetic relationships of the entire Baetidae, and thus evaluate the proposed classification schemes, using morphological evidence.

Materials and methods

Taxon sampling

Outgroups were selected from families considered to be closely related to Baetidae, based on shared diagnostic characters and on the results of previous studies. The phylogenetic tree was rooted with *Siphuriscus chinensis* Ulmer (Siphuriscidae). The molecular evidence indicates that this family is a sister group of all other mayfly clades, and Baetidae is supported as a lineage near the base of the tree (Ogden *et al.*, 2009, 2019). *Isonychia bicolor* (Walker) (Isonychiidae) was included because this family was identified as closely related to Baetidae in some topologies in molecular-based phylogenetic analyses (Ogden & Whiting, 2005; Ogden *et al.*, 2009). *Siphlaenigma janae* Penniket was included because Siphlaenigmatidae has historically been considered to be the sister group of Baetidae (Landa & Soldan, 1985; Staniczek, 1997; Kluge, 2004) and has been included as such in a number of recent analyses (Nieto & Richard, 2008; Nieto, 2010; Salles *et al.*, 2015). According to McCafferty's (1991) arrangement, Baetidae is placed in the suborder Pisciforma and, in the Rank-Free System, the suborder Tridentiseta. The family Ameletidae (*Ameletus subnotatus*

Eaton) is in the same suborder in the two arrangements and is included in our analysis. *Leptophlebia marginata* (Linnaeus) (Leptophlebiidae) was chosen because species in Leptophlebiidae have a compound eye with dorsal and ventral faceted surfaces in male imagoes. As a general rule, we attempted to achieve a balanced representation of all available main lineages of Baetidae, and we favoured species with detailed morphological descriptions. It is important to allow the reproducibility of analyses as well the possibility for the matrix to be properly verified. Four criteria were adopted for inclusion of taxa in the present phylogenetic analysis: (i) the descriptions and illustrations of the species must be of adequate quality or, alternatively, access to material housed in collections for direct observation, (ii) species with imagoes described have preference, (iii) the type species of the genus is included, as long as the description (or redescription) and illustrations are adequate and (iv) data on the larvae are available (for extant species). The second and third criteria (data on the adult stage) are especially important given the diagnostic role of the adults in both systems. Given this, 98 of the 105 extant baetid genera, plus one extinct genus, were included in the present analysis, with a total of 164 species sampled (some genera were represented by more than one species). In order to permit a reliable comparison of results between lines of investigations, species from the molecular analyses of Monaghan *et al.* (2005) and Gattolliat *et al.* (2008) were purposely included. All taxa are presented in File S2.

Higher-level classification and taxon sampling

The concepts of Protapatellata and Anteropatellata are mainly based on one binary character (Kluge, 1997; Kluge & Novikova, 2011, 2014, 2016). This approach makes the application of the two names easy for any species with properly described larvae. The concepts of Baetinae (Figs 1C, E, H) and Cloeoninae (Figs 1A, B) proposed by Gillies (1991) (following Kazlauskas, 1972) were formulated based on a combination of larval and adult characters. Thus, many taxa cannot be allocated to any subfamily, either because of the lack of adults, or because of the lack of correlation between the three characteristics. In the last case, only the correlation between intercalary veins and the tuft of setae on the mandible was adopted. This is due to, according to Gillies (1991), the denticles on the tarsal claws correlate less closely with the adult venational, probably because the claws have been highly modified in response to adaptive pressures. Even accepting such a premise, some taxa could not be attributed to any subfamily.

The a priori application of the higher classification was based on species morphology, rather than being based on previous proposals, diagnosis of genera or molecular analyses (Kluge & Novikova, 1992; Gattolliat *et al.*, 2008). The main reason to prefer the specific rather than the generic approach is that many genera have been shown to be either paraphyletic or polyphyletic (Gattolliat *et al.*, 2008; Kluge & Novikova, 2014; Salles *et al.*, 2015). Adopting such an approach, the monophyly of the subfamilies can be tested either considering the subfamilies restricted to a geographical area

(Africa+Madagascar) or considering them as global lineages, both by the original diagnostic characters (Kazlauskas, 1972) or revised by Gillies (1991).

The subfamily Callibaetinae contains only *Callibaetis* (Fig. 1D); and two species of the genus were included in our study. There is no doubt about the monophyly of the genus (Cruz *et al.*, 2016), but its status as a subfamily needs to be verified. For purposes of this study, there is no difference in the effect of considering the genus *Callibaetis* to be in this subfamily or to be in an undetermined subfamily. However, as pointed by Kluge (2017), when one marginal intercalary is located either anteriorly, or posteriorly of the middle of a space, an eccentric single marginal intercalary of this type is secondary. This is the case for some species in *Cloodes* Traver (Fig. 1C), *Callibaetis* and *Centroptilum elongatum* Suter. Based on this finding, the three taxa were treated a priori as Baetinae. The extinct species *Palaeocloeon taimyricum* Kluge was described based on the imago and subimago of both sexes and was treated as Palaeocloeoninae Kluge. The remaining extinct subfamily Vetuformosinae Poinar (2011) only has the female imago described and thus was not included in the analyses. Considering the aspects mentioned above, taxa were also selected based on the applicability or inapplicability of subdivision concepts.

Characters

The matrix included 245 morphological characters, of which 207 were derived from larval stages and 38 from the adult (imago). All characters were considered to be non-additive (unordered). To separate neomorphic and transformational components effectively in the case of taxa that lack a particular structure, the transformational character states were coded as inapplicable, indicated by ‘-’ (Serenó, 2007). All characters and states were codified from the literature and from the Index of Characters available on the Phylogenetic System of Ephemeroptera website (Kluge, 2019). In some cases, characters or states were not described for a specific species, but were present in photographs and/or illustrations of the description; consequently, these characters were utilized. Finally, for some taxa that are insufficiently or incorrectly described, direct observations of the specimens were performed. Autapomorphic characters, although non-informative, were kept in the present study because they represent essential information for future studies at lower taxonomic levels. The list of characters and states is presented in File S1; list of species, literature or collection is present in File S2; list of tree indexes (tcomp and sprdiff) is presented in File S3; matrix is presented in File S4; Tcomp and sprdiff scripts are presented in File S5; list of adjusted homoplasy index for each character is presented in File S6; and evolution of selected characters in topology are presented in File S7.

Terminology

The morphological terminology used in this paper follows Hubbard (1995) for terms commonly found in the mayfly literature, such as prostheca, gills, legs, etc. Some more recently

proposed terms (Kluge, 2004, 2012; Kluge & Novikova, 2011) were also adopted. Names are employed according to the most recent literature and only pre-existing names were used. This caution is justified by the preliminary nature of the phylogenetic hypothesis and to avoid the proliferation of new names.

Analyses

All phylogenetic analyses were performed with TNT (Goloboff *et al.*, 2008) under extended implied weighting (Goloboff, 2014) (TNT default) following the script published by Mirande *et al.* (2013). The extended implied weighting method prevents the characters with many missing entries from having artificially high weights (Goloboff, 2014). The analyses provide 21 values of K and their respective topologies. For each K-value, under extended implied weighting, extra searches were carried out using parsimony ratchet (Nixon, 1999), sectorial searches, tree fusing and tree drifting (Goloboff, 1999) until the best fit was obtained three times, all using TNT default. Trees obtained for each K-value, were reduced to a strict consensus (if more than one). These searches resulted in 21 trees. As in Mirande (2009, 2010) and Mirande *et al.* (2011, 2013), stability was used as a criterion to select topologies to produce a strict consensus considered to represent the most stable solutions under different parameters. The measures of stability used were the distortion coefficient ('tcomp') (Farris, 1989) and the SPR distance ('sprdiff') (Goloboff, 2008). The strict consensus trees obtained for each K-value (21) were included in TNT memory (RAM) and the distortion coefficient and SPR distance were found using the scripts in File S5. The simple average for each reference tree was calculated (File S3). Those with the two best averages of 'tcomp' were selected to compose the set of topologies. Inside the best 'tcomp', the two-best average of 'sprdiff' was selected to compose the final set of topologies. In order to produce a stable final hypothesis, the unstable taxa were investigated using the commands 'pcrprune' and 'prunnelsen'.

Support was estimated through frequency differences expressed as GC values (Goloboff *et al.*, 2003). These values were calculated with 200 replicates by symmetrical resampling of the original matrix. Each replicate was calculated by 10 random addition sequences plus SPR, saving 10 trees per replicate. The characters and states were plotted in trees using WinClada 1.00.08.

Results and discussion

The final tree produced (Figs 2, 3, 4) has a total fit of 94.22, adjusted homoplasy of 133.77 and 2589 steps.

Evolutionary trends of key characters

According to Kluge (2011, 2012) and Kluge & Novikova (2011), the absence of the patella-tibial suture on the forelegs of the larvae (Char. 133) is plesiomorphic. The absence of the suture on the forelegs of the larvae in our study was also recovered as plesiomorphic, with some changes (7 steps) in different clades (Fig. 3 in File S7), thus resulting in a high homoplastic index (0.66).

Edmunds (1975), Morihara & McCafferty (1979) and Gillies (1991) all interpreted the presence of a setal tuft on the right mandible (Char. 49), the two rows of denticles on the claw (Char. 147) and paired marginal intercalary veins (Char. 211) as ancestral traits within Baetidae. Here, the presence of a setal tuft on the right mandible was recovered as apomorphic, with many changes (16 steps) in different clades (Fig. 1 in File S7), resulting in a high homoplastic value (0.83). The presence of two rows of denticles on the claws was recovered as plesiomorphic, also with many changes (12 steps) in different clades (Fig. 2 in File S7), thus resulting in a high homoplastic value (0.75). The single marginal intercalary vein (Fig. 3 in File S7) was recovered as plesiomorphic, only with two steps (one change to paired and one reversal), therefore resulting in a one of the lowest homoplastic value (0.25). The reversal occurred in the clade that comprises *C. elongatum* + *Callibaetis* (present in species of *Callibaetis* not included in the analysis). Based on the arguments of Kluge (2017), this is not a true single vein, since, when a single marginal intercalary is located either anteriorly or posteriorly of the middle of the space, the eccentric single marginal intercalary is secondary. In some *Callibaetis* species examined microscopically, traces of a second vein were observed (PVC personal observation). In some *Callibaetis*, *Cloeodes* and *Callibaetoides* Cruz, Sales & Hamada, the difference in intercalary veins is expressed as a sexual dimorphism (Cruz *et al.*, 2013, 2014; Kluge, 2017).

The shape of gonovectes (Char. 241) followed the same distribution in topology as the intercalary veins, with one step and the best homoplastic value (0.00) (Fig. 3 in File S7). The arched shape (and probably movable) was recovered as an apomorphy. Unfortunately, for many species the male imago either remains unknown, or its descriptions lack the gonovectes. The type of microlepid on subimago tarsi (Char. 225) followed almost the same distribution in topology as the intercalary veins and the shape of the gonovectes, with three steps and a low homoplastic value (0.25) (Fig. 3 in File S7). The blunt microlepid were recovered as apomorphic. As stated for the previous character, many species are either not described at the subimaginal stage or their descriptions did not include the microlepid.

Taking into account the findings described above, and the comparisons of the adjusted homoplasy index for each character (see all in File S6), many larval characters had high homoplastic values (≥ 0.50) and required many steps, while the imaginal characters have lower homoplastic values (< 0.50) and fewer steps. In a larger context and in the attempt to investigate broader lineages, the imaginal characters were recovered as more stable (less homoplastic) than those from larvae. This result can be plausibly attributed to the higher selective pressures on larvae than on imagoes (leading to many convergences), as in the variation of claws pointed out by Gillies (1991).

Gillies' revision of the Kazlauskas system

While attempting to apply the subfamilial concept to the species, several of them could not be allocated in any of the groups (combination of characters does not match – green in

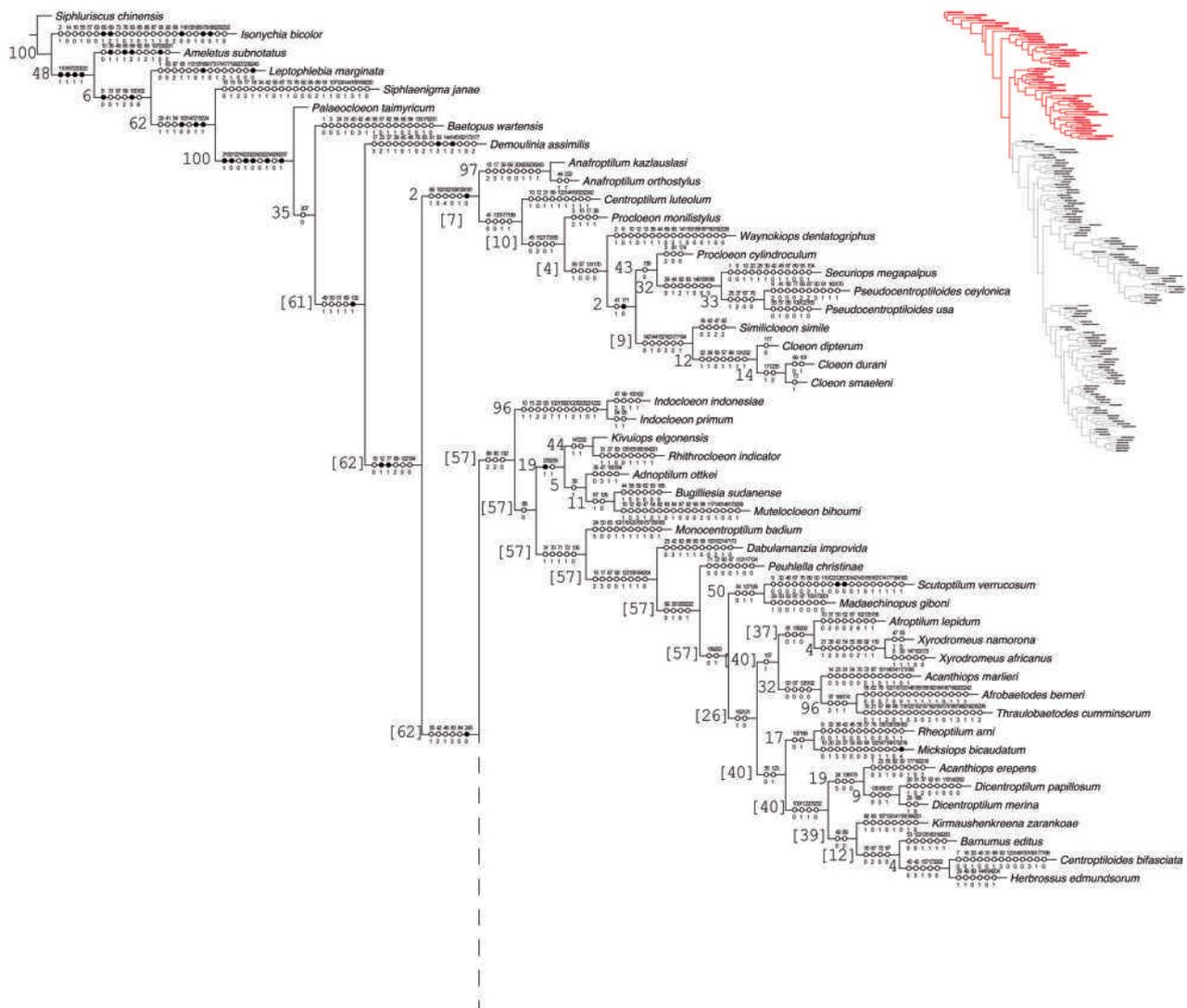


Fig. 2. Phylogeny of Baetidae (part). Black circles indicate synapomorphies, white circles represent non-unique changes in the tree, either forward or reverse; above are the character numbers and below are the character states. The values on left of branches correspond to Frequencies differences. [Colour figure can be viewed at wileyonlinelibrary.com.]

Fig. 5B), even for some species with the male imago described. This inapplicability is related to the homoplastic nature of the setal tuft on the right mandible (Fig. 1 in File S7), and the rows of denticles on the tarsal claws (Fig. 2 in File S7). However, surprisingly, all species a priori classified as Baetinae (*sensu* Gillies), were recovered in a single, large monophyletic group (clade A in Figs 3, 4). This clade is characterized by (Fig. 3) three unambiguous characters: mainly blunt microlepidies on the subimago foretarsi (Char. 225), the double marginal intercalary veins (Char. 211) (as proposed originally by Kazlauskas) and arched gonovectes (and probably movable) (Char. 241). Almost all species in this clade share the presence of a patella-tibial suture (Char. 133). Therefore, based on these discoveries, clade A is recognized as Baetinae, but newly circumscribed.

The subfamily Baetinae also contains the former Callibaetinae. In our results, Callibaetinae was recovered as a monophyletic group, nested with genera from New World (*Callibaetoides*) and Australia (*C. elongatum*), calling into question its status as a separate subfamily. In a cladistic reconstruction of South American Baetidae, Nieto (2010) also did not recover Callibaetinae as a distinct subfamily. The close relationship of these genera was also recovered by Cruz *et al.* (2016), and proposed by Kluge (2019) and Jacobus & Wiersema (2014). The inclusion of *Callibaetis* and *C. elongatum* in Baetinae (as well some species of *Cloeodes*) expands the concept of Baetinae to include those taxa that have secondarily lost one of the paired intercalary veins (in these taxa, as mentioned previously, the position of this vein is lateral and not central as in Cloeoninae).

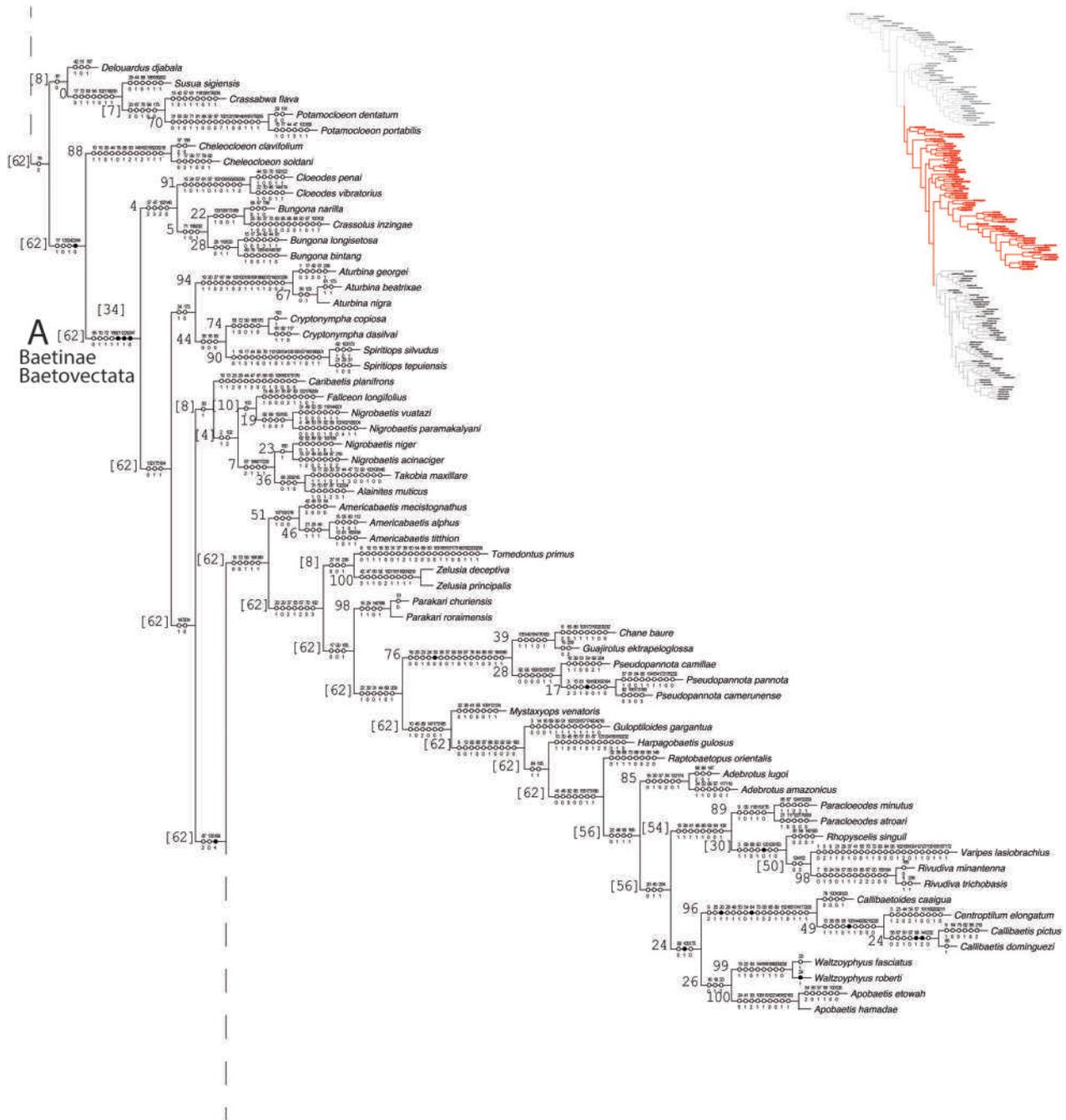


Fig. 3. Phylogeny of Baetidae (continued). Black circles indicate synapomorphies, white circles represent non-unique changes in the tree, either forward or reverse; above are the character numbers and below are the character states. The values on left of branches correspond to Frequencies differences. [Colour figure can be viewed at wileyonlinelibrary.com].

All remaining groups, which together include all species classified a priori as Cloeoninae, were polyphyletic and recovered in at least four distinct clades (Figs 2, 3, 5). The results of our study are similar to those based on molecular data of Monaghan *et al.* (2005) and Gattolliat *et al.* (2008). In both molecular studies, the topology recovered Baetinae as monophyletic,

while Cloeoninae was recovered as monophyletic in Monaghan *et al.* (2005), and monophyletic (maximum likelihood) or paraphyletic (direct optimization) in Gattolliat *et al.* (2008). Regarding the position of taxa in higher lineages, herein and in both molecular studies, almost all species and genera were recovered in the same groups (Baetinae or Cloeoninae). For example,

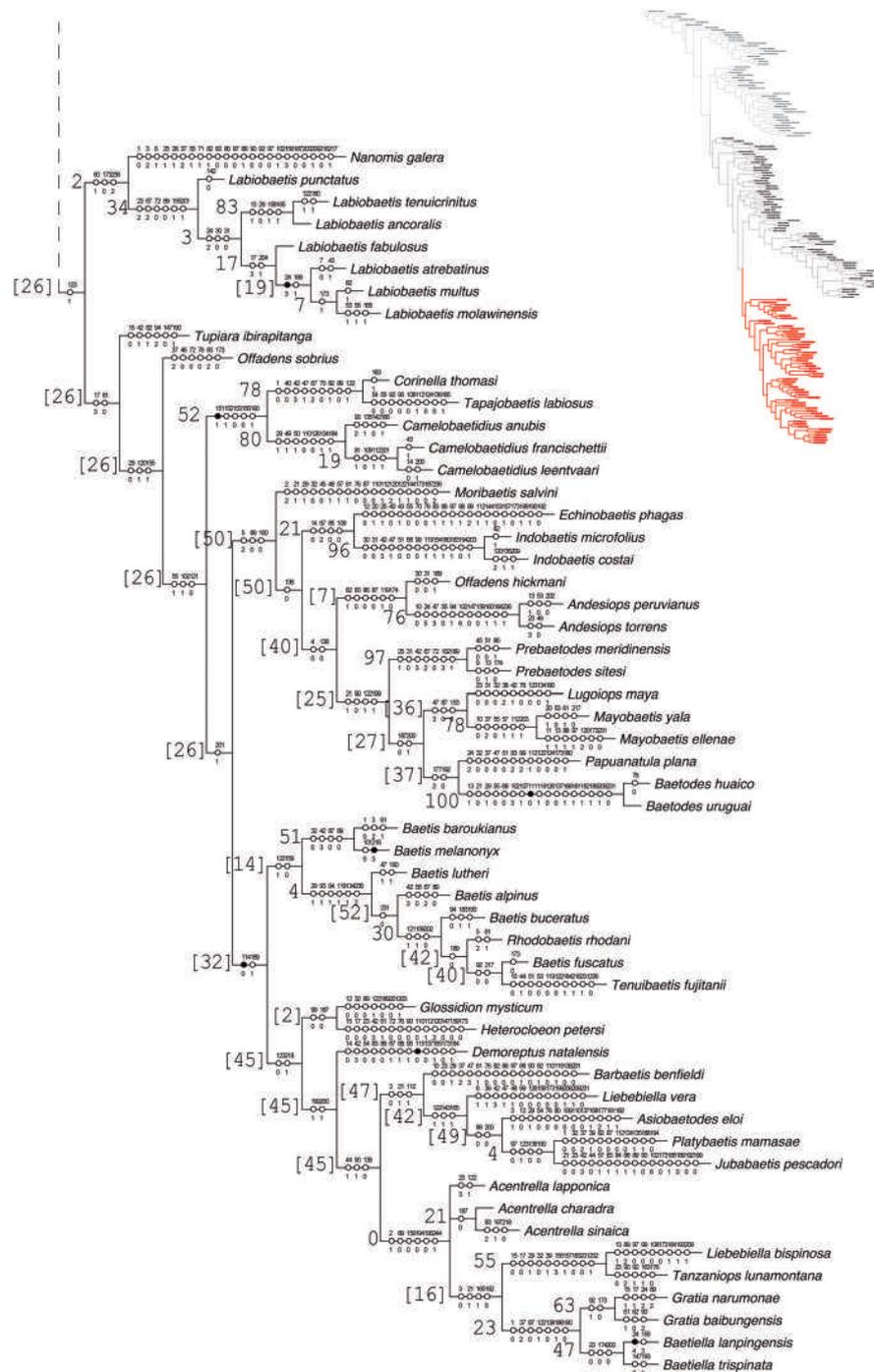


Fig. 4. Phylogeny of Baetidae (continued). Black circles indicate synapomorphies, white circles represent non-unique changes in the tree, either forward or reverse; above are the character numbers and below are the character states. The values on left of branches correspond to Frequencies differences. [Colour figure can be viewed at wileyonlinelibrary.com].

African representatives of *Cloeodes* l., now recognized as *Potamocloeon* (Salles *et al.*, 2015), were recovered in Cloeoninae here and in both molecular analyses, while the South American representatives of *Cloeodes* belong to Baetinae (Fig. 3).

The congruence of results between molecular and morphological analyses appear likely due to the inclusion of imaginal characters, which are confirmed to be more stable and therefore more useful in investigations of the evolution of higher lineages.



Fig. 5. Representatives of subdivisions of Baetidae distributed in recovered topology. (A) Rank-free System; (B) Gillies' revision of the Kazlauskas System. [Colour figure can be viewed at wileyonlinelibrary.com].

Rank-free system

The group concepts, applied to the species on topology (Fig. 5A), did not recover Protopatellata and Anteropatellata as monophyletic. This is directly related to the homoplastic nature

of the patella-tibial suture on the forelegs of the larvae (Fig. 2 in File S7).

The absence of patella-tibial suture is one of six states attributed to Protopatellata, such as the setae on the inner margin of the mandibles (Chars 49 and 50), the pointed microlepid

on tarsi of the subimago (Char. 225), subimaginal gonostylus buds under the cuticle of the mature larva (Char. 198) in the 'Afroptilum-Cloeon' type, larval abdominal terga usually with scales (Char. 190) and a single marginal intercalary vein on the forewing (Char. 211). Even if the concept of Protopatellata were changed to include only the last five of these character states, thus excluding patella-tibial suture from the concept, the group continues to be non-monophyletic, as was originally proposed in the Rank-free system (Kluge, 2004).

Anteropatellata was erected based on the presence of a patella-tibial suture on foreleg of larva. Its presence, as mentioned previously, is high homoplastic, being present in distinct clades (Fig. 5; Fig. 2 in File S7), thus does not corroborate the group as monophyletic. The subsequent group in the system is Baetovectata (or Baetis/fg6), which was recovered in almost the same concept as in the original proposition (Kluge & Novikova, 2011), and has the same concept as Baetinae (Clade A) here recovered (monophyletic) (Figs 3, 4, 5).

Characters, clade support and implications

Our results are congruent with other higher-level cladistics investigations based on morphology (Manuel *et al.*, 2003; Michat *et al.*, 2017). The best-supported clades are more derived lineages (most of them corresponding to the genus level), with many ambiguous synapomorphies, while deeper clades have low support and few, although unambiguous, synapomorphies. The most significant case is the clade A (Baetinae/Baetovectata), which has low support (Fig. 3) but is sustained by meaningful synapomorphies (low homoplastic values) from adults (Chars 211, 225 and 241). A plausible assumption is that a low clade support is related to the many missing entries in these key characters (percentage of missing data: Char. 211 = 27.4%; Char. 241 = 50%; Char. 225 = 67%; missing from total data 30% adults and 9% larvae). In this sense, description of the adult stages is imperative in order to test the hypothesis formulated here.

Under these circumstances, we do not consider it appropriate to propose any formal modifications of existing classifications, or new classification, until missing data on the (sub)imagoes can be included.

Conclusions

The reconstruction of evolutionary relationships is a challenge by itself, but the reconstruction of higher lineages is particularly problematic. At a higher level, it is difficult to find unambiguous characters that are not strongly homoplastic and thus phylogenetically robust. The results recovered the subimaginal and imaginal characters with lowest homoplastic values and, therefore, the best for understanding the deeper relationships. Consequently, the largest monophyletic lineage (Baetinae/Baetovectata – Clade A) is diagnosed by few unambiguous subimaginal and imaginal characters, with low clade support.

The two previously proposed systems are not convergent, with their main groups recovered as strongly polyphyletic (Cloeoninae, Anteropatellata and Protopatellata). However, Baetinae and Baetovectata were recovered monophyletic (Clade A), surprisingly with the same circumscription, and in agreement with molecular reconstructions (related to group composition).

This study is the first global cladistic approach in the systematics of Baetidae, and must be considered as a first step toward a more integrated approach using morphological and molecular data. Combination of both approaches is necessary in order to strengthen the reliability of the reconstruction and propose a robust classification within Baetidae.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

File S1. List of characters and states.

File S2. List of material examined.

File S3. List of tree indexes (Tcomp and Sprdiff).

File S4. Matrix.

File S5. Tcomp and sprdiff scripts.

File S6. List of the adjusted homoplasy index for each character.

File S7. Figs 1–3. Evolution of selected characters in topology.

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Data availability statement

The data that supports the findings of this study are available in the supplementary material of this article.

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