MARCELA MIRANDA DE LIMA

PHYLOGENY OF THE ORDER EPHEMEROPTERA HYATT & ARMS, 1891 (INSECTA) BASED ON MORPHOLOGICAL CHARACTERS

Thesis submitted to the Entomology Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae*.

Adviser: Frederico Falcão Salles

Co-advisers: Pedro Seyferth R. Romano
Michel Sartori

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APPROVED: July 27, 2023.

Assent:

Marcela Miranda de Lima

Marcela Miranda de Lima
Author

Frederico Falcão Salles
Adviser

To my family.

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Doctor Frederico Falcão Salles I must thank twice.

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“Anyone who has never made a mistake has never tried anything new.”

(Albert Einstein)

ABSTRACT

LIMA, Marcela Miranda de, D.Sc., Universidade Federal de Viçosa, July, 2023. **Phylogeny of the order Ephemeroptera Hyatt & Arms, 1891 (Insecta) based on morphological characters.** Adviser: Frederico Falcão Salles. Co-advisers: Pedro Seyferth R. Romano and Michel Sartori.

The suprafamilial classification of Ephemeroptera has still not reached consensus. Of the few publications that have addressed the phylogeny of the entire order in the last 20 years, only one has performed a cladistic analysis based on parsimony to analyze morphological data. In the first chapter of this thesis, we attempt to understand this lack of consensus in the classification of Ephemeroptera. To do so, we have provided a commented and contextualized review of the methods used to date to infer relationships among the major groups and discuss ways to improve knowledge of the phylogeny of Ephemeroptera and to achieve a more comprehensive classification of the group. We conclude that it is imperative that more scientists devote themselves to the study of the phylogeny of the group on the basis of methods that can be replicated so that a larger number of phylogenetic hypotheses can be tested and a robust classification can be proposed for the group. In the second chapter, a phylogenetic analysis of the order was conducted based on morphological data from nymphs and adults. A matrix consisting of 106 taxa and 131 morphological characters was analyzed using parsimony under Implied Weighting with five different K values (8, 9, 10, 11 and 12). The analyzes yielded five different topologies, one for each K values. The consensus of all analyzes was unresolved for the deep relationship within Ephemeroptera. In the general consensus, monophyly is supported for Caenotergaliae (=Caenoidea), Ephemerella/Fg1 (=Ephemerelloidea), Eusetisura, Fimbriatotergaliae, Fossoriae, Heptagennota, and Posteritorna (=Carapacea). Anteritorna, Branchitergaliae, and Tridentiseta were not supported as monophyletic. Five families were not found to be monophyletic in any of our analyzes: Ephemerellidae, Ephemeridae, Nesameletidae, Potamanthidae, and Teloganodidae. The evolution of the number of dentisetae on the maxilla, the presence and shape of maxillary gills, the degree of fusion of the forewing pads, and the presence of a double row of long filtering hairs on anterior femora and tibiae, all important characters for the systematics of mayflies is presented and discussed.

Keywords: Aquatic insects. Mayflies. Classification. Parsimony. Cladistic analysis. Character evolution.

RESUMO

LIMA, Marcela Miranda de, D.Sc., Universidade Federal de Viçosa, julho de 2023. **Filogenia da ordem Ephemeroptera Hyatt & Arms, 1891 (Insecta) com base em caracteres morfológicos.** Orientador: Frederico Falcão Salles. Coorientadores: Pedro Seyferth R. Romano e Michel Sartori.

A classificação suprafamiliar de Ephemeroptera ainda não é consensual e das poucas publicações que abordaram a filogenia de toda a ordem nos últimos 20 anos, em apenas uma foi realizada uma análise cladística com base em parcimônia para dados morfológicos. No primeiro capítulo desta tese, procuramos compreender esta falta de consenso na classificação. Para isso, preparamos uma revisão comentada e contextualizada dos métodos usados até o momento para inferir relações entre os principais grupos e discutimos formas de melhorar o conhecimento da filogenia do grupo para alcançar uma classificação mais robusta. Concluímos que é imprescindível que mais cientistas se dediquem ao estudo da filogenia do grupo com base em métodos que possam ser replicados para que um maior número de hipóteses filogenéticas possam ser testadas e uma classificação robusta possa ser proposta. No segundo capítulo, foi realizada uma análise filogenética da ordem com base em dados morfológicos de ninfas e adultos. A matriz composta por 106 táxons e 131 caracteres morfológicos foi analisada usando parcimônia com pesagem implícita e cinco valores de K diferentes (8, 9, 10, 11 e 12). As análises resultaram em cinco diferentes topologias, uma para cada valor de K. O consenso das árvores obtidas não tem resolução para o relacionamento profundo em Ephemeroptera. A monofilia é suportada para Caenotergaliae (=Caenoidea), Ephemerella/Fg1 (=Ephemerelloidea), Eusetisura, Fimbriatotergaliae, Fossoriae, Heptagennota e Posteritorna (=Carapacea). Anteritorna, Branchitergaliae e Tridentiseta não foram suportadas como grupos monofiléticos. Cinco famílias não foram consideradas monofiléticas: Ephemerellidae, Ephemeridae, Nesameletidae, Potamanthidae e Teloganodidae. A evolução do número de dentisetas na maxila, a presença e forma das brânquias maxilares, o grau de fusão das tecas alares anteriores e a presença de uma dupla fileira de pelos filtradores nos fêmures e tíbias anteriores, todos caracteres importantes para a sistemática de Ephemeroptera é apresentada e discutida de acordo com as diferentes hipóteses obtidas.

Palavras-chave: Insetos aquáticos. Efeméridas. Classificação. Parcimônia. Análise cladística. Evolução de caracteres.

SUMMARY

GENERAL INTRODUCTION

Mayfly higher classification has been the subject of study since the 19th century, and up until around 2005, most proposed classifications were in agreement with each other (Ogden and Whiting, 2005; Soldán, 2003). The situation began to shift with the introduction of molecular data, as these findings contradicted many previously proposed groups based on morphological characteristics (Ogden, 2019; Ogden et al., 2009; Ogden and Whiting, 2005).

While there is a general agreement on certain lineages (Carapacea, Furcatergalia, Fossoriae, Pannota, Caenoidea and Ephemerelloidea) (Ogden et al., 2009), there remains disagreement regarding the relationship between these taxa and their nomenclature (Brittain and Sartori, 2009; Salles et al., 2018; Sartori and Brittain, 2015).

The initial chapter of this thesis seeks to assess the methodologies employed in determining relationships between Ephemeroptera families, as well as existing classification proposals. This objective is achieved by examining historical context to understand why specialists in this field have not yet reached a consensus. Based on these findings, potential avenues for improving understanding of Ephemeroptera phylogeny were identified with the ultimate goal of developing a more comprehensive classification system for the group.

The second chapter of this thesis aimed to conduct a phylogenetic analysis of the order Ephemeroptera based on morphological characters for adult and nymph. The first step involved improving the pre-existing set of characters through semantic redescription, elimination of dependent and non-informative characters, adjustment of character transformation series, and introduction of new characters. In the second step, each species was coded for all relevant characters to conduct a cladistic analysis and develop a new hypothesis regarding homology in Ephemeroptera.

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CHAPTER I

MAYFLY (INSECTA, EPHEMEROPTERA) MODERN CLASSIFICATION: AN OVERVIEW ON PRINCIPLES AND METHODS

Mayfly (Insecta, Ephemeroptera) modern classification: an overview on principles and method application

Marcela Miranda de Lima, Michel Sartori, Pedro Seyferth R. Romano and Frederico Falcão Salles

ABSTRACT

Given the current situation on mayfly systematics, specially the lack of consensus about the recognition, diagnosis and classification of the superfamilies of the order, we present a commented and contextualized review of methodologies used so far to infer relationships among Ephemeroptera major groups. We discuss ways to improve the knowledge concerning the phylogeny of Ephemeroptera and, therefore, achieve a more robust classification for the group. So far, there are few comprehensive phylogenies based on strong (tested) evidence for the order. Despite homology hypotheses for morphological characters being relatively well set, we believe that there is still room for more studies that involve data gathered from available semaphoronts, also including its internal anatomy, as well as more combined analysis of morphological and molecular data. We believe it is necessary that more Ephemeroptera specialists dedicate themselves to studying the phylogeny of the group, mostly because most "traditional" systematic frameworks published to the order so far are based on methodological approaches difficult to replicate or lack strong evidence for homology hypothesis of characters used. As a consequence, few efforts were focused on evaluating the robustness of published phylogenies. This increased effort will help us to obtain a better view of the evolution of the group, of kinship relations between families and between major groups, and in this way establish a well-founded modern classification for the order.

Keywords: phylogeny, history, methodology, phylogenetic classification, review, systematics.

Introduction

Although man has classified living beings for a long time, the way in which this classification is carried out has undergone numerous philosophical and methodological changes over time (Richards, 2016). Thus, in order to understand the current classification of a group of organisms, we need to understand the history of this classification, at what historical moment it was carried out, and on what methods it was based.

The modern classification of organisms began to take shape when naturalists started to ask themselves what makes a classification system "natural" and what a classification should represent. In light of the evolutionary perspective brought by Charles Darwin, Alfred Wallace and other nineteenth century evolutionists, the classification started to aim at reflecting the ancestral relationships between organisms (Richards, 2016). Over time, different ideas emerged about how to combine the knowledge of the relationships between organisms with classification, leading to the formulation of three competing theories of classification during the XX century; evolutionary systematics, phenetic, and cladistic (Brower and Schuh, 2021; Richards, 2016).

In evolutionary systematics, organisms were classified on the basis of phylogenetic branching, as well as the amount and nature of evolutionary change between branch points (in other words, representing cladogenetic *and* anagenetic events). Proponents of phenetic systematics have opposed an evolutionary basis for classification, claiming that we do not have sufficient knowledge of the evolutionary past, and have proposed classification based on "general similarity" among organisms. In turn, proponents of cladistics argue that classification should exclusively reflect ancestral relationships between organisms (cladogenesis), rejecting the use of assumptions about the evolutionary process to reconstruct the past (Brower and Schuh, 2021; Richards, 2016).

Although both evolutionary systematics and cladistics propose their classifications based on the hypothesized phylogeny, methodological approaches used to reconstruct this phylogeny have also changed over time. Lukhtanov (2010) classifies the methodologies used for phylogenetic inference as: "Traditional Approach", "Hennigian Cladistics" and "Parsimonious Cladistics", in addition to the "modern" statistical methods "Method of Maximum Likelihood" and "Bayesian Method". According to this author, in the traditional approach, which dates back to E. Haeckel and its predecessors, there is a deep analysis of characters, with assessment of the direction of morphological series, followed by an empirical phylogenetic analysis. The Hennigian approach follows the proposal of W. Hennig (1950, 1966) and is described by Lukhtanov (2010) as a multistep process of proposing and testing phylogenetic hypotheses based on a small number of "reliable" synapomorphies. Conflicts between potential synapomorphies are solved by distinguishing false synapomorphies from true ones by means of re-examination of the material and the study of more characters and taxa. In the "parsimonious cladistics", phylogenetic hypotheses are based on the maximum possible number of potential synapomorphies and conflicts are solved with the use of the parsimony method. The more recent methods, Maximum Likelihood and Bayesian methods are based on statistical analysis and are based on the assumption of models for character evolution (Lukhtanov, 2010).

Among the aquatic insects orders, Ephemeroptera comprise 3778 species and 478 genera (Jacobus et al., 2021) and between 41 and 42 families (differs depending on the classification system; Barber-James et al., 2013; Sartori and Brittain, 2015). The order has a global distribution, with the exception of Antarctica and some very remote islands (Jacobus et al., 2019). Mayfly life cycle comprises the egg, the immature stage called nymph with a variable number of instars, and the adult stage, which has two instars unlike any other pterygote, the subimago and the imago (Brittain and Sartori, 2009; Kamsoi et al., 2021). Nymphs live in fresh and brackish water environments and have different forms of feeding: the subimago has developed wings and loses the mouthparts; the imago stage has a short duration and is highly specialized in the dispersal and reproduction (see Jacobus et al. (2019) for an overview of the biology and ecological importance of Ephemeroptera).

Extant Ephemeroptera belong to an old lineage of insects (Ephemerida), which date back to the Carboniferous (Sartori and Brittain, 2015; Willmann, 2007). Modern mayflies constitute a well defined clade, supported by several synapomorphies, such as: vestigial mouthparts in adults, enlarged compound eyes in males, Palmén’s organ (extension of head tracheae), males with additional joint between tibia and proximal tarsomere, midgut filled with air, hindwing reduced in size, anal region of wings reduced, and naiads with lateral abdominal tracheal gills (Beutel et al., 2013). For Kluge (2004a), however, there is no clear exclusive character state diagnostic to the group "Ephemeroptera sensu stricto", for which he lists two characteristics as "Characters of unclear phylogenetic status", namely: anteromotorism and loss of the gills (tergalia) on segments VIII-IX.

The relationship of Ephemeroptera with the other winged groups of insects, Odonata and Neoptera, is still one of the major unsolved problems in entomology (Beutel et al., 2013; Ogden and Whiting, 2003; Simon et al., 2018; Whitfield and Kjer, 2008). Competing hypotheses have been proposed based on different data (molecular and/or morphological): Ephemeroptera as sister group of Metapterygota (Odonata + Neoptera); Odonata as sister group of Chiastomyaria (Ephemeroptera + Neoptera) and Ephemeroptera as sister group of Odonata, constituting the clade Palaeoptera, which is the sister group of Neoptera (Beutel et al., 2013; Ogden and Whiting, 2003). Recent molecular or phylogenomic (Simon et al., 2018) and morphologic data (Blanke et al., 2012) offer stronger support for the Palaeoptera hypothesis. One of the causes for the difficulty in understanding these relationships may be the huge amount of time since the radiation of those groups when compared with the time of their radiation, which may cause an "overwritting" of the synapomorphies that appeared a long time ago (Whitfield and Kjer, 2008). Also, the lack of an outgroup in which wings are present, may hamper the polarization of wing related characteristics (Blanke et al., 2012; Whitfield and Kjer, 2008).

Current higher classification

Higher classification of mayflies has been studied since the XIX century and until around 2005 the two main classifications in use (Kluge, 2004; McCafferty (see Ogden and Whiting, 2005)) were concordant with each other in most of the groups formed, although the relations within groups and between groups were not (Ogden and Whiting, 2005). The classification started to change with the first works using molecular data as these data did not support many of the groups anteriorly proposed based on morphological characters (Ogden, 2019; Ogden et al., 2009; Ogden and Whiting, 2005).

Essentially, although there is consensus on some lineages (Carapacea, Furcatergalia, Fossoriae, Pannota, Caenoidea, and Ephemerelloidea) (Ogden, 2009), there is still no agreement on the relationship between these taxa or their names (Brittain and Sartori, 2009; Salles et al., 2018; Sartori and Brittain, 2015). Examples of the lack of consensus in the taxonomy of the group, Domínguez et al. (2006) and Salles et al. (2018) presented the families of mayfly in alphabetical order, intentionally avoiding the use of nomenclature above family level. Bauernfeind and Soldán (2012), followed "delimitation and sequence″ of the taxa after Hubbard (1990) with modifications following McCafferty (1991a), also ignoring the more recent literature that changed the internal classification of Ephemeroptera.

Objective

Considering the current situation on mayfly systematics, the objective of this work is to access the methodologies used to infer the relationships between Ephemeroptera families so far and the proposals of classifications for the group, contextualizing them historically in order to understand the lack of consensus among specialists in the area. Based on this background, we aim to point out ways to improve knowledge about the phylogeny of Ephemeroptera and, therefore, achieve a more robust classification for the group.

Material and Methods

We included in our overview the works that presented Ephemeroptera phylogenies and/or classification above the family level, focusing in the period between 1950 and 2019. The works of Eaton (1888), Lameere (1917) and Spieth (1933), from the period before 1950, were included in order to exemplify earlier attempts to classify mayflies based on an evolutionary perspective. For more information on the early years of mayfly classification (1880-1935), under a historical perspective, we strongly suggest Eaton (1888), Burks (1953) and Landa & Soldán (1985).

In order to properly understand the historical context of the work carried out on Ephemeroptera taxonomy, a parallel was drawn with what was being developed synchronously with the study of insect taxonomy. For such a parallel, the review made by Engel and Kristensen (2013) on the history of entomological classification was used as a basis.

For each paper, as long as available or traceable based on other publications, we attempted to analyze some parameters that could help us to better understand the methodology employed by the author(s) for hypothesizing the relationship between groups. Also, the analysis of these parameters for all papers allowed us to better visualize the "big picture" of how hypotheses of relationship and proposals of classifications were made for the group by looking at similarities and differences between proposals.

The following parameters were observed, whenever possible:

A. Focus of the work (classification; phylogeny; morphology or biogeography);

B. Semaphoronts analyzed (egg, nymph, adult (subimago and/or imago));

C. Taxon sampling (fossil, extant or both);

D. Characters analyzed (external morphology, internal anatomy, behavior, habitat, molecular);

E. Form of presentation of the characters (description, matrix, GenBank numbers);

F. Assumptions about character evolution (reversibility, weighting, optimization criteria);

G. Method used to establish the character polarization (fossil, outgroup);

H. Nature of phylogenetic inference (nomenclature according to Lukhtanov (2010)): "Traditional Approach", "Hennigian Cladistics", "Parsimonious Cladistics", "Maximum Likelihood", and "Bayesian Method";

I. Theoretical background for classification; Evolutionary, Phenetic or Phylogenetic.

Results

Some data on the fundamental works on classification and/or phylogeny of higher groups of Ephemeroptera is summarized in the following table. More information can be obtained in the text below.

Table 1 - Summary of data gathered on the fundamental works on classification and/or phylogeny of higher groups of Ephemeroptera.

Focus: Classification (C); Phylogeny (P); Morphology (M); Biogeography (B)

Semaphoronts analyzed: Egg (E); Nymph (N); Adult (A); Molecular data - semaphoronts not discriminated (M)

Character type: External Morphology (EM); Internal Anatomy (IA); Behavior (B); Molecular (M)

Character presentation: Description (D); Matrix (M); Genbank Accession Numbers (GN)

Phylogeny Method: - Traditional (T); Hennigian Cladistic (H); Parsimonious Cladistic (P); Maximum Likelihood (ML); Bayesian Inference (B)

Reference	Taxon	Focus	Semaphoronts	Character type	Character presentation	Phylogeny Method
Eaton (1883-1888)	Ephemerida	C	N - A	EM	-	-
Lameere (1917)	Ephemeroptera	C	N - A	EM	D	-
Spieth (1933)	Ephemerida (USA)	P	N - A	EM	D	T
Burks (1953)	Ephemeroptera (Illinois)	C - P	N - A	-	-	T
Demoulin (1958)	Ephemeroptera	C - P	N - A	EM	D	T
Edmunds (1962)	Ephemeroptera	C - P	-	-	-	T
Tshernova (1970)	Ephemeroptera	C	N - A	EM	D	-
Edmunds (1972)	Ephemeroptera	B - P	E - N - A	EM - IA	D	T
Edmunds (1973)	Ephemeroptera	C - P	N - A	EM - IA - B	D	T
Riek (1973)	Ephemeroptera	C - P	N - A	EM - IA	D	T
Landa (1973)	Ephemeroptera	P	N - A	EM - IA	D	T
Koss and Edmunds (1974)	Ephemeroptera	M - P	E	EM	D	T
McCafferty and Edmunds (1979)	Palingeniidae	C - P	N - A	EM	D	H
McCafferty and Edmunds (1979)	Ephemeroptera	C - P	N - A	EM - IA	D	T
McCafferty (1979)	Ephemeroidea	C - P	E - N - A	EM - IA	D	H
Landa and Soldan (1985)	Ephemeroptera	C - P	N	IA	D	T
Kluge (1989)	Ephemeroptera	M - P	N - A	EM	D	T
Tomka e Elpers (1991)	Ephemeroptera focus on Heptageniidae	P	E - N - A	EM - IA	D	H
McCafferty (1991)	Heptagenioidea	C - P	N - A	EM - IA	D	H-P
McCafferty (1991)	Ephemeroptera	C - P	N - A	EM - IA	D	H
Kluge et al. (1995)	Siphlonuroidea	C - P	E - N - A	EM	D	H
Bae and McCafferty (1995)	Ephemeroidea	M - P	N	EM	D	H-P
Kluge (1997)	Furcatergalia	P	N - A	EM	D	H
Kluge (1998)	Ephemeroptera	C - P	N - A	EM	D	H
McCafferty and Wang (2000)	Pannota	C - P	N - A	EM	-	H-P
Molineri and Dominguez (2003)	Ephemerelloidea	C - P	E - N - A	EM	M	P
Kluge (2003)	Ephemeroidea (Pinnatitergaliae)	P	E - N - A	EM	D	H
Kluge (2004)	Ephemeroptera	C - P	E - N - A	EM - IA	D	H
Ogden and Whiting (2005)	Ephemeroptera	P	M	M	GN	P + ML
Sun et al. (2006)	Ephemeroptera	P	M	M	-	P
Jacobus and McCafferty	Pannota	C - P	E - N - A	EM	M	P

Reference	Taxon	Focus	Semaphoronts	Character type	Character presentation	Phylogeny Method
(2006)						
Ogden et al. (2009)	Ephemeroptera	P	N-A + M	EM - M	M + GN	P + ML + B
Miller et al. (2018)	Burrowing mayflies	P	M	M	-	ML + B
Ogden (2019)	Ephemeroptera (partial)	P	M	M	-	B

When all the mayflies were part of a single family, Ephemeridae, Eaton proposed their division into three groups, based on the habits and structures of the nymphs; 1) the tusked and burrowing forms, 2) the flattened and crawling forms, and 3) the more elongated forms with a swimming habit (Eaton, 1869). Later, his classification became more complex and he proposed a classification based on the characters of adults with modifications based on the study of nymphs (Eaton, 1888). While still having a linear view of relationships between genera, the author was clear that only by analyzing the points of difference and agreement on the characters of adults and nymphs could these relations be demonstrated adequately. At this moment, the author proposes a classification organized into 3 groups,9 series and 14 sections, which he considers provisional, to serve as a basis for the study of affinities between the genera.

> "*Until such comparisons can be and shall have been carried out, the whole question of their arrangement can only be dealt with in a tentative and experimental manner; and it will be fortunate if error be avoided in the necessary grouping of the genera into provisional alliances of apparently kindred forms, preparatory to the study of their affinities.*" (Eaton, 1888, p. 18)

In the classification of insects, according to Engel and Kristensen (2013), the work that represents the first great conceptual leap towards the substantial application of evolutionary thinking dates from this period (Brauer, 1885). Among the works focusing on Ephemeroptera, the first to be highlighted, for having explicitly an approach or at least a concern to reflect the evolution of the group, are for us Lameere (1917) and Spieth (1933).

In Hexapoda studies, since the second half of the 19th century there has been an increase in the influence of studies in paleontology as evidence to understand the relationships between living groups and help in classification, with fossils being seen at the time as precursors of current species (Engel and Kristensen, 2013).

In 1917 Lameere made the first classification of Ephemeroptera taking into account the paleontological data for the group. Lameere (1917) praised the work of Eaton (1888) while highlighting the artificiality of the groups he formed. According to the author, by basing his classification mainly on the characters of adults, Eaton ended up creating groups that are not supported by the characters of nymphs, thus forming artificial groupings.

According to Lameere, from the classification proposed by Eaton, only the sections were kept, without any connection between them. Lameere proposes that for the classification of the group all available characters of nymphs and adults should be used, so that the disagreements evidenced by the characters of adults and nymphs would be indicative of convergence (evolutionary or random) and that these should be revised accordingly in order to find the true evolutionary history of the group.

In this context, in his proposal Lameere raises characters from the external morphology of nymphs and adults from fossil and extant groups to establish a classification and even differentiates some of these characters into monogenetic (single origin) and polygenetic (multiple origins). The groupings are suggested by the presence of similar characters. Based on the hypothesis of how the characters evolved, the evolutionary history of the group is inferred. Following the vision of the time, Lameere considers fossils as the current species precursors. Thus, the evolutionary history of the group is inferred as a function of known characters in fossils (considered primitive) and their complexity (simple to complex).

Spieth (1933) adopted a methodological proposal very similar to that of Lameere, but investigated the phylogeny of the Ephemeridae families present in the United States. The author also evaluates external morphological characters of nymphs and adults, and points out that all the similarities found are useful to infer the relationships between groups. Characters are polarized based on fossil record data and its complexity. In his proposal, the North American Ephemeroptera are divided into 4 superfamilies. As Lameere, Spieth points out that the natural classification of the group (one that expresses its evolution) will only be achieved when the characters of adults and nymphs are worked together. According to Bae and McCafferty:

> "*Spieth (1933), being a student of one of the pioneers of phylogenetic research, A. C. Kinsey, attempted the first study of mayfly phylogeny per se, but*

> *his analysis was restricted to the North American fauna and was phenetically based.*" (Bae and McCafferty, 1991, p. 80)

Following these historical hypotheses, Burks (1953) proposed a classification concerned with reflecting the degree of divergence from the ancestral Ephemeroptera based on an assessment of available characters from nymphs and adults. Based on the similarities between the groups, the author hypothesizes relationships between the proposed families. This work also brings a summary of several previous classifications since Eaton.

Edmunds and Traver (1954) suggested a new classification for the living members of the order, dividing it into 5 superfamilies, but they did not explain its bases. The text is short and shows no concern for the evolutionary history of the group.

In 1958, Demoulin proposed a classification, according to himself, "governed essentially by phylogenetic considerations" (Demoulin, 1958). Like Lameere, he also used characters from the external morphology of nymphs and adults from fossil and extant groups to infer their relationships. As in previous works, there was a concern to infer the transformation of characters to determine the evolutionary history of the group, with the idea of an "évolution spécialisatrice" (evolution towards specialization). Demoulin also demonstrates a view of fossils as precursors of current taxa, which can be seen when he states that it was not yet known or perhaps they were not able to distinguish among the fossils known at the time, the representatives of ancestral forms in common with Ephemeroptera and Archodonata (a group of fossil insects from the Carboniferous-Permian with great similarity to Ephemeroptera; these two groups were united by the author because they have a median caudal filament as long as the cercus, in addition to intercalary veins on the wings). Demoulin also claims that some authors (not cited) believed they could claim that current and fossil forms had in common only one community of origin, which he considered an "easy way out" to the question of determining the relationship between these groups. For Demoulin, nymphs evolved more quickly than adults, and thus, the characters of the adults have priority over those of the nymphs in case of doubt (Demoulin, 1958).

From the 1940s onwards, two new approaches to the classification of living beings began to be outlined: numerical systematics and evolutionary systematics. At least for studies on the higher classification of Ephemeroptera, numerical systematics did not have any enthusiasts, with evolutionary taxonomy being the school followed by numerous researchers in the area. This classification philosophy later became known as the gradist school and had as main proponents Ernst Mayr (1904–2005) and George Gaylord Simpson (1902–1984) (Richards, 2016). For Mayr, higher taxa should be "monophyletic", which for him meant that they should contain only descendants of the same ancestor, but not necessarily all of their descendants. In this way, the formation of groups known today as paraphyletic was admitted and, in this way, the classification was based on phylogeny, without necessarily reflecting the same. Based on Darwin's proposals, evolutionary taxonomists proposed the delimitation of genera and families using the concept of gap in morphology between individuals necessary to delimit them. Evolutionary classification was adopted in many studies of the order Ephemeroptera from the 1960s onwards, as we will demonstrate herein, by several authors.

In 1962, Edmunds proposed the first phylogenetic representation for the order (Figure 1), but without explaining how he did it, the main focus of this work was the formulation of a classification based on the proposed phylogeny (Edmunds, 1962). Following a clearly gradist reasoning, Edmunds states that in Ephemeroptera the genera should be marked by a distinct gap in the characters of both nymphs and adults. For him, when the gap was distinct only for one of the semaphoronts, the category of subgenera should be adopted for this group. Also, the gaps between families should be large enough so that relationships could only be perceived with a more detailed study, while gaps within families should be small enough so that relationships could be easily perceived. The author admits that these criteria are subjective, but argues that they are also practical.

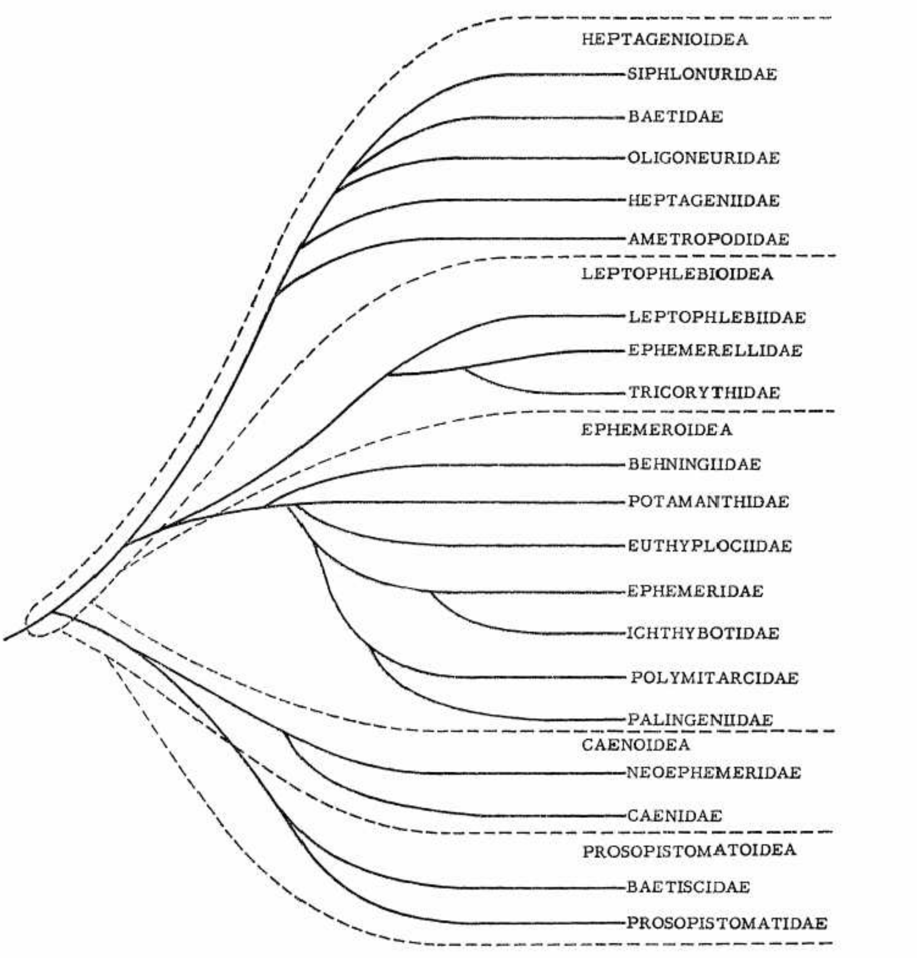

Figure 1 - Hypothesis presented by Edmunds (1962)

In 1965, Edmunds published a short theoretical text where he stated, contrary to what was proposed by Demoulin (1958), that it is not possible to formulate a rule about which semaphoront evolves more quickly (Edmunds, 1965). Thus, for him, the classification should take into account the characters of adults as well as nymphs. There is a concern to delimit the families through gaps, but these can be different in the two semaphoronts.

In 1966, Edmunds and Allen again emphasized the importance of nymphs in the systematics of Ephemeroptera (Edmunds and Allen, 1966). They stressed that, despite the importance of nymphs in clarifying the relationships between groups and the inclusion of nymph characters since the first sound classification of Ephemeroptera proposed by Eaton (1888), adults were better known at the time, thus highlighting the importance of rearing nymphs in the laboratory for association with adults.

The innovative vision and terminologies disseminated by Willi Hennig in his original article (1950), especially after his extensively revised English translation published in 1966 (Hennig, 1966), gave rise to a third line within systematics, which was developed throughout the 20th century and became the most widely deployed and accepted among taxonomists since. Edmunds (1972), was the first article on mayfly systematics to quote Hennig and it also is the first to bring a concern in determining which character state is plesiomorphic or apomorphic. This author understood that it was necessary, in order to determine the relationships, to find the groups that shared apomorphies. The purpose of this study was to trace the evolution of the group and make a study of biogeography, without proposing a classification. Edmunds (1972) uses internal anatomy characters proposed anteriorly by Landa (1973) (cited as "in press") and external morphology mainly of nymphs and adults (with some reference to egg characters) to propose a phylogeny for the order. According to him, *"The curse of phyleticists, parallelism, is avoidable because it is unlikely that similar selection pressures will occur in two or more life history stages."* (Edmunds, 1972, p. 21). The author does not explain how the determination of the polarity of the characters used was carried out, only that this determination "must be done with caution".

Fossils still had an important role for the author, who searched the fossil record for intermediates between higher categories, time of occurrence of evolutionary grades within phyletic lines, and indications of general evolutionary trends in addition to distributions of phyletic lines in geographic regions where they were extinct. The cladogram presented (Figure 2) shows groups starting from a plesiomorphic central point to the sides, where the "more apomorphic" groups are placed. In general, we can say that although the author demonstrates an affinity with the cladistic school, his methodology and conclusions are still those of the gradist school.

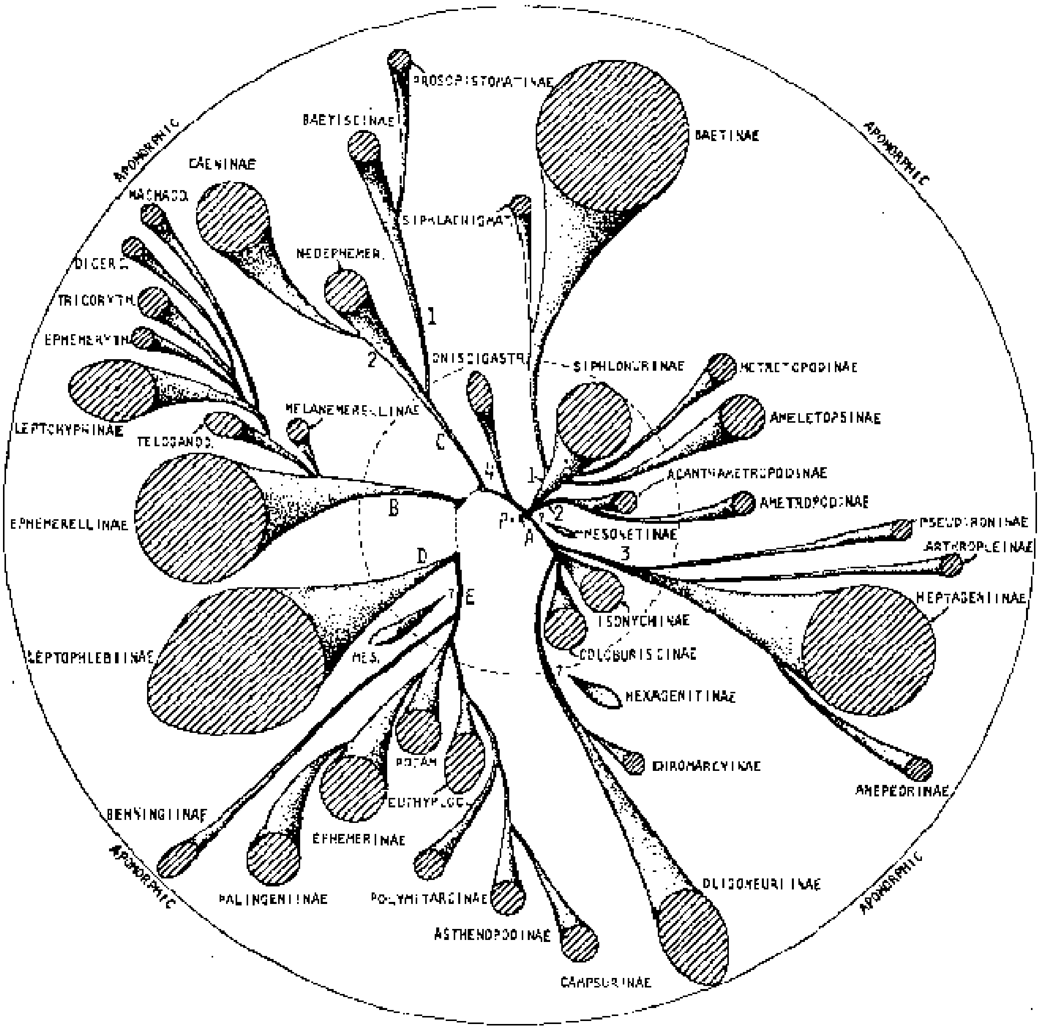

Figure 2 - Phylogenetic representation by Edmunds (1972)

Edmunds (1973) discussed the relationships between some families for which there was new data. Although the author discusses some classification issues, the work is more focused on discussing the relationships between the groups. Edmunds uses characters from the external morphology of nymphs and adults, as well as some internal anatomy characters proposed by Landa. While demonstrating the intention to form groups based on synapomorphies, and in some cases even highlighting the synapomorphy of a group, in other cases he speaks only of shared characters (which could be symplesiomorphies). Also, character polarization seems to be intuitive as in "*No extant mayfly retains what I believe to be the primitive (plesiomorphic) characters of the Ephemeroptera*" (Edmunds, 1973, p. 145) and also in: "*It is uncertain whether the ancestral Siphlonuridae had a fibrilliform tuft and a* *gill plate or just a plate. I am inclined to concur with PENNIKET (1966) who believed that a tuft plus a plate was the primitive form.*" (Edmunds, 1973, p. 146). The author considers Siphlonuridae as the most primitive family among Ephemeroptera and from which the other families of the group would have derived. He discusses pre-group and post-group, indicating an understanding of an ancestrality relationship between living organisms. There is still great concern in the evolutionary grades necessary for the elaboration of mayfly classification, indicating a view that still mixes gradist concepts with the cladistic proposal.

Koss and Edmunds (1974) published together a proposal for a phylogeny based solely on data of eggs, some of them already presented previously by Koss (1968). They also compared their results with those from analysis of nymphs and adults and, in those cases where data seemed to be "in striking disharmony" with phylogenies derived from other data, the evidence was discussed. The authors mention the importance of apomorphic characters for the delimitation of groups, but the proposal is clearly evolutionary and intends to trace the evolution of characters to infer the evolutionary history of the group, as can be seen in the excerpt below:

> "*If the sequence of character-states can be established with reasonable certainty for a variety of characters, the sequence of branching and amount of divergence can be hypothesized with reasonable certainty.*" (Koss and Edmunds, 1974, p. 269)

Character polarization was done intuitively and some character states are taken as "obviously apomorphic" and an apparent evolutionary advantage of the character state could also be used as an argument to infer their hypothesis.

> *"The apparent survival advantage of having attachment structures concentrated at the poles lends support to the suggestion that polar concentrations of attachment structures are apomorphic to a complete coverage of the egg by the same." (Koss and Edmunds, 1974, p. 278)*

The hypothesized phylogeny based on egg characters was found to be similar to the phylogenies proposed previously by Edmunds (Edmunds, 1972, 1962; Edmunds et al., 1963; Edmunds and Traver, 1954).

Concurrent to the works authored or co-authored by Edmunds, in the early 1970s, other authors also addressed the classification of Ephemeroptera, such as Tshernova (1970), Landa (1973) and Riek (1973).

Tshernova (1970) proposes a classification containing 10 superfamilies for the order encompassing both fossil and extant organisms. Basically, aspects of the external morphology of nymphs and adults were evaluated. Tshernova attributes the differences in the classifications of Demoulin (1958) and Edmunds (1962) to the inclusion or not of fossil organisms, in addition to the greater importance given to the characters of adults or nymphs by each of the authors. Families are grouped based on shared similarities, without distinguishing ancestral characters from those derived.

Landa's work (1973) is the result of a long period of studies where the author studied and systematized data on the internal anatomy of Ephemeroptera nymphs with information from more than 127 species from 94 genera. The tracheal system, the Malpighian tubules and the nervous cord of these insects were comparatively studied, and based on these studies, hypotheses for the evolution of each system were drawn up. The author considers paleontological data as "direct information" to build knowledge about the evolution of the order, while morphological data is seen as a kind of "indirect information". Based on internal morphology and paleontological data added to the external morphology of nymphs and adults, the author proposes a hypothesis about the evolution of the order in the form of a phylogenetic tree (Figure 3).

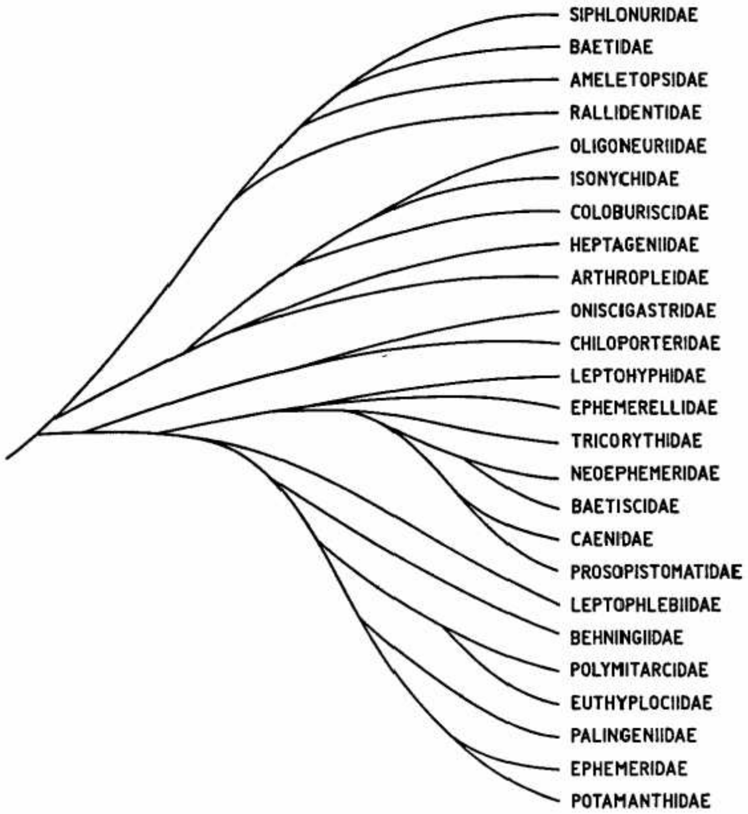

Fig. 1. Hypothetical scheme of the evolution of Ephemeroptera.

Figure 3 - Phylogenetic diagram presented by Landa (1973)

In 1973, Riek proposed a classification based on characters from the external morphology of nymphs and adults, as well as characters from the internal anatomy of nymphs (Riek, 1973). Riek, unlike Demoulin (1958) and Tshernova (1970), gave greater weight to the characters of nymphs than those of adults. Thus, in the case of character conflicts, the relationship indicated by the characters of the nymphs was considered at the expense of that indicated by the characters of the adults. The author presents a phylogenetic tree of the order (Figure 4), with the apomorphies marked in some branches and for each branch delimited by a synapomorphy, another branch is delimited by the corresponding plesiomorphic state(s), contradicting Hennigs idea that

> *"... in a phylogenetic system which must contain only monophyletic groups, every group formation, irrespective of the rank to which it belongs, must be established by demonstration of derivative ("apomorph") characters in its ground plan." (Hennig, 1965 p. 106)*

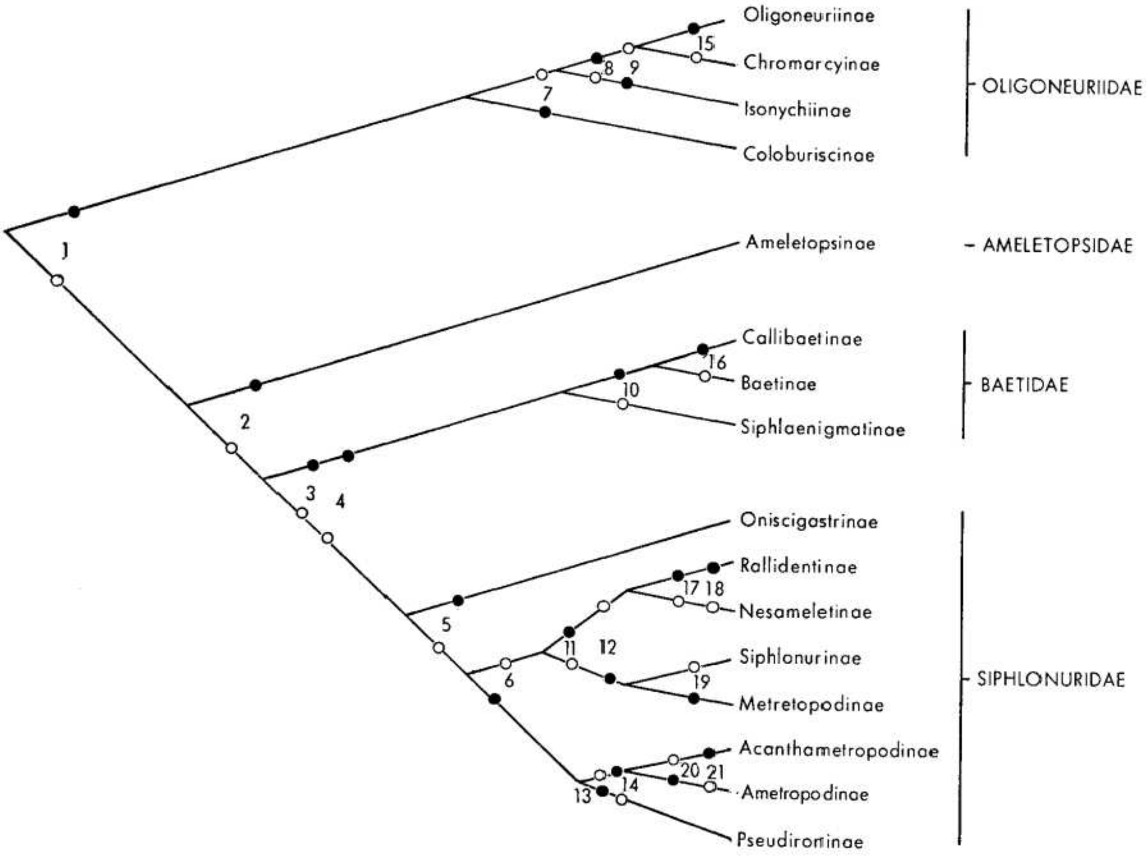

FIGURE 2. Phylogeny of the Baetoidea. Apomorphic state, represented by a blackened circle, defined.

Figure 4 - Phylogenetic diagram presented by Riek (1973)

Despite the concern to form groups that share apomorphies, the text is permeated by references to similarities and differences between the groups. Character polarization is not clear for all characters, but for some he considers the character state present in fossils to be the ancestor. The author traces an evolutionary history of the shape of the gills, and based on this history, he proposes his hypothesis that the Siphlonuridae type would be the ancestor of the other types, with a simple gill and a swimming body.

In the second half of the 1970s, Edmunds and colleagues (1976) published the book "The Mayflies of North and Central America" where they discuss some reasons for the differences in classification proposals of the same group, making it clear that, although different classifications can be correct (because classification depends not only on factual data, but also on the principles used while constructing the classification), some will be clearly incorrect once they group together distantly related organisms, excluding most closely related forms. The book follows a classification system modified from Edmunds and Traver (1954), which did not explain the characters used to infer the proposed hypothesis.

McCafferty and Edmunds (1976) focused on the classification of Pentageniidae, but the work as a whole is quite enlightening with regard to the methodology used in the elaboration of the classification proposed by these authors. At this time, the intent of the authors was to elaborate an evolutionary classification that would combine data on relationships between groups (phyletic) and similarity (phenetic) in order to be *"expressive of phylogeny as far as possible without becoming biologically impractical"*. In this paper, McCafferty and Edmunds made recommendations for the evolutionary classification of genera which are phylogenetically intermediate between family groups. One of the criteria proposed is that, when a genus is intermediate relative to two families, being more similar to one family at one stage of life and more similar to the other family at another stage, then the genus should be classified within the family that represents the most derived group. However, if there was no phenetic gap between the genus and its parental family (either in nymphal or adult stage), then it should be classified with the parental family, regardless of the phylogenetic relationship. This subjective rule shows that priority was still given to phenetics in detriment of phylogenetic relationships between groups.

McCafferty and Edmunds (1979) proposed for the first time to group living superfamilies into two suborders, Pannota (presumably monophyletic) and Schistonota (admittedly paraphyletic). The assumed maintenance of a large paraphyletic group was justified as to avoid the fragmentation of classification. The suborder Pannota includes the families in which the fusion of the nymphal wingpad occurs along the mesonotum and there is a general widening of the mesonotum. Schistonota was designated to comprise the rest of the families, which would have retained the ancestral condition of the thorax. Landa's (1973) internal anatomy data were used to support the Pannota hypothesis, since the groups contained in this suborder would form a lineage with an "improved" tracheal system. The authors have a still linear and progressive view of evolution as can be seen in: *"Thus, within the Pannota there are three progressive evolutionary gradations expressed by the 3 superfamilies."* (McCafferty and Edmunds, 1979, p. 8). Although there is concern about character polarization, since monophyletic groups are defined on the basis of shared derived characters, the criteria used to infer character polarization is unclear. Throughout the text, there is reference to reconstructions made by other authors of "primitive" nymphs that would have little or no fusion of the wingpad, thus being the plesiomorphic type.

There is also mention of the use of similarities for inference of relationship between groups as in: *"Because of behavioral and gill structure similarities, its most probable common ancestry was with the Siphlonurus-Parameletus cluster..."*. The families Siphlonuridae and Leptophlebiidae (extant) are considered "Stem-groups" that would have given origin to other families and the authors consider that all extant Ephemeroptera have their origin in Siphlonuridae. *"All modern mayflies have an origin traceable to within the Siphlonuridae."* (McCafferty and Edmunds, 1979, p. 9). In this work, the authors maintain the same proposal of an evolutionary classification proposed previously (McCafferty and Edmunds, 1976) in order to avoid a "taxonomic inflation" in the group while somehow reflecting evolution.

From the late 1970s onwards, more authors began to explicitly address the search for synapomorphies as a criterion for the classification of Ephemeroptera. The first of these authors, McCafferty, in his first single work addressing the phylogeny of large groups (McCafferty, 1979), explores the evolution of the Ephemeroidea superfamily. The author draws on characters from nymphs and adults, mainly of external morphology, but also uses data from the internal anatomy of nymphs to validate his results or solve "problems". The ancestry hypotheses are based on the study of characters derived in common (synapomorphies) as proposed by Ross (1974).

McCafferty was concerned with the use of synapomorphies to infer relationships, but he did not make explicit how he defined which character was plesiomorphic or apomorphic. Ross (1974), referred to as his theoretical basis, has suggested three different criteria that will give *"the most likely answer"* for determining ancestral and derivative states; fossil sequences, comparisons with related groups ("comparison of ex-groups") and "group trends". Thus, it is not possible to state which of the criteria McCafferty adopted to determine the ancestral status of the characters in each case. The author defines apomorphies and establishes groups *"clearly derived from a common ancestor"* but seems to consider the possibility of evolutionary convergences only in some cases and not in others.

The 1980s have few works involving phylogeny and these works continue to adopt an evolutionary classification. In an extensive and detailed work, which also includes a good review of previous classifications, Landa and Soldan (1985) present a proposal for phylogeny and classification based on the internal anatomy of nymphs. The justification given for using only nymphs is the considerable reduction of structures in adults, whereas nymphs have, in addition to the systems found in adults, already developed gonads (in mature nymphs). The proposal is based on the reconstruction of the evolution of each organ system, tracing what the authors call "anagenetic trend". The authors describe the polarization of characters in statements such as: *"Without any doubt, tubules entering individually alimentary channel represent the most plesiomorphic situation."* (Landa and Soldán, 1985, p. 55), and it is not clear which criterion was used to carry out this polarization. Despite this, the article is the first to describe in detail the characters used, as well as the states found in families and/or subfamilies, in the form of text accompanied by illustrations.

The phylogeny proposed by Landa and Soldan (1985) is inferred based on the presumed evolution of characters in the groups and the evolutionary classification with paraphyletic groups is again adopted to avoid their fragmentation. The authors make an evaluation of the internal anatomy characters for the suborders created by McCafferty and Edmunds (1979) and consider that Pannota presents homogeneity in the characters, while Schistonota is comparatively more heterogeneous. They also claim that the convergences found by McCafferty and Edmunds (1979) are not correlated with convergences in the internal systems of these organisms.

In the late 1980s, Kluge (1989) published an article in which he proposed some changes in the phylogeny of the order to support his discussion of the gills (or tergalia, according to him) of Ephemeroptera. His proposal was based on characters from the external morphology of nymphs and adults, and also mentions the nervous system. Based on characters present in each group, the author traces the evolutionary history of the order in a diagram showing relationships between the groups (Figure 5). In his hypothesis of the relationship between the superfamilies of Ephemeroptera, he gets to a proposal which is, according to him, similar to that of Edmunds (1972).

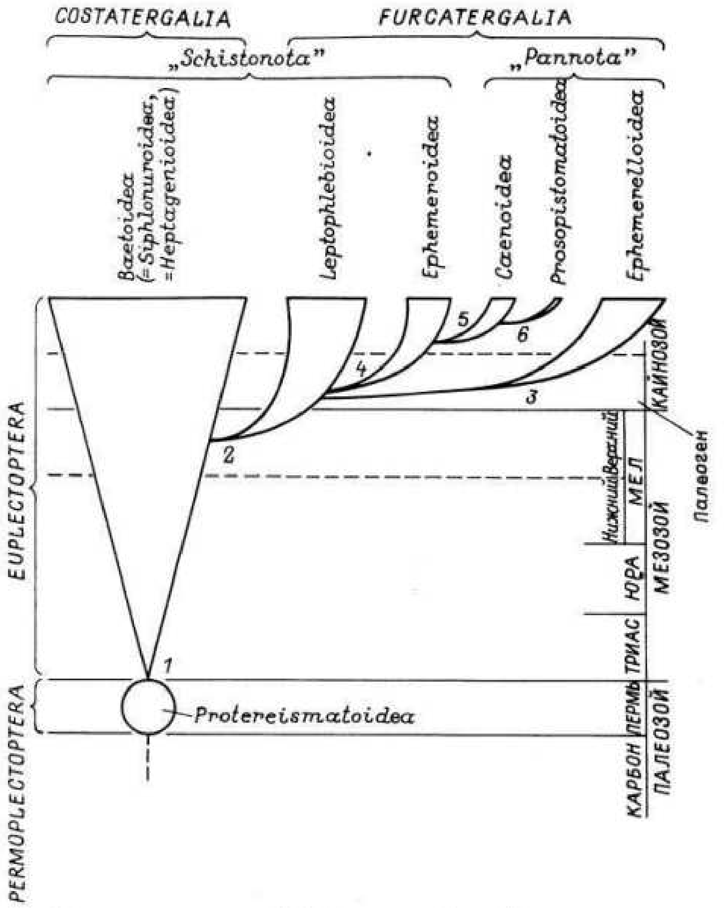

Figure 5 - Phylogenetic diagram presented by Kluge (1989)

After criticizing McCafferty and Edmunds (1979) for creating equal-category (suborder) taxa in which one of the two taxa is simultaneously larger and more heterogeneous, (Kluge, 1989) proposes the division of living mayflies into two suborders of *"approximately equal volume"*, Furcatergalia and Costatergalia, this second group being paraphyletic. The author does not make clear the method used to infer his phylogenetic proposal, and similarities are pointed out to infer kinship relationships, without clearly distinguishing derived and ancestral characters.

Tomka and Elpers (1991) focused on the family Heptageniidae, but also presented a proposal for the phylogeny of large groups based on a compilation of characters from the literature and some new ones on external morphology of eggs, nymphs and adults, and internal anatomy of nymphs. The authors were explicitly concerned with forming groups united by synapomorphies, but once again character polarization is not clear, they just say: *"To find the proper adelphotaxa we look for synapomorphic characters by carefully avoiding* *symplesiomorphic ones."* (Tomka and Elpers, 1991, p. 116). They presented in a table the characters considered apomorphic used in the construction of cladograms and also drawings and photos of them. The sentence *"The usefulness of a character is inversely proportional to the number of involved convergences."* (Tomka and Elpers, 1991, p. 116) suggests a lower weight given to characters that appear as convergences. There is no proposal for classification in this work.

In 1991, McCafferty published two papers addressing the phylogeny of higher groups of Ephemeroptera, the first of them focused on Heptagenioidea (McCafferty, 1991b) and the second focused on a discussion on the classification of the order with a phylogenetic basis (McCafferty, 1991a).

In his study of Heptagenioidea, McCafferty (1991b) performed the first cladistic analysis using parsimony to assess supra familial relationships within the order. External and internal nymph morphology data were used, as well as adult wing venation, totalizing 36 characters. Character polarization was performed based on comparison with the external group Siphlonurinae (historically considered to represent mayfly goundplan). This was also the first work that came closer to the presentation of a matrix, with a table of characters and their respective states. However, the author does not describe the state considered for each group, and, very importantly, McCafferty adopts the character state present in the taxon he considers most primitive within the group as representative of that taxon.

Furthermore, when faced with a character state present in two distinct lineages, McCafferty considered "*a very remote possibility*" that it had arisen twice, with a single emergence and further loss being more likely. Thus, in elaborating his hypothesis, a different weight was given to the gain and loss of a character in a group. Regarding the classification suggested in this work, it was the first proposal strictly concerned with the phylogeny of the group, without the formation of paraphyletic groups.

In his second work on the phylogeny of large groups published in the same year, McCafferty (1991a) addressed the phylogeny of the order and discussed its classification, drawing attention to the need for a classification that would strictly reflect phylogenetic relationships. The author also presented and discussed the positions of advocates of phylogenetic and evolutionary classifications in general and discussed the reluctance of ephemeropterists to adopt phylogenetic classifications for the order, although the discussion in this regard had been going on since the 1970s. According to him, the main reason for this reluctance would be the adoption of the gap criterion, which he recognized as being subjective and linked to the specialist's knowledge of the studied group. Another reason would be the excessive increase in the number of taxonomic categories believed to be necessary for a phylogenetic classification of the order. McCafferty further claims that a defined methodology was necessary for the validation of systematics as a reproducible science and that this is not the case in evolutionary systematics. By pointing out the need for change, he brings the metaphor of metamorphosis:

> *"I view my rejection of evolutionary classification and adoption of phylogenetic classification as a necessary metamorphosis and take some comfort in Gould's (1989) reminder that "obsolescence is a fate devoutly to be wished, lest science stagnate and die.*" (McCafferty, 1991a, p. 347).

He argued that, although evolutionary classification was an outdated method, some criticized aspects of the method, such as its dependence on the investigator's intuition, experience or art, could contribute to the investigation of phylogeny.

In this work, three suborders are proposed (Rechtracheata, Setisura and Pisciforma), similar to what is found in a diagram previously proposed by Landa (1973). Rechtracheata was divided into 3 infraorders (Vetulata, Lanceolata and Pannota) and Pisciforma was divided into two infraorders (Arenata and Imprimata). However, some points did not follow his previous proposal of a phylogenetic classification, for example, by proposing the infraorder Imprimata, for which *"synapomorphies must be found to validate it as a holophyletic taxon*". In this way, although calling for a phylogenetic classification, the proposal was still, in part, evolutionary.

In 1992, Kluge made a proposal that was not published until six years later (Kluge, 1998), and was cited as "in press" throughout that period. As the author proposed new groups that will be mentioned from now on, we will mention it out of chronological order. In this proposal, Ephemeroptera were grouped into two suborders: Permoplectoptera (fossil group - paraphyletic) and Euplectoptera (living taxa - polyphyletic or holophyletic). The suborder Euplectoptera, in turn, was divided into Posteriorna and Anteritorna, based on a character of venation of the fore wing. Anteritorna was divided into two groups based on the number of "dentisetae" three (Tridentiseta) or two (Bidentiseta). Tridentiseta (according to the author himself a possibly paraphyletic taxon) was divided into Tetramerotarsata and Siphlonuroidea. And finally, Bidentiseta was divided into two infraorders, Branchitergaliae and Furcatergalia.

His proposal was based on external characters from nymphs, subimagos and imagos, and also characters from the internal anatomy of nymphs. The author defined the characters considered synapomorphies and "proved" the existence of a group based on these characteristics defined by him as synapomorphies as in: *"The monophyly of the posteritorna, which include Baetisca and Prosopistoma, can be proved by the following synapomorphies: ... "* (Kluge, 1998, p. 258). The author accepted the formation of groups known to be paraphyletic, but drew attention to the size of the paraphyletic taxon compared to the monophyletic taxon in a classification. For Kluge, a paraphyletic taxon could be accepted in a classification when it is smaller than the taxa he called Holophyletic.

Bae and McCafferty (1995) studied the evolution of tusks (anteriorly directed mandibular processes) in Ephemeroidea. They assessed tusk characters in 54 species from the seven families belonging to the superfamily. The characters and their states are presented in the work in two tables, one for the eight characters used to infer subfamilial relationships of Polymitarcyidae and one for the seven characters used to infer relationships between the subfamilies of Ephemeridae. The methodology used is not explained. One example of how character polarization was inferred is given in the quote below:

> *"Since Polymitarcyidae tusks are all convergent anteriorly, Potamanthidae tusks are convergent anteriorly (at least somewhat convex laterally or concave medially), and those of the plesiotypic subfamily Ichthybotinae of the Ephemeridae are also convergent, it is a fair assumption that the ancestral tusks were convergent."* (Bae and McCafferty, 1995, p. 383).

These authors bring up three reasons why the cladograms obtained would not be so reliable: first, character polarization "subject to interpretation" since outgroups do not have tusks; second, the high rate of evolutionary convergence found in the external morphology of the mouthparts in Ephemeroptera and third, the restricted character set used. The authors finalize the paper drawing up a hypothesis about the trends in the evolution of tusk form and in the evolution of burrowing behaviour and habitat adaptation in Ephemereoidea.

In the same year, Kluge and collaborators (1995) proposed the superfamily Siphlonuroidea based on morphological characters of eggs, nymphs, adults, and also pointed out characters exclusive to subimagos. However, the authors already mentioned the possibility that the group would be paraphyletic:

> *"Siphlonuroidea is probably paraphyletic and the most primitive group of recent Ephemeroptera, most of its representatives having a complex of plesiomorphies."* (Kluge et al., 1995 p. 107).

They considered that each of the families belonging to Siphlonuroidea was possibly holophyletic based on the presence of unique characters that could be apomorphies, however, the relationships between the families were considered uncertain.

Kluge (1997) analyzed the relationships within the infraorder Furcatergalia. He noted that older authors, analyzing mainly characters from imagos, arrived at different relationship hypotheses from those reached by more recent authors, who gave priority to characters from nymphs. After surveying the synapomorphies that could unite each set of taxa into groups, he concluded that:

> *"The combinations of adult and larval characters of Neoephemeridae, Caenidae, Tricorythidae and Ephemerellidae do not allow the construction of a phylogenetic tree in which all branches would be directed from the plesiomorphic to the apomorphic states of characters, and each complex of apomorphies would appear only once." (Kluge, 1997, p. 525)*

According to the author, to "solve" this paradoxical situation, the occurrence of evolutionary convergence in characters that do not have an adaptive role would have to be admitted, which he considers very unlikely. He then suggests two alternative hypotheses. One of them would be the reversion of more specialized characters to more primitive ones. The other would be transduction - the same family would have originated simultaneously from two different families, one providing the characters of the adult and the other providing the characters of the nymphs. This article does not make it clear which would be the best hypothesis for the author.

In this work Kluge begins to mention that some characters are unique to a certain clade and some characters are not unique, but the importance of this information for him is not very clear at this point. However, he discusses the Pannota infraorder suggested by McCafferty and Edmunds (1979) and refutes the synapomorphy proposed by these authors (the fusion of the wingpads) with the argument that other families outside Pannota present that fusion (the character would not be unique to the group) and that some families considered as Pannota do not have that character.

Wang, McCafferty and Bae (1997) presented a new character found in adults of Caenidae and Neoephemeridae, the ommation. Since this characteristic was considered by the authors as possibly non-adaptive and being unique in Ephemeroptera, they suggested, based on it, a strong support for the monophyly hypothesis of Caenoidea, a group originally proposed by Edmunds and Traver (1954) based on nymph characters.

McCafferty and Wang (2000) analyzed the phylogeny of major lineages within the Pannota infraorder using cladistic methodology. The analysis was performed analyzing 51 characters of external morphology of nymphs and adults for 16 UTOs represented by groupings of genera. The relationship hypotheses were generated based on synapomorphies and the polarity was determined by outgroup analysis:

> "*Essentially all non-pannote mayflies are available as the outgroup. However, other Furcatergalia (especially Leptophlebiidae and plesiotypic Ephemeroidea such as Potamanthidae) took priority when the outgroup character state varied among non-pannote mayflies.*" (McCafferty and Wang, 2000, p. 14)

In this analysis, when there was more than one character state in the same OTU, it was considered the character state of the "most plesiotypic" species within the group. Character inconsistencies were resolved using the parsimony criterion, while losses as well as convergent structural gains were accepted in the analysis. Finally, the classification proposal presented tried to accurately represent the phylogenetic relationship hypothesis found. McCafferty and Wang's (2000) data corroborate Pannota's division into two superfamilies, Caenoidea and Ephemerelloidea. Also in this work, the authors excluded Prosopistomatidae and Baetiscidae from the infraorder.

In the year 2000, Kluge published a book on insect systematics in Russian (Kluge, 2000) which is partially available translated into English on his online page (Kluge, 2004b). Although this is not a reference focused on Ephemeroptera, the available translated chapter "General Principles Of Biological Systematics" is important to understand some aspects not fully explained in his articles. Some of these aspects are related to his method of performing the phylogenetic reconstruction, the principles used to carry out the classification, as well as the principles of his nomenclatural system (Dual Nomenclature System), which is an important part of his method of phylogenetic reconstruction. According to the author, his method (Cladoendesis) was used by him since 1999 (Kluge, n.d.), although this term appeared for the first time in the literature only in 2011. According to Kluge,

> *"The main principle of cladoendesis is that all the data are entered in a classification in which all the characters are attributed to hierarchically subordinated taxa. The new data are added to the already existing classification, which is expanded and improved in the process, or corrected if errors are revealed."* (Kluge, 2012, p. 624)

Thus, although he did not use this term in his 2000 book (Kluge, 2000), this would already be his way of inferring phylogenetic relationships at this time. In a later work, the author makes a point of stressing that his method is opposed to methods based on the elaboration of matrices and that the principle of parsimony would be inconsistent with the scientific methodology (Kluge, 2012).

Kluge (2004b), states that conclusions about ancestral-descendant relationships can only be inferred based on what he considers to be a cladistic analysis. In this chapter, in addition to describing in general terms his method of "scientific cladistic analysis" denominated "Cladoendesis", Kluge harshly criticizes what he calls "numerical cladistic analysis" (according to him, analyzes commonly performed using matrices, computer programs and parsimony). However, it is clear from the author's own words that he does not understand how an analysis is done using the help of a computer:

> *"The first way is to "reconstruct" the phylogeny by such computer method which is not understood by anybody and is grounded on nothing (see I.1.3 above), so that even the author of this "phylogeny" can not explain what are his arguments and refers only to a computer program written by somebody; because of this his arguments can not be disproved." (Kluge, 2004b, sec. I.2.3.3.4)*

His analyses are based on the assumption that evolutionary changes are unique and irreversible (Dollo's law). Thus, if different species share at least one derived character, this is used to infer that they are part of the same phylogenetic branch.

> *"In order to prove that several taxa form a common phylogenetic branch, it is necessary to find out at least one their synapomorphy, which at the same time would be an autapomorphy for all this branch." (Kluge, 2004b, sec. 1.1.2.2)*

The polarity is inferred based on the "out group" criterion, being considered apomorphies those characters that are not found outside the group being studied. He draws attention to what he calls "independently appeared homologous characters" which he assumes not being an inheritance of the phenotypic character itself, but of the genes that encode it, and thus it would be an homologous character, but not a synapomorphy and should be excluded from the analysis.

Kluge (2000) creates several new groups to express the kinship relationships inferred in his analyses, among them, some still used by the author himself, such as Fossoriae and Caenotergaliae. These taxa, like others formerly erected by the author, follow his own nomenclature system (Dual Nomenclature System), and may refer to new taxa, such as Fossoriae, or to taxa already existing in the traditional system and renamed by the author. This is the case of Caenotergaliae, which is equivalent to the superfamily Caenoidea.

The use of computers to assist in the process of reconstructing insect phylogenies matured throughout the 1980s and at the end of the decade the first analyzes for large groups of insects are carried out using molecular data, mostly using ribosomal DNA data (Engel and Kristensen, 2013). The first computer-aided suprafamilial group analysis for Ephemeroptera was performed by Molineri and Domínguez (2003).

Molineri and Domínguez (2003) described the nymphs (male and female) and the egg of *Melanemerella*, a genus that had been described based only on the adult female and that had already been considered in 3 different families. In this work, the authors performed a cladistic analysis to elucidate its relationship with the other families within Ephemerelloidea. Representatives of the South American genera of Ephemerelloidea were analyzed, as well as representatives of the families considered in the superfamily by McCafferty and Wang (2000). *Neoephemera* (Neoephemeridae) was used as an outgroup, and the genus *Massartella* (Leptophlebioidea, Leptophlebiidae) was included in the analysis because it was previously hypothesized as the group to which Melanemerella would belong. The 32-character matrix was analyzed in PIWE (Parsimony With Implied Weightings), and the implicit weighing was used in the analysis. The results supported the creation of the Malanemerellidae family to house the monotypic genus Melanemerella.

In the same year, Kluge (2003) discusses the clade Pinnatitergaliae, created by him previously (Kluge, 2000) based on egg, nymph, subimago and imago characters and composed by the families Potamantidae, Euthyplociidae and Fossoriae. The phylogenetic relationship between these taxa is unclear. The author raises unique apomorphies and autapomorphies that "prove" that certain taxa are holophyletic as in: *"Cryptoprosternata is a holophyletic taxon, which is provided by following unique autapomorphies."* (Kluge, 2003, p. 149). In cases where characters from groups proposed by other authors contradict his phylogenetic hypothesis, Kluge reanalyses the character proposed by the other author and shows that they are actually different characters or says that it is more likely than "single characters" originated independently in the two groups.

In 2004 Kluge published the book "The Phylogenetic System of Ephemeroptera" containing his proposed phylogeny for the entire Ephemeroptera order with the exception of Baetidae and part of Leptophlebiidae (Kluge, 2004a). In this publication, the author lists the characters of each taxon divided into: autapomorphies, plesiomorphies and characters of unclear phylogenetic status. Overall, the book maintains its previous classification (Kluge, 1998) where the order is divided into Posteritorna and Anteritorna and uses his previously explained methodology (Kluge, 2004b, 2000). Pinnatitergaliae and Costatergaliae are joined into a new taxon called Fimbriatotergaliae and new taxa are also created to put together other members of Furcatergaliae (Tricoryptera, Pantricorythi and Afrotricorythi). Kluge also launches the term "plesiomorphon" used by the author to designate taxa characterized only by plesiomorphies:

> *"Phylogenetic (cladistic) principle of systematics declares that all taxa should be holophyletic only, but never polyphyletic or paraphyletic. Instead, here is suggested a new term "plesiomorphon" (in English plural "plesiomorphons", but not Greek "plesiomorphy"): plesiomorphon is a taxon characterized by plesiomorphies only; thus, its holophyly is not proven, and possibly (or probably) this taxon is paraphyletic, but its paraphyly is also not proven yet.* (Kluge, 2004a, p. 16)

The infraorder Scapphodonta (Ephemeroidea) was once more studied by McCafferty (2004). The author analyzed previous proposals dealing with the group, and based on an analysis of the synapomorphies raised in these proposals and on an apparently subjective analysis of them, he proposes a new cladogram and a new classification for the group:

> "*Considering all of the above, certain phylogenetic modifications can now be made to the cladogram of Scapphodonta originally offered by McCafferty (1991).*" (McCafferty, 2004, p. 87)

Jacobus and McCafferty (2006) reassessed the phylogeny of the infraorder Pannota by performing a cladistic analysis based on morphological data. They evaluated 46 characters (eggs, nymphs and adults) from 34 OTUs (most genera and some larger subfamilies represented by the type genus). Five characters were considered irreversible, but it is unclear whether the Camim Sokal or Dollo optimization was used. The analysis was performed under the criterion of parsimony using the software PAUP. *Rhoenanthus* (Potamanthidae) was used as an outgroup, because they considered it to be the most plesiotypic genus within the more plesiotypic family of Scapphodonta (sister group to Pannota). The authors propose a new classification for Pannota based on the phylogeny proposed for the group, keeping the two superfamilies already in use, Caenoidea and Ephemerelloidea.

The first phylogenetic analysis based on molecular data for the order Ephemeroptera was carried out by Ogden and Whiting (2005). In this paper, in addition to a brief historical review of the main phylogeny studies for the group, the authors present a figure comparing the phylogenies proposed so far by Kluge and McCafferty, the two main proposals so far. Data from five genes were analyzed through direct optimization in the POY software. A majority consensus tree of the most parsimonious topologies found was calculated using the PAUP software. Alignments, datasets, and implicit trees are available. Nine species of Odonata and 5 species of apterigotes were used as the outgroup and polarization was made with reference to the family Hypogastruridae (Collembola). The results showed similarities and differences in relation to the results obtained from morphological data. Baetidae was recovered as a sister group to the other Ephemeroptera groups and while the superfamilies Ephemerelloidea and Caenoidea were supported as monophyletic, Baetoidea, Siphlonuroidea, Ephemeroidea and Heptagenioidea were not. The authors do not propose a new classification based on their analysis.

Sun et al. (2006) performed an analysis using the 18S molecular marker of ribosomal DNA. The work, which does not present the phylogeny obtained in the publication, was heavily criticized in a reply made by Ogden et al. (2008). The criticism points out methodological flaws, the non-use of data already available at the time, among others, and concludes that many of the main conclusions of Sun et al. (2006) regarding relationships within Ephemeroptera are not supported by their own data or by analyzes made using more data.

The first and unique matrix of morphological characters for the order Ephemeroptera as well as the first work to gather morphological and molecular data for the entire order was carried out by Ogden et al. (2009). Furthermore, in this work the cladistic analysis performed was clearly presented by the authors. A total of 101 morphological characters were surveyed for 113 taxa, including representatives of all families of the order. Of the 101 characters evaluated, 31 refer to imagos and 70 to nymphs. Despite the absence of data from eggs and internal anatomy, the authors mention that "all pertinent characters" used in previous analyzes were included in the matrix. Of the characters, 9 were coded as ordered, 11 as unordered, 24 were coded as Dollo and 57 as irreversible (Sokal). The reason for choosing each optimization was not explained. The morphological data matrix was analyzed under parsimony criteria using the PAUP software. A 50% majority consensus tree was built based on the 512.247 most parsimonious trees found.

For the molecular analysis, 5 genes were evaluated for 84 species of Ephemeroptera, including representatives of 91% of the families. The total molecular data were analyzed in PAUP under maximum parsimony and a reduced molecular dataset was analyzed under the maximum likelihood criterion. Furthermore, molecular data and morphological characters were analyzed combined using Bayesian inference. Siphluriscidae, which had not been included in the analysis by Ogden & Whiting (2005), was included in this analysis and appears in the results as the sister group to the remaining Ephemeroptera. Furcatergalia, Carapacea, Fossoriae and Pannota lineages, as well as Ephemerelloidea and Caenoidea superfamilies were recovered as monophyletic according to the total combined data analysis. Based on their results, the authors claim that previous phylogenetic reconstructions were hampered by similarities in external morphology (high level of homoplasy), particularly in the nymphs. There is no proposition for a new classification based on the results.

The relationship between burrowing Ephemeroptera (Ephemeroidea) was analyzed using phylogenomic techniques by Miller et al. (2018). The authors evaluated 23 species, 19 belonging to the interest group (Behningiidae, Ephemeridae, Euthyplociidae, Ichthybotidae, Palingiidae, Polymitarcyidae and Potamanthidae). As external groups, species belonging to four closely related families (Ephemerellidae, Tricorythidae, Leptophlebiidae and Nesameletidae) were used. Maximum likelihood and Bayesian analysis were performed, which gave identical results.

The burrowing Ephemeroptera were recovered as a monophyletic group with Behningiidae within the group. In this way, the reconstruction supports the hypothesis of the tusks gain in the excavators group with a subsequent loss in Behningiidae. This hypothesis corroborates what was previously proposed (Kluge, 2004a; Ogden et al., 2009) and is contrary to what was proposed by McCafferty (2004), for whom Behningiidae would be a sister group of the tusked mayflies.

In 2018, the year of the most recent "International Conference on Ephemeroptera and Plecoptera", preliminary data from an extensive work on phylogeny based on molecular data (phylogenomics) for the order were presented and later published (Ogden, 2019). The authors analyzed genera representing 35 of the 41 Ephemeroptera families and used as an external group representatives from Odonata, Zygentoma and Archaeognatha.

It is interesting to point out that although the data are preliminary, their results show that many of the lineages proposed by McCafferty and Kluge would not be monophyletic. Lineages Caenoidea/ Caenotergaliae, Ephemerelloidea, Fimbriatotergaliae, Heptagenota, Caparacea/ Posteritorna and Tricoryptera were recovered as monophyletic. However, some families were not included and some lineages found at this moment to be monophyletic may no longer be so when more taxa are included.

Discussion

In general, considering the classifications presented for the order within the evaluated period (1917 - 2021), we can consider that until 1979 the classifications were evolutionary, giving priority to the similarity between organisms (phenetic criterion) to the detriment of information about the relationship between the species (phyletic criterion). Beginning with McCafferty and Edmunds (1976), some authors began to show concern with forming monophyletic groups based on synapomorphies, however, concerned about not fragmenting the superior classification, they continued to admit paraphyletic groups and the classification continued without having as a premise to reflect the phylogeny. In 1991, the situation began to change, with the first analysis using the criterion of parsimony for large groups in Ephemeroptera (McCafferty, 1991b) and, in the same year, the proposal by the same author to abandon the evolutionary classification for the group (McCafferty, 1991a).

We analyzed a total of 36 works. Of that total, only six papers presented tested hypotheses of homology (Jacobus and McCafferty, 2006; Miller et al., 2018; Molineri and Domínguez, 2003; Ogden, 2019; Ogden et al., 2009; Ogden and Whiting, 2005), while the others present hypotheses of homology that were not tested. Classification proposals for the order were all based on the studies where the hypotheses were not tested. On the other hand, most authors who tested their hypotheses did not propose classifications, except for Molineri and Domínguez (2003) and Jacobus and Maccafferty (2006), who worked on smaller groups (Ephemerelloidea and Pannota respectively).

In the review of the semaphoronts used in the analyses we observed that most of the studies used characters from nymphs and adults (18) or from eggs, nymphs and adults together (8). Few studies had a more restricted data set when we thought about the representation of different semaphoronts. Koss and Edmunds (1974) used only data from eggs and two works used only data from nymphs (Bae and McCafferty, 1995; Landa and Soldán, 1985) being therefore older works. Four more recent works used molecular data and the semaphoronts are not stated.

Among the studies analyzed, we found that in the vast majority only data from external morphology (16) or external morphology combined with internal anatomy (10), and only one study was based exclusively on internal anatomy data (Landa and Soldán, 1985).

We consider that despite the vast amount of work done based on morphology for Ephemeroptera there is still room for improvement as morphological data is still an important source of data for phylogenetic analysis (Beutel et al., 2013). It is important to highlight that, of the 11 studies that used internal anatomy data, only Kluge (2004) is more recent, the others being prior to 1991. So far, we have 3 studies on phylogeny of the order that are based exclusively on molecular data (Ogden, 2019; Ogden and Whiting, 2005; Sun et al., 2006). And finally, Ogden et al. (2009) stands out for being the only work that integrates morphological data with molecular data.

Although there are efforts to carry out new works involving molecular data and their integration with morphological data, one of the most active authors in taxonomic studies of the order today, Nikita J. Kluge, elaborates his phylogenetic hypotheses using his own method, the "Cladoendesis". This author is strongly opposed to the cladistic approach currently used, carried out with the aid of computers and following the criterion of parsimony, in addition to not considering the use of molecular data.

We consider a low number of works with data that are likely to be re-evaluated since most works do not present the data on which they based their hypothesis clearly. Only the most recent works present a matrix showing the characters, their states, and how the coding for each species was performed (Jacobus and McCafferty, 2006; Molineri and Domínguez, 2003; Ogden et al., 2009) and to date there is only one matrix of morphological data that covers the entire order (Ogden et al., 2009). Even with the large number of taxa involved in the works, the first computer-aided analysis to assess suprafamilial relationships within the order was carried out only in 2003 (Molineri and Domínguez, 2003).

Regarding the treatment of characters, we analyzed whether there was some form of weighing the characters and whether the characters were considered reversible or not. For the weight given to the characters, it was possible to observe that although Lameere (1917) and Spieth (1933) had already called attention to the importance of observing both adult and nymphal characters, authors who came later had different views, prioritizing characters of adults over those of the nymphs (Demoulin, 1958; Tshernova, 1970) or prioritizing the characters of the nymphs over those of the adults (Riek, 1973).

Different authors attributed less weight to characters that appeared as evolutionary convergences (Edmunds et al., 1963; McCafferty, 1991b; Tomka and Elpers, 1991). Likewise, Kluge et al. (1995) did not accept as a synapomorphy of a group a state of character that was also present in another group. They considered only the unique synapomorphies, thus giving them greater weight in inferring phylogeny.

Finally, considering works that investigated suprafamilial relationships, only one used implicit character weighing (Molineri and Domínguez, 2003). Implicit character weighing can be important to reduce the weight of a large number of homoplastic characters in the analysis of morphological data, thus improving the support of the analysis (Goloboff et al., 2008). Given the large number of homoplasies found, especially in the morphological characters of Ephemeroptera nymphs (Ogden et al., 2009), the adoption of implicit weighing as performed by Molineri and Domínguez (2003) for groups of Ephemerelloidea is very promising. This type of weighing, however, has not yet been adopted in an analysis for the whole order.

In more recent works, the attribution of weight to different characters is less evident or not so explicit, but, on the other hand, the criteria of reversibility or irreversibility of characters appear. McCafferty and Wang (2000) accepted the reversibility of a character, while Kluge (2000) considers that evolutionary changes are unique and irreversible. In more recent works, mixed criteria of reversibility appear, with some characters being considered reversible and others irreversible (Jacobus and McCafferty, 2006; Ogden et al., 2009). We believe that this issue, although it is very important and can greatly affect the results of the analysis, is not clearly stated in the papers.

Conclusions

Internal relationships in Ephemeroptera remain uncertain and there is no consensus for most clades. The elaboration of relationship hypotheses for organisms from very old groups can be complicated by the large amount of time elapsed since cladogenetic events, which allows the emergence of many characters by evolutionary convergence. In the case of Ephemeroptera, the situation is complicated by the short lifespan of the adult (reducing the selective pressure in this instar) and the aquatic way of life of the nymphs (which tends to be a strong selective pressure). Despite these difficulties, it is possible to study the history of these groups through modern phylogenetic methods and practices, and from these, create classifications that reflect the relationships between the groups.

However, there are few comprehensive phylogenies based on tested evidence for the order and there is no classification for the order based on a tested hypothesis of homology. Despite homology hypotheses for morphological characters being relatively well set, we believe that there is still room for further studies with morphological and molecular data. Just as other groups have benefited from morphological data for the elaboration of phylogenetic hypotheses (Beutel et al., 2013; Lawrence et al., 2011), and consequently for their classification, the same can happen for Ephemeroptera as long as modern methodologies and based on scientific criteria are carried out.

In the last 30 years we had basically 3 groups working with the higher groups phylogeny of mayflies: McCafferty and collaborators, Kluge, and Ogden and collaborators. In face of that we consider that is also important that more scientists dedicate themselves to studying the phylogeny of the group and that they do so based on methodologies that can be replicated, so that we have a greater number of phylogenetic hypotheses that can be evaluated as to their robustness. This increased effort will help us to obtain a better view of the evolution of the group, of kinship relations between families and between major groups, and in this way establish a well-founded modern classification for the order.

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CHAPTER II

PHYLOGENY OF THE ORDER EPHEMEROPTERA (INSECTA) BASED ON MORPHOLOGICAL CHARACTERS

Phylogeny of the Order Ephemeroptera (Insecta) Based on Morphological Characters

Marcela Miranda de Lima, Pedro Seyferth Ribeiro Romano, Michel Sartori and Frederico Falcão Salles

Abstract

A cladistic analysis of the order Ephemeroptera is presented. The only existing coded matrix for the order was reviewed and reanalysed. The matrix consisting of 106 taxa and 131 morphological characters was analyzed under Implied Weighting with five different K values (8, 9,10,11 and 12). The analyzes yield five different topologies, one for each K value. The consensus of all analyzes was unresolved for the deep relationship within Ephemeroptera. Monophyly is supported for Caenotergaliae (=Caenoidea), Ephemerella/Fg1 (=Ephemerelloidea), Eusetisura, Fimbriatotergaliae, Fossoriae, Heptagennota, and Posteritorna (=Carapacea). Anteritorna, Branchitergaliae, and Tridentiseta were not supported as monophyletic. Five families were not found to be monophyletic in any of our analyzes: Ephemerellidae, Ephemeridae, Nesameletidae, Potamanthidae, and Teloganodidae. The evolution of the number of dentisetae on the maxilla, the presence and shape of maxillary gills, the degree of fusion of the forewing pads, and the presence of a double row of long filtering hairs on anterior femora and tibiae, all important characters for the systematics of mayflies, are presented and discussed according to the different hypotheses found.

Keywords: Parsimony, Cladistic analysis, Character evolution, Mayflies, Pterygota

Introduction

Extant Ephemeroptera are part of an old lineage of Pterygota (Insecta) called Ephemerida, having its origin in the late carboniferous (Willmann, 2007). Ephemeroptera is currently included in a taxon called Heptabranchia, together with Coxoplectoptera, its sister group. Their larvae share the presence of 7 pairs of abdominal gills, the presence of a single tarsal segment, and a single pretarsal claw (Staniczek et al., 2011).

The relationships of Ephemerida and the remaining winged insects (Protodonata and Neoptera), often referred to simply as the relationship of Ephemeroptera, Odonata and Neoptera or the Palaeoptera Problem (Blanke et al., 2013; Hovmoller et al., 2002; Ogden and Whiting, 2003; Simon et al., 2018), is still an unresolved question in the domains of insect evolution and systematics. There are three primary hypotheses regarding their correlation (Figure 1), each supported by distinct morphological traits. Meanwhile, different molecular research has validated all three proposals (Yu et al., 2021).

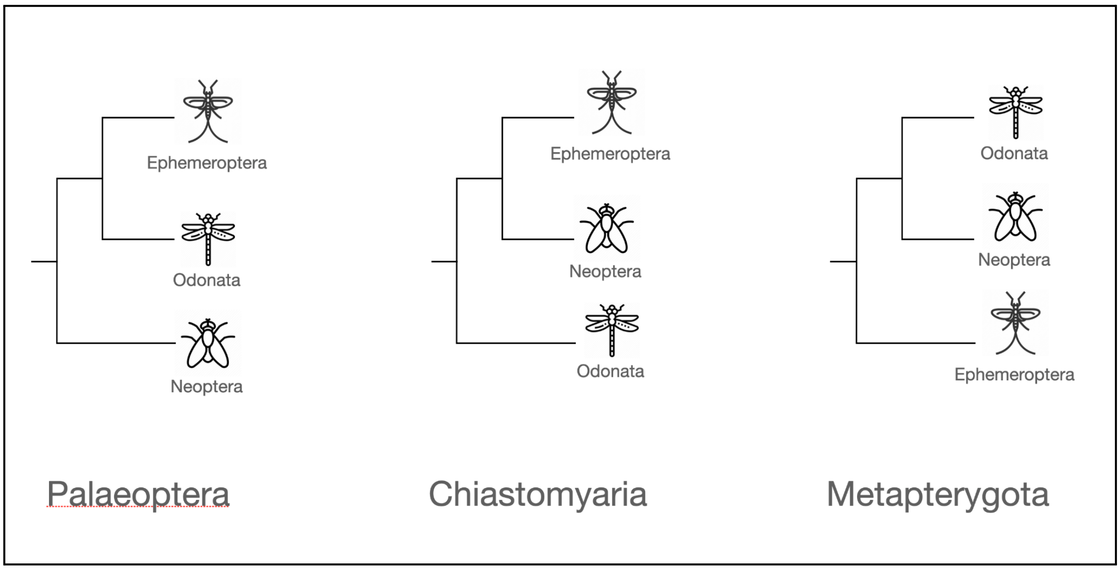
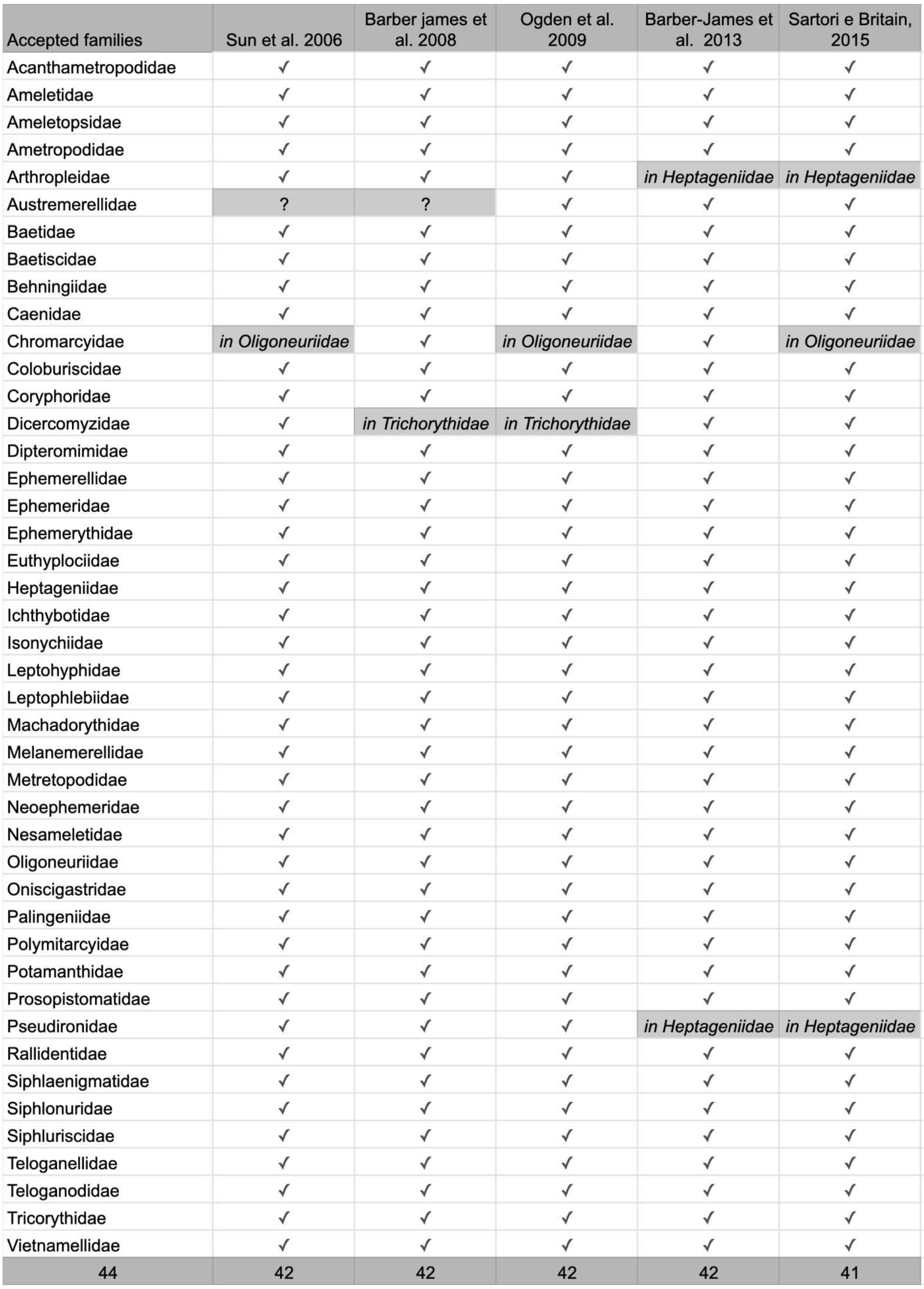


Figure 1. Different hypotheses of relationship between pterygote insects.

Ephemeroptera is a small insect order comprising less than 4000 described species in 389 genera (Jacobus et al., 2021). There are currently 44 recognised families, but no author accepts them all (Barber-James et al., 2013, 2008; Ogden et al., 2009; Sartori and Brittain, 2015; Sun et al., 2006). This gives different numbers of families accepted depending on the author, ranging from 41 to 42, and sometimes the same number comprises different families (table 1). Kluge is not referenced in that table because he does not use the traditional rank-based nomenclature, using a different system of nomenclature for the taxa.

Table 1. Families of Ephemeroptera accepted by different authors



Accepted families	Sun et al. 2006	Barber james et al. 2008	Ogden et al. 2009	Barber-James et al. 2013	Sartori e Britain, 2015
Acanthametropodidae	✓	✓	✓	✓	✓
Ameletidae	✓	✓	✓	✓	✓
Ameletopsidae	✓	✓	✓	✓	✓
Ametropodidae	✓	✓	✓	✓	✓
Arthropleidae	✓	✓	✓	*in Heptageniidae*	*in Heptageniidae*
Austremerellidae	?	?	✓	✓	✓
Baetidae	✓	✓	✓	✓	✓
Baetiscidae	✓	✓	✓	✓	✓
Behningiidae	✓	✓	✓	✓	✓
Caenidae	✓	✓	✓	✓	✓
Chromarcyidae	*in Oligoneuriidae*	✓	*in Oligoneuriidae*	✓	*in Oligoneuriidae*
Coloburiscidae	✓	✓	✓	✓	✓
Coryphoridae	✓	✓	✓	✓	✓
Dicercomyzidae	✓	*in Trichorythidae*	*in Trichorythidae*	✓	✓
Dipteromimidae	✓	✓	✓	✓	✓
Ephemerellidae	✓	✓	✓	✓	✓
Ephemeridae	✓	✓	✓	✓	✓
Ephemerythidae	✓	✓	✓	✓	✓
Euthyplociidae	✓	✓	✓	✓	✓
Heptageniidae	✓	✓	✓	✓	✓
Ichthybotidae	✓	✓	✓	✓	✓
Isonychiidae	✓	✓	✓	✓	✓
Leptohyphidae	✓	✓	✓	✓	✓
Leptophlebiidae	✓	✓	✓	✓	✓
Machadorythidae	✓	✓	✓	✓	✓
Melanemerellidae	✓	✓	✓	✓	✓
Metretopodidae	✓	✓	✓	✓	✓
Neoephemeridae	✓	✓	✓	✓	✓
Nesameletidae	✓	✓	✓	✓	✓
Oligoneuriidae	✓	✓	✓	✓	✓
Oniscigastridae	✓	✓	✓	✓	✓
Palingeniidae	✓	✓	✓	✓	✓
Polymitarcyidae	✓	✓	✓	✓	✓
Potamanthidae	✓	✓	✓	✓	✓
Prosopistomatidae	✓	✓	✓	✓	✓
Pseudironidae	✓	✓	✓	*in Heptageniidae*	*in Heptageniidae*
Rallidentidae	✓	✓	✓	✓	✓
Siphlaenigmatidae	✓	✓	✓	✓	✓
Siphlonuridae	✓	✓	✓	✓	✓
Siphluriscidae	✓	✓	✓	✓	✓
Teloganellidae	✓	✓	✓	✓	✓
Teloganodidae	✓	✓	✓	✓	✓
Tricorythidae	✓	✓	✓	✓	✓
Vietnamellidae	✓	✓	✓	✓	✓
44	42	42	42	42	41

There is a growing number of papers describing new taxa in Ephemeroptera (Jacobus et al., 2021), and many studies addressing the phylogeny of its families (e.g. Bae and McCafferty, 1998; Barber-James, 2009; Cruz et al., 2021; Jacobus and McCafferty, 2006a; Molineri, 2006; Monjardim et al., 2020; Wang and McCafferty, 2004) or groups of families (e.g. Jacobus and McCafferty, 2006b; McCafferty, 1991a; McCafferty and Wang, 2000; Miller et al., 2018). Nonetheless, few publications have addressed the phylogeny of the entire order (Kluge, 2004; McCafferty, 1991b; Ogden, 2019; Ogden et al., 2009; Ogden and Whiting, 2005). An overview of older works historically contextualized may be found in Lima et al. (in prep., chapter 1 of the present thesis).

In the first attempt to make a phylogenetic hypothesis based on molecular data, Ogden & Whiting (2005) formulated a summary tree with the two most current hypotheses regarding the group. According to these authors, McCafferty's more actual hypothesis was constructed in parts (Jacobus and McCafferty, 2006b; McCafferty, 1997, 1991a, 1991b; McCafferty and Wang, 2000; Wang and McCafferty, 1995) being the combination of those parts added of personal communication with McCafferty used to construct the tree. This combined hypothesis is partly congruent with that presented by Kluge (2004), being both based on morphological data. The nomenclature is somewhat confusing since Kluge uses a hierarchy-based nomenclature developed by himself, so even if a taxon is accepted by both authors, the name may be different (e.g. Superfamily Heptagenioidea = Branchitergalia; Superfamily Caenoidea = Caenotergaliae).

In the McCafferty's hypothesis as compiled by Ogden e Whiting (2005), the families are organized in four suborders; Carapacea, Furcatergalia, Setisura and Pisciforma, and six superfamilies: Baetoidea, Caenoidea, Heptagenioidea, Ephemerelloidea, Siphlonuroidea, and Ephemeroidea. The four suborders are recognized by both authors, with different names and relationship between them, and, in the case of Furcatergalia and Setisura, with different internal arrangements between the families (Figure 2).

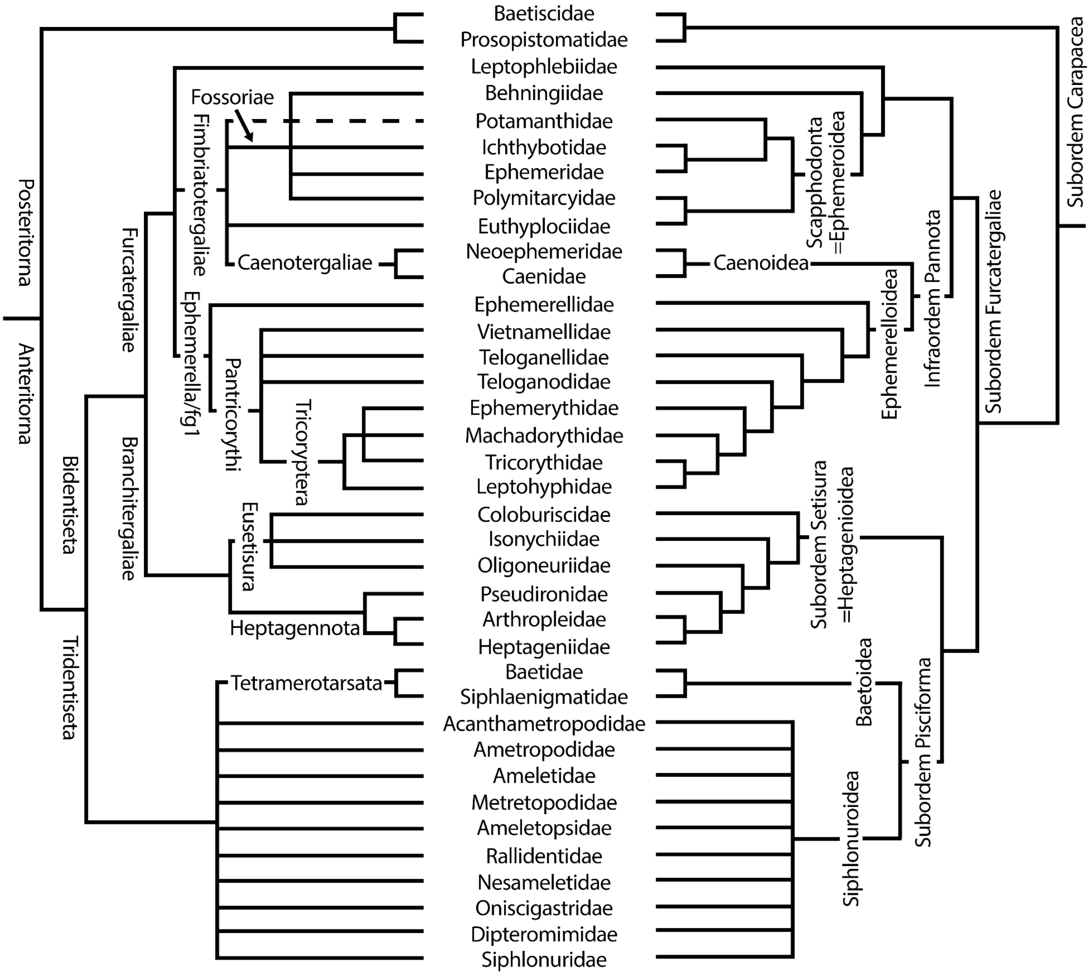


Figure 2. Comparison of Kluge and McCafferty's phylogenetic hypothesis for the order Ephemeroptera as compiled by Ogden et al. (2005).

Baetoidea is accepted by Kluge (2004) as Tetramerotarsata, and Caenoidea as Caenotergaliae, Heptagenioidea and Ephemerelloidea are equivalent in taxon composition to Branchitergalia and Ephemerella/fg1, respectively. The families composing Siphlonuroidea form, for both authors, a polytomy with no relationship proposed between the families. Ephemeroidea is the sole superfamily where the authors disagree on which families are included. The families Baetiscidae and Prosopistomatidae, are grouped in a distinct suborder, Carapacea, which is considered the sister group of the remaining mayflies in both proposals. This group was formerly called Prosopistomatoidea, but its name was changed once it became a suborder.

In the first molecular phylogeny of the order, Ogden and Whiting (2005) included 31 of the 42 families and found support for the suborders Furcatergalia and Carapacea, and also for the Infraorder Pannota (*sensu* McCafferty and Wang, 2000), a clade that comprises Caeneoidea + Ephemerelloidea.

Ogden et al. (2009) performed the first and only cladistic analysis combining molecular and morphological data for the whole order. Their combined analysis supported the suborders Carapacea and Furcatergalia, as well as the infraorder Pannota. Their parsimony analysis of morphological data, recovered Carapacea, Baetoidea, Caenoidea and Ephemeroidea. Caenoidea was recovered in a clade with a paraphyletic Ephemerelloidea and Carapacea, but still corroborates Pannota.

Despite the numerous studies on the phylogeny of Ephemeroptera (in prep., chapter 1 of the present thesis), the first matrix of morphological characters for the order was not presented until 2009 by Ogden and coworkers in a study that combined molecular and morphological data. Their morphological dataset consisted of 101 coded characters for 108 taxa. Of these, 24 were optimized as Dollo characters (Farris, 1977), weighted so that a derived state can occur only once but can be lost multiple times thereafter. However, this postulate is considered suitable for complex structures (Brower and Schuh, 2021), but was used for simple characters, and this could determine the outcome of the analysis. Another important point to consider is that the analysis yielded 512.247 most parsimonious topologies and the result was the fifty percent majority-rule consensus of these trees.

Therefore, considering that there are practically no quantitative analyzes based on morphological data for the order, considering that there is already a matrix that has been filled in by various specialists in the group with a relevant set of data, and also considering that this single existing matrix has some problems that may have affected the results obtained, it is imperative to reanalyze this pre-existing collection of morphological characters. Thus, before developing a new homology hypothesis for the group and revising its current systematics based on morphological characters, certain steps must be taken. These include semantic redescription of characters, removal of dependent and non-informative ones, adjustment of the transformation series of characters, redefinition of optimization for each character, and proposal of new characters. The aim of this study is to perform a phylogenetic analysis of Ephemeroptera using a scrutinized set of morphological data.

Material and methods

Taxon sampling

A total of 105 taxa were included in this study, mainly the same taxa treated by Ogden et al. (2009). In some cases, when available descriptions and figures were absent, incomplete or too sparse in the literature, species were replaced by congeneric taxa. The genus *Vietnamella* was added to the matrix in order to represent the monogeneric family Vietnamellidae. In a few cases, when all character information was not found for a single species, data from other species of the same genus were used, thus composing a OTU.

One species of Odonata (*Epiophlebia superstes*) was defined as the outgroup for character polarization. We also included two fossil genera of the stem group Protereismatidae (Permoplectoptera), *Kukalova americana* and *Protereisma permianum*, and *Mickoleitia longimanus* (Coxoplectoptera), considered the sister group of the order Ephemeroptera (Staniczek et al., 2011).

Character coding

A new general matrix for Ephemeroptera was built from the reinterpretation, exclusion, combination and redefinition of the morphological characters previously proposed by Ogden et al. (2009) and the addition of new characters. Furthermore, all character statements were reviewed and modified following Sereno (2007) in order to distinguish between neomorphic and transformational character statements. Character statements were also modified to ensure a more consistent language by explicitly stating the locators and variables of each character statement. The index of characters from Kluge's (2004) book, containing summarized information with respect to character variation observed in Ephemeroptera was consulted in order to complement character states.

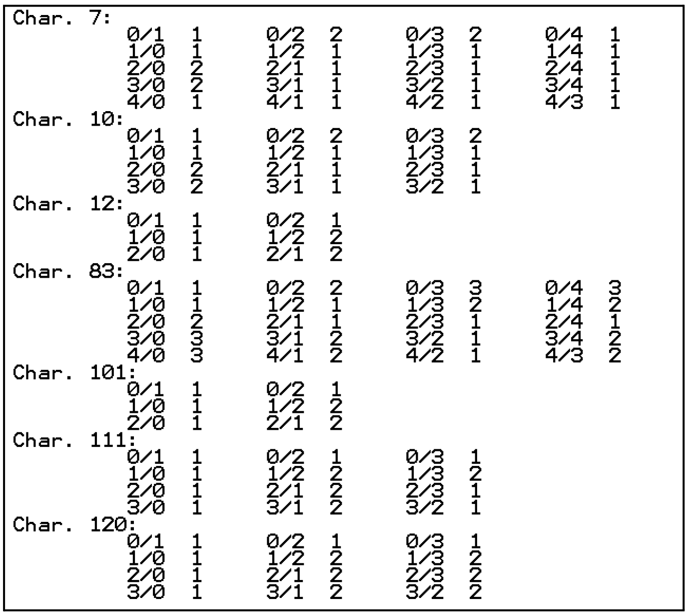
Information for the codification of the characters was mostly gathered in the literature (text and/or pictures). This was possible thanks to two databases with most of the older and recent publications of the group digitized and cataloged: Ephemeroptera of the world (Kluge, 2023) and Ephemeroptera Galactica (Hubbard and Staniczek, 2012). Information from literature was complemented by direct observation of specimens available at the Musée Cantonal de Zoologie at Lausanne (Switzerland) and Museu de Entomologia at the Federal University of Viçosa (Brazil). When information concerning the state of a character could not be retrieved with confidence in the literature and/or observation of specimens and pictures, it was retrieved from the original matrix (Ogden et al., 2009). Of the 5374 character states coded, 649 were scored as unknown (?) and 1522 as inapplicable (-).

Few characters, not commonly described in the literature, were mostly coded according to information presented by Kluge (2004). In instances where information about a specific genus was unavailable, we consulted the higher groups established by the author until we obtained the necessary data. The author's highly systematized and constantly actualized website (Kluge, 2009) was utilized to facilitate our search for updated information on the taxa. There, each group hierarchy is shown with links in the upper part of the page, giving easy access to all the information. We also utilized this method when coding other characters for certain species.

For two taxa, characters related to the hindwing were encoded based on the female's wing: *Melanemerella brasiliana* (male imago not described) and *Potamanthellus caenoides* (male described, but no image of its wings was found). For *Mickoleitia longimanus* we do not know if the description is based on a male or a female, but the data was used for characters of the hindwing.

Character ordering

From the 34 multistate characters, 7 were treated as non-additive or maximally connected (Slowinski, 1993) when the degree of similarity was considered to be the same between all states. This was the case for all the meristic characters. Some of the multistate characters, however, were considered to have some states more similar to each other than to a third one, so degrees of homology between the character states were hypothesized based on their similarity (Lipscomb, 1992). This was done by establishing different transformation costs for the changes between the states in TNT (Figure 3) (Goloboff, 2022). Twelve characters were treated as additive or minimally connected (Slowinski, 1993). In the seven cases where the relationships of similarity between states could be specified hierarchically as a tree, the characters were considered as "moderately connected" (Slowinski, 1993) and the costs were defined using the "character state tree" option in TNT (Goloboff, 2022). Transformation costs hypothesized for each of these characters can be seen on figure 3.



```
Char. 7:
        0/1  1     0/2  2     0/3  2     0/4  1
        1/0  1     1/2  1     1/3  1     1/4  1
        2/0  2     2/1  1     2/3  1     2/4  1
        3/0  2     3/1  1     3/2  1     3/4  1
        4/0  1     4/1  1     4/2  1     4/3  1
Char. 10:
        0/1  1     0/2  2     0/3  2
        1/0  1     1/2  1     1/3  1
        2/0  2     2/1  1     2/3  1
        3/0  2     3/1  1     3/2  1
Char. 12:
        0/1  1     0/2  1
        1/0  1     1/2  2
        2/0  1     2/1  2
Char. 83:
        0/1  1     0/2  2     0/3  3     0/4  3
        1/0  1     1/2  1     1/3  2     1/4  2
        2/0  2     2/1  1     2/3  1     2/4  1
        3/0  3     3/1  2     3/2  1     3/4  2
        4/0  3     4/1  2     4/2  1     4/3  2
Char. 101:
        0/1  1     0/2  1
        1/0  1     1/2  2
        2/0  1     2/1  2
Char. 111:
        0/1  1     0/2  1     0/3  1
        1/0  1     1/2  2     1/3  2
        2/0  1     2/1  2     2/3  1
        3/0  1     3/1  2     3/2  1
Char. 120:
        0/1  1     0/2  1     0/3  1
        1/0  1     1/2  2     1/3  2
        2/0  1     2/1  2     2/3  2
        3/0  1     3/1  2     3/2  2
```

Figure 3 - Costs of transformation between states hypothesized based on degree of similarity using character state trees

Phylogenetic analysis

Tree searches

Morphological data were analyzed using parsimony methods in TNT 1.6 (Goloboff and Morales, 2023). All searches were done using heuristic methods (table 2). The first searches were done using random addition sequences (RAS) followed by tree-bisection reconnection (TBR) using only prior weights. The consensus of the analysis done using prior weights only will not be taken into account here, but it may be found in the appendix (Appendix 1). After finding the Most Parsimonious Trees (MPTs) with traditional search with 1000 replications and 200 trees saved per replicate (analysis 1 - table 2), searches were conducted using New Technology under Implied Weighting (from here on IW). Driven search was set up to find the minimum length 15 times. Searches were done using sectorial search and trees fusing using default parameters for both, random seed zero and collapsing trees after search. As the consensus trees obtained through the second method had identical scores and were also identical, we conducted subsequent searches using New Technologies with 15 hits to expedite the process.

Table 2 - Tree searches performed in the phylogenetic analysis detailing settings used.

Analysis	Search	Algorithm	Score	Hits	Trees	Time
1	Heuristic	RAS + TBR 1000 repl/ 200 trees save per repl. Collapse trees after search Random seed = 0	1104	15	500	12:31
2	Heuristic	Driven Search (15 hits) New technology default parameters Set Search + Tree fusing Collapse trees after search/ Random seed = 0	1104	15	47	00:37
3	Heuristic	Driven Search (15 hits) New technology default parameters Set Search + Tree fusing Collapse trees after search/ Random seed = 0 **IW K=8**	52,725	15	1	12:24
4	Heuristic	Driven Search (15 hits) New technology default parameters Set Search + Tree fusing Collapse trees after search/ Random seed = 0 **IW K=9**	49,616	15	1	12:15
5	Heuristic	Driven Search (15 hits) New technology default parameters Set Search + Tree fusing Collapse trees after search/ Random seed = 0 **IW K=10**	46,880	15	1	06:21
6	Heuristic	Driven Search (15 hits) New technology default parameters Set Search + Tree fusing Collapse trees after search/ Random seed = 0 **IW K=11**	44,449	4 (stopped)	1	34:04:00
7	Heuristic	Driven Search (15 hits) New technology default parameters Set Search + Tree fusing Collapse trees after search/ Random seed = 0 **IW K=12**	42,275	14 (stopped)	1	01:49:45

Clade Support

Combined Bremer support (CBS) was estimated using TBR from existing trees as implemented in TNT (Goloboff, 2022). For the analyses using IW, calculation of Bremer support was not possible for the strict consensus tree because the analysis must be done under the same IW the analysis was done. The supports were then calculated separately for each K value, using the correspondent MPT.

The jackknifing used symmetric resampling, intended for use in cases where character weights or state transformation weights are not uniform (Goloboff, 2022). Tree searches for resampling were done using traditional search (default parameters but with 100 replicates) in

100 replicates. The results were obtained in terms of frequency differences (CG), best suited to measure support for groups of low support (Goloboff, 2022).

Results

A total of 131 discrete characters were considered in the new matrix. From those, 102 are at least based on existing characters from Ogden et al. (2009), whereas the other 29 are new or based on other sources (Table 3). A total of 50 characters are from imagos and 81 are from nymphs. Some characters were excluded from the matrix of Ogden et al. (2009) (18, 21, 26, 32, 33, 36, 37, 38, 47, 52, 54, 61, 63, 83, 90 and 97). The major reason was the difficulty to retrieve information from the literature on the state of character for each taxa, but also some characters were considered to be non-informative. Characters 128, 129 and 130 were added during the analysis so, to keep the organization in terms of instar and anatomy, their numbers are not following the sequence.

Table 3 - Characters statements with their respective origin, modifications made in the original character statement (when it was the case), character type and optimization used in each case.

Character number	Character statement	Source of character	Original	Modification	Source for addition or modification	Character type	Optimization
Imago							
0	Female, subimago moult; 0) absent, 1) present (at least partially)	Ogden et al. (2009)	1 - Female subimago 0) moults at least partially 1) does not moult	Splitting/ semantics	-	Discrete - neomorphic	non-additive
1	Female, subimago moult, attribute (contingent to 0(1)); 0) parcial, 1) complete				-	Discrete - transformational / binary	non-additive
2	Male, compound eyes, size relative to female's eyes; 0) subequal, 1) enlarged.	Ogden et al. (2009)	2 - Male compound eyes 0) of same size as in female 1) enlarged and undivided 2) enlarged and partly divided 3) turbinate eyes	Splitting/ semantics	-	Discrete - transformational / binary	non-additive
3	Compound eyes of male, division; 0) undivided, 1) divided, without elevated portion, 2) totally divided, with elevated portion (turbinated eye)				-	Discrete - transformational / multistate	additive linear
4	Mesothorax, medial region, ommation; 0) absent, 1) present	Ogden et al. 2009	3 - Mesothorax medially 0) without ommation 1) with ommation	Semantics	-	Discrete - neomorphic	non-additive
5	Mesothorax, anterior paracoxal suture; 0)	Kluge (2004)	Not applicable	NEW	Index of characters	Discrete - transformational / binary	non-additive

	incomplete; 1) complete				[2.2.19]/ Kluge 2004		
6	Mesothorax, furcasternal protuberances; 0) contiguous; 1) not contiguous	Kluge (2004)	Not applicable	NEW	Index of characters [2.2.23]/ Kluge 2004	Discrete - transformational / binary	non-additive
7	Male, fore legs, claws, shape; 0) both hooked, 1) one hooked and one blunt, 2) both blunt, 3) single blunt, 4) single hooked	Ogden et al. (2009)	4 - Claws of male forelegs 0) both hooked 1) one hooked and one blunt 2) both blunt	New character state/ semantics	Index of characters [2.2.77] / Kluge 2004	Discrete - transformational / multistate	additive nonlinear - character-sate-tree
8	Middle legs, tarsomere I and tibia, fusion: 0) not fused 1) partially or completely fused	Ogden et al. (2009)	5 - Middle leg with 0) five free tarsomeres 1) four free tarsomeres, but tarsomere I is still distinctive 2) four free tarsomeres (or less), tarsomere I is entirely fused with tibia	Semantics / deletion of one character state	-	Discrete - transformational / binary	non-additive
9	Middle and hind legs, claws; 0) absent, 1) present (may be vestigial)	Kluge (2004)	Not applicable	NEW	Index of characters [2.2.85] / Kluge 2004	Discrete - neomorphic	non-additive
10	Male, Middle and hind legs, claws, shape; 0) both hooked, 1) one hooked and one blunt, 2) both blunt, 3) single blunt	Ogden et al. (2009)	6 - Claws of middle legs 0) both hooked 1) one hooked and one blunt	New character state/ semantics	Index of characters [2.2.85] / Kluge 2004	Discrete - transformational / multistate	additive nonlinear - character-sate-tree
11	Females, hind legs, development; 0) developed, 1) atrophied	Ogden et al. (2009)	7 - Hind legs of females 0) functional 1) atrophied	Semantics	-	Discrete - transformational / binary	non-additive
12	Male, fore legs, size relative to mid and hind legs; 0) similar, 1) elongated, 2) shortened	Kluge (2004)	Not applicable	NEW	Index of characters [2.2.74] / Kluge 2004	Discrete - transformational / multistate	additive nonlinear - character-sate-tree
13	Male, forewings, hairs at posterior margin; 0) absent, 1) present	Ogden et al. (2009)	8 - Wings of male imago 0) without hairs at posterior margin 1) with hairs at posterior margin	Semantics	-	Discrete - neomorphic	non-additive
14	Forewings, hind margin, short free intercalaries on most spaces; 0) absent, 1) present	Ogden et al. (2009)	9 - Hind margin of forewing 0) without short free intercalaries 1) with short free intercalaries	Semantics	-	Discrete - neomorphic	non-additive
15	Forewings, hind margin, short free intercalaries, number/ space (contingent to 14 (1)); 0) 1, 1) 2, 2) variable	Kluge (2004)	Not applicable	NEW	Index of characters [2.2.55] / Kluge 2004	meristic	non-additive

16	Forewings, hind margin, shape: 0) slightly curved, tornus absent, 1) strongly curved, tornus present	Ogden et al. (2009)	10 - Forewing 0) without tornus 1) anteritorn condition 2) posteritorn condition	Splitting/ semantics	-	Discrete - transformational / binary	non-additive
17	Forewings, distance between CuA and CuP at the margin of the wing in relation to CuP length (ratio); 0) < 0.3, 1) >0.4	NEW		NEW	-	Discrete - transformational / binary	non-additive
18	Forewings, longitudinal veins, gemination; 0) absent, 1) present	Ogden et al. (2009)	11 - Forewings 0) without pairs of approximated longitudinal veins 1) with pairs of approximated longitudinal veins	Reinterpretation	Index of characters [2.2.28] / Kluge 2004	Discrete - neomorphic	non-additive
19	Forewings, gemination (contingent to 18(1)); 0) between major veins only, 1) between major veins and intercalaries	NEW	Not applicable	NEW	-	Discrete - transformational / binary	non-additive
20	Forewings, MA2, relation to MA1; 0) directly attached, forming a bifurcation, 1) detached or attached by a transversal vein	Ogden et al. (2009)	12 - Forewings 0) MA forked 1) MA2 detached	Semantics	-	Discrete - transformational / binary	non-additive
21	Forewings, MA bifurcation, position (contingent to 20 (0)); 0) middle to distal half, 1) basal half	Ogden et al. (2009)	13 - Forewings 0) MA forked in its distal or in the middle half 1) MA forked in its basal half	Semantics	-	Discrete - transformational / binary	non-additive
22	Forewings, MP2, relation to MP1; 0) directly attached, forming a bifurcation, 1) detached or attached by a transversal vein	NEW	Not applicable	NEW	-	Discrete - transformational / binary	non-additive
23	Forewings, MP2 length relative to MP1; 0) nearly as long as (more than 0,8); 1) shorter than (no longer than 0,8).	NEW	Not applicable	NEW	-	Discrete - transformational / binary	non-additive
24	Forewings, CUa, bifurcation; 0) absent; 1) present	NEW	Not applicable	NEW	-	Discrete - neomorphic	non-additive
25	Forewings, CUa, basal half, shape; 0) not arched towards CuP; 1) arched towards CuP	Ogden et al. (2009)	14 - Forewings 0) MP2 basally not arched 1) MP2 basally arched	Semantics	-	Discrete - transformational / binary	non-additive

26	Forewings, area between CuA and CuP, main intercalary veins; number; 0) 0, 1) 1, 2) 2, 3) 3, 4) 4 (two pairs), 5) more than 4	Ogden et al. (2009)	15 - Cubital field of forewings 0) with veins running from CuA to hind margin 1) more than two long intercalary veins 2) one pair of long intercalary veins 3) without long intercalary veins	Splitting/ New character state/ semantics	-	meristic	non-additive
27	Forewings, area between CUa and Cup, more than two veins running from CuA to margin; 0) absent, 1) present				-	Discrete - neomorphic	non-additive
28	Forewings, fusion of ICu1 and CuP at base ("tricorythid fork"); 0) absent, 1) present	Ogden et al. (2009)	16 - Forewings 0) ICu1 basally not fused with CuP (not forming the typical tricorythid fork) 1) ICu1 basally fused with CuP (forming the typical tricorythid fork)	Semantics	-	Discrete - neomorphic	non-additive
29	Forewings, CuP, shape; 0) not strongly curved, 1) strongly curved, diverging from CuA	Ogden et al. (2009)	17 - Forewings 0) CuP basally not strongly curved and not approximating CuA 1) CuP strongly curved and approximating CuA	Semantics	-	Discrete - transformational / binary	non-additive
128	Forewings, vein AA, fork 0) absent, 1) present	Ogden et al. (2009)	18 - Forewings 0) 1A unforked 1) 1A forked	Semantics	-	Discrete - neomorphic	non-additive
129	Forewings, vein AA, fork shape; 0) symmetrical, 1) forward, 2) backward	NEW	Not applicable	NEW	-	Discrete - transformational / multistate	non-additive
30	Hindwings; (0) present in both sexes; (1) present in male, absent in female; (2) absent in both sexes.	Ogden et al. (2009) / Molineri (2006)	19 - Hindwings of males 0) with more than four longitudinal veins 1) at most four longitudinal veins 2) absent	Splitting/ New character state/ semantics	-	Discrete - neomorphic	non-additive
31	Male, hindwings, number of longitudinal veins; 0) more than six, 1) at most four					Discrete - transformational / binary	non-additive
32	Male, hindwings, Sc termination point; 0) at apex of costal margin, 1) at middle of costal margin	NEW	Not applicable	NEW	Index of characters [2.2.63] / Kluge (2004)	Discrete - transformational / binary	non-additive
33	Male, hindwings, MA fork (contingent to 32 (0)); 0) absent; 1) present	NEW	Not applicable	NEW	Index of characters [2.2.67] / Kluge (2004)	Discrete - neomorphic	non-additive
34	Male, hindwings, MP fork (contingent	Ogden et al. (2009)	22 - MP of male hind wing 0)	Semantics	-	Discrete - neomorphic	non-additive

	to 37 (0)); 0) absent; 1) present		forked 1) unforked				
35	Male, hindwings, MP fork, location (contingent to 40 (1)); 0) closer to base; 1) close to the middle; 2) closer to the margin (after the middle)	NEW	Not applicable	NEW	Index of characters [2.2.69] / Kluge (2004)	Discrete - transformational / multistate	non-additive
36	Male, hindwings, costal projection: 0) absent, 1) present	Ogden et al. (2009)	20 - Hindwings of males 0) without costal projection 1) with obtuse projection 2) with acute costal projection	Splitting/ semantics	-	Discrete - neomorphic	non-additive
37	Male, hindwings, costal projection, shape; 0) broadly rounded, 1) obtuse angled (>=90), 2) acute angled (<90)			Splitting/ New character state/ semantics	-	Discrete - transformational / multistate	additive linear
38	Male, hindwings, apex of costal projection, position; 0) proximal, 1) middle, 2) distal	NEW	-	NEW	Index of characters [2.2.62] / Kluge (2004)	Discrete - transformational / multistate	additive linear
39	Hindwing, size relative to forewing (ratio); 0) small (0.05 - 0.29), 1) medium (0.30 - 0.45), 2) large (0.46 - 1)	Molineri 2006	16. Ratio length of fore and hind wings (male): (0) 0.25 or more; (1) 0.20 or less.	Semantics / transformed in continuous	-	Discrete - transformational / multistate	additive linear
40	Abdomen, posterolateral projections; 0) absent, 1) present (at least in one segment)	Ogden et al. (2009)	23 - Posterolateral projections on abdomen 0) absent 1) present	Semantics	-	Discrete - neomorphic	non-additive
41	Styliger plate, shape; 0) completely divided (two separate parts), 1) partially divided (split on more than half length), 2) entire (may have a median cleft)	Ogden et al. (2009)	24 - Styliger plate 0) completely divided 1) partially divided (split on more than half length) 2) entire	Semantics	-	Discrete - transformational / multistate	additive linear
42	Forceps, distal short segments, number; 0) 0, 1) 1, 2) 2, 3) more than 2	Ogden et al. (2009)	25 - Number of terminal segments of forceps 0) more than 2 1) 2 segments 2) 1 segment 3) 0 segment	Semantics	-	meristic	non-additive
43	Penis, lobe, structure; 0) entire, 1) divided	NEW	28 - Parameres 0) distinctly separated (fused less than half length) 1) at least fused on half length to mesomeres 2) absent	Reinterpretation / semantics	Ogden et al. (2009)	Discrete - transformational / binary	non-additive
44	Penis lobes, fusion degree; 0) no fusion, 1) fused at maximum in basal		29 - Mesomeres 0) distinctly separated (fused less than half		Ogden et al. (2009)	Discrete - transformational / multistate	additive linear

	half, 2) fused in more than 3/4		length) 1) at least fused on half length to each other 2) absent				
45	Penis, size; 0) shorter than styliger plate 1) extending beyond styliger plate but shorter than length of forceps 2) extending beyond end of forceps	Ogden et al. (2009)	27 - Penis 0) external but shorter than styliger plate 1) external and extending beyond styliger plate but shorter than length of forceps 2) extending beyond end of forceps	Semantics	-	Discrete - transformational / multistate	additive linear
46	Female, posterior margin of sternite VII, subgenital plate; 0) absent, 1) present	Ogden et al. (2009)	30 - Female subgenital extension 0) absent 1) present	Semantics	-	Discrete - neomorphic	non-additive
47	Female, paracercus, size in relation to cerci; 0) similar or reduced at most to one fourth of its length, 1) smaller than one fourth of cerci length	Ogden et al. (2009)	31 - Terminal filament of females 0) equal or reduced at most to one fourth of length of cerci 1) almost or completely reduced	Semantics	-	Discrete - transformational / binary	non-additive
Nymph							
48	Frontal sutures, position relative to lateral ocelli; 0) ventral (anterior), 1) dorsal or lateral (posterior)	Ogden et al. (2009)	34 - Lateral ocelli located 0) lateral or ventral of the frontal sutures 1) dorsal of the frontal sutures	Semantics	-	Discrete - transformational / binary	non-additive
49	Antenna, length in relation to head length; 0) shorter or subequal than twice its size, 1) longer than twice its size.	Ogden et al. (2009)	35 - Length of antenna 0) shorter than twice the length of head 1) longer than twice the length of head	Semantics	-	Discrete - transformational / binary	non-additive
50	Right mandible, incisivi organization; 0) separated in two groups, 1) together in one group	Ogden et al. (2009)	39 - Incisivi of right mandible 0) in two separate groups 1) in two approximated groups 2) in one group	Semantics / deletion of one character state	-	Discrete - transformational / binary	non-additive
51	Prostheca, right mandible; 0) absent, 1) present	Ogden et al. (2009)	40 - Prostheca of right mandible 0) well developed 1) reduced to two or one bristles 2) prostheca absent	Splitting/ semantics	-	Discrete - neomorphic	non-additive
52	Prostheca, right mandible, development; 0) well developed, 1) reduced to one or two bristles				-	Discrete - transformational / binary	non-additive
53	Right mandible, area between mola and prostheca, row of setae; 0) absent, 1) present	Ogden et al. (2009)	41 - Row of setae between mola and prostheca in the right mandible 0) present 1) absent	Semantics	-	Discrete - neomorphic	non-additive
54	Mandibular tusks; 0) absent, 1) present	Ogden et al. (2009)	42 - Ratio tusk length to head	Splitting/ semantics	-	Discrete - neomorphic	non-additive

55	Mandibular tusks length, head length, ratio; 0) small (< 0.49), 1) medium (0.5 < x < 2.9), 2) large (> 3.0)		length mandibular tusks 0) tusk absent 1) < 0.5 2) between 0.5 to 3 3) > than 3.0		-	Discrete - transformational / multistate	additive linear
56	Mandibular tusks, outer margin, many long and dense hair; 0) absent, 1) present	Ogden et al. (2009)	43 - Outer margin of mandibular tusks 0) bare or with few scattered hairs 1) with many long and dense hairs	Semantics	-	Discrete - neomorphic	non-additive
57	Mandibular tusks, outer margin, teeth; 0) absent, 1) present	Ogden et al. (2009)	44 - Outer margin of mandibular tusks 0) without teeth 1) with teeth	Semantics	-	Discrete - neomorphic	non-additive
58	Mandibular tusks, direction of curvature; 0) medially, 1) laterally	Kluge (2004)	Not applicable	NEW	Index of character [1.1.20] / Kluge (2004)	Discrete - transformational / binary	non-additive
59	Mandible, outer surface, oval area of dense hairs; 0) absent, 1) present	Ogden et al. (2009)	45 - Outer surface of mandible 0) without oval area of dense hairs 1) with oval area of dense hairs	Semantics	-	Discrete - neomorphic	non-additive
60	Mandible, outer margin, dense row of long hairs; 0) absent, 1) present	Ogden et al. (2009)	46 - Lateral margin of mandible 0) without dense row of long hairs 1) with dense row of long hairs	Semantics	-	Discrete - neomorphic	non-additive
61	Maxilla, dentisetae, number; 0) 0, 1) 1, 2) 2, 3) 3, 4) more than 3, 5) variable (2 or 3)	Ogden et al. (2009)	48 - Maxilla with 0) more than three dentisetae 1) three dentisetae 2) two dentisetae 3) one dentisetae 4) without dentisetae	New character state/ semantics	-	meristic	non-additive
62	Maxilla, canines, number; 0) 0, 1) 1, 2) 2, 3) 3, 4) indeterminate	Ogden et al. (2009)	49 - Apex of maxilla with 0) three incisors 1) two incisors 2) one incisor 3) without incisor	New character state/ semantics	-	meristic	non-additive
63	Maxilla, galealacinea, shape; 0) truncated with canine (s) on medial margin, 1) truncated with canine(s) on outer margin, 2) pointed	Ogden et al. (2009)	50 - Apex of maxilla 0) pointed 1) truncate	New character state/ semantics	-	Discrete - transformational / multistate	non-additive
64	Maxilla, outer side, area between cardo and stipes, maxillary gills; 0) absent, 1) present	Ogden et al. (2009)	51 - Posterior side of maxilla between cardo and stipes 0) without maxillary gill 1) with single tubular gill 2) with slightly branched gill 3) with fibrilliform gill	Splitting / Change in character states/ semantics	-	Discrete - neomorphic	non-additive
65	Maxilla, maxillary gills, shape (contingent to 69 (1)); 0) single or double and tubular,				-	Discrete - transformational / binary	non-additive

	1) branched to fibrilliform						
66	Maxilla, galealacinea, base of canine (s), small tuft of setae; 0) absent; 1) present	NEW	Not applicable	NEW	-	Discrete - neomorphic	non-additive
67	Maxilla, galelacinia, distal margin, comb-like setae; 0) absent, 1) present	Ogden et al. (2009)	55 - Setae between palp and apex of maxilla 0) not comb-like 1) comb-like	Semantics	-	Discrete - neomorphic	non-additive
68	Maxillary palp; 0) absent, 1) present	Ogden et al. (2009)	53 - Maxillary palp 0) with three distinctive segments 1) with three segments of which segment two and three are partially fused 2) with two segments 3) one segment 4) absent	Splitting / deletion of one character state / semantics	-	Discrete - neomorphic	non-additive
69	Maxillary palps, segments, number (contingent to 73 (1)); 0) 1, 1) 2, 2) 3, 3) multisegmented				-	meristic	non-additive
70	Maxillary palp, length relative to gelealacinea (ratio); 0) small (<1), 1) medium (1< x <2), 2) large (2.1< x <3.9), 3) very large (>4.0)	NEW	Not applicable	NEW	-	Discrete - transformational / multistate	additive linear
71	Postmentum width, glossae + paraglossae width, ratio; 0) Extremely narrow (< 1.0), 1) Narrow (1.0 < 1.60), 2) Broad (> 1.8)	Ogden et al. (2009)	59 - Postmentum 0) not expanded or elongated 1) laterally expanded 2) basally elongated	Change in character states/ semantics	Baumgardner (2008)	Discrete - transformational / multistate	non-additive
72	Glossae, fusion; 0)not totally fused, 1) totally fused	Ogden et al. (2009)	60 - Glossae 0) medially separated 1) medially fused	Semantics	-	Discrete - transformational / binary	non-additive
73	Glossae, fusion to paraglosssae, fusion; 0) not totally fused, 1) totally fused	Ogden et al. (2009)	62 - Paraglossae 0) medially separated 1) medially fused	Semantics	-	Discrete - transformational / binary	non-additive
74	Labial palps, segments, number; 0) 1, 1) 2, 2) 3, 3) multisegmented	Ogden et al. (2009)	56 - Labial palp 0) 3 - segmented or more 1) 2 - segmented 2) 1 - segmented	New character state/ semantics	-	meristic	non-additive
75	Labial palp, last segment, margins; 0)parallel or subparallel (cylindrical), 1) divergent from the base (ovoid or almond), 2) distally divergent (opened), 3) distally convergent (pointed), 4)	Ogden et al. (2009)	58 - Labial palp distally 0) enlarged 1) of same width 2) narrowed	Change in character states/ semantics	-	Discrete - transformational / multistate	non-additive

	convergent from de base (semicircular)						
76	Three segmented labial palp, last segment, size relative to second segment, ratio; 0) smaller (< 0.79), 1) subequal (0.8 <x < 1.2) 2) larger (1.21 < x < 2.99), 3) much larger (>3.0)				-	Discrete - transformational / multistate	additive linear
77	Labial palp, basal segment, medial notch ; 0) absent, 1) present	Ogden et al. (2009)	65 - Basal segment of labial palp 0) without medial notch 1) with medial notch	Semantics	-	Discrete - neomorphic	non-additive
78	Labial palp, orientation; 0) laterally, 1) ventrally	Ogden et al. (2009)	57 - Labial palp 0) oriented laterally 1) orientated ventrally	Semantics	-	Discrete - transformational / binary	non-additive
79	Forewing pads, degree of fusion; 0) not fused to notum, 1) fused with notum at most up to the end of scutellum, 2) fused by mesal plate posterior to scutellum	Ogden et al. (2009)	66 - Pads of forewings 0) medially separated 1) at least partially fused	New character state/ semantics	Index of character [1.2.6] Kluge (2004)	Discrete - transformational / multistate	additive linear
80	Thorax, dorsal shield (fusion of pro and mesonotum); 0) absent, 1) present	Ogden et al. (2009)	67 - Pronotum 0) not expanded posteriorly 1) pronotum expanded to form a dorsal shield covering all gills	Reinterpretation / semantics	-	Discrete - neomorphic	non-additive
81	Collar on pronotum; 0) absent, 1) present	NEW	Not applicable	NEW	Index of character [1.2.2] Kluge (2004)	Discrete - neomorphic	non-additive
82	Collar on mesonotum; 0) absent, 1) present	NEW	Not applicable	NEW	Index of character [1.2.5] Kluge (2004)	Discrete - neomorphic	non-additive
83	Tibia, patella-tibial suture; 0) absent on all legs, 1) present on all legs, 2) present on middle and hind legs only, 3) present on middle legs only, 4) present on hind legs only	Ogden et al. (2009)	68 - Intratibial suture 0) present in all tibiae 1) present in middle and hind tibia 2) not present	New character state/ semantics	Index of character [1.2.18] Kluge (2004)	Discrete - transformational / multistate	additive nonlinear - character-sate-tree
84	Fore tibia, proeminent distomedial non-articulated spine-like projection; 0) absent, 1) present	Ogden et al. (2009)	69 - Fore tibia 0) without spine-line projections 1) with prominent non-articulated spineline projections	Semantics	-	Discrete - neomorphic	non-additive
85	Forelegs, coxal gills, 0) absent, 1) present	Ogden et al. (2009)	70 - Forelegs 0) without coxal gills 1) with coxal gills	Semantics	-	Discrete - neomorphic	non-additive

86	Forelegs, coxal gills, shape; 0) single or double and tubular, 1) branched to fibrilliform	NEW	Not applicable	NEW	-	Discrete - transformational / binary	non-additive
87	Fore femora, dorsal/anterior surface, transverse row of **setae**; 0) absent, 1) present.	Ogden et al. (2009)	71 - Fore femora 0) without transverse row of spines 1) with transverse row of spines	Splitting / semantics	-	Discrete - neomorphic	non-additive
88	Fore femora, dorsal/anterior surface, transverse row of **setae**, position (contingent to 90 (1)); 0) proximal, 1) middle to distal.	NEW		NEW	-	Discrete - transformational / binary	non-additive
89	Fore femora and tibia, inner margin, double row of long filtering hairs; 0) absent, 1) present	Ogden et al. (2009)	72 - Ventral margin of fore femora and fore tibiae 0) without row of filtering hairs 1) with two long longitudinal rows of filtering hairs	Semantics	-	Discrete - neomorphic	non-additive
90	Middle femora, inner margin, longitudinal row of filtering hairs; 0) absent, 1) present	Ogden et al. (2009)	73 - Ventral margin of middle femora and middle tibiae 0) without row of filtering hairs 1) with two long longitudinal rows of filtering hairs	Semantics	-	Discrete - neomorphic	non-additive
91	Femora, apex of external margin, ventral extension; 0) absent, 1) present	Ogden et al. (2009)	74 - Apex of dorsal lobe of femora 0) not expanded ventrally 1) expanded ventrally	Semantics	-	Discrete - neomorphic	non-additive
92	Forelegs, tarsal claws; 0) absent, 1) present	NEW	Not applicable	NEW	Index of character [1.2.20] Kluge (2004)	Discrete - neomorphic	non-additive
93	Forelegs, tarsal claws, shape; 0) undivided, 1) bifid	Ogden et al. (2009)	75 - Tarsal claws of forelegs 0) normal 1) bifid	Semantics	-	Discrete - transformational / binary	non-additive
94	Forelegs, Tarsal claws, inner side, denticles; 0) absent, 1) present	Ogden et al. (2009)	76 - Tarsal claws 0) without denticle 1) with one row of denticles 2) with two rows of denticles	Splitting / semantics	-	Discrete - neomorphic	non-additive
95	Forelegs, Tarsal claws, inner side, denticles, number of rows (contingent to 97 (1)); 0) 1, 1) 2	Ogden et al. (2009)			-	Discrete - transformational / binary	non-additive
96	Abdominal terga, posterior margin, spines; 0) absent, 1) present	Ogden et al. (2009)	77 - Hind margin of abdominal terga 0) with spines 1) without spines	Semantics	-	Discrete - neomorphic	non-additive
97	Abdominal terga, posterior margin,	Ogden et al. (2009)	78 - Abdominal terga 0) without	Splitting / semantics	-	Discrete - neomorphic	non-additive

	tubercule (median projections); 0) absent, 1) present		paired tubercles 1) with paired tubercles				
98	Abdominal terga, posterior margin, tubercule (median projections) per tergite, number (contingent to 100 (1)); 0) 1 or 2 (unpaired), 1) 2 (paired)	NEW			-	Discrete - transformational / binary	non-additive
99	Abdominal segments, posterolateral projections, segments II-IV; 0) absent, 1) present	Ogden et al. (2009)	79 - Posterolateral projections on abdominal segments IV 0) present 1) absent	Semantics	-	Discrete - neomorphic	non-additive
100	Gill I; 0) absent, 1) present	Ogden et al. (2009)	80 - Gill I 0) present 1) absent	Reordering of states	-	Discrete - neomorphic	non-additive
101	Gill I, size relative to remaining gills (contingent to 103 (1)); 0) similar (including semi-operculate), 1) reduced (to less than 1/3 of gill2), 2) larger (including opercular)	Ogden et al. (2009)	82 - Gill I 0) not modified 1) semi-operculate 2) operculate	Reinterpretation	-	Discrete - transformational / multistate	additive nonlinear - character-sate-tree
102	Gill I, shape (contingent to 103 (1)); 0) finger-like, 1) plate like single , 2) plate-like bifid	Ogden et al. (2009)	81 - Gill I 0) not finger-like 1) finger-like	New character state/ semantics	-	Discrete - transformational / multistate	non-additive
103	Gill I, attachment position (contingent to 103 (1)); 0) dorso-laterally, 1) ventrally	Ogden et al. (2009)	84 - Position of Gill I 0) laterally 1) dorsally 2) ventrally	Change in character states/ semantics	-	Discrete - transformational / binary	non-additive
104	Gill II; 0) absent, 1) present	Ogden et al. (2009)	85 - Gill II 0) present 1) absent	Reordering of states	-	Discrete - neomorphic	non-additive
105	Gill II, shape relative to following gills (contingent to 107 (1)); 0) similar (including semi-operculate), 1) modified (operculate)	Ogden et al. (2009)	86 - Gill II 0) not modified 1) semi-operculate 2) operculate	Reinterpretation	-	Discrete - transformational / binary	non-additive
130	Gill II (operculate), secondary ribs (contingent to 105 (1)); 0) Absent, 1) Present	NEW	Not applicable	NEW	-	Discrete - neomorphic	non-additive
106	Gill II, attachment position (contingent to 105 (1)); 0) dorso-laterally, 1) ventrally	Ogden et al. (2009)	87 - Position of Gill II 0) laterally 1) dorsally 2) ventrally	Change in character states/ semantics	-	Discrete - transformational / binary	non-additive
107	Gill III; 0) absent; 1) present	NEW	Not applicable	NEW	-	Discrete - neomorphic	non-additive
108	Gill III, shape (contingent to 107 (1)); 0) similar to	NEW	Not applicable	NEW	Index of character	Discrete - transformational / binary	non-additive

	remaining gills, 1) operculate				[1.3.45] Kluge (2004)		
109	Gill IV, number of lamellae; 0)1, 1)2	Ogden et al. (2009)	88 - Gill IV 0) without lower branch 1) with plate-like 2) multilobed 3) fibrilliform lower branch	Spliting (see character 122) / semantics	-	Discrete - transformational / binary	non-additive
110	Gill IV, unique lamella, fold (contingent to 109 (0)); 0) absent, 1) present	NEW	Not applicable	NEW	-	Discrete - neomorphic	non-additive
111	Gill IV, unique or dorsal lamella, shape; 0) plate like; 1) fibriliform; 2) semi-operculate; 3) operculate	NEW	Not applicable	NEW	Index of character [1.3.50] Kluge (2004)	Discrete - transformational / multistate	additive nonlinear - character-sate-tree
112	Gill IV, unique or dorsal lamella, margin; 0) entire; 1) fringed	NEW	Not applicable	NEW	-	Discrete - transformational / binary	non-additive
113	Gill IV, unique or dorsal lamella, costal rib (=anterior margin); 0) absent, 1) present	Ogden et al. (2009)	91 - Anterior margin of gill IV 0) not enforced 1) enforced	Reinterpretation	Index of character [1.3.27] Kluge (2004)	Discrete - neomorphic	non-additive
114	Gill IV, unique or dorsal lamella, anal rib; 0) absent, 1) present	Ogden et al. (2009)	93 - Gill IV 0) without posterior reinforcement along hind margin 1) with posterior reinforcement	Reinterpretation	Index of character [1.3.28] Kluge (2004)	Discrete - neomorphic	non-additive
115	Gill IV, unique or dorsal lamella, anal rib, position; 0) on anal margin, 1) shifted medially, 2) bifurcated	Ogden et al. (2009)	94 - Gill IV 0) without posterior reinforcement within gill membrane 1) with posterior reinforcement within gill membrane	Reinterpretation		Discrete - transformational / multistate	non-additive
116	Gill IV, unique or dorsal lamella, tracheation; 0) absent, 1) present	Ogden et al. (2009)	92 - Tracheation of gill IV 0) palmate 1) pinnate 2) absent	Splitting / deletion of one character state / semantics	-	Discrete - neomorphic	non-additive
117	Gill IV, unique or dorsal lamella, tracheation, shape; 0) palmate, 1) pinnate, 2) linear (unbranched)	Ogden et al. (2009)			-	Discrete - transformational / multistate	non-additive
118	Gill IV, ventral lamella, shape; 0) plate like, 1) fibriliform	Ogden et al. (2009)	88 - Gill IV 0) without lower branch 1) with plate-like 2) multilobed 3) fibrilliform lower branch	Splitting (see character 123)/ semantics	-	Discrete - transformational / binary	non-additive
119	Gill IV, ventral lamellae, division (contingent to 118	NEW	Not applicable	NEW	-	Discrete - transformational / binary	non-additive

	(0)); 0) entire, 1) divided						
120	Gill IV, ventral lamella, outer margin (contingent to 118 (0)); 0) entire, 1) fringed, 2) lobed, 3) dissected	Ogden et al. (2009)	88 - Gill IV 0) without lower branch 1) with plate-like 2) multilobed 3) fibrilliform lower branch 89 - Lower branch of Gill IV 0) not fringed 1) fringed	Reorganization / addition of one character state / semantics	-	Discrete - transformational / multistate	additive nonlinear - character-sate-tree
121	Gill VI, 0) absent, 1) present	Ogden et al. (2009)	95 - Gill VI 0) present 1) absent	Semantics	-	Discrete - neomorphic	non-additive
122	Gill VII; 0) absent, 1) present	Ogden et al. (2009)	96 - Gill VII 0) present 1) strongly reduced 2) absent	Deletion of one character state / semantics	-	Discrete - neomorphic	non-additive
123	Cercus, spine-like setae; 0) absent, 1) present	Ogden et al. (2009)	98 - Setae of cercus 0) only bristle-like 1) spine-like (with or without bristle like)	Semantics		Discrete - neomorphic	non-additive
124	Paracercus, size in relation to cercus (ratio); 0) reduced (<0,70), 1) developed (>0,75)	Ogden et al. (2009)	101 - Paracercus 0) developed 1) reduced to few segments	Semantics / change of character states		Discrete - transformational / binary	non-additive
125	Primary swimming setae; 0) absent, 1) present	Ogden et al. (2009)	99 - Setae of cerci 0) in rows on inner side 1) in rows on inner and outer side 2) in whorls with or without setae on side	Reinterpretation	Index of character [1.3.66] Kluge (2004)	Discrete - neomorphic	non-additive
126	Secondary swimming setae; 0) absent, 1) present	Ogden et al. (2009)			Index of character [1.3.67] Kluge (2004)	Discrete - neomorphic	non-additive
127	Cercus, length in relation to body (ratio); 0) smaller (< 0.79), 1) subequal (0.8 < x < 1.2) 2) larger (> 1.21)	Ogden et al. (2009)	100 - Length of cercus 0) less than half of body length 1) at least two third of body length	Categories turned into continuous	-	Discrete - transformational / multistate	additive linear

Each analysis using implied weight (K = 8, 9, 10, 11 and 12) resulted in one MPT. Each tree and its respective synapomorphies may be found in the appendix (appendices 2-11). Our analyzes resulted in 5 distinct topologies, one for each K value analyzed; the topologies were similar for K values of 8, 9, 11 and 12 (consensus in figure 4), and a very different one for a K value of 10 (figure 5).

The strict consensus of the 5 trees (figure 6) was unresolved for the deep relationship inside Ephemeroptera but seven supra familial groups proposed by Kluge (2004) were retrieved; Caenotergaliae, Ephemerella/Fg1, Eusetisura, Fimbriatotergaliae, Fossoriae, Heptagennota, and Posteritorna. Considering McCafferty's proposal, according to Ogden et al.

(2005), the taxa retrieved in our analyses were surborder Carapacea (=Posteritorna), superfamilies Ephemerelloidea (= Ephemerella/fg1) and Caenoidea (= Caenotergaliae).

Groups that were recovered with some changes in taxa composition are Pantricorythi, from which Austremerella and Vietnamella were removed, while *Coryphorus* was added, and Tricoryptera (in which *Coryphorus* was added). Some taxa were recovered in one analysis but not in the others: Bidentiseta and Furcatergalia were only recovered with k=10 (figure 6, table 4), and Tetramerotarsata was recovered only with K=11 (appendix 8, table 4). Anteritorna, Branchitergaliae and Tridentiseta were not recovered as monophyletic in any of the analyses under IW (table 4).

Of the 25 families represented by two or more genera in this study, 16 were found as monophyletic, 5 were not retrieved in any of the trees (Ephemerellidae, Ephemeridae, Nesameletidae, Potamanthidae and Teloganodidae), while 4 families were recovered as monophyletic or not depending on the value of K (Acanthametropodidae, Oniscigastridae, Protereismatidae and Siphlonuridae). A summary of the taxa found in each analysis is presented for comparison (table 4).

The values found for Combined Bremer Support (CBS) and Symmetric Resampling (SR) found in each analysis, without IW (Prior weights only) and with IW under different values of K are presented in table 5. The taxa had, in general, a low degree of support. For suprafamilial taxa, Posteritorna had supports (CBS and SR) above 50 for all analyses and Caenotergaliae had only values of Symmetric resampling above 50. Considering the support for the families that were recovered as monophyletic in this study, some of them got values above 50 for Symmetric Resampling independently of the value of K used (Ameletidae, Ameletopsidae, Baetidae, Behningiidae, Caenidae, Coloburiscidae, Leptohyphidae, Metretopodidae, Neoephemeridae, Oligoneuriidae and Palingeniidae). The remaining families had values of SR below 50 in one or more analyses. Values of combined Bremer support were above 50 only in some of the analyses for Behningiidae, Caenidae, and in one analysis for Polymitarcyidae.

Figure 4 - Strict consensus of 4 MPT found using New Technology under IW (4 MPT from each analysis with K values 8, 9, 11 and 12). Numbers in parenthesis indicate the original number of taxa in the group followed by the number of added taxa indicated by a (+) and the number of subtracted taxa indicated by a (-).

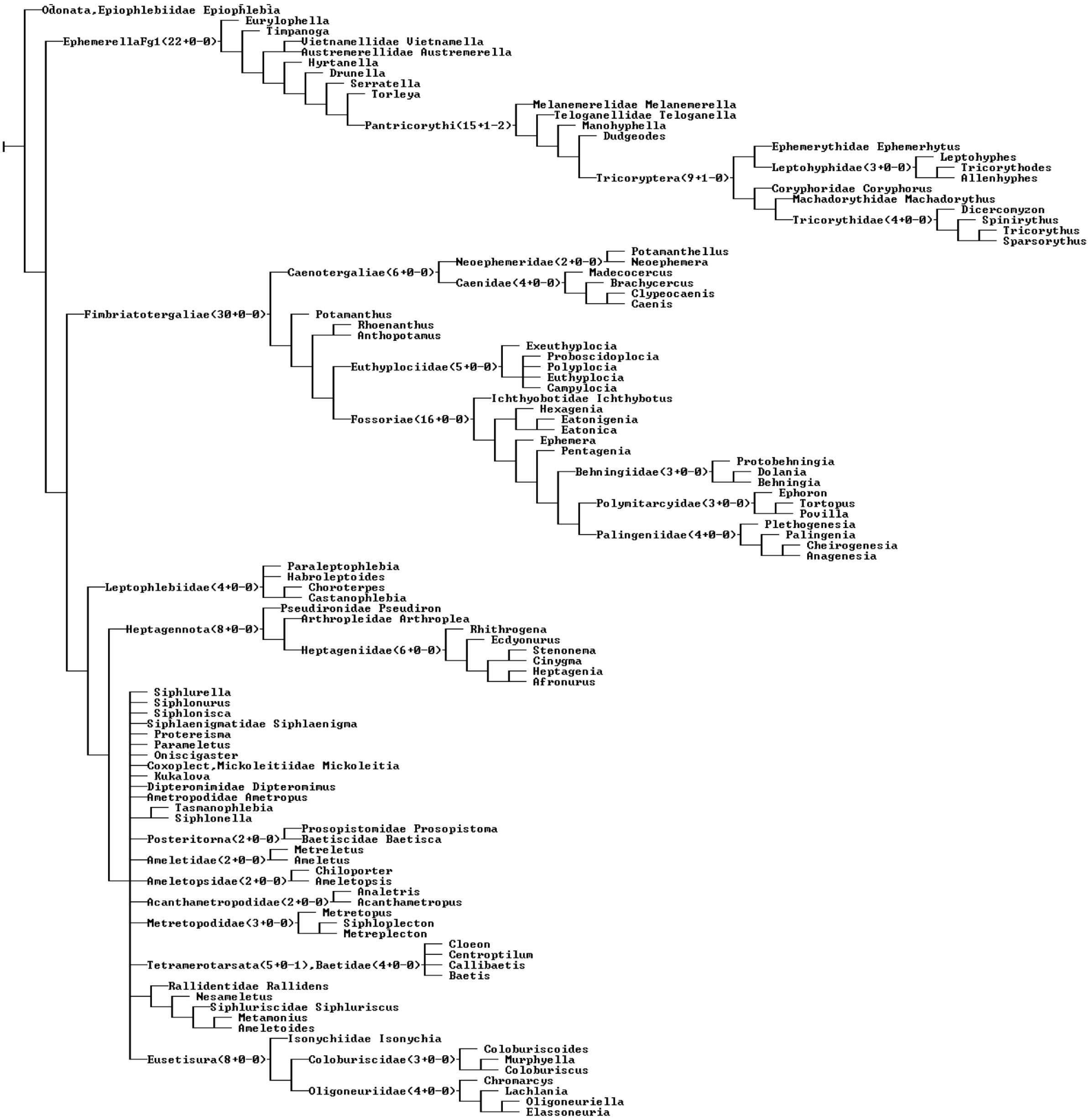


Figure 5- Most parsimonious tree found using New Technology under IW (K = 10). Numbers in parenthesis indicate the original number of taxa in the group followed by the number of added taxa indicated by a (+) and the number of subtracted taxa indicated by a (-).

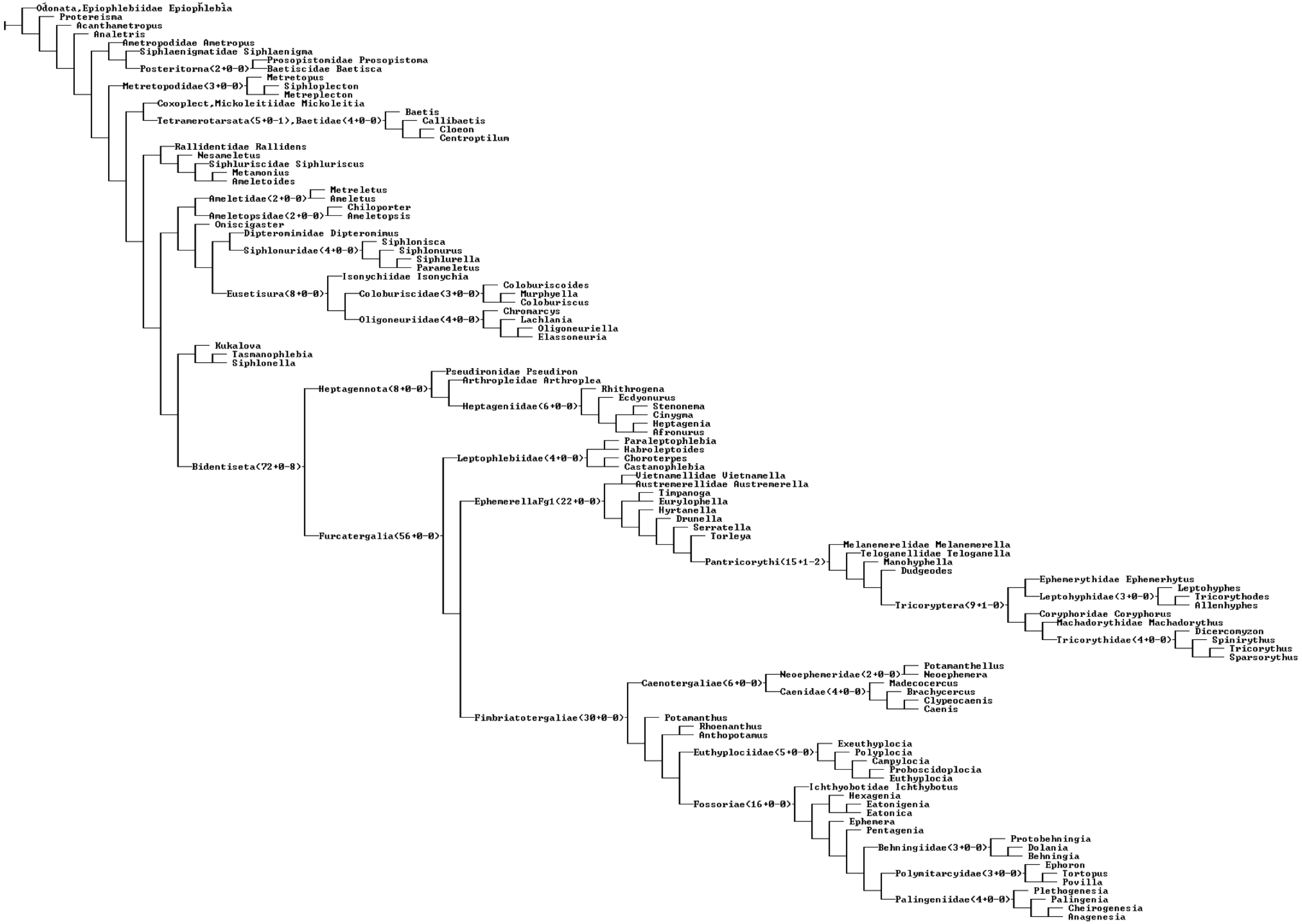


Figure 6 - Strict consensus of 5 MPT found using New Technology under IW (1 MPT from each analysis with K values 8, 9, 10, 11 and 12). Numbers in parenthesis indicate the original number of taxa in the group followed by the number of added taxa indicated by a (+) and the number of subtracted taxa indicated by a (-).

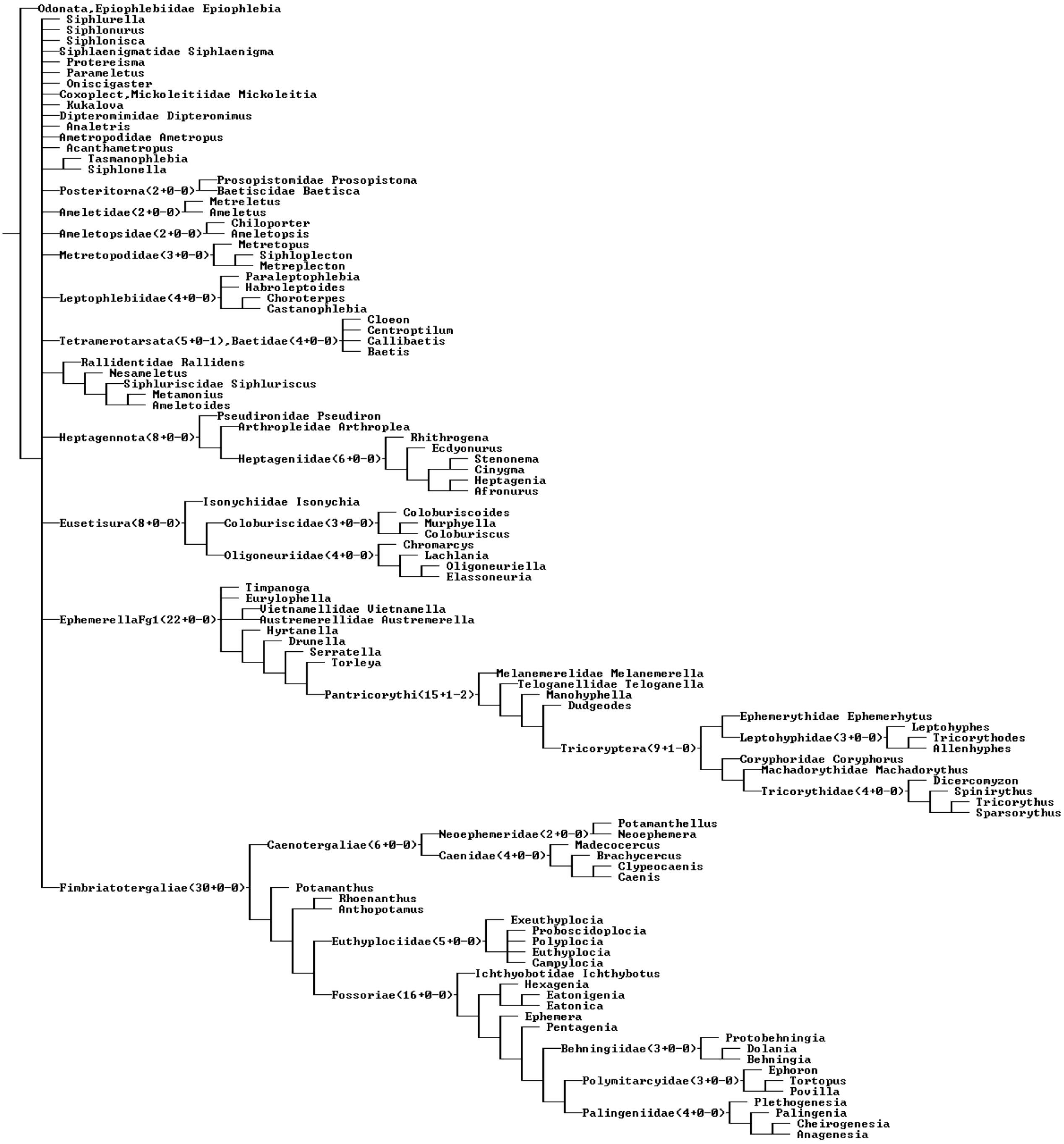


Table 4 - Summary of the taxa found in each analysis. The first part of the table refers to the supra familial groups and the second section refers to families represented by more than two genera in the present study. Lines marked in yellow indicate taxa that were recovered differently in at least one of the analyzes. Lines marked in green indicate the taxa for which the result was the same independently of the concavity assumed in analyzes under IW. (-) and (+) indicates taxa subtracted or added to the original group.

	New Technology IW **K=8**	New Technology IW **K=9**	New Technology IW **K=10**	New Technology IW **K=11**	New Technology IW **K=12**
Suprafamiliar Groups					
Caenotergaliae (6 taxa)	monophyletic	monophyletic	monophyletic	monophyletic	monophyletic
Ephemerella/Fg1 (22 taxa)	monophyletic	monophyletic	monophyletic	monophyletic	monophyletic
Eusetisura (8 taxa)	monophyletic	monophyletic	monophyletic	monophyletic	monophyletic
Fimbriatotergaliae (30 taxa)	monophyletic	monophyletic	monophyletic	monophyletic	monophyletic
Fossoriae (16 taxa)	monophyletic	monophyletic	monophyletic	monophyletic	monophyletic
Heptagennota (8 taxa)	monophyletic	monophyletic	monophyletic	monophyletic	monophyletic
Posteritorna (2 taxa)	monophyletic	monophyletic	monophyletic	monophyletic	monophyletic
Pantricorythi (15 taxa)	(-) Austremerella (-) Vietnamella (+) Coryphorus	(-) Austremerella (-) Vietnamella (+) Coryphorus	(-) Austremerella (-) Vietnamella (+) Coryphorus	(-) Austremerella (-) Vietnamella (+) Coryphorus	(-) Austremerella (-) Vietnamella (+) Coryphorus
Tricoryptera (9 taxa)	(+) Coryphorus	(+) Coryphorus	(+) Coryphorus	(+) Coryphorus	(+) Coryphorus
Anteritorna	x	x	x	x	x
Branchitergaliae (16 taxa)	x	x	x	x	x
Tridentiseta (28 taxa)	x	x	x	x	x
Bidentiseta (72 taxa)	x	x	(-) Eusetisura	x	x
Furcatergaliae (56 taxa)	x	x	monophyletic	x	x
Tetramerotarsata (5 taxa)	x	x	x	monophyletic	x
Families represented by two or more genera					
Acanthametropodidae (2 taxa)	monophyletic	monophyletic	x	monophyletic	monophyletic
Ameletidae (2 taxa)	monophyletic	monophyletic	monophyletic	monophyletic	monophyletic
Ameletopsidae (2 taxa)	monophyletic	monophyletic	monophyletic	monophyletic	monophyletic
Baetidae (4 taxa)	monophyletic	monophyletic	monophyletic	monophyletic	monophyletic
Behningiidae (3 taxa)	monophyletic	monophyletic	monophyletic	monophyletic	monophyletic
Caenidae (4 taxa)	monophyletic	monophyletic	monophyletic	monophyletic	monophyletic
Coloburiscidae (3 taxa)	monophyletic	monophyletic	monophyletic	monophyletic	monophyletic
Ephemerellidae (6 taxa)	x	x	x	x	x
Ephemeridae (5 taxa)	x	x	x	x	x
Euthyplociidae (5 taxa)	monophyletic	monophyletic	monophyletic	monophyletic	monophyletic
Heptageniidae (6 taxa)	monophyletic	monophyletic	monophyletic	monophyletic	monophyletic
Leptohyphidae (3 taxa)	monophyletic	monophyletic	monophyletic	monophyletic	monophyletic
Leptophlebiidae (4 taxa)	monophyletic	monophyletic	monophyletic	monophyletic	monophyletic
Metretopodidae (3 taxa)	monophyletic	monophyletic	monophyletic	monophyletic	monophyletic
Nesameletidae (3 taxa)	x	x	x	x	x
Neoephemeridae (2 taxa)	monophyletic	monophyletic	monophyletic	monophyletic	monophyletic
Oligoneuriidae (4 taxa)	monophyletic	monophyletic	monophyletic	monophyletic	monophyletic
Oniscigastridae (3 taxa)	x	x	x	monophyletic	monophyletic
Palingeniidae (4 taxa)	monophyletic	monophyletic	monophyletic	monophyletic	monophyletic
Polymitarcyidae (3 taxa)	monophyletic	monophyletic	monophyletic	monophyletic	monophyletic
Potamanthidae (3 taxa)	x	x	x	x	x
Protereismatidae (2 taxa)	x	x	x	monophyletic	monophyletic
Siphlonuridae (4 taxa)	monophyletic	monophyletic	monophyletic	(+) Mickoleitia	monophyletic
Teloganodidae (2 taxa)	x	x	x	x	x
Tricorythidae (4 taxa)	monophyletic	monophyletic	monophyletic	monophyletic	monophyletic

Table 5 - Values of Combined Bremer Support (CBS) and symmetric resampling (SR) found. In the cases where the family was not recovered as monophyletic in the analysis a (x) was used, and in the cases where the taxon was recovered but had no support it was indicated by a (-).

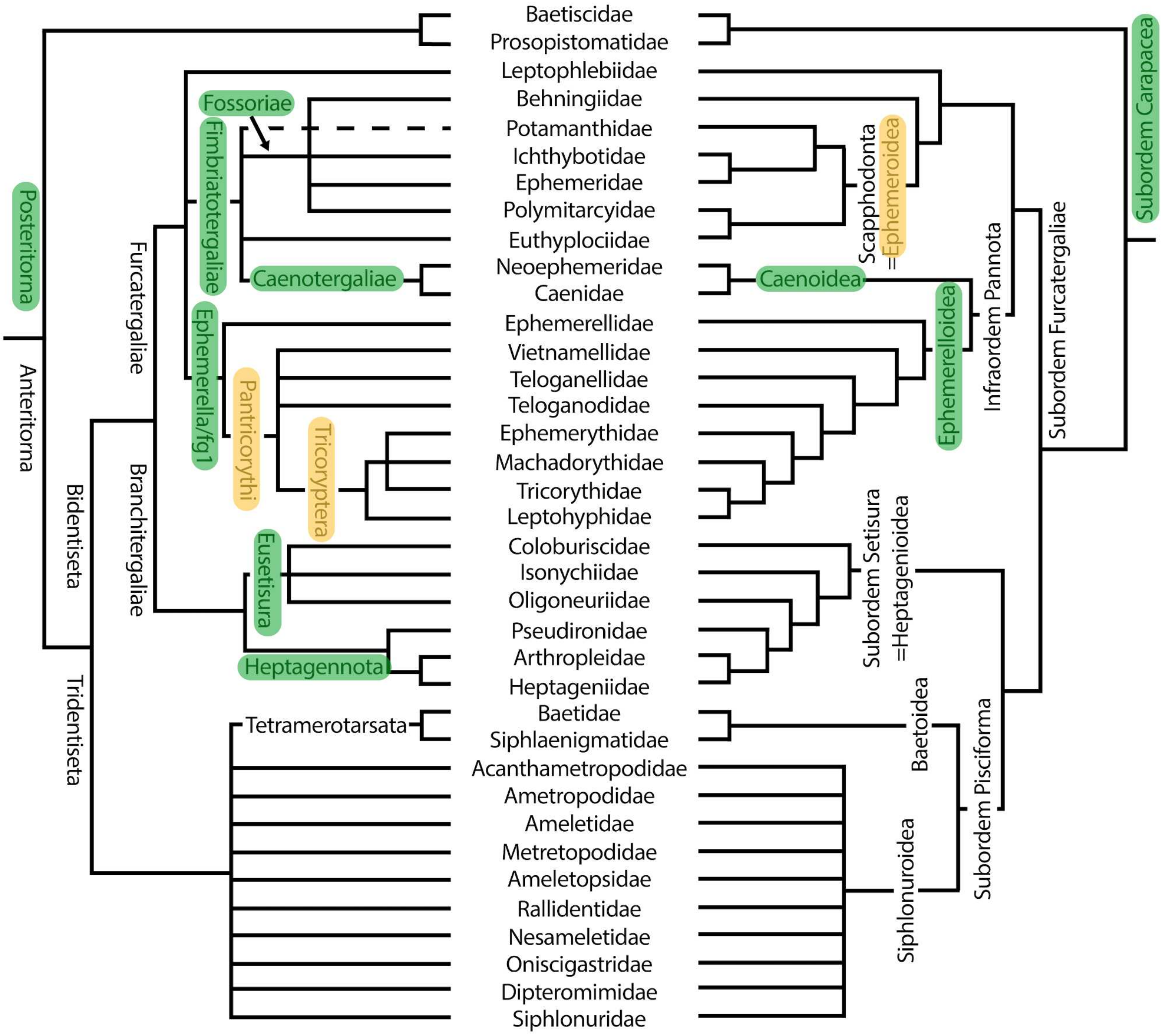
	New technology IW K=8		New technology IW K=9		New technology IW K=10		New technology IW K=11		New technology IW K=12	
	Suprafamiliar Groups									
Taxon	CBS	SR	CBS	SR	CBS	SR	CBS	SR	CBS	SR
Bidentiseta (72 taxa)	x	x	x	x	11	-	x	x	x	x
Caenotergaliae (6 taxa)	18	79	22	75	29	74	41	70	42	69
Ephemerella/Fg1 (22 taxa)	9	26	12	35	24	39	22	40	26	48
Eusetisura (8 taxa)	22	40	19	39	23	43	23	46	20	45
Fimbriatotergaliae (30 taxa)	20	17	22	21	29	21	32	23	37	21
Fossoriae (16 taxa)	10	0	11	-	14	-	13	6	14	6
Furcatergaliae (56 taxa)	x	x	x	x	22	-	x	x	x	x
Heptagennota (8 taxa)	15	30	13	37	15	33	9	41	14	31
Pantricorythi (15 taxa)*	6	-	9	-	5	-	11	-	11	-
Posteritorna (2 taxa)	59	89	63	90	56	85	65	84	56	84
Tetramerotarsata (5 taxa)	x	x	x	x	x	x	5	-	x	x
Tricoryptera (9 taxa)	10	9	12	7	7	12	15	9	11	13
	Families represented by two or more genera									
Taxon	CBS	SR	CBS	SR	CBS	SR	CBS	SR	CBS	SR
Acanthametropodidae (2 taxa)	16	44	21	46	x	x	19	49	14	48
Ameletidae (2 taxa)	15	56	15	60	17	64	12	60	16	65
Ameletopsidae (2 taxa)	5	77	2	72	1	74	1	75	3	71
Baetidae (4 taxa)	17	70	15	69	15	70	27	69	19	68
Behningiidae (3 taxa)	26	96	27	91	41	91	65	93	66	91
Caenidae (4 taxa)	43	100	49	98	58	100	64	100	71	98
Coloburiscidae (3 taxa)	34	99	36	96	42	97	36	97	41	97
Euthyplociidae (5 taxa)	14	52	15	45	17	53	18	55	20	57
Heptageniidae (6 taxa)	18	38	18	35	25	39	17	45	18	41
Leptohyphidae (3 taxa)	1	60	3	65	6	65	8	67	11	75
Leptophlebiidae (4 taxa)	8	42	6	49	7	51	12	52	6	50
Metretopodidae (3 taxa)	22	67	21	63	27	61	36	61	44	58
Neoephemeridae (2 taxa)	9	56	10	59	2	59	10	63	11	62
Oligoneuriidae (4 taxa)	20	69	18	65	39	69	25	70	27	73
Oniscigastridae (3 taxa)	x	x	x	x	x	x	0	4	0	8
Palingeniidae (4 taxa)	29	68	31	69	34	72	36	70	37	69
Polymitarcyidae (3 taxa)	27	18	31	26	37	28	40	28	57	39
Protereismatidae (2 taxa)	x	x	x	x	x	x	0	-	0	-
Siphlonuridae (4 taxa)	8	26	4	35	2	36	1	-	0	35
Tricorythidae (4 taxa)	0	-	0	-	1	-	1	-	2	-

Discussion

Phylogenetic relationships

The main suprafamilial groups in Ephemeroptera are discussed below, as shown in figure 7.

Figure 7 - Taxa found to be monophyletic in all analyzes under IW. The groups circled in green were recovered with the same taxa composition as proposed by Kluge (2004) on the left side or by McCafferty (apud Ogden and Whiting, 2005). Taxa circled in orange had one more taxon added to the original proposal of the authors (see table 4).



Posteritorna / Suborder Carapacea / Prosopistomatoidea

The suborder Posteritorna was proposed to comprise Baetiscidae and Prosopistomatidae being the synapomorphy proposed for the group the tornus of the wing between the ends of CuA and CuP (Kluge et al., 1995). The same group was also proposed by McCafferty (McCafferty, 1997) under the name of Carapacea. In their latest proposition, both authors consider Posteritorna to be the sister group of all remaining mayflies families, a taxon named Anteritorna by Kluge.

Posteritorna was recovered as monophyletic in the present study with good support (CBS> 56 and SR> 84, table 5). Several synapomorphies unite these taxa (appendices 12 and 13; Node 149), two of them unique. One is the distance between CuA and CuP at the margin of the wing in relation to CuP length being smaller than 0.3 (equivalent to the "posteritorna condition"), and the presence of a dorsal shield (fusion of the pro and mesonotum). Ogden et al. (2009) also found Carapacea as monophyletic but while the combined analysis indicated it to be the sister group of Oligoneuriidae, the morphological analysis recuperated Carapacea as sister group of Caenoidea, differently of the proposed by Kluge (2004) and McCafferty (apud Ogden and Whiting, 2005).

In the strict consensus, Posteritorna was recovered in a polytomy, without information about its relationship with other taxa. It was recovered as the sister group of *Ametropus* for K = 9 and 11 and of Siphlaenigma for K = 8, 10 and 12 (synapomorphies for the groups found in each tree may be found in appendices). That relationship with *Siphlaenigma* is in accordance with Ogden & Whiting (2005) which, in their molecular phylogeny of the Order, found *Siphlaenigma* nested in an unresolved node together with Baetiscidae and Prosopistomatidae.

Anteritorna

Anteritorna (Kluge, 1998) has as proposed autapomorphy the tornus of the forewing situated between the apices of CuA and CUP veins. Since Posteritorna was not recovered as the sister group to all remaining mayflies, the concept of Anteritorna as a group (Kluge, 2004) is not supported in the present study. Molecular (Ogden and Whiting, 2005) and combined analysis (Ogden et al., 2009) did not recover Anteritorna as a taxa either.

Tridentiseta / Suborder Pisciforma

For Kluge (2004), Tridentiseta is a plesiomorphon[1] where he put together all taxa in Anteritorna not belonging to Bidentiseta (Siphlonuridae, Dipteromimidae, Ameletidae, Metretopodidae, Acanthametropodidae, Ametropodidae, Siphluriscidae, Nesameletidae, Oniscigastridae, Ameletopsidae, Rallidentidae and the taxon Tetramerotarsata (Baetidae + Siphlaenigmatide). The suborder Pisciforma, as originally proposed by McCafferty (1991b) closely resembles the circumscription Tridentiseta, but at that time included Pseudironidae and did not include Oniscigastridae. Pseudironidae was later removed from Pisciforma (Wang and McCafferty, 1995) and Oniscigastridae was moved from the no longer accepted suborder Rechtracheata to Pisciforma - according to the synthesis formulated by Ogden & Whiting (2005). Both taxa were initially proposed without inferring a relationship between its members. Later, Kluge (2004) inferred that Siphluriscidae and Nesameletidae are close groups, and they were put together in the taxon Nesameletus/f1=Metamonius/g1.

In the present study, Tridentiseta was not recovered as monophyletic (figure 6, table 4), in agreement with more recent studies based on molecular data (Ogden and Whiting, 2005), combined analysis (Ogden et al., 2009) and phylogenomics (Ogden, 2019).

The only recovered relationship between families belonging to Tridentiseta in the consensus was *Siphluriscus* (Siphluriscidae) being found nested inside Nesameletidae, and *Rallidens* (Rallidentidae) as its sister group (figure 6). One of the synapomorphies found for this group (Rallidentidae + (Sipluriscidae / Nesameletidae) (appendices 12 and 13 - Node 130) is the presence of maxillary gills single or double and tubular. Staniczek (2010), studying accessory gills of different mayfly families, reported the similarity of these structures in Rallidentidae and Nesameletidae based on their position, shape and spatial orientation. Kluge (2004) also hypothesized the maxillary gills of *Rallidens* to be a synapomorphy with that of Nesameletus/f1=Metamonius/g1, but considered the maxillary gill of Nesameletidae and Siphluriscidae a character of "unclear phylogenetic status".

Tetramerotarsata/ Baetoidea (?)

Tetramerotarsata (Kluge, 1997) comprises the families Baetidae and Siphlaenigmatidae. The proximity of these two families was proposed by several authors

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[1] Plesiomorphon, according to Kluge (2004) is "... a taxon characterized by plesiomorphies only; thus, its holophyly is not proven, and possibly (or probably) this taxon is paraphyletic, but its paraphyly is also not proven yet. Plesiomorphon can be a taxon of any size and systematic position in ranking or non-ranking classification, living or fossil, with any name (ranking, hierarchical, circumscriptional or other)."

based on different synapomorphies (Kluge, 1997; Kluge et al., 1995; McCafferty and Edmunds, 1979; Staniczek, 1997). Although McCafferty & Edmunds (1979) proposed Siphlaenigma to be part of Baetidae as a subfamily, the name Baetoidea, present in McCafferty's combined hypothesis by Ogden & Whiting (2005, figure 1), was not found in any work of McCafferty nor in his website (https://www.entm.purdue.edu/mayfly/).

Tetramerotarsata was not recovered in our consensus tree, being recovered in only one of the trees (k=11) (appendix 8, table 4). Ogden et al. (2009) recovered Tetramerotarsata in the consensus (50% majority rule) in the morphological analysis, but not in the molecular analysis nor in the total combined analysis. The phylogenomic analysis (Ogden, 2019) did not recover the taxon either.

Bidentiseta

Bidentiseta was firstly proposed by Kluge in 1993 (in russian) and is still present in the author's most recent works. The taxon currently comprises Furcatergaliae and Branchitergaliae and has no equivalent in McCafferty's proposal, since the authors did not agree on the position of the latter, which is equivalent to McCafferty's Setisura. According to (McCafferty, 1997) Setisura is more related to Pisciforma. Herein, and in agreement with Ogden & Whiting (2005), Ogden et al. (2009) and Ogden et al. (2019), Bidentiseta was not recovered as monophyletic in the consensus (figure 6).

Only one tree, resulting from the analysis under Implied Weight with K = 10 recovered Bidentiseta (without Eusetisura) (appendix 6 - Node 115; table 4). The synapomorphies found for the group in this analysis are anterior paracoxal suture incomplete, presence of two dentisetae, and gill IV with 2 lamellae. The presence of two dentisetae is considered by Kluge (2004) as the autapomorphy of the group and the gill with two lamellas is cited as a character of unclear phylogenetic status. Bidentiseta was also recovered without Eusetisura in the analysis made without IW. The synapomorphies for the group in this analysis are anterior paracoxal suture incomplete, MP2 attached to MP1 forming a bifurcation, costal projection of hindwing obtuse angled, and presence of two dentisetae.

Our results are not conclusive with respect to the evolution of the number of dentisetae. The results found in analysis under IW with k=10 suggests that taxa with two dentisetae evolved from an ancestor with tree dentisetae. From the lineage with two dentisetae, some taxa would have lost one more dentisetae (e.g. Leptophlebiidae, Tricorythidae and Palingeniidae) or both dentisetae (*Spinirythus*, *Protobehningia* and *Povilla*).

Conversely, the results obtained from the analysis under IW with K = 8, 9, 11 and 12 (figure 4) suggest that taxa with three dentisetae may have evolved from an ancestor with two dentisetae. If this happened, then Oligoneuriidae would have lost one dentiseta, Ameletidae would have lost two dentisetae and Ameletopsidae would have lost all their dentisetae. Coloburiscidae, by its turn, retained a variable number of dentisetae.

Branchitergaliae / Suborder Setisura /Heptagenioidea

Branchitergaliae (Kluge, 1998) groups the same families as Heptagenioidea (Wang and McCafferty, 1995): Coloburiscidae, Isonychiidae, Oligoneuriidae, Pseudironidae, Arthropleidae and Heptageniidae, but with a distinct topology. Kluge (2004), classified Branchitergaliae into Eusetisura and Heptagennota. While both of these groups were found to be monophyletic in our study (figure 6, table 4), they were not recovered together in any of the analyses conducted. Branchitergaliae was also not recovered in studies based on molecular data (Ogden, 2019; Ogden and Whiting, 2005) nor with morphological and molecular data together (Ogden et al., 2009). However, it's important to point out that not all synapomorphies attributed to the group by Kluge (2004) were included in our matrix.

Heptagennota was proposed by Kluge in 2000 and at present groups the families Pseudironidae, Arthropleidae and Heptageniidae (Kluge, 2004) and is equivalent to what Wang and McCafferty (1995) called the "Heptageniidae complex". Both authors agree on Pseudironidae to be the sister group of Arthropleidae + Heptageniidae.

In the current study, Heptagennota was found to be monophyletic in all analyses under IW (figure 6, table 4) and even when only prior weights were used (see appendix 1). This indicates that it is a very stable group, despite its support values not being high (table 5). The synapomorphies found for the group in all the trees obtained under IW are the presence of two pairs of main intercalary veins between CuA and CuP, the lack of prostheca on the right mandible, and labial palp 2 segmented (appendices 12 and 13 - Node 113).

The familial relationship identified in this study is consistent with the one proposed by Kluge (2004) and Wang & McCafferty (1995). Ogden et al. (2009) also found a close relationship between these three taxa on the morphological analysis, but with Arthropleidae being the sister group of Pseudironidae + Heptageniidae. Studies based on molecular data also found these taxa to be closely related, but found Heptageniidae paraphyletic in relation to Pseudironidae and Arthropleidae (Ogden, 2019; Ogden et al., 2009; Ogden and Whiting, 2005). Therefore, the present study agrees both with the placement of *Arthroplea* and *Pseudiron* in separate families or with both genera as lineages within Heptageniidae.

Eusetisura was proposed by Kluge (1998) to include Coloburiscidae, Isonychiidae and Oligoneuriidae and the group has no equivalent in McCafferty's system (According to Ogden and Whiting, 2005). Eusetisura was recovered as monophyletic in the consensus tree for the analyses under IW (figure 6, table 4) and was also recovered in the analysis without the use of IW (appendix 1). The synapomorphies for the group recovered on all trees under IW (appendices 12 and 13 - Node 162) are the reduction from 3 to 2 segments in maxillary palps, the reduction from 3 to 2 segments in labial palps, and the presence of a double row of long filtering hairs on the inner margin of the fore femora and tibiae. Our findings differ from studies based on molecular data, which did not recover this taxon (Ogden, 2019; Ogden et al., 2009; Ogden and Whiting, 2005). We found Isonychiidae as the sister group of Coloburiscidae + Oligoneuriidae, in accordance with the results found by Massariol, Takiya and Salles (2019) in their analysis. Synapomorphies found for Coloburiscidae and Oligoneuriidae (appendices 12 and 13 - Node 161) were the degree of fusion of the wing pads and the loss of tracheation on the dorsal lamella of the gill IV. Kluge (2004) does not present a proposal of relationship between the tree taxa.

Furcatergaliae / Surborder Furcartergalia

The group **Furcatergaliae** was originally proposed by Kluge (1989) but then the same author changed its circumscription (Kluge, 1998). Herein we are considering the more recent proposal, which comprises the same group of families denominated suborder Furcatergalia by McCafferty and Wang (2000) in a distinct topology. Furcatergalia encompass the superfamilies Leptophlebioidea, Ephemeroidea, Caenoidea, and Ephemerelloidea.

The three groups that constitute Furcatergaliae according to Kluge (2004) classification, namely Leptophlebiidae, Fimbriatotergaliae and Ephemerella/Fg1 were all found to be monophyletic. However, there was no consensus regarding their relationship among them and with other groups, so Furcatergaliae was not retrieved in our consensus tree for analyses under IW (figure 6, table 4). Our results differ from Ogden & Whiting (2005), Ogden et al. (2009) and Ogden et al. (2019), which recovered Furcatergaliae as monophyletic.

Although the group was not recovered in the consensus, it was recovered in the analysis under implied weight with K=10 (appendices 6 and 7) and in the analysis under prior weights only (appendix 1). Some characters proposed by Kluge (2004) as synapomorphies of the group related to modification of pleura of prothorax and shortening of imago and subimago first tarsal segment were not included in this analysis and should be considered in future research.

Ephemerella/Fg1/ Ephemerelloidea

Ephemerella/Fg1 (Kluge, 2004) and Ehemerelloidea (Jacobus and McCafferty, 2006b) are equivalent taxa in terms of family composition, but with different proposals for internal relationship. These taxa were found as monophyletic in our strict consensus for the analyses under IW (figure 6, table 4) and also, with the addition of Caenidae, for the consensus under prior weights only (appendix 1).

Kluge (2004) considers Ephemerellidae as the sister group of the remaining families of the group, and this hypothesis was corroborated by our result. Even if Ephemerellidae was recovered as paraphyletic, all Ephemerellidae were recovered "outside" of the remaining families. That is, all the remaining members of Ephemerella/Fg1 or Ephemerelloidea are descendants, according to this hypothesis, of an Ephemerellidae ancestor.

In a study of Pannota based on morphological characters, Jacobus and McCafferty (2006b) found Vietnamellidae as the sister group of Austremerellidae + remaining groups. In our consensus, Austremerelidae and Vietnamellidae were found together and the unique synapomorphy found for the group in all trees (appendices 12 and 13 - Node 147) was the presence of a transverse row of setae on dorsal surface of fore femora. Other characters were found in other trees, such as the presence of primary swimming setae (character 125, present in trees with K= 8, 9, 11 and 12), which is in agreement with McCafferty and Wang (1997).

Pantricorythi (Kluge, 2004) comprises Austremerellidae, Vietnamellidae, Melanemerellidae, Teloganellidae, Teloganodidae and Tricoryptera (see below). In the strict consensus of all analysis under IW (figure 6, table 4) we found Pantricorythi monophyletic, but without Austremerellidae and Vietnamellidae and with the addition of Coryphoridae (considered by Kluge (2004) as Incertae sedis in Ephemerella/Fg1). The synapomorphies for Pantricorythi (appendices 12 and 13 - Node 121) are the margins of the last segment of the labial palp parallel or subparallel, presence of gill II and absence of the gill VII. Pantricorythi was also recuperated in the total combined analysis (Ogden et al., 2009) and although Ogden et al. (2019) analyzed only a few taxa of Pantricorythi, the results found do not contradict the group as monophyletic.

Tricoryptera (Kluge, 2004), a clade comprising Ephemerythidae, Machadorythidae, Tricorythidae, and Leptohyphidae, was found as monophyletic with the inclusion of Coryphoridae in the strict consensus of all analysis under IW (figure 6, table 4). The synapomorphies for the group (appendices 12 and 13 - Node 117) are MP2 detached or attached by a transversal vein to MP1, and middle to distal position of the transversal row of setae on the fore femora. Trichoryptera was also retrieved including Coryphoridae in other studies (Jacobus and McCafferty, 2006b; Molineri, 2006; Molineri and Domínguez, 2003), in agreement with our findings. All these authors, however, found Coryphoridae as the sister group of Leptohyphidae whereas in our study it was found closer to Machadorythidae + Tricorythidae (appendices 12 and 13 - Node 166). The synapomorphies found in our study for this group are the absence of hindwings in both sexes, the fusion of glossae medially and with paraglossae, and the loss of the transverse row of setae on the fore femora. The summary hypothesis of Ogden et al. (2009) presents unresolved relationships for Pantrichorythi, not recovering Trichoryptera. In their study, Ogden et al. (2019) only considered Tricorythidae and Leptohyphidae from the families of Tricoryptera, which were found to form a monophyletic group.

Fimbriatotergaliae

Fimbriatotergaliae (Kluge, 2004), a taxon that comprehends Fossoriae, Potamanthidae, Euthyplociidae and Caenotergaliae, was recovered as monophyletic in the strict consensus, and so were Fossoriae and Caenotergaliae (figure 6, table 4). Caenotergaliae was recovered as the sister group of the remaining taxa. Several synapomorphies were found for Fimbriatotergaliae (appendices 12 and 13 - Node 145), being 4 of them present in the 5 trees from the different analysis under IW. Those were: 1) length of MP2 nearly as long as MP1, 2) presence of a collar in the pronotum, 3) dorsal lamella of gill IV with fringed margin and 4) ventral lamella of gill IV with fringed margin.

Both Potamanthidae and Euthyplociidae were recovered outside Fossoriae, as proposed by Kluge (2004). McCafferty (2004) in his review of the Scapphodonta (=Ephemeroidea *sensu* McCafferty (1991b)) phylogeny also had a very similar proposal, with Potamanthidae and Euthyplociidae as more external groups in relation to the remaining taxa, which is even more coincident with our findings. However, different from McCafferty's hypothesis, we found Behningiidae within the Fossoriae, in accordance with Kluge (2004), being the loss of the tusk one of the autapomorphies for the family (appendices 12 and 13 - Node 151- character 54). This finding is supported by studies based on molecular data that also recovered Behningidae together with the other burrowing mayflies (Miller et al., 2018; Ogden, 2019; Ogden et al., 2009; Ogden and Whiting, 2005). Also in Fossoriae, the close relationship found between Polymitarcyidae and Palingeniidae was already pointed out in morphologic based studies (Kluge, 2004; McCafferty, 2004) but was not found in studies based on molecular data (Miller et al., 2018; Ogden, 2019).

The composition of **Caenotergaliae** (Kluge, 2000) and Caenoidea (Edmunds and Traver, 1954) is the same, being both taxa composed of Caenidae + Neoephemeridae. The group is well supported and was recovered in the studies of Ogden & Whiting (2005), Ogden et al. (2009) and Ogden et al. (2019). More recently, Li et al. (2021), analyzing mitochondrial genome, also found the sister-group relationship of Caenidae and Neoephemeridae as well supported. Interestingly, in our study, Caenotergaliae was recuperated as monophyletic in all the analysis under IW, but not in the analysis under prior weights only (appendix 1). Without downweighting the homoplasies, Caenidae appeared close to the Ephemerelloids, whereas Neoephemeridae was retrieved in Fimbriatotergaliae.

Although Caenotergaliae has been supported by numerous studies, the unanswered question pertains to its position within Ephemeroptera, as for Kluge it belongs to Furcatergaliae, and for McCafferty to Pannota. Fimbriatotergaliae was not recovered by Ogden & Whiting (2005) nor by Ogden et al., (2009) using molecular data, as they found Caenotergaliae to be more closely related to the Ephemerellloids. That relationship was firstly proposed by McCafferty and Edmunds (1979) with the suborder **Pannota**, based mainly on the fusion of the larval wing pads and an enlargement of the mesonotum. More recently, however, Ogden et al. (2019) recovered Fimbriatotergaliae in their partial phylogenomic analysis of the order, which implies the finding of a closer relationship of Caenatotergaliae with the Ephemeroids, similar to our result. Li et al. (2021), in a partial analysis with few clades, also found Caenotergaliae closer to Ephemeridae and Potamanthidae (representing the Ephemeroidea).

Familial monophyly

Of the 25 families represented by two or more genera in this study, 16 families were found as monophyletic. This was not the case for the families Ephemerellidae, Ephemeridae, Nesameletidae, Potamanthidae, and Teloganodidae. Of these families, Ephemeridae and Teloganodidae were recovered as non-monophyletic in at least one work done with parsimonious cladistics (table 6).

Table 6 -Families not recovered as monophyletic in our study; Ephemerellidae, Ephemeridae, Nesameletidae Potamanthidae and Teloganodidae. Are they monophyletic in other studies?

	Ephemerellidae	Ephemeridae	Nesameletidae	Potamanthidae	Teloganodidae
Ogden & Whiting (2005)	yes	no	yes	yes	-
Ogden et al. (2009) total combined dataset	yes	no	yes	yes	no
Ogden et al. (2009) morphological	yes	yes	yes	yes	yes
Miller et al. 2018	-	no	-	yes	-
Ogden et al. (2019)	yes	no	yes	yes	**

* Teloganodidae not sampled by Ogden et al. (2005);

** Teloganodidae represented by only one family in Ogden et al. (2019)

Miller et al. (2018) found strong support for *Hexagenia* being separate from Ephemeridae, thus suggesting Ephemeridae is not a monophyletic family. McCafferty and Wang (2000) included Ephemerellidae and Teloganodidae in their analysis as OTUs, so their monophyly was not tested. Considering this, Ephemerellidae, Nesameletidae and Potamanthidae may deserve a re-examination on their codes in our matrix and/or the addition of new characters that are important for those groups and may not be present in the matrix. Ephemeridae and Teloganodidae deserve more studies to confirm their monophyly.

Some families were recovered as monophyletic or not depending on the value of K used in the analysys. Acanthametropodidae and Siphlonuridae were recovered in four out of five of the analyses, indicating support for their monophyly, specially Siphlonuridae, which was considered non monophyletic because of the insertion of *Mickoleitia* (Coxoplectoptera) in one analysys. Considering only extant families, Dipteromimidae was recovered as the sister group of Siphlonuridae in all analyses, differently from Ogden et al. (2009), who found Dipteromimidae to be part of Siphlonuridae.

Oniscigastridae was found as monophyletic in two out of five analyses (K = 11 and 12, table 4), and in two others they were considered non-monophyletic because of the insertion of *Kukalova†* (K = 8 and 9, table 4). Only in the analysis with K=10 *Oniscigaster* was recovered distant from the other members of the family, *Siphlonella* and *Tasmanophlebia*. Considering these results, we also consider that the monophyly of the group was supported. The monophyly of Oniscigastridae has also been supported by molecular data (Ogden, 2019; Ogden et al., 2009; Ogden and Whiting, 2005), but not by morphological data (Ogden et al., 2009).

Protereismatidae, which is a fossil taxon represented by only two families and with considerable missing data, was recovered in two out of the five analyses, so although it was not supported in our analysis, it was not refuted neither.

Evolution of selected characters

As some important characters for the systematics of mayflies had an independent origin according to our hypotheses, they will be addressed here. We will discuss the consensus tree of the analyzes with K = 8, 9, 11 and 12 (figure 4) separately from the tree found in the analysis using K = 10 (figure 5), since the first ones were similar to each other and very different from the latter.

The **number of dentisetae on the maxilla** is variable in mayflies, ranging from zero to six in different taxa, and in some genera the number may also vary. For the tree recovered under IW with K=10 (figure 5), the plesiomorphic state is unknown (234), with three groups independently losing one dentisetae (*Acanthametropus*, Oligoneuriidae, and Bidentisetae), several groups independently losing two or all dentisetae, and some taxa gaining more dentisetae (Coloburiscidae and *Baetisca*). On the other hand, a different scenario emerges for the consensus tree of the analyses under IW with values of K = 8, 9, 11, and 12 (figure 4). In this case, the plesiomorphic state would be two dentisetae with only one change to three dentisetae in the group that includes all taxa with three dentisetae plus Eusetisura. The presence of two dentisetae in Eusetisura, therefore, represents a reversal. This group of taxa with three dentisetae includes all the representatives of Tridentisetae / Pisciforma, the fossil taxa included in the analyzes, and Posteritorna and one of the synapomorphies of the group is the presence of three dentisetae in the maxilla. Although Kluge (2004) considers Tridentiseta to be a plesiomorphon, four of the five analyzes under IW resulted in a grouping of taxa with three dentisetae, with the addition of Eusetisura. However, these results have not been found in other studies to date.

The **maxillary gills**, according to our hypothesis, have three independent origins, the plesiomorphic condition being the absence of gills on the maxillae. Maxillary gills are present in Rallidentidae, Siphluriscidae, Nesameletidae, Eusetisura, and at least some Polymitarcyidae (*Tortopus* and *Povilla*). *Tortopsis*, another genus of Polymitarcyidae not included in our study, also has a finger-like gill at the base of the maxilla (Molineri et al., 2021). Our hypothesis is consistent with the information of Kluge (2004), according to which the attachment of the gills to the maxilla is different in Eusetisura, where they originate from the articular membrane at the base of the maxilla, and in Rallidentidae + Siphluriscidae + Nesameletidae, where they are located on the lateral side of the maxilla. However, the evolution of character 65 - shape of maxillary gills - shows that in the group Rallidentidae + Siphluriscidae + Nesameletidae the gills were originally single or double and subsequently changed to a more complex branched to fibrilliform shape in *Siphluriscus*. In Eusetisura the gills appeared branched to fibrilliform, and changed to single/double in Coloburiscidae.

As to the **degree of fusion of the forewing pads**, in mayflies the wing pads may be fused to the notum at most to the end of the scutellum, or may be fused by a mesal plate behind the scutellum. This difference has led McCafferty and Edmunds (1979) to divide Ephemeroptera into two groups: the Schistonota, in which the wing pads are fused to no more than half their length; and the Pannota, which are characterized by the wing pads being free less than half their length. Although Schistonota is no longer recognized, Pannota was present in the last works published by McCafferty (Jacobus and McCafferty, 2008, 2006b). The group was also recovered as monophyletic with molecular data (Ogden et al., 2009; Ogden and Whiting, 2005), and is also recognized by other authors (Martynov et al., 2022; Monjardim et al., 2020). However, Pannota was not recovered by Ogden et al., (2019) and is considered paraphyletic by Kluge. Our hypothesis supports three independent origins of forewing pads fusion; one for Prosopistomatidae, one for Coloburiscidae and Oligoneuriidae, and a third for Ephemerella/fg1.

Some Ephemeroptera present a **double row of long filtering hairs on the inner margin of the anterior femora and tibiae.** Despite their similarity in Eusetisura and in the group formed by Polymitarcyidae and Palingeniidae, our analyzes suggest two different origins for these setal rows in these two groups. Our results corroborates Kluge (2004), who has already pointed out that these setae rows, besides similar, are an autapomorphy of Eusetisura and also a synapomorphy for Polymitarcyidae and Palingeniidae wich are grouped together in a clade named Cryptosternata.

Analyzing the **evolution of gill characters** in Ephemeroptera in the context of our hypotheses, we find that gill one was present in the ancestors and was independently lost in *Murphyella*, which also lost the remaining gills, and in part of Ephemerella/fg1, *Teloganella* and *Manohyphella*. In the tree obtained using IW with K = 10 (figure 5), there are two independent losses of the first gill in Ephemerella/fg1; one for *Vietnamella* and one for part of Ephemerellidae and remaining taxa.

In the analysis with K = 8, 9, 11 and 12 (figure 4), gill one is hypothesized to be reduced in the ancestral and have posteriorly attained a size similar to the other gills in two groups (Behningiidae and Leptophlebiidae + Heptageniidae + Eusetisura + Prosopistomatidae + "Tridentisetae"). For this same analysis, strangely, gill two would be absent in the ancestral Ephemeroptera, having appeared independently three times (one for *Austremerella* + *Vietnamella*, one for *Torleya* + Pantricorythi and a third one for the remaining Ephemeroptera - Ephemerella/fg1. In the analysis with K = 10 however, an opposite and much more plausible scenario would have happened. The ancestor would have had similar sized gills, with a posterior reduction in Furcatergaliae - Leptophlebiidae and a reversion in Behningiidae. For the second gill, the ancestor would have gill two present with two independent losses and a reversal in part of Ephemerella/fg1.

The transformation of the second gill in opercular is hypothesized to have occurred four times independently of the value of K used in the analysis (one for Prosopistomatidae, one for *Melanemerella*, one for *Dudgeodes* + Ephemerythidae + Leptohyphidae + Coryphoridae + Machadorythidae and a last one for Caenotergaliae).

With respect to the number of lamellae, both results suggest an origin with two lamellae and posterior losses and also reversals in several groups.

The general shape of the gills was examined using the fourth gill. The fringed margins on the gill lamellae is a derived condition in all the analysis under IW. Also, the fringed margins of both dorsal and ventral lamellae were found as an autapomorphy for

Fimbriatotergaliae in agreement with the proposed by Kluge (2004). There were also some independent origins of fringed margins for other groups for either the ventral or the dorsal lamella.

Conclusions

We found two very different topologies with different K values in our analyzes: a similar one for K values 8, 9, 11, and 12 and a very different one for a K value of 10. The consensus of all analyzes was unresolved for the deep relationship within Ephemeroptera.

Monophyly is supported for 7 suprafamilial groups proposed by Kluge (2004): Caenotergaliae, Ephemerella/Fg1, Eusetisura, Fimbriatotergaliae, Fossoriae, Heptagennota, and Posteritorna. Three suprafamilial groups attributed to McCafferty were also recovered: Carapacea (= Posteritorna), Ephemerelloidea (= Ephemerella/Fg1), and Caenoidea (= Caenotergaliae). Pannota, Anteritorna, Branchitergaliae, and Tridentiseta were not recovered as monophyletic in any of the analyses under IW.

Five families were not found to be monophyletic in any of our analyzes: Ephemerellidae, Ephemeridae, Nesameletidae, Potamanthidae, and Teloganodidae.

Considering the evolution of characters, especially the presence and absence of gills one and two in the groups, the hypothesis under K10 supporting Furcatergalia as monophyletic seems more plausible than the other hypotheses, with K 8, 9, 11, and 12.

Although our results are not resolved for deep relationships, several important groups have been recovered, some of which are the same groups supported by the most recent research in the phylogeny of Ephemeroptera (Ogden et al., 2019). We believe that a next step will be to combine the morphological data we reviewed with data that has been gathered on phylogenomics to gain a better understanding of relationships in the group and hopefully propose a new classification system for Ephemeroptera.

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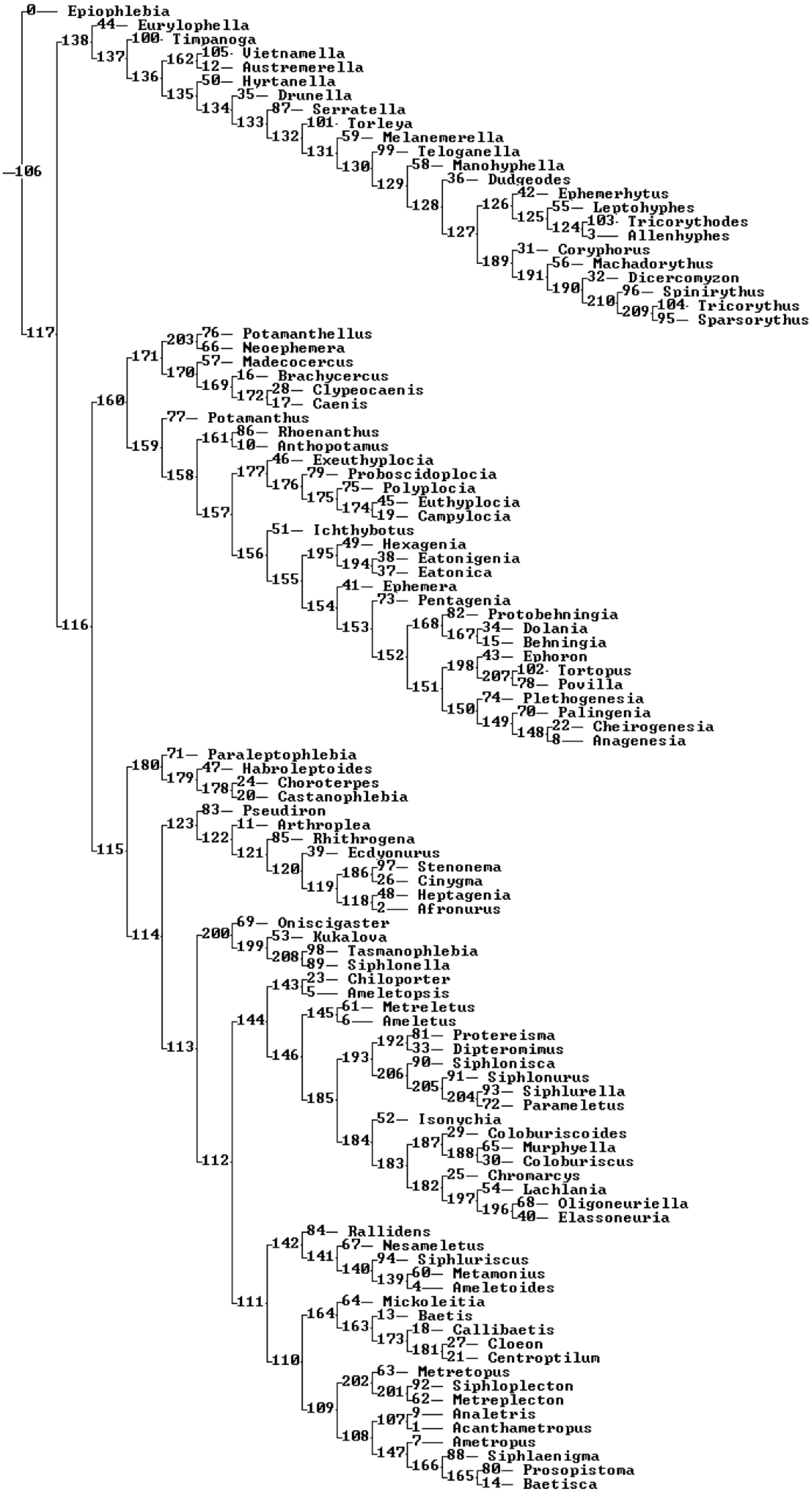
Appendices

Appendix 1 - Strict consensus of 47 MPT found using New Technology under prior weights only

Appendix 2 - Nodes on tree of analysis under IW (k=8)



Appendix 3 - Synapomorphies for groups above family in tree found under IW with k = 8

Node 107 :
Char. 27: 0 --> 1
Char. 36: 1 --> 0
Char. 39: 1 --> 2
Char. 51: 1 --> 0
Char. 109: 0 --> 1
Node 108 :
Char. 47: 1 --> 0
Char. 94: 1 --> 0
Node 109 :
Char. 6: 1 --> 0
Char. 62: 3 --> 2
Node 110 :
Char. 26: 0 --> 2
Char. 27: 1 --> 0
Char. 37: 1 --> 2
Char. 118: 1 --> 0
Node 111 :
Char. 95: 0 --> 1
Node 112 :
Char. 35: 1 --> 0
Char. 46: 0 --> 1
Char. 113: 0 --> 1
Node 113 :
Char. 5: 0 --> 1
Char. 61: 2 --> 3
Char. 109: 1 --> 0
Node 114 :
Char. 47: 0 --> 1
Char. 75: 3 --> 0
Char. 118: 0 --> 1
Char. 125: 0 --> 1
Node 115 :
Char. 82: 1 --> 0
Char. 96: 0 --> 1
Char. 101: 1 --> 0
Node 116 :
Char. 36: 0 --> 1
Char. 71: 2 --> 0
Char. 104: 0 --> 1
Char. 107: 0 --> 1
Node 117 :
No synapomorphies
Node 118 :
Char. 127: 1 --> 2
Node 119 :
Char. 125: 1 --> 0
Node 120 :
Char. 35: 1 --> 0
Char. 63: 1 --> 0
Node 121 :
Char. 67: 0 --> 1
Char. 75: 0 --> 1
Char. 77: 0 --> 1
Char. 127: 0 --> 1
Node 122 :
Char. 46: 0 --> 1
Char. 53: 0 --> 1
Char. 69: 2 --> 1
Node 123 :
Char. 26: 0 --> 4
Char. 51: 1 --> 0
Char. 74: 2 --> 1
Node 124 :
Char. 69: 2 --> 1
Char. 99: 0 --> 1
Char. 120: 2 --> 0
Node 125 :
Char. 13: 0 --> 1
Char. 38: 1 --> 0
Char. 68: 0 --> 1
Char. 76: 0 --> 1
Char. 114: 0 --> 1
Char. 123: 1 --> 0
Node 126 :
Char. 2: 1 --> 0
Char. 62: 1 --> 3
Char. 97: 1 --> 0
Node 127 :
Char. 22: 0 --> 1
Char. 88: 0 --> 1
Node 128 :
Char. 32: 0 --> 1
Char. 105: 0 --> 1
Char. 127: 0 --> 1
Node 129 :
Char. 14: 1 --> 0
Char. 37: 01 --> 2
Char. 62: 3 --> 1
Char. 98: 1 --> 0
Node 130 :
Char. 16: 1 --> 0
Char. 31: 0 --> 1
Char. 68: 1 --> 0
Char. 121: 1 --> 0
Node 131 :
Char. 75: 3 --> 0
Char. 104: 0 --> 1
Char. 122: 1 --> 0
Node 132 :
Char. 26: 0 --> 1
Char. 27: 1 --> 0
Char. 33: 0 --> 1
Char. 36: 0 --> 1
Char. 87: 0 --> 1
Node 133 :
Char. 22: 1 --> 0
Char. 40: 1 --> 0
Node 134 :
Char. 14: 0 --> 1
Node 135 :
Char. 38: 0 --> 1
Node 136 :
Char. 22: 0 --> 1
Char. 107: 0 --> 1
Node 137 :
Char. 35: 1 --> 0
Char. 39: 1 --> 0
Char. 46: 0 --> 1
Node 138 :
Char. 3: 0 --> 1
Char. 40: 0 --> 1
Char. 66: 0 --> 1
Char. 79: 1 --> 2
Char. 97: 0 --> 1
Char. 99: 0 --> 1
Node 139 :
Char. 22: 0 --> 1
Char. 37: 0 --> 1
Node 140 :
Char. 52: 0 --> 1
Node 141 :
Char. 7: 1 --> 0
Char. 10: 1 --> 0
Char. 50: 0 --> 1
Char. 71: 0 --> 1
Node 142 :
Char. 37: 1 --> 0
Char. 64: 0 --> 1
Char. 114: 0 --> 1
Char. 115: 0 --> 2
Node 143 :
Char. 44: 1 --> 2
Char. 61: 3 --> 0
Char. 69: 2 --> 3
Char. 74: 2 --> 3
Char. 96: 1 --> 0
Char. 126: 0 --> 1
Node 144 :
Char. 40: 0 --> 1
Node 145 :
Char. 3: 0 --> 1
Char. 61: 3 --> 1
Char. 67: 0 --> 1
Node 146 :
Char. 6: 1 --> 0
Char. 114: 0 --> 1
Node 147 :
Char. 22: 0 --> 1
Char. 34: 1 --> 0
Node 148 :
Char. 56: 0 --> 1
Char. 128: 0 --> 1
Node 149 :
Char. 12: 1 --> 0
Char. 47: 0 --> 1
Char. 124: 1 --> 0
Node 150 :
Char. 18: 0 --> 1
Char. 24: 0 --> 1
Node 151 :
Char. 59: 0 --> 1
Char. 69: 2 --> 1
Char. 89: 0 --> 1

```
Node 152 :
Char. 0: 1 --> 0
Char. 10: 1 --> 3
Char. 21: 0 --> 1
Char. 22: 0 --> 1
Char. 27: 1 --> 0
Char. 128: 1 --> 0
Node 153 :
Char. 62: 3 --> 1
Char. 75: 2 --> 1
Char. 99: 0 --> 1
Node 154 :
Char. 37: 0 --> 1
Char. 56: 1 --> 0
Node 155 :
Char. 58: 0 --> 1
Node 156 :
Char. 63: 0 --> 2
Char. 74: 2 --> 1
Char. 78: 0 --> 1
Char. 82: 1 --> 0
Node 157 :
Char. 37: 1 --> 0
Char. 56: 0 --> 1
Char. 102: 0 --> 2
Node 158 :
Char. 55: 1 --> 2
Node 159 :
Char. 6: 1 --> 0
Char. 35: 1 --> 0
Char. 54: 0 --> 1
Char. 63: 1 --> 0
Char. 76: 1 --> 2
Char. 123: 1 --> 0
Char. 125: 0 --> 1
Char. 126: 0 --> 1
Node 160 :
Char. 23: 1 --> 0
Char. 81: 0 --> 1
Char. 94: 1 --> 0
Char. 112: 0 --> 1
Char. 120: 0 --> 1
Node 161 :
Char. 129: 2 --> 0
Node 162 :
Char. 87: 0 --> 1
Char. 104: 0 --> 1
Char. 125: 0 --> 1
Node 163 :
Char. 31: 0 --> 1
Char. 39: 1 --> 0
Node 164 :
Char. 20: 0 --> 1
Char. 49: 0 --> 1
Node 165 :
Char. 17: 1 --> 0
Char. 37: 2 --> 0
Char. 79: 1 --> 2
Char. 80: 0 --> 1
Char. 83: 2 --> 0
Char. 105: 0 --> 1
Char. 112: 0 --> 1
Char. 117: 1 --> 0
Char. 122: 1 --> 0
Char. 128: 1 --> 0
Node 166 :
Char. 62: 2 --> 3
Char. 99: 1 --> 0
Char. 113: 1 --> 0
Node 167 :
Char. 24: 0 --> 1
Char. 25: 1 --> 0
Char. 39: 1 --> 2
Char. 49: 0 --> 1
Char. 53: 0 --> 1
Char. 92: 1 --> 0
Node 168 :
Char. 7: 2 --> 3
Char. 11: 0 --> 1
Char. 37: 1 --> 2
Char. 42: 2 --> 0
Char. 45: 1 --> 2
Char. 54: 1 --> 0
Char. 74: 1 --> 2
Char. 75: 1 --> 3
Char. 101: 1 --> 0
Char. 103: 0 --> 1
Char. 106: 0 --> 1
Node 169 :
Char. 49: 0 --> 1
Char. 63: 1 --> 2
Char. 109: 1 --> 0
Node 170 :
Char. 2: 1 --> 0
Char. 13: 0 --> 1
Char. 16: 1 --> 0
Char. 20: 0 --> 1
Char. 22: 0 --> 1
Char. 26: 0 --> 2
Char. 27: 1 --> 0
Char. 30: 0 --> 2
Char. 42: 2 --> 0
Char. 71: 0 --> 1
Char. 75: 3 --> 0
Char. 83: 2 --> 0
Node 171 :
Char. 4: 0 --> 1
Char. 8: 1 --> 0
Char. 46: 0 --> 1
Char. 99: 0 --> 1
Char. 105: 0 --> 1
Char. 122: 1 --> 0
Node 172 :
Char. 94: 0 --> 1
Node 173 :
Char. 44: 1 --> 2
Char. 53: 0 --> 1
Char. 91: 0 --> 1
Node 174 :
Char. 55: 2 --> 3
Node 175 :
Char. 10: 1 --> 0
Node 176 :
Char. 8: 1 --> 0
Node 177 :
Char. 2: 1 --> 0
Char. 21: 0 --> 1
Char. 42: 2 --> 0
Node 178 :
Char. 32: 0 --> 1
Char. 127: 0 --> 1
Node 179 :
Char. 16: 1 --> 0
Char. 34: 1 --> 0
Char. 39: 1 --> 0
Char. 62: 3 --> 0
Node 180 :
Char. 3: 0 --> 1
Char. 26: 0 --> 2
Char. 61: 2 --> 1
Char. 63: 1 --> 0
Node 181 :
Char. 75: 0 --> 2
Node 182 :
Char. 11: 0 --> 1
Char. 12: 1 --> 2
Char. 18: 0 --> 1
Char. 40: 1 --> 0
Char. 46: 1 --> 0
Char. 83: 2 --> 0
Char. 96: 1 --> 0
Char. 113: 1 --> 0
Node 183 :
Char. 79: 1 --> 2
Char. 99: 0 --> 1
Char. 116: 1 --> 0
Node 184 :
Char. 35: 0 --> 2
Char. 64: 0 --> 1
Char. 69: 2 --> 1
Char. 74: 2 --> 1
Char. 89: 0 --> 1
Char. 109: 0 --> 1
Node 185 :
Char. 63: 1 --> 2
Node 186 :
Char. 3: 0 --> 1
Char. 114: 0 --> 1
Node 187 :
Char. 59: 0 --> 1
Char. 62: 1 --> 4
Char. 63: 2 --> 0
Char. 65: 1 --> 0
Char. 70: 1 --> 2
Char. 75: 0 --> 1
Char. 78: 0 --> 1
Char. 87: 0 --> 1
Char. 90: 0 --> 1
Node 188 :
Char. 22: 0 --> 1
```

```
Char. 29: 0 --> 1
Char. 35: 2 --> 0
Char. 37: 1 --> 2
Char. 69: 1 --> 2
Char. 125: 1 --> 0
Node 189 :
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Char. 73: 0 --> 1
Char. 87: 1 --> 0
Node 190 :
Char. 61: 2 --> 1
Char. 62: 1 --> 0
Char. 97: 1 --> 0
Char. 105: 1 --> 0
Node 191 :
Char. 12: 1 --> 0
Char. 68: 0 --> 1
Char. 83: 2 --> 0
Node 192 :
Char. 16: 1 --> 0
Char. 40: 1 --> 0
Node 193 :
Char. 7: 1 --> 0
Char. 70: 1 --> 2
Char. 94: 1 --> 0
Char. 115: 0 --> 1
Node 194 :
Char. 21: 0 --> 1
Char. 42: 2 --> 1
Char. 126: 1 --> 0
Node 195 :
Char. 62: 3 --> 2
Node 196 :
Char. 43: 0 --> 1
Node 197 :
Char. 1: 1 --> 0
Char. 10: 1 --> 2
Char. 21: 0 --> 1
Char. 24: 0 --> 1
Char. 27: 1 --> 0
Char. 31: 0 --> 1
Char. 39: 1 --> 2
Char. 45: 0 --> 1
Char. 103: 0 --> 1
Node 198 :
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Char. 41: 1 --> 0
Char. 58: 1 --> 0
Char. 62: 1 --> 3
Char. 63: 2 --> 0
Node 199 :
Char. 63: 1 --> 2
Char. 101: 0 --> 2
Node 200 :
Char. 97: 0 --> 1
Node 201 :
Char. 128: 1 --> 0
Node 202 :
Char. 44: 1 --> 2
Char. 74: 2 --> 1
Char. 75: 0 --> 2
Char. 93: 0 --> 1
Node 203 :
Char. 3: 0 --> 1
Char. 97: 0 --> 1
Char. 116: 1 --> 0
Node 204 :
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Node 205 :
Char. 67: 0 --> 1
Node 206 :
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Char. 35: 0 --> 1
Char. 43: 0 --> 1
Char. 99: 0 --> 1
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Char. 11: 0 --> 1
Char. 42: 2 --> 0
Char. 47: 0 --> 1
Char. 64: 0 --> 1
Char. 75: 1 --> 0
Char. 99: 1 --> 0
Node 208 :
Char. 121: 1 --> 0
Char. 122: 1 --> 0
Node 209 :
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Char. 22: 1 --> 0
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Node 210 :
Char. 13: 0 --> 1
Char. 60: 0 --> 1
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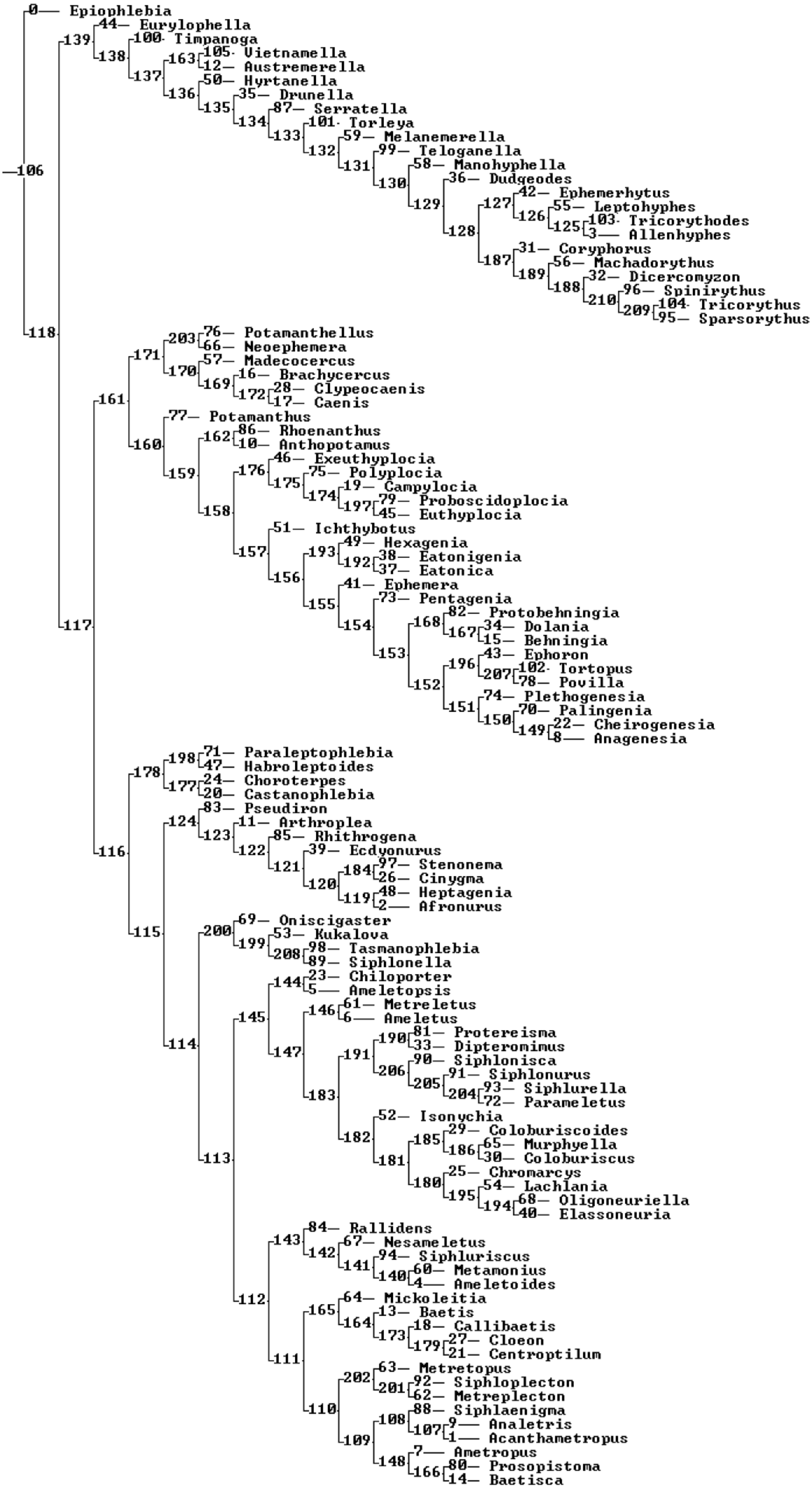
Appendix 4 - Nodes on tree of analysis under IW (k=9)



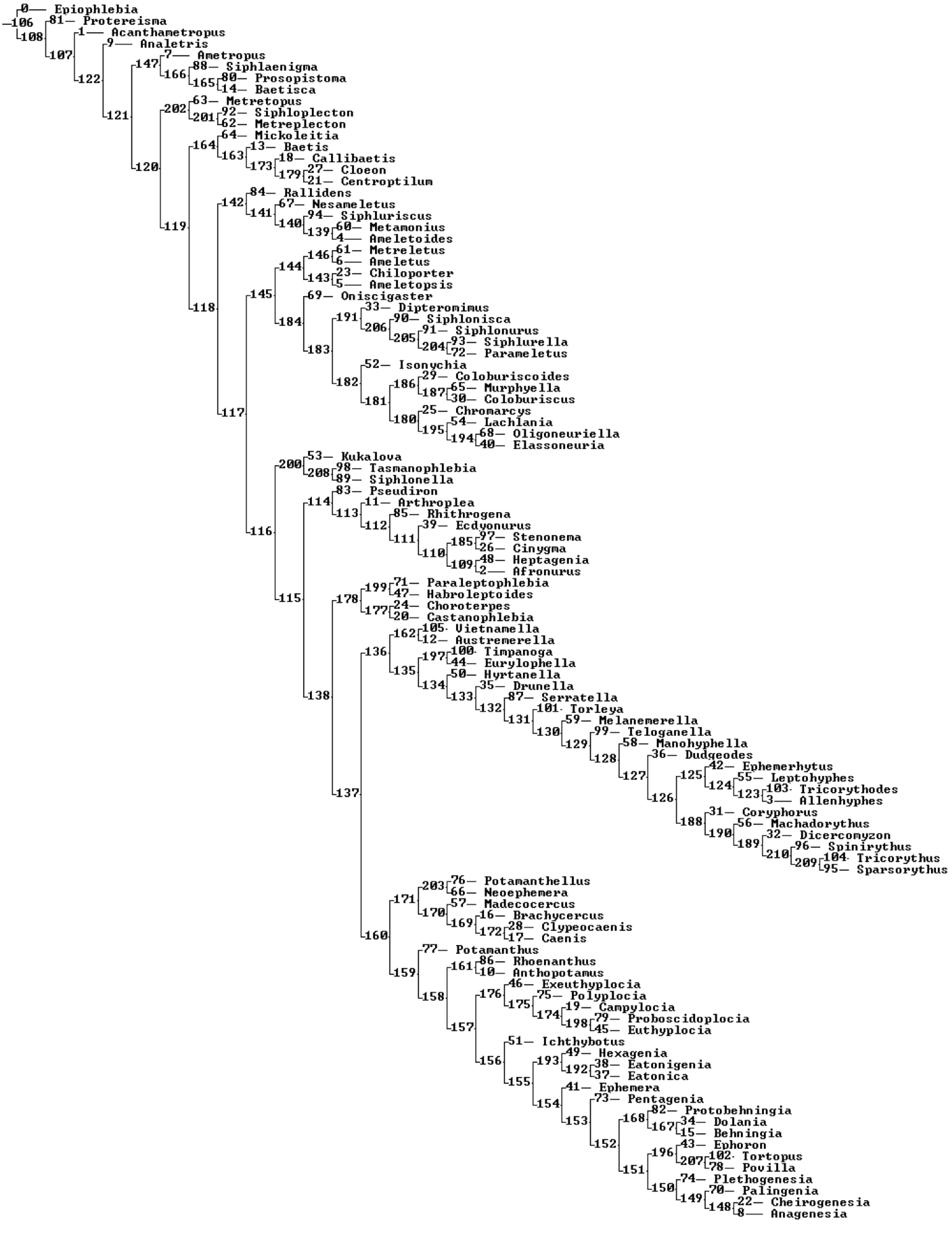
Appendix 5 - Synapomorphies for groups above family in tree found under IW with k = 9

Node 107 :
Char. 27: 0 --> 1
Char. 36: 1 --> 0
Char. 39: 1 --> 2
Char. 51: 1 --> 0
Node 108 :
Char. 43: 0 --> 1
Char. 109: 0 --> 1
Node 109 :
Char. 7: 1 --> 2
Char. 47: 1 --> 0
Node 110 :
Char. 6: 1 --> 0
Node 111 :
Char. 26: 0 --> 2
Char. 27: 1 --> 0
Char. 37: 1 --> 2
Char. 118: 1 --> 0
Node 112 :
Char. 95: 0 --> 1
Node 113 :
Char. 35: 1 --> 0
Char. 46: 0 --> 1
Char. 113: 0 --> 1
Node 114 :
Char. 5: 0 --> 1
Char. 61: 2 --> 3
Char. 109: 1 --> 0
Node 115 :
Char. 33: 0 --> 1
Char. 47: 0 --> 1
Char. 75: 3 --> 0
Char. 118: 0 --> 1
Char. 125: 0 --> 1
Node 116 :
Char. 82: 1 --> 0
Char. 96: 0 --> 1
Char. 101: 1 --> 0
Node 117 :
Char. 36: 0 --> 1
Char. 71: 2 --> 0
Char. 104: 0 --> 1
Char. 107: 0 --> 1
Node 118 :
No synapomorphies
Node 119 :
Char. 127: 1 --> 2
Node 120 :
Char. 125: 1 --> 0
Node 121 :
Char. 35: 1 --> 0
Char. 63: 1 --> 0
Node 122 :
Char. 67: 0 --> 1
Char. 75: 0 --> 1
Char. 77: 0 --> 1
Char. 127: 0 --> 1
Node 123 :
Char. 46: 0 --> 1
Char. 53: 0 --> 1
Char. 69: 2 --> 1
Node 124 :
Char. 26: 0 --> 4
Char. 51: 1 --> 0
Char. 70: 1 --> 2
Char. 74: 2 --> 1
Node 125 :
Char. 69: 2 --> 1
Char. 99: 0 --> 1
Char. 120: 2 --> 0
Node 126 :
Char. 13: 0 --> 1
Char. 38: 1 --> 0
Char. 68: 0 --> 1
Char. 76: 0 --> 1
Char. 114: 0 --> 1
Char. 123: 1 --> 0
Node 127 :
Char. 2: 1 --> 0
Char. 62: 1 --> 3
Char. 97: 1 --> 0
Node 128 :
Char. 22: 0 --> 1
Char. 88: 0 --> 1
Node 129 :
Char. 32: 0 --> 1
Char. 105: 0 --> 1
Char. 127: 0 --> 1
Node 130 :
Char. 14: 1 --> 0
Char. 37: 01 --> 2
Char. 62: 3 --> 1
Char. 98: 1 --> 0
Node 131 :
Char. 16: 1 --> 0
Char. 31: 0 --> 1
Char. 68: 1 --> 0
Char. 121: 1 --> 0
Node 132 :
Char. 75: 3 --> 0
Char. 104: 0 --> 1
Char. 122: 1 --> 0
Node 133 :
Char. 26: 0 --> 1
Char. 27: 1 --> 0
Char. 33: 0 --> 1
Char. 36: 0 --> 1
Char. 87: 0 --> 1
Node 134 :
Char. 22: 1 --> 0
Char. 40: 1 --> 0
Node 135 :
Char. 14: 0 --> 1
Node 136 :
Char. 38: 0 --> 1
Node 137 :
Char. 22: 0 --> 1
Char. 107: 0 --> 1
Node 138 :
Char. 35: 1 --> 0
Char. 39: 1 --> 0
Char. 46: 0 --> 1
Node 139 :
Char. 3: 0 --> 1
Char. 40: 0 --> 1
Char. 66: 0 --> 1
Char. 79: 1 --> 2
Char. 97: 0 --> 1
Char. 99: 0 --> 1
Node 140 :
Char. 22: 0 --> 1
Char. 37: 0 --> 1
Node 141 :
Char. 52: 0 --> 1
Node 142 :
Char. 7: 1 --> 0
Char. 10: 1 --> 0
Char. 50: 0 --> 1
Char. 71: 0 --> 1
Node 143 :
Char. 37: 1 --> 0
Char. 64: 0 --> 1
Char. 114: 0 --> 1
Char. 115: 0 --> 2
Node 144 :
Char. 44: 1 --> 2
Char. 61: 3 --> 0
Char. 69: 2 --> 3
Char. 74: 2 --> 3
Char. 96: 1 --> 0
Char. 126: 0 --> 1
Node 145 :
Char. 40: 0 --> 1
Node 146 :
Char. 3: 0 --> 1
Char. 61: 3 --> 1
Char. 67: 0 --> 1
Node 147 :
Char. 6: 1 --> 0
Char. 114: 0 --> 1
Node 148 :
Char. 33: 1 --> 0
Char. 126: 0 --> 1
Node 149 :
Char. 56: 0 --> 1
Char. 128: 0 --> 1
Node 150 :
Char. 12: 1 --> 0
Char. 47: 0 --> 1
Char. 124: 1 --> 0
Node 151 :
Char. 18: 0 --> 1
Char. 24: 0 --> 1
Node 152 :

Char. 59: 0 --> 1
Char. 69: 2 --> 1
Char. 89: 0 --> 1
Node 153 :
Char. 0: 1 --> 0
Char. 10: 1 --> 3
Char. 21: 0 --> 1
Char. 22: 0 --> 1
Char. 27: 1 --> 0
Char. 128: 1 --> 0
Node 154 :
Char. 62: 3 --> 1
Char. 75: 2 --> 1
Char. 99: 0 --> 1
Node 155 :
Char. 37: 0 --> 1
Char. 56: 1 --> 0
Node 156 :
Char. 58: 0 --> 1
Node 157 :
Char. 63: 0 --> 2
Char. 74: 2 --> 1
Char. 78: 0 --> 1
Char. 82: 1 --> 0
Node 158 :
Char. 37: 1 --> 0
Char. 56: 0 --> 1
Char. 102: 0 --> 2
Node 159 :
Char. 55: 1 --> 2
Node 160 :
Char. 6: 1 --> 0
Char. 35: 1 --> 0
Char. 54: 0 --> 1
Char. 63: 1 --> 0
Char. 76: 1 --> 2
Char. 123: 1 --> 0
Char. 125: 0 --> 1
Char. 126: 0 --> 1
Node 161 :
Char. 23: 1 --> 0
Char. 76: 0 --> 1
Char. 81: 0 --> 1
Char. 94: 1 --> 0
Char. 112: 0 --> 1
Char. 120: 0 --> 1
Node 162 :
Char. 129: 2 --> 0
Node 163 :
Char. 87: 0 --> 1
Char. 104: 0 --> 1
Char. 125: 0 --> 1
Node 164 :
Char. 31: 0 --> 1
Char. 39: 1 --> 0
Node 165 :
Char. 20: 0 --> 1
Char. 49: 0 --> 1
Node 166 :
Char. 17: 1 --> 0
Char. 37: 2 --> 0
Char. 71: 0 --> 2
Char. 79: 1 --> 2
Char. 80: 0 --> 1
Char. 83: 2 --> 0
Char. 105: 0 --> 1
Char. 112: 0 --> 1
Char. 113: 1 --> 0
Char. 117: 1 --> 0
Char. 122: 1 --> 0
Char. 128: 1 --> 0
Node 167 :
Char. 24: 0 --> 1
Char. 25: 1 --> 0
Char. 39: 1 --> 2
Char. 49: 0 --> 1
Char. 53: 0 --> 1
Char. 92: 1 --> 0
Node 168 :
Char. 7: 2 --> 3
Char. 11: 0 --> 1
Char. 37: 1 --> 2
Char. 42: 2 --> 0
Char. 45: 1 --> 2
Char. 54: 1 --> 0
Char. 74: 1 --> 2
Char. 75: 1 --> 3
Char. 101: 1 --> 0
Char. 103: 0 --> 1
Char. 106: 0 --> 1
Node 169 :
Char. 49: 0 --> 1
Char. 63: 1 --> 2
Char. 109: 1 --> 0
Node 170 :
Char. 2: 1 --> 0
Char. 13: 0 --> 1
Char. 16: 1 --> 0
Char. 20: 0 --> 1
Char. 22: 0 --> 1
Char. 26: 0 --> 2
Char. 27: 1 --> 0
Char. 30: 0 --> 2
Char. 42: 2 --> 0
Char. 71: 0 --> 1
Char. 75: 3 --> 0
Char. 83: 2 --> 0
Node 171 :
Char. 4: 0 --> 1
Char. 8: 1 --> 0
Char. 46: 0 --> 1
Char. 99: 0 --> 1
Char. 105: 0 --> 1
Char. 122: 1 --> 0
Node 172 :
Char. 94: 0 --> 1
Node 173 :
Char. 53: 0 --> 1
Char. 91: 0 --> 1
Node 174 :
Char. 55: 2 --> 3
Node 175 :
Char. 10: 1 --> 0
Char. 84: 0 --> 1
Node 176 :
Char. 2: 1 --> 0
Char. 21: 0 --> 1
Char. 42: 2 --> 0
Node 177 :
Char. 32: 0 --> 1
Char. 127: 0 --> 1
Node 178 :
Char. 3: 0 --> 1
Char. 26: 0 --> 2
Char. 38: 0 --> 1
Char. 61: 2 --> 1
Char. 63: 1 --> 0
Node 179 :
Char. 75: 0 --> 2
Node 180 :
Char. 11: 0 --> 1
Char. 12: 1 --> 2
Char. 18: 0 --> 1
Char. 40: 1 --> 0
Char. 46: 1 --> 0
Char. 83: 2 --> 0
Char. 96: 1 --> 0
Char. 113: 1 --> 0
Node 181 :
Char. 79: 1 --> 2
Char. 99: 0 --> 1
Char. 116: 1 --> 0
Node 182 :
Char. 35: 0 --> 2
Char. 64: 0 --> 1
Char. 69: 2 --> 1
Char. 74: 2 --> 1
Char. 89: 0 --> 1
Char. 109: 0 --> 1
Node 183 :
Char. 63: 1 --> 2
Node 184 :
Char. 3: 0 --> 1
Char. 114: 0 --> 1
Node 185 :
Char. 59: 0 --> 1
Char. 62: 1 --> 4
Char. 63: 2 --> 0
Char. 65: 1 --> 0
Char. 70: 1 --> 2
Char. 75: 0 --> 1
Char. 78: 0 --> 1
Char. 87: 0 --> 1
Char. 90: 0 --> 1
Node 186 :
Char. 22: 0 --> 1
Char. 29: 0 --> 1
Char. 35: 2 --> 0
Char. 37: 1 --> 2
Char. 69: 1 --> 2

```
Char. 125: 1 --> 0
Node 187 :
Char. 30: 0 --> 2
Char. 72: 0 --> 1
Char. 73: 0 --> 1
Char. 87: 1 --> 0
Node 188 :
Char. 61: 2 --> 1
Char. 62: 1 --> 0
Char. 97: 1 --> 0
Char. 105: 1 --> 0
Node 189 :
Char. 12: 1 --> 0
Char. 68: 0 --> 1
Char. 83: 2 --> 0
Node 190 :
Char. 16: 1 --> 0
Char. 40: 1 --> 0
Node 191 :
Char. 7: 1 --> 0
Char. 70: 1 --> 2
Char. 94: 1 --> 0
Char. 115: 0 --> 1
Node 192 :
Char. 21: 0 --> 1
Char. 42: 2 --> 1
Char. 126: 1 --> 0
Node 193 :
Char. 62: 3 --> 2
Node 194 :
Char. 43: 0 --> 1
Node 195 :
Char. 1: 1 --> 0
Char. 10: 1 --> 2
Char. 21: 0 --> 1
Char. 24: 0 --> 1
Char. 27: 1 --> 0
Char. 31: 0 --> 1
Char. 39: 1 --> 2
Char. 45: 0 --> 1
Char. 103: 0 --> 1
Node 196 :
Char. 26: 0 --> 2
Char. 39: 1 --> 2
Char. 41: 1 --> 0
Char. 58: 1 --> 0
Char. 62: 1 --> 3
Char. 63: 2 --> 0
Node 197 :
Char. 39: 1 --> 2
Char. 42: 0 --> 1
Node 198 :
Char. 41: 2 --> 1
Char. 49: 0 --> 1
Char. 70: 1 --> 2
Char. 76: 0 --> 2
Node 199 :
Char. 63: 1 --> 2
Char. 101: 0 --> 2
Node 200 :
Char. 97: 0 --> 1
Node 201 :
Char. 128: 1 --> 0
Node 202 :
Char. 74: 2 --> 1
Char. 75: 0 --> 2
Char. 93: 0 --> 1
Node 203 :
Char. 3: 0 --> 1
Char. 97: 0 --> 1
Char. 116: 1 --> 0
Node 204 :
Char. 40: 1 --> 0
Node 205 :
Char. 67: 0 --> 1
Node 206 :
Char. 23: 1 --> 0
Char. 35: 0 --> 1
Char. 43: 0 --> 1
Char. 99: 0 --> 1
Node 207 :
Char. 0: 0 --> 1
Char. 11: 0 --> 1
Char. 42: 2 --> 0
Char. 47: 0 --> 1
Char. 64: 0 --> 1
Char. 75: 1 --> 0
Char. 99: 1 --> 0
Node 208 :
Char. 121: 1 --> 0
```

Appendix 6 - Nodes on tree of analysis under IW (k=10)



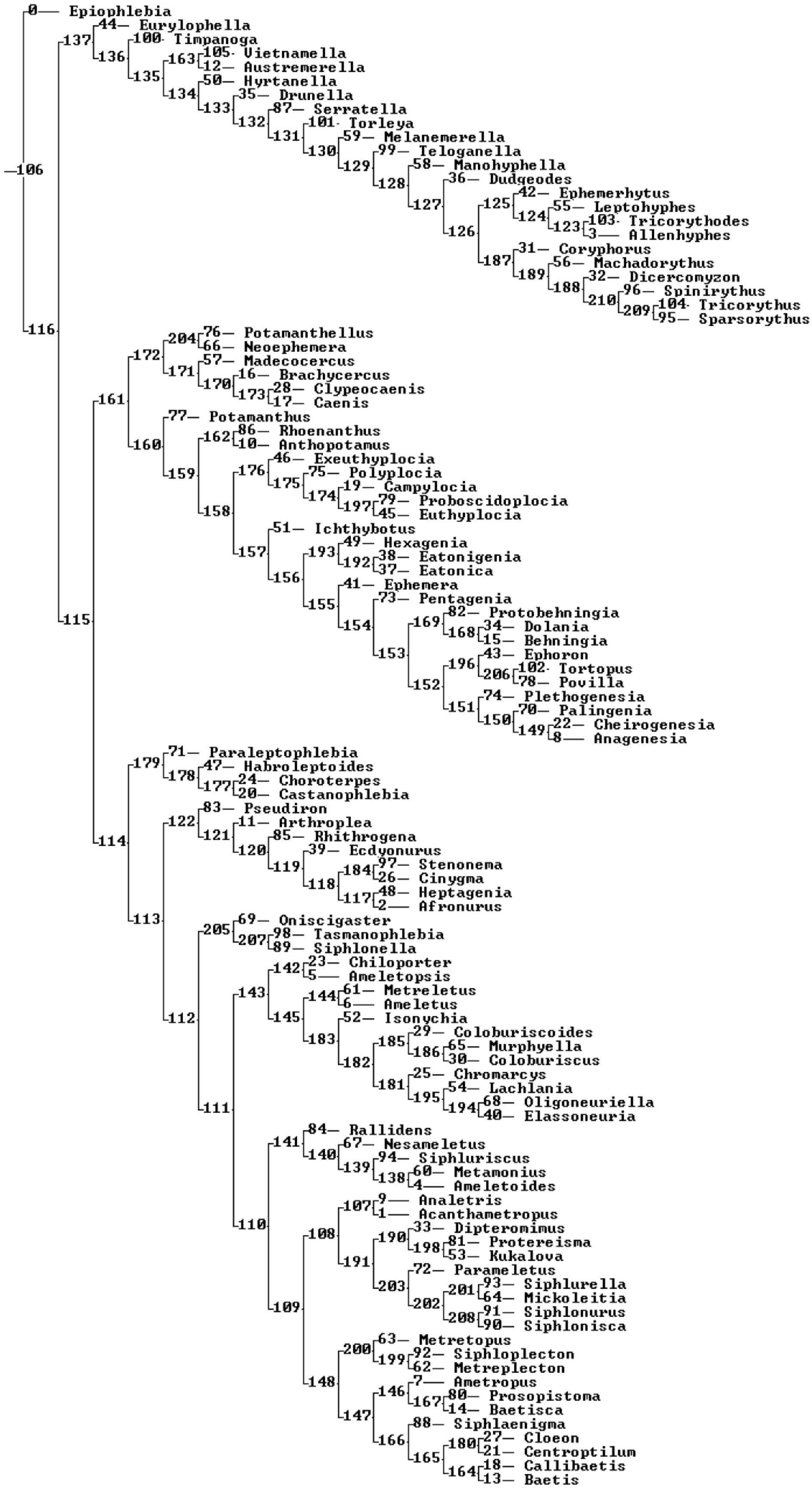
Appendix 7 - Synapomorphies for groups above family in tree found under IW with k = 10

Node 107 :
Char. 7: 0 --> 1
Char. 10: 0 --> 1
Char. 16: 0 --> 1
Node 108 :
No synapomorphies
Node 109 :
Char. 127: 1 --> 2
Node 110 :
Char. 125: 1 --> 0
Node 111 :
Char. 63: 1 --> 0
Node 112 :
Char. 67: 0 --> 1
Char. 75: 0 --> 1
Char. 77: 0 --> 1
Char. 127: 0 --> 1
Node 113 :
Char. 53: 0 --> 1
Char. 69: 2 --> 1
Node 114 :
Char. 26: 0 --> 4
Char. 51: 1 --> 0
Char. 70: 1 --> 2
Char. 74: 2 --> 1
Node 115 :
Char. 5: 1 --> 0
Char. 61: 3 --> 2
Char. 109: 0 --> 1
Node 116 :
Char. 113: 1 --> 0
Node 117 :
Char. 95: 1 --> 0
Node 118 :
Char. 26: 2 --> 0
Char. 27: 0 --> 1
Char. 37: 2 --> 1
Char. 118: 0 --> 1
Node 119 :
Char. 6: 0 --> 1
Char. 62: 2 --> 3
Node 120 :
Char. 47: 0 --> 1
Char. 94: 0 --> 1
Node 121 :
Char. 12: 0 --> 1
Char. 26: 0 --> 2
Char. 27: 1 --> 0
Char. 36: 0 --> 1
Char. 39: 2 --> 1
Char. 51: 0 --> 1
Char. 109: 1 --> 0
Node 122 :
Char. 69: 0 --> 2
Node 123 :
Char. 69: 2 --> 1
Char. 99: 0 --> 1
Char. 120: 2 --> 0
Node 124 :
Char. 13: 0 --> 1
Char. 38: 1 --> 0
Char. 68: 0 --> 1
Char. 76: 0 --> 1
Char. 114: 0 --> 1
Char. 123: 1 --> 0
Node 125 :
Char. 2: 1 --> 0
Char. 62: 1 --> 3
Char. 97: 1 --> 0
Node 126 :
Char. 22: 0 --> 1
Char. 88: 0 --> 1
Node 127 :
Char. 32: 0 --> 1
Char. 105: 0 --> 1
Char. 127: 0 --> 1
Node 128 :
Char. 14: 1 --> 0
Char. 37: 01 --> 2
Char. 62: 3 --> 1
Char. 98: 1 --> 0
Node 129 :
Char. 16: 1 --> 0
Char. 31: 0 --> 1
Char. 68: 1 --> 0
Char. 121: 1 --> 0
Node 130 :
Char. 75: 3 --> 0
Char. 104: 0 --> 1
Char. 122: 1 --> 0
Node 131 :
Char. 26: 0 --> 1
Char. 27: 1 --> 0
Char. 33: 0 --> 1
Char. 36: 0 --> 1
Char. 87: 0 --> 1
Node 132 :
Char. 40: 1 --> 0
Node 133 :
Char. 14: 0 --> 1
Node 134 :
Char. 38: 0 --> 1
Char. 100: 1 --> 0
Node 135 :
Char. 104: 1 --> 0
Node 136 :
Char. 36: 1 --> 0
Char. 42: 2 --> 1
Char. 66: 0 --> 1
Char. 70: 1 --> 0
Char. 71: 0 --> 2
Char. 79: 1 --> 2
Char. 97: 0 --> 1
Char. 98: 0 --> 1
Char. 116: 1 --> 0
Char. 119: 0 --> 1
Char. 120: 0 --> 2
Char. 128: 1 --> 0
Node 137 :
Char. 82: 0 --> 1
Char. 96: 1 --> 0
Char. 101: 0 --> 1
Node 138 :
Char. 33: 1 --> 0
Char. 39: 1 --> 0
Char. 47: 1 --> 0
Char. 75: 0 --> 3
Char. 118: 1 --> 0
Node 139 :
Char. 22: 0 --> 1
Char. 37: 0 --> 1
Node 140 :
Char. 52: 0 --> 1
Node 141 :
Char. 7: 1 --> 0
Char. 10: 1 --> 0
Char. 50: 0 --> 1
Char. 71: 0 --> 1
Node 142 :
Char. 37: 1 --> 0
Char. 64: 0 --> 1
Char. 114: 0 --> 1
Char. 115: 0 --> 2
Node 143 :
Char. 44: 1 --> 2
Char. 69: 2 --> 3
Char. 74: 2 --> 3
Char. 96: 1 --> 0
Char. 123: 1 --> 0
Char. 126: 0 --> 1
Node 144 :
Char. 22: 0 --> 1
Char. 51: 1 --> 0
Char. 62: 3 --> 0
Node 145 :
Char. 40: 0 --> 1
Node 146 :
Char. 6: 1 --> 0
Char. 67: 0 --> 1
Char. 114: 0 --> 1
Node 147 :
Char. 7: 1 --> 2
Char. 22: 0 --> 1
Char. 34: 1 --> 0
Node 148 :
Char. 56: 0 --> 1
Char. 128: 0 --> 1
Node 149 :
Char. 12: 1 --> 0
Char. 47: 0 --> 1
Char. 124: 1 --> 0
Node 150 :
Char. 18: 0 --> 1
Char. 24: 0 --> 1

```
Node 151 :
Char. 59: 0 --> 1
Char. 69: 2 --> 1
Char. 89: 0 --> 1
Node 152 :
Char. 0: 1 --> 0
Char. 10: 1 --> 3
Char. 21: 0 --> 1
Char. 22: 0 --> 1
Char. 27: 1 --> 0
Char. 128: 1 --> 0
Node 153 :
Char. 62: 3 --> 1
Char. 75: 2 --> 1
Char. 99: 0 --> 1
Node 154 :
Char. 37: 0 --> 1
Char. 56: 1 --> 0
Node 155 :
Char. 58: 0 --> 1
Node 156 :
Char. 63: 0 --> 2
Char. 74: 2 --> 1
Char. 78: 0 --> 1
Char. 82: 1 --> 0
Node 157 :
Char. 37: 1 --> 0
Char. 56: 0 --> 1
Char. 102: 0 --> 2
Node 158 :
Char. 55: 1 --> 2
Node 159 :
Char. 6: 1 --> 0
Char. 39: 0 --> 1
Char. 46: 1 --> 0
Char. 54: 0 --> 1
Char. 63: 1 --> 0
Char. 76: 1 --> 2
Char. 123: 1 --> 0
Char. 126: 0 --> 1
Node 160 :
Char. 23: 1 --> 0
Char. 76: 0 --> 1
Char. 81: 0 --> 1
Char. 94: 1 --> 0
Char. 112: 0 --> 1
Char. 120: 0 --> 1
Node 161 :
Char. 129: 2 --> 0
Node 162 :
Char. 87: 0 --> 1
Node 163 :
Char. 31: 0 --> 1
Char. 39: 1 --> 0
Node 164 :
Char. 20: 0 --> 1
Char. 49: 0 --> 1
Node 165 :
Char. 17: 1 --> 0
Char. 37: 2 --> 0
Char. 79: 1 --> 2
Char. 80: 0 --> 1
Char. 83: 2 --> 0
Char. 105: 0 --> 1
Char. 112: 0 --> 1
Char. 117: 1 --> 0
Char. 122: 1 --> 0
Char. 128: 1 --> 0
Node 166 :
Char. 62: 2 --> 3
Char. 99: 1 --> 0
Char. 113: 1 --> 0
Node 167 :
Char. 24: 0 --> 1
Char. 25: 1 --> 0
Char. 39: 1 --> 2
Char. 49: 0 --> 1
Char. 53: 0 --> 1
Char. 92: 1 --> 0
Node 168 :
Char. 7: 2 --> 3
Char. 11: 0 --> 1
Char. 37: 1 --> 2
Char. 42: 2 --> 0
Char. 45: 1 --> 2
Char. 54: 1 --> 0
Char. 74: 1 --> 2
Char. 75: 1 --> 3
Char. 101: 1 --> 0
Char. 103: 0 --> 1
Char. 106: 0 --> 1
Node 169 :
Char. 49: 0 --> 1
Char. 63: 1 --> 2
Char. 109: 1 --> 0
Node 170 :
Char. 2: 1 --> 0
Char. 13: 0 --> 1
Char. 16: 1 --> 0
Char. 20: 0 --> 1
Char. 22: 0 --> 1
Char. 26: 0 --> 2
Char. 27: 1 --> 0
Char. 30: 0 --> 2
Char. 42: 2 --> 0
Char. 71: 0 --> 1
Char. 75: 3 --> 0
Char. 83: 2 --> 0
Node 171 :
Char. 4: 0 --> 1
Char. 8: 1 --> 0
Char. 105: 0 --> 1
Char. 122: 1 --> 0
Node 172 :
Char. 94: 0 --> 1
Node 173 :
Char. 44: 1 --> 2
Char. 53: 0 --> 1
Char. 91: 0 --> 1
Node 174 :
Char. 55: 2 --> 3
Node 175 :
Char. 10: 1 --> 0
Char. 84: 0 --> 1
Node 176 :
Char. 2: 1 --> 0
Char. 21: 0 --> 1
Char. 42: 2 --> 0
Node 177 :
Char. 32: 0 --> 1
Char. 127: 0 --> 1
Node 178 :
Char. 26: 0 --> 2
Char. 38: 0 --> 1
Char. 61: 2 --> 1
Char. 63: 1 --> 0
Node 179 :
Char. 75: 0 --> 2
Node 180 :
Char. 11: 0 --> 1
Char. 12: 1 --> 2
Char. 18: 0 --> 1
Char. 40: 1 --> 0
Char. 46: 1 --> 0
Char. 83: 2 --> 0
Char. 96: 1 --> 0
Char. 113: 1 --> 0
Node 181 :
Char. 79: 1 --> 2
Char. 116: 1 --> 0
Node 182 :
Char. 35: 1 --> 2
Char. 62: 3 --> 1
Char. 64: 0 --> 1
Char. 69: 2 --> 1
Char. 74: 2 --> 1
Char. 89: 0 --> 1
Char. 109: 0 --> 1
Node 183 :
Char. 6: 1 --> 0
Char. 63: 1 --> 2
Char. 114: 0 --> 1
Node 184 :
Char. 35: 0 --> 1
Char. 45: 1 --> 0
Node 185 :
Char. 3: 0 --> 1
Char. 114: 0 --> 1
Node 186 :
Char. 59: 0 --> 1
Char. 62: 1 --> 4
Char. 63: 2 --> 0
Char. 65: 1 --> 0
Char. 70: 1 --> 2
Char. 75: 0 --> 1
Char. 78: 0 --> 1
Char. 87: 0 --> 1
Char. 90: 0 --> 1
Node 187 :
Char. 22: 0 --> 1
```

```
Char. 29: 0 --> 1
Char. 35: 2 --> 0
Char. 37: 1 --> 2
Char. 69: 1 --> 2
Char. 125: 1 --> 0
Node 188 :
Char. 30: 0 --> 2
Char. 72: 0 --> 1
Char. 73: 0 --> 1
Char. 87: 1 --> 0
Node 189 :
Char. 61: 2 --> 1
Char. 62: 1 --> 0
Char. 97: 1 --> 0
Char. 105: 1 --> 0
Node 190 :
Char. 12: 1 --> 0
Char. 68: 0 --> 1
Char. 83: 2 --> 0
Node 191 :
Char. 7: 1 --> 0
Char. 70: 1 --> 2
Char. 94: 1 --> 0
Char. 115: 0 --> 1
Node 192 :
Char. 21: 0 --> 1
Char. 42: 2 --> 1
Char. 126: 1 --> 0
Node 193 :
Char. 62: 3 --> 2
Node 194 :
Char. 43: 0 --> 1
Node 195 :
Char. 1: 1 --> 0
Char. 10: 1 --> 2
Char. 21: 0 --> 1
Char. 24: 0 --> 1
Char. 27: 1 --> 0
Char. 31: 0 --> 1
Char. 39: 1 --> 2
Char. 45: 0 --> 1
Char. 103: 0 --> 1
Node 196 :
Char. 26: 0 --> 2
Char. 39: 1 --> 2
Char. 41: 1 --> 0
Char. 58: 1 --> 0
Char. 62: 1 --> 3
Char. 63: 2 --> 0
Node 197 :
Char. 107: 1 --> 0
Node 198 :
Char. 39: 1 --> 2
Char. 42: 0 --> 1
Node 199 :
Char. 41: 2 --> 1
Char. 49: 0 --> 1
Char. 70: 1 --> 2
Char. 76: 0 --> 2
Node 200 :
Char. 63: 1 --> 2
Char. 97: 0 --> 1
Char. 101: 0 --> 2
Node 201 :
Char. 128: 1 --> 0
Node 202 :
Char. 44: 1 --> 2
Char. 74: 2 --> 1
Char. 75: 0 --> 2
Char. 93: 0 --> 1
Node 203 :
Char. 97: 0 --> 1
Char. 116: 1 --> 0
Node 204 :
Char. 40: 1 --> 0
Node 205 :
Char. 67: 0 --> 1
Node 206 :
Char. 23: 1 --> 0
Char. 43: 0 --> 1
Node 207 :
Char. 0: 0 --> 1
Char. 11: 0 --> 1
Char. 42: 2 --> 0
Char. 47: 0 --> 1
Char. 64: 0 --> 1
Char. 75: 1 --> 0
Char. 99: 1 --> 0
Node 208 :
Char. 110: 0 --> 1
Char. 121: 1 --> 0
Char. 122: 1 --> 0
Node 209 :
Char. 0: 1 --> 0
Char. 22: 1 --> 0
Char. 53: 0 --> 1
Node 210 :
Char. 13: 0 --> 1
Char. 60: 0 --> 1
```

Appendix 8 - Nodes on tree of analysis under IW (k=11)



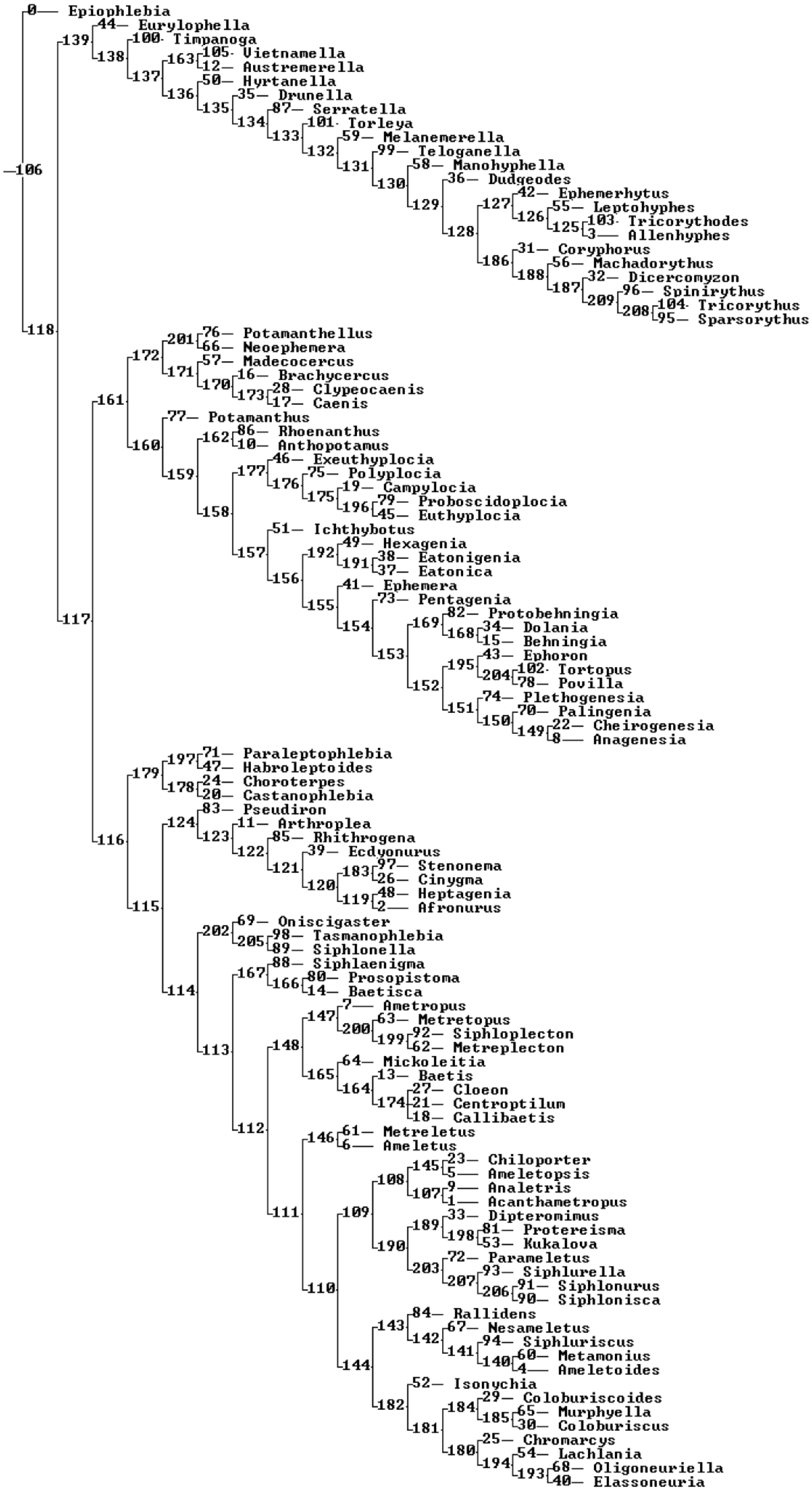
Appendix 9 - Synapomorphies for groups above family in tree found under IW with k = 11

Node 107 :
Char. 36: 1 --> 0
Char. 51: 1 --> 0
Char. 62: 3 --> 2
Char. 109: 0 --> 1
Node 108 :
Char. 39: 1 --> 2
Char. 94: 1 --> 0
Char. 96: 1 --> 0
Char. 123: 1 --> 0
Node 109 :
Char. 6: 1 --> 0
Char. 118: 1 --> 0
Node 110 :
Char. 95: 0 --> 1
Node 111 :
Char. 35: 1 --> 0
Char. 46: 0 --> 1
Char. 113: 0 --> 1
Node 112 :
Char. 5: 0 --> 1
Char. 109: 1 --> 0
Node 113 :
Char. 47: 0 --> 1
Char. 75: 3 --> 0
Char. 118: 0 --> 1
Char. 125: 0 --> 1
Node 114 :
Char. 82: 1 --> 0
Char. 96: 0 --> 1
Char. 101: 1 --> 0
Node 115 :
Char. 36: 0 --> 1
Char. 71: 2 --> 0
Char. 104: 0 --> 1
Char. 107: 0 --> 1
Node 116 :
No synapomorphies
Node 117 :
Char. 127: 1 --> 2
Node 118 :
Char. 125: 1 --> 0
Node 119 :
Char. 35: 1 --> 0
Char. 63: 1 --> 0
Node 120 :
Char. 67: 0 --> 1
Char. 75: 0 --> 1
Char. 77: 0 --> 1
Char. 127: 0 --> 1
Node 121 :
Char. 46: 0 --> 1
Char. 53: 0 --> 1
Char. 69: 2 --> 1
Node 122 :
Char. 26: 0 --> 4
Char. 51: 1 --> 0
Char. 74: 2 --> 1
Node 123 :
Char. 69: 2 --> 1
Char. 99: 0 --> 1
Char. 120: 2 --> 0
Node 124 :
Char. 13: 0 --> 1
Char. 38: 1 --> 0
Char. 68: 0 --> 1
Char. 76: 0 --> 1
Char. 114: 0 --> 1
Char. 123: 1 --> 0
Node 125 :
Char. 2: 1 --> 0
Char. 62: 1 --> 3
Char. 97: 1 --> 0
Node 126 :
Char. 22: 0 --> 1
Char. 88: 0 --> 1
Node 127 :
Char. 32: 0 --> 1
Char. 105: 0 --> 1
Char. 127: 0 --> 1
Node 128 :
Char. 14: 1 --> 0
Char. 37: 01 --> 2
Char. 62: 3 --> 1
Char. 98: 1 --> 0
Node 129 :
Char. 16: 1 --> 0
Char. 31: 0 --> 1
Char. 68: 1 --> 0
Char. 121: 1 --> 0
Node 130 :
Char. 75: 3 --> 0
Char. 104: 0 --> 1
Char. 122: 1 --> 0
Node 131 :
Char. 26: 0 --> 1
Char. 27: 1 --> 0
Char. 33: 0 --> 1
Char. 36: 0 --> 1
Char. 87: 0 --> 1
Node 132 :
Char. 22: 1 --> 0
Char. 40: 1 --> 0
Node 133 :
Char. 14: 0 --> 1
Node 134 :
Char. 38: 0 --> 1
Node 135 :
Char. 22: 0 --> 1
Char. 107: 0 --> 1
Node 136 :
Char. 35: 1 --> 0
Char. 39: 1 --> 0
Char. 46: 0 --> 1
Node 137 :
Char. 3: 0 --> 1
Char. 40: 0 --> 1
Char. 66: 0 --> 1
Char. 79: 1 --> 2
Char. 97: 0 --> 1
Char. 99: 0 --> 1
Node 138 :
Char. 22: 0 --> 1
Char. 37: 0 --> 1
Node 139 :
Char. 52: 0 --> 1
Node 140 :
Char. 7: 1 --> 0
Char. 10: 1 --> 0
Char. 50: 0 --> 1
Char. 71: 0 --> 1
Node 141 :
Char. 64: 0 --> 1
Char. 114: 0 --> 1
Char. 115: 0 --> 2
Node 142 :
Char. 44: 1 --> 2
Char. 69: 2 --> 3
Char. 74: 2 --> 3
Char. 96: 1 --> 0
Char. 126: 0 --> 1
Node 143 :
Char. 40: 0 --> 1
Char. 62: 3 --> 0
Node 144 :
Char. 67: 0 --> 1
Node 145 :
Char. 6: 1 --> 0
Char. 114: 0 --> 1
Node 146 :
Char. 33: 1 --> 0
Char. 94: 1 --> 0
Char. 126: 0 --> 1
Node 147 :
Char. 22: 0 --> 1
Char. 34: 1 --> 0
Node 148 :
Char. 26: 0 --> 2
Char. 27: 1 --> 0
Char. 37: 01 --> 2
Char. 44: 1 --> 2
Node 149 :
Char. 56: 0 --> 1
Char. 128: 0 --> 1
Node 150 :
Char. 12: 1 --> 0
Char. 47: 0 --> 1
Char. 124: 1 --> 0
Node 151 :
Char. 18: 0 --> 1
Char. 24: 0 --> 1
Node 152 :
Char. 59: 0 --> 1
Char. 69: 2 --> 1

Char. 89: 0 --> 1
Node 153 :
Char. 0: 1 --> 0
Char. 10: 1 --> 3
Char. 21: 0 --> 1
Char. 22: 0 --> 1
Char. 27: 1 --> 0
Char. 128: 1 --> 0
Node 154 :
Char. 62: 3 --> 1
Char. 75: 2 --> 1
Char. 99: 0 --> 1
Node 155 :
Char. 37: 0 --> 1
Char. 56: 1 --> 0
Node 156 :
Char. 58: 0 --> 1
Node 157 :
Char. 63: 0 --> 2
Char. 74: 2 --> 1
Char. 78: 0 --> 1
Char. 82: 1 --> 0
Node 158 :
Char. 37: 1 --> 0
Char. 56: 0 --> 1
Char. 102: 0 --> 2
Node 159 :
Char. 55: 1 --> 2
Node 160 :
Char. 6: 1 --> 0
Char. 35: 1 --> 0
Char. 54: 0 --> 1
Char. 63: 1 --> 0
Char. 76: 1 --> 2
Char. 123: 1 --> 0
Char. 125: 0 --> 1
Char. 126: 0 --> 1
Node 161 :
Char. 23: 1 --> 0
Char. 81: 0 --> 1
Char. 94: 1 --> 0
Char. 112: 0 --> 1
Char. 120: 0 --> 1
Node 162 :
Char. 129: 2 --> 0
Node 163 :
Char. 87: 0 --> 1
Char. 104: 0 --> 1
Char. 125: 0 --> 1
Node 164 :
Char. 15: 0 --> 1
Char. 69: 2 --> 1
Node 165 :
Char. 3: 0 --> 2
Char. 6: 0 --> 1
Char. 14: 0 --> 1
Char. 16: 1 --> 0
Char. 20: 0 --> 1
Char. 31: 0 --> 1
Char. 41: 2 --> 0
Char. 42: 2 --> 1
Char. 45: 1 --> 0
Char. 48: 1 --> 0
Char. 49: 0 --> 1
Char. 83: 2 --> 1
Char. 128: 1 --> 0
Node 166 :
Char. 39: 1 --> 0
Char. 53: 0 --> 1
Node 167 :
Char. 17: 1 --> 0
Char. 37: 2 --> 0
Char. 71: 0 --> 2
Char. 79: 1 --> 2
Char. 80: 0 --> 1
Char. 83: 2 --> 0
Char. 99: 1 --> 0
Char. 105: 0 --> 1
Char. 112: 0 --> 1
Char. 113: 1 --> 0
Char. 117: 1 --> 0
Char. 122: 1 --> 0
Char. 123: 1 --> 0
Char. 128: 1 --> 0
Node 168 :
Char. 24: 0 --> 1
Char. 25: 1 --> 0
Char. 39: 1 --> 2
Char. 49: 0 --> 1
Char. 53: 0 --> 1
Char. 92: 1 --> 0
Node 169 :
Char. 7: 2 --> 3
Char. 11: 0 --> 1
Char. 37: 1 --> 2
Char. 42: 2 --> 0
Char. 45: 1 --> 2
Char. 54: 1 --> 0
Char. 74: 1 --> 2
Char. 75: 1 --> 3
Char. 101: 1 --> 0
Char. 103: 0 --> 1
Char. 106: 0 --> 1
Node 170 :
Char. 49: 0 --> 1
Char. 63: 1 --> 2
Char. 109: 1 --> 0
Node 171 :
Char. 2: 1 --> 0
Char. 13: 0 --> 1
Char. 16: 1 --> 0
Char. 20: 0 --> 1
Char. 22: 0 --> 1
Char. 26: 0 --> 2
Char. 27: 1 --> 0
Char. 30: 0 --> 2
Char. 42: 2 --> 0
Char. 71: 0 --> 1
Char. 75: 3 --> 0
Char. 83: 2 --> 0
Node 172 :
Char. 4: 0 --> 1
Char. 8: 1 --> 0
Char. 46: 0 --> 1
Char. 99: 0 --> 1
Char. 105: 0 --> 1
Char. 122: 1 --> 0
Node 173 :
Char. 94: 0 --> 1
Node 174 :
Char. 55: 2 --> 3
Node 175 :
Char. 10: 1 --> 0
Char. 84: 0 --> 1
Node 176 :
Char. 2: 1 --> 0
Char. 21: 0 --> 1
Char. 42: 2 --> 0
Node 177 :
Char. 32: 0 --> 1
Char. 127: 0 --> 1
Node 178 :
Char. 16: 1 --> 0
Char. 34: 1 --> 0
Char. 39: 1 --> 0
Char. 62: 3 --> 0
Node 179 :
Char. 3: 0 --> 1
Char. 26: 0 --> 2
Char. 61: 2 --> 1
Char. 63: 1 --> 0
Node 180 :
Char. 75: 04 --> 2
Node 181 :
Char. 11: 0 --> 1
Char. 12: 1 --> 2
Char. 18: 0 --> 1
Char. 40: 1 --> 0
Char. 46: 1 --> 0
Char. 83: 2 --> 0
Char. 96: 1 --> 0
Char. 113: 1 --> 0
Node 182 :
Char. 79: 1 --> 2
Char. 99: 0 --> 1
Char. 116: 1 --> 0
Node 183 :
Char. 35: 0 --> 2
Char. 45: 1 --> 0
Char. 62: 0 --> 1
Char. 63: 1 --> 2
Char. 64: 0 --> 1
Char. 69: 2 --> 1
Char. 74: 2 --> 1
Char. 89: 0 --> 1
Char. 109: 0 --> 1
Node 184 :
Char. 3: 0 --> 1
Char. 114: 0 --> 1
Node 185 :

```
Char. 59: 0 --> 1
Char. 62: 1 --> 4
Char. 63: 2 --> 0
Char. 65: 1 --> 0
Char. 70: 1 --> 2
Char. 75: 0 --> 1
Char. 78: 0 --> 1
Char. 87: 0 --> 1
Char. 90: 0 --> 1
Node 186 :
Char. 22: 0 --> 1
Char. 29: 0 --> 1
Char. 35: 2 --> 0
Char. 37: 1 --> 2
Char. 69: 1 --> 2
Char. 125: 1 --> 0
Node 187 :
Char. 30: 0 --> 2
Char. 72: 0 --> 1
Char. 73: 0 --> 1
Char. 87: 1 --> 0
Node 188 :
Char. 61: 2 --> 1
Char. 62: 1 --> 0
Char. 97: 1 --> 0
Char. 105: 1 --> 0
Node 189 :
Char. 12: 1 --> 0
Char. 68: 0 --> 1
Char. 83: 2 --> 0
Node 190 :
Char. 16: 1 --> 0
Char. 49: 0 --> 1
Node 191 :
Char. 7: 1 --> 0
Char. 10: 1 --> 0
Char. 63: 1 --> 2
Char. 70: 1 --> 2
Char. 76: 0 --> 12
Char. 114: 0 --> 1
Node 192 :
Char. 21: 0 --> 1
Char. 42: 2 --> 1
Char. 126: 1 --> 0
Node 193 :
Char. 62: 3 --> 2
Node 194 :
Char. 43: 0 --> 1
Node 195 :
Char. 1: 1 --> 0
Char. 10: 1 --> 2
Char. 21: 0 --> 1
Char. 24: 0 --> 1
Char. 27: 1 --> 0
Char. 31: 0 --> 1
Char. 39: 1 --> 2
Char. 45: 0 --> 1
Char. 103: 0 --> 1
Node 196 :
Char. 26: 0 --> 2
Char. 39: 1 --> 2
Char. 41: 1 --> 0
Char. 58: 1 --> 0
Char. 62: 1 --> 3
Char. 63: 2 --> 0
Node 197 :
Char. 39: 1 --> 2
Char. 42: 0 --> 1
Node 198 :
Char. 24: 0 --> 1
Node 199 :
Char. 128: 1 --> 0
Node 200 :
Char. 62: 3 --> 2
Char. 74: 2 --> 1
Char. 75: 0 --> 2
Char. 93: 0 --> 1
Node 201 :
Char. 110: 0 --> 1
Node 202 :
Char. 96: 0 --> 1
Char. 117: 1 --> 0
Char. 123: 0 --> 1
Node 203 :
Char. 23: 1 --> 0
Char. 45: 1 --> 0
Node 204 :
Char. 3: 0 --> 1
Char. 97: 0 --> 1
Char. 116: 1 --> 0
Node 205 :
Char. 97: 0 --> 1
Char. 110: 0 --> 1
Node 206 :
Char. 0: 0 --> 1
Char. 11: 0 --> 1
Char. 42: 2 --> 0
Char. 47: 0 --> 1
Char. 64: 0 --> 1
Char. 75: 1 --> 0
Char. 99: 1 --> 0
Node 207 :
Char. 35: 1 --> 2
Char. 39: 1 --> 2
Char. 63: 1 --> 2
Char. 101: 0 --> 2
Char. 121: 1 --> 0
Char. 122: 1 --> 0
Node 208 :
Char. 35: 0 --> 1
Char. 40: 0 --> 1
Node 209 :
Char. 0: 1 --> 0
Char. 22: 1 --> 0
Char. 53: 0 --> 1
Node 210 :
Char. 13: 0 --> 1
Char. 60: 0 --> 1
```

Appendix 10 - Nodes on tree of analysis under IW (k=12)



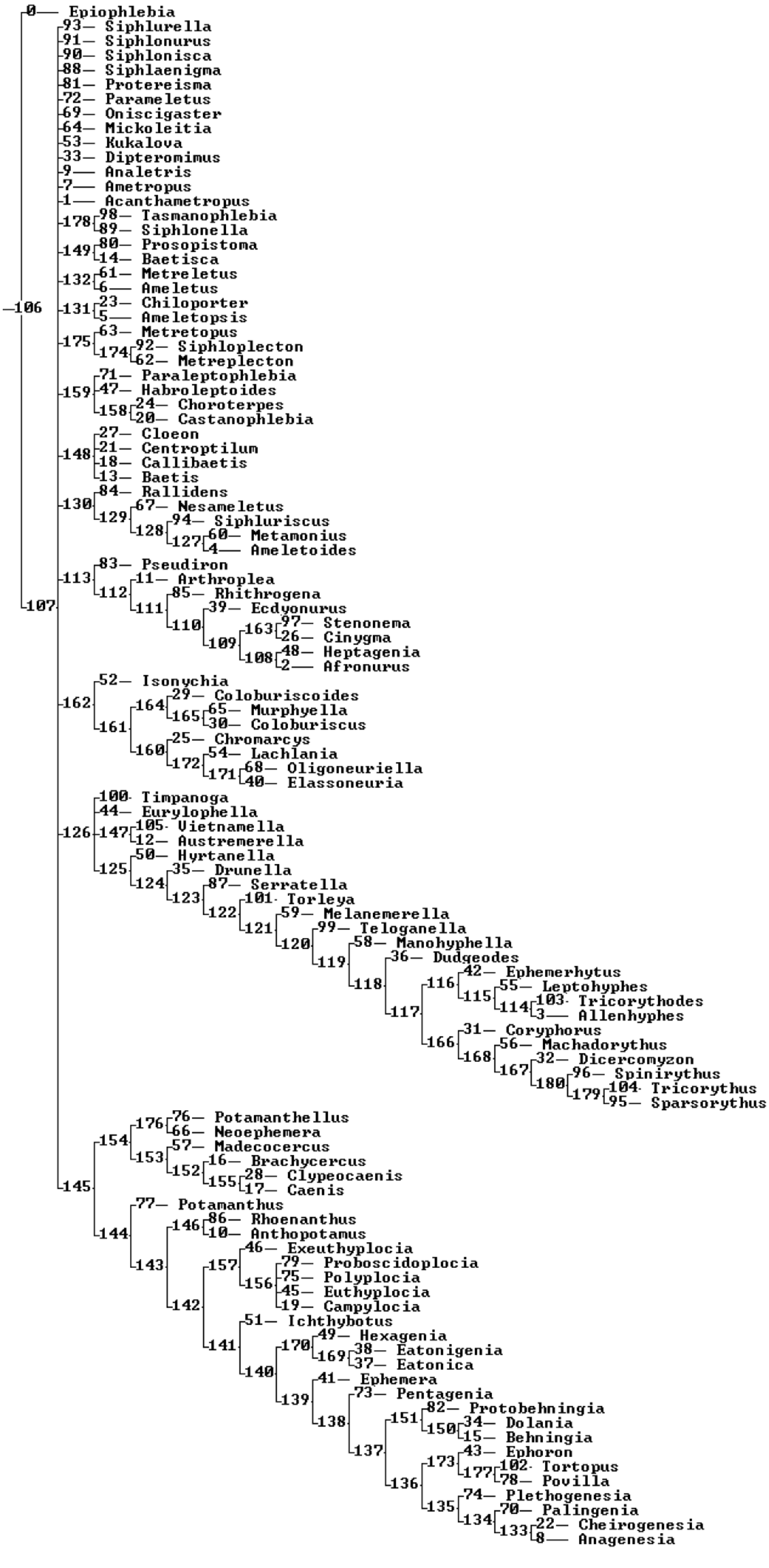
Appendix 11 - Synapomorphies for groups above family in tree found under IW with k = 12

- Node 107 :
  - Char. 36: 1 --> 0
  - Char. 99: 0 --> 1
- Node 108 :
  - Char. 51: 1 --> 0
  - Char. 114: 1 --> 0
- Node 109 :
  - Char. 39: 1 --> 2
  - Char. 94: 1 --> 0
  - Char. 96: 1 --> 0
- Node 110 :
  - Char. 22: 1 --> 0
- Node 111 :
  - Char. 27: 0 --> 1
- Node 112 :
  - Char. 35: 1 --> 0
  - Char. 46: 0 --> 1
  - Char. 113: 0 --> 1
- Node 113 :
  - Char. 6: 1 --> 0
  - Char. 22: 0 --> 1
- Node 114 :
  - Char. 5: 0 --> 1
  - Char. 61: 2 --> 3
  - Char. 109: 1 --> 0
- Node 115 :
  - Char. 33: 0 --> 1
  - Char. 47: 0 --> 1
  - Char. 75: 3 --> 0
  - Char. 125: 0 --> 1
- Node 116 :
  - Char. 27: 1 --> 0
  - Char. 82: 1 --> 0
  - Char. 96: 0 --> 1
  - Char. 101: 1 --> 0
- Node 117 :
  - Char. 36: 0 --> 1
  - Char. 71: 2 --> 0
  - Char. 104: 0 --> 1
  - Char. 107: 0 --> 1
- Node 118 :
  - No synapomorphies
- Node 119 :
  - Char. 127: 1 --> 2
- Node 120 :
  - Char. 125: 1 --> 0
- Node 121 :
  - Char. 35: 1 --> 0
  - Char. 63: 1 --> 0
- Node 122 :
  - Char. 67: 0 --> 1
  - Char. 75: 0 --> 1
  - Char. 77: 0 --> 1
  - Char. 127: 0 --> 1
- Node 123 :
  - Char. 46: 0 --> 1
  - Char. 53: 0 --> 1
  - Char. 69: 2 --> 1
- Node 124 :
  - Char. 26: 02 --> 4
  - Char. 51: 1 --> 0
  - Char. 70: 1 --> 2
  - Char. 74: 2 --> 1
- Node 125 :
  - Char. 69: 2 --> 1
  - Char. 99: 0 --> 1
  - Char. 120: 2 --> 0
- Node 126 :
  - Char. 13: 0 --> 1
  - Char. 38: 1 --> 0
  - Char. 68: 0 --> 1
  - Char. 76: 0 --> 1
  - Char. 114: 0 --> 1
  - Char. 123: 1 --> 0
- Node 127 :
  - Char. 2: 1 --> 0
  - Char. 62: 1 --> 3
  - Char. 97: 1 --> 0
- Node 128 :
  - Char. 22: 0 --> 1
  - Char. 88: 0 --> 1
- Node 129 :
  - Char. 32: 0 --> 1
  - Char. 105: 0 --> 1
  - Char. 127: 0 --> 1
- Node 130 :
  - Char. 14: 1 --> 0
  - Char. 37: 01 --> 2
  - Char. 62: 3 --> 1
  - Char. 98: 1 --> 0
- Node 131 :
  - Char. 16: 1 --> 0
  - Char. 31: 0 --> 1
  - Char. 68: 1 --> 0
  - Char. 121: 1 --> 0
- Node 132 :
  - Char. 75: 3 --> 0
  - Char. 104: 0 --> 1
  - Char. 122: 1 --> 0
- Node 133 :
  - Char. 26: 0 --> 1
  - Char. 27: 1 --> 0
  - Char. 33: 0 --> 1
  - Char. 36: 0 --> 1
  - Char. 87: 0 --> 1
- Node 134 :
  - Char. 22: 1 --> 0
  - Char. 40: 1 --> 0
- Node 135 :
  - Char. 14: 0 --> 1
- Node 136 :
  - Char. 38: 0 --> 1
- Node 137 :
  - Char. 22: 0 --> 1
  - Char. 107: 0 --> 1
- Node 138 :
  - Char. 35: 1 --> 0
  - Char. 39: 1 --> 0
  - Char. 46: 0 --> 1
- Node 139 :
  - Char. 3: 0 --> 1
  - Char. 40: 0 --> 1
  - Char. 66: 0 --> 1
  - Char. 79: 1 --> 2
  - Char. 97: 0 --> 1
  - Char. 99: 0 --> 1
- Node 140 :
  - Char. 22: 0 --> 1
  - Char. 37: 0 --> 1
- Node 141 :
  - Char. 52: 0 --> 1
- Node 142 :
  - Char. 7: 1 --> 0
  - Char. 10: 1 --> 0
  - Char. 50: 0 --> 1
  - Char. 71: 0 --> 1
- Node 143 :
  - Char. 6: 0 --> 1
  - Char. 65: 1 --> 0
- Node 144 :
  - Char. 64: 0 --> 1
- Node 145 :
  - Char. 6: 0 --> 1
  - Char. 44: 1 --> 2
  - Char. 61: 3 --> 0
  - Char. 69: 2 --> 3
  - Char. 74: 2 --> 3
  - Char. 126: 0 --> 1
- Node 146 :
  - Char. 3: 0 --> 1
  - Char. 51: 1 --> 0
  - Char. 61: 3 --> 1
  - Char. 62: 3 --> 0
  - Char. 67: 0 --> 1
- Node 147 :
  - Char. 62: 3 --> 2
  - Char. 70: 1 --> 2
  - Char. 99: 0 --> 1
- Node 148 :
  - Char. 37: 1 --> 2
  - Char. 75: 0 --> 2
- Node 149 :
  - Char. 56: 0 --> 1
  - Char. 128: 0 --> 1
- Node 150 :
  - Char. 12: 1 --> 0
  - Char. 47: 0 --> 1
  - Char. 124: 1 --> 0
- Node 151 :
  - Char. 18: 0 --> 1
  - Char. 24: 0 --> 1
- Node 152 :
  - Char. 59: 0 --> 1
  - Char. 69: 2 --> 1

```
  Char. 89: 0 --> 1
Node 153 :
  Char. 0: 1 --> 0
  Char. 10: 1 --> 3
  Char. 21: 0 --> 1
  Char. 22: 0 --> 1
  Char. 27: 1 --> 0
  Char. 128: 1 --> 0
Node 154 :
  Char. 62: 3 --> 1
  Char. 75: 2 --> 1
  Char. 99: 0 --> 1
Node 155 :
  Char. 37: 0 --> 1
  Char. 56: 1 --> 0
Node 156 :
  Char. 58: 0 --> 1
Node 157 :
  Char. 63: 0 --> 2
  Char. 74: 2 --> 1
  Char. 78: 0 --> 1
  Char. 82: 1 --> 0
Node 158 :
  Char. 37: 1 --> 0
  Char. 56: 0 --> 1
  Char. 102: 0 --> 2
Node 159 :
  Char. 55: 1 --> 2
Node 160 :
  Char. 6: 1 --> 0
  Char. 35: 1 --> 0
  Char. 54: 0 --> 1
  Char. 63: 1 --> 0
  Char. 76: 1 --> 2
  Char. 123: 1 --> 0
  Char. 125: 0 --> 1
  Char. 126: 0 --> 1
Node 161 :
  Char. 23: 1 --> 0
  Char. 76: 0 --> 1
  Char. 81: 0 --> 1
  Char. 94: 1 --> 0
  Char. 112: 0 --> 1
  Char. 120: 0 --> 1
Node 162 :
  Char. 129: 2 --> 0
Node 163 :
  Char. 87: 0 --> 1
  Char. 104: 0 --> 1
  Char. 125: 0 --> 1
Node 164 :
  Char. 31: 0 --> 1
  Char. 39: 1 --> 0
Node 165 :
  Char. 20: 0 --> 1
  Char. 49: 0 --> 1
Node 166 :
  Char. 17: 1 --> 0
  Char. 33: 1 --> 0
  Char. 37: 1 --> 0
  Char. 79: 1 --> 2
  Char. 80: 0 --> 1
  Char. 83: 2 --> 0
  Char. 94: 1 --> 0
  Char. 105: 0 --> 1
  Char. 112: 0 --> 1
  Char. 122: 1 --> 0
  Char. 126: 0 --> 1
  Char. 128: 1 --> 0
Node 167 :
  Char. 7: 1 --> 2
  Char. 123: 1 --> 0
Node 168 :
  Char. 24: 0 --> 1
  Char. 25: 1 --> 0
  Char. 39: 1 --> 2
  Char. 49: 0 --> 1
  Char. 53: 0 --> 1
  Char. 92: 1 --> 0
Node 169 :
  Char. 7: 2 --> 3
  Char. 11: 0 --> 1
  Char. 37: 1 --> 2
  Char. 42: 2 --> 0
  Char. 45: 1 --> 2
  Char. 54: 1 --> 0
  Char. 74: 1 --> 2
  Char. 75: 1 --> 3
  Char. 101: 1 --> 0
  Char. 103: 0 --> 1
  Char. 106: 0 --> 1
Node 170 :
  Char. 49: 0 --> 1
  Char. 63: 1 --> 2
  Char. 109: 1 --> 0
Node 171 :
  Char. 2: 1 --> 0
  Char. 13: 0 --> 1
  Char. 16: 1 --> 0
  Char. 20: 0 --> 1
  Char. 22: 0 --> 1
  Char. 26: 0 --> 2
  Char. 27: 1 --> 0
  Char. 30: 0 --> 2
  Char. 42: 2 --> 0
  Char. 71: 0 --> 1
  Char. 75: 3 --> 0
  Char. 83: 2 --> 0
Node 172 :
  Char. 4: 0 --> 1
  Char. 8: 1 --> 0
  Char. 46: 0 --> 1
  Char. 99: 0 --> 1
  Char. 105: 0 --> 1
  Char. 122: 1 --> 0
Node 173 :
  Char. 94: 0 --> 1
Node 174 :
  Char. 46: 1 --> 0
  Char. 53: 0 --> 1
  Char. 91: 0 --> 1
  Char. 95: 0 --> 1
  Char. 99: 0 --> 1
Node 175 :
  Char. 55: 2 --> 3
Node 176 :
  Char. 10: 1 --> 0
  Char. 84: 0 --> 1
Node 177 :
  Char. 2: 1 --> 0
  Char. 21: 0 --> 1
  Char. 42: 2 --> 0
Node 178 :
  Char. 32: 0 --> 1
  Char. 127: 0 --> 1
Node 179 :
  Char. 3: 0 --> 1
  Char. 38: 0 --> 1
  Char. 61: 2 --> 1
  Char. 63: 1 --> 0
Node 180 :
  Char. 11: 0 --> 1
  Char. 12: 1 --> 2
  Char. 18: 0 --> 1
  Char. 46: 1 --> 0
  Char. 83: 2 --> 0
  Char. 96: 1 --> 0
  Char. 113: 1 --> 0
Node 181 :
  Char. 79: 1 --> 2
  Char. 116: 1 --> 0
Node 182 :
  Char. 45: 1 --> 0
  Char. 62: 3 --> 1
  Char. 63: 1 --> 2
  Char. 69: 2 --> 1
  Char. 74: 2 --> 1
  Char. 89: 0 --> 1
Node 183 :
  Char. 3: 0 --> 1
  Char. 114: 0 --> 1
Node 184 :
  Char. 59: 0 --> 1
  Char. 62: 1 --> 4
  Char. 63: 2 --> 0
  Char. 65: 1 --> 0
  Char. 70: 1 --> 2
  Char. 75: 0 --> 1
  Char. 78: 0 --> 1
  Char. 87: 0 --> 1
  Char. 90: 0 --> 1
Node 185 :
  Char. 22: 0 --> 1
  Char. 29: 0 --> 1
  Char. 35: 2 --> 0
  Char. 37: 1 --> 2
  Char. 69: 1 --> 2
  Char. 125: 1 --> 0
Node 186 :
  Char. 30: 0 --> 2
```

```
  Char. 72: 0 --> 1
  Char. 73: 0 --> 1
  Char. 87: 1 --> 0
Node 187 :
  Char. 61: 2 --> 1
  Char. 62: 1 --> 0
  Char. 97: 1 --> 0
  Char. 105: 1 --> 0
Node 188 :
  Char. 12: 1 --> 0
  Char. 68: 0 --> 1
  Char. 83: 2 --> 0
Node 189 :
  Char. 16: 1 --> 0
  Char. 49: 0 --> 1
Node 190 :
  Char. 7: 1 --> 0
  Char. 10: 1 --> 0
  Char. 63: 1 --> 2
  Char. 70: 1 --> 2
  Char. 76: 0 --> 12
Node 191 :
  Char. 21: 0 --> 1
  Char. 42: 2 --> 1
  Char. 126: 1 --> 0
Node 192 :
  Char. 62: 3 --> 2
Node 193 :
  Char. 43: 0 --> 1
Node 194 :
  Char. 1: 1 --> 0
  Char. 10: 1 --> 2
  Char. 21: 0 --> 1
  Char. 24: 0 --> 1
  Char. 27: 1 --> 0
  Char. 31: 0 --> 1
  Char. 39: 1 --> 2
  Char. 45: 0 --> 1
  Char. 103: 0 --> 1
Node 195 :
  Char. 26: 0 --> 2
  Char. 39: 1 --> 2
  Char. 41: 1 --> 0
  Char. 58: 1 --> 0
  Char. 62: 1 --> 3
  Char. 63: 2 --> 0
Node 196 :
  Char. 39: 1 --> 2
  Char. 42: 0 --> 1
Node 197 :
  Char. 41: 2 --> 1
  Char. 49: 0 --> 1
  Char. 70: 1 --> 2
  Char. 76: 0 --> 2
Node 198 :
  Char. 24: 0 --> 1
Node 199 :
  Char. 128: 1 --> 0
Node 200 :
  Char. 22: 1 --> 0
  Char. 74: 2 --> 1
  Char. 93: 0 --> 1
Node 201 :
  Char. 3: 0 --> 1
  Char. 97: 0 --> 1
  Char. 116: 1 --> 0
Node 202 :
  Char. 27: 0 --> 1
  Char. 97: 0 --> 1
  Char. 99: 0 --> 1
  Char. 110: 0 --> 1
Node 203 :
  Char. 23: 1 --> 0
  Char. 43: 0 --> 1
  Char. 45: 1 --> 0
Node 204 :
  Char. 0: 0 --> 1
  Char. 11: 0 --> 1
  Char. 42: 2 --> 0
  Char. 47: 0 --> 1
  Char. 64: 0 --> 1
  Char. 75: 1 --> 0
  Char. 99: 1 --> 0
Node 205 :
  Char. 35: 1 --> 2
  Char. 39: 1 --> 2
  Char. 63: 1 --> 2
  Char. 101: 0 --> 2
  Char. 121: 1 --> 0
  Char. 122: 1 --> 0
Node 206 :
  Char. 40: 0 --> 1
Node 207 :
  Char. 96: 0 --> 1
  Char. 99: 0 --> 1
  Char. 117: 1 --> 0
  Char. 123: 0 --> 1
Node 208 :
  Char. 0: 1 --> 0
  Char. 22: 1 --> 0
  Char. 53: 0 --> 1
Node 209 :
  Char. 13: 0 --> 1
  Char. 60: 0 --> 1
```

Appendix 12- Nodes on Strict consensus of 5 MPT found using New Technology under IW (1 MPT from each analysis with K values 8, 9, 10, 11 and 12).



Appendix 13 - Common synapomorphies to the strict consensus of 5 MPT found using New Technology under IW (1 MPT from each analysis with K values 8, 9, 10, 11 and 12). Nodes relative to genus were excluded from the list.

Node 107 :
  All trees:
    No synapomorphies

Node 108 :
  All trees:
    Char. 127: 1 --> 2

Node 109 :
  All trees:
    Char. 125: 1 --> 0

Node 110 :
  All trees:
    Char. 63: 1 --> 0
  Some trees:
    Char. 35: 1 --> 0

Node 111 :
  All trees:
    Char. 67: 0 --> 1
    Char. 75: 0 --> 1
    Char. 77: 0 --> 1
    Char. 127: 0 --> 1

Node 112 :
  All trees:
    Char. 53: 0 --> 1
    Char. 69: 2 --> 1
  Some trees:
    Char. 46: 0 --> 1
    Char. 94: 01 --> 1

Node 113 :
  All trees:
    Char. 26: 0 --> 4
    Char. 51: 1 --> 0
    Char. 74: 2 --> 1
  Some trees:
    Char. 5: 1 --> 0
    Char. 6: 0 --> 1
    Char. 27: 1 --> 0
    Char. 61: 3 --> 2
    Char. 70: 1 --> 2
    Char. 113: 1 --> 0
    Char. 118: 0 --> 1

Node 114 :
  All trees:
    Char. 69: 2 --> 1
    Char. 99: 0 --> 1
    Char. 120: 2 --> 0

Node 115 :
  All trees:
    Char. 13: 0 --> 1
    Char. 38: 1 --> 0
    Char. 68: 0 --> 1
    Char. 76: 0 --> 1
    Char. 114: 0 --> 1
    Char. 123: 1 --> 0

Node 116 :
  All trees:
    Char. 2: 1 --> 0
    Char. 62: 1 --> 3
    Char. 97: 1 --> 0

Node 117 :
  All trees:
    Char. 22: 0 --> 1
    Char. 88: 0 --> 1

Node 118 :
  All trees:
    Char. 32: 0 --> 1
    Char. 105: 0 --> 1
    Char. 127: 0 --> 1

Node 119 :
  All trees:
    Char. 14: 1 --> 0
    Char. 37: 01 --> 2
    Char. 62: 3 --> 1
    Char. 98: 1 --> 0

Node 120 :
  All trees:
    Char. 16: 1 --> 0
    Char. 31: 0 --> 1
    Char. 68: 1 --> 0
    Char. 121: 1 --> 0

Node 121 :
  All trees:
    Char. 75: 3 --> 0
    Char. 104: 0 --> 1
    Char. 122: 1 --> 0

Node 122 :
  All trees:
    Char. 26: 0 --> 1
    Char. 27: 1 --> 0
    Char. 33: 0 --> 1
    Char. 36: 0 --> 1
    Char. 87: 0 --> 1

Node 123 :
  All trees:
    Char. 40: 1 --> 0
  Some trees:
    Char. 22: 1 --> 0

Node 124 :
  All trees:
    Char. 14: 0 --> 1

Node 125 :
  All trees:
    Char. 38: 0 --> 1
  Some trees:
    Char. 100: 1 --> 0

Node 126 :
  All trees:
    Char. 66: 0 --> 1
    Char. 79: 1 --> 2
    Char. 97: 0 --> 1
  Some trees:
    Char. 3: 0 --> 1
    Char. 5: 1 --> 0
    Char. 6: 0 --> 1
    Char. 29: 0 --> 1
    Char. 33: 1 --> 0
    Char. 36: 1 --> 0
    Char. 37: 1 --> 0
    Char. 39: 1 --> 0
    Char. 40: 0 --> 1
    Char. 42: 2 --> 1
    Char. 44: 12 --> 2
    Char. 47: 1 --> 0
    Char. 61: 3 --> 2
    Char. 70: 1 --> 0
    Char. 71: 0 --> 2
    Char. 75: 02 --> 3
    Char. 82: 0 --> 1
    Char. 96: 1 --> 0
    Char. 98: 0 --> 1
    Char. 99: 01 --> 1
    Char. 101: 0 --> 1
    Char. 102: 1 --> 0
    Char. 104: 1 --> 0
    Char. 109: 0 --> 1
    Char. 113: 1 --> 0
    Char. 116: 1 --> 0
    Char. 119: 0 --> 1
    Char. 120: 0 --> 2
    Char. 125: 1 --> 0
    Char. 128: 1 --> 0

Node 127 :
  All trees:
    Char. 22: 0 --> 1
    Char. 37: 0 --> 1

Node 128 :
  All trees:
    Char. 52: 0 --> 1

Node 129 :
Char. 71: 0 --> 1

Node 130 :
Some trees:
Char. 6: 0 --> 1
Char. 37: 1 --> 0
Char. 64: 0 --> 1
Char. 65: 1 --> 0
Char. 114: 0 --> 1
Char. 115: 01 --> 2

Node 131 :
All trees:
Char. 44: 1 --> 2
Char. 69: 2 --> 3
Char. 74: 2 --> 3
Char. 126: 0 --> 1
Some trees:
Char. 6: 0 --> 1
Char. 51: 1 --> 0
Char. 61: 3 --> 0
Char. 62: 3 --> 0
Char. 96: 1 --> 0
Char. 123: 1 --> 0

Node 132 :
All trees:
Char. 67: 0 --> 1
Some trees:
Char. 3: 0 --> 1
Char. 6: 1 --> 0
Char. 22: 0 --> 1
Char. 51: 1 --> 0
Char. 61: 3 --> 1
Char. 62: 3 --> 0
Char. 99: 01 --> 0
Char. 114: 0 --> 1

Node 133 :
All trees:
Char. 56: 0 --> 1
Char. 128: 0 --> 1

Node 134 :
All trees:
Char. 12: 1 --> 0
Char. 47: 0 --> 1
Char. 124: 1 --> 0

Node 135 :
All trees:
Char. 18: 0 --> 1
Char. 24: 0 --> 1

Node 136 :
All trees:
Char. 59: 0 --> 1
All trees:
Char. 7: 1 --> 0
Char. 69: 2 --> 1
Char. 89: 0 --> 1

Node 137 :
All trees:
Char. 0: 1 --> 0
Char. 10: 1 --> 3
Char. 21: 0 --> 1
Char. 22: 0 --> 1
Char. 27: 1 --> 0
Char. 128: 1 --> 0

Node 138 :
All trees:
Char. 62: 3 --> 1
Char. 75: 2 --> 1
Char. 99: 0 --> 1

Node 139 :
All trees:
Char. 37: 0 --> 1
Char. 56: 1 --> 0

Node 140 :
All trees:
Char. 58: 0 --> 1

Node 141 :
All trees:
Char. 63: 0 --> 2
Char. 74: 2 --> 1
Char. 78: 0 --> 1
Char. 82: 1 --> 0

Node 142 :
All trees:
Char. 37: 1 --> 0
Char. 56: 0 --> 1
Char. 102: 0 --> 2

Node 143 :
All trees:
Char. 55: 1 --> 2

Node 144 :
All trees:
Char. 54: 0 --> 1
Char. 63: 1 --> 0
Char. 76: 1 --> 2
Char. 123: 1 --> 0
Char. 126: 0 --> 1
Some trees:
Char. 6: 1 --> 0
Char. 35: 1 --> 0
Char. 39: 0 --> 1
Char. 46: 1 --> 0
Char. 99: 1 --> 0
Char. 125: 0 --> 1
Char. 10: 1 --> 0
Char. 50: 0 --> 1

Node 145 :
All trees:
Char. 23: 1 --> 0
Char. 81: 0 --> 1
Char. 112: 0 --> 1
Char. 120: 0 --> 1
Some trees:
Char. 5: 1 --> 0
Char. 33: 1 --> 0
Char. 47: 1 --> 0
Char. 61: 3 --> 2
Char. 76: 0 --> 1
Char. 82: 0 --> 1
Char. 94: 01 --> 0
Char. 96: 1 --> 0
Char. 101: 0 --> 1
Char. 102: 1 --> 0
Char. 109: 0 --> 1
Char. 113: 1 --> 0

Node 146 :
All trees:
Char. 129: 2 --> 0

Node 147 :
All trees:
Char. 87: 0 --> 1
Some trees:
Char. 22: 0 --> 1
Char. 104: 0 --> 1
Char. 125: 0 --> 1

Node 148 :
All trees:
Char. 31: 0 --> 1
Some trees:
Char. 3: 0 --> 2
Char. 6: 01 --> 1
Char. 14: 0 --> 1
Char. 16: 1 --> 0
Char. 20: 0 --> 1
Char. 22: 0 --> 1
Char. 26: 0 --> 2
Char. 27: 1 --> 0
Char. 39: 1 --> 0
Char. 41: 2 --> 0
Char. 42: 2 --> 1
Char. 44: 1 --> 2
Char. 45: 01 --> 0
Char. 48: 1 --> 0
Char. 49: 0 --> 1
Char. 53: 0 --> 1
Char. 83: 2 --> 1
Char. 91: 0 --> 1
Char. 94: 01 --> 1
Char. 95: 0 --> 1
Char. 128: 1 --> 0

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Node 149 :
 All trees:
  Char. 17: 1 --> 0
  Char. 37: 12 --> 0
  Char. 79: 1 --> 2
  Char. 80: 0 --> 1
  Char. 83: 2 --> 0
  Char. 105: 0 --> 1
  Char. 112: 0 --> 1
  Char. 122: 1 --> 0
  Char. 128: 1 --> 0
 Some trees:
  Char. 22: 0 --> 1
  Char. 27: 1 --> 0
  Char. 33: 1 --> 0
  Char. 71: 0 --> 2
  Char. 94: 01 --> 0
  Char. 99: 01 --> 0
  Char. 113: 1 --> 0
  Char. 117: 01 --> 0
  Char. 123: 1 --> 0
  Char. 126: 0 --> 1

Node 150 :
 All trees:
  Char. 24: 0 --> 1
  Char. 25: 1 --> 0
  Char. 39: 1 --> 2
  Char. 49: 0 --> 1
  Char. 53: 0 --> 1
  Char. 92: 1 --> 0

Node 151 :
 All trees:
  Char. 7: 2 --> 3
  Char. 11: 0 --> 1
  Char. 37: 1 --> 2
  Char. 42: 2 --> 0
  Char. 45: 1 --> 2
  Char. 54: 1 --> 0
  Char. 74: 1 --> 2
  Char. 75: 1 --> 3
  Char. 101: 1 --> 0
  Char. 103: 0 --> 1
  Char. 106: 0 --> 1

Node 152 :
 All trees:
  Char. 49: 0 --> 1
  Char. 63: 1 --> 2
  Char. 109: 1 --> 0

Node 153 :
 All trees:
  Char. 2: 1 --> 0
  Char. 13: 0 --> 1
  Char. 16: 1 --> 0
  Char. 20: 0 --> 1
  Char. 22: 0 --> 1
  Char. 26: 0 --> 2
  Char. 27: 1 --> 0
  Char. 30: 0 --> 2
  Char. 42: 2 --> 0
  Char. 71: 0 --> 1
  Char. 83: 2 --> 0
 Some trees:
  Char. 75: 3 --> 0

Node 154 :
 All trees:
  Char. 4: 0 --> 1
  Char. 8: 1 --> 0
  Char. 105: 0 --> 1
  Char. 122: 1 --> 0
 Some trees:
  Char. 6: 01 --> 1
  Char. 44: 1 --> 2
  Char. 46: 01 --> 1
  Char. 99: 01 --> 1
  Char. 125: 01 --> 0

Node 155 :
 All trees:
  Char. 94: 0 --> 1

Node 156 :
 Some trees:
  Char. 8: 1 --> 0
  Char. 10: 1 --> 0
  Char. 84: 0 --> 1

Node 157 :
 All trees:
  Char. 2: 1 --> 0
  Char. 21: 0 --> 1
  Char. 42: 2 --> 0

Node 158 :
 All trees:
  Char. 32: 0 --> 1
  Char. 127: 0 --> 1

Node 159 :
 All trees:
  Char. 61: 23 --> 1
  Char. 63: 12 --> 0
 Some trees:
  Char. 3: 0 --> 1
  Char. 5: 1 --> 0
  Char. 6: 01 --> 1
  Char. 26: 0 --> 2
  Char. 27: 1 --> 0
  Char. 29: 0 --> 1
  Char. 38: 0 --> 1
  Char. 46: 1 --> 0
  Char. 47: 1 --> 0
  Char. 94: 01 --> 1
  Char. 99: 01 --> 0
  Char. 109: 0 --> 1
  Char. 113: 1 --> 0
  Char. 117: 01 --> 2
  Char. 125: 1 --> 0

Node 160 :
 All trees:
  Char. 11: 0 --> 1
  Char. 12: 1 --> 2
  Char. 18: 0 --> 1
  Char. 46: 1 --> 0
  Char. 83: 2 --> 0
  Char. 96: 1 --> 0
  Char. 113: 1 --> 0
 Some trees:
  Char. 40: 1 --> 0

Node 161 :
 All trees:
  Char. 79: 1 --> 2
  Char. 116: 1 --> 0
 Some trees:
  Char. 99: 01 --> 1

Node 162 :
 All trees:
  Char. 69: 2 --> 1
  Char. 74: 2 --> 1
  Char. 89: 0 --> 1
 Some trees:
  Char. 35: 0 --> 2
  Char. 45: 1 --> 0
  Char. 62: 03 --> 1
  Char. 63: 12 --> 2
  Char. 64: 0 --> 1
  Char. 109: 0 --> 1
  Char. 114: 0 --> 1
  Char. 118: 0 --> 1

Node 163 :
 All trees:
  Char. 3: 0 --> 1
  Char. 114: 0 --> 1

Node 164 :
 All trees:
  Char. 59: 0 --> 1
  Char. 62: 1 --> 4
  Char. 63: 2 --> 0
  Char. 65: 1 --> 0
  Char. 70: 1 --> 2
  Char. 75: 0 --> 1
  Char. 78: 0 --> 1
  Char. 87: 0 --> 1
  Char. 90: 0 --> 1

Node 165 :
 All trees:
  Char. 22: 0 --> 1
  Char. 29: 0 --> 1
  Char. 35: 2 --> 0
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    Char. 37: 1 --> 2
    Char. 69: 1 --> 2
    Char. 125: 1 --> 0

Node 166 :
  All trees:
    Char. 30: 0 --> 2
    Char. 72: 0 --> 1
    Char. 73: 0 --> 1
    Char. 87: 1 --> 0

Node 167 :
  All trees:
    Char. 61: 2 --> 1
    Char. 62: 1 --> 0
    Char. 97: 1 --> 0
    Char. 105: 1 --> 0

Node 168 :
  All trees:
    Char. 12: 1 --> 0
    Char. 68: 0 --> 1
    Char. 83: 2 --> 0

Node 169 :
  All trees:
    Char. 21: 0 --> 1
    Char. 42: 2 --> 1
    Char. 126: 1 --> 0

Node 170 :
  All trees:
    Char. 62: 3 --> 2

Node 171 :
  All trees:
    Char. 43: 0 --> 1

Node 172 :
  All trees:
    Char. 1: 1 --> 0
    Char. 10: 1 --> 2
    Char. 21: 0 --> 1
    Char. 24: 0 --> 1
    Char. 27: 1 --> 0
    Char. 31: 0 --> 1
    Char. 39: 1 --> 2
    Char. 45: 0 --> 1
    Char. 103: 0 --> 1

Node 173 :
  All trees:
    Char. 26: 0 --> 2
    Char. 39: 1 --> 2
    Char. 41: 1 --> 0
    Char. 58: 1 --> 0
    Char. 62: 1 --> 3
    Char. 63: 2 --> 0

Node 174 :
  All trees:
    Char. 128: 1 --> 0

Node 175 :
  All trees:
    Char. 74: 2 --> 1
    Char. 93: 0 --> 1
  Some trees:
    Char. 22: 1 --> 0
    Char. 44: 1 --> 2
    Char. 62: 3 --> 2
    Char. 75: 02 --> 2
    Char. 94: 01 --> 1

Node 176 :
  All trees:
    Char. 97: 0 --> 1
    Char. 116: 1 --> 0
  Some trees:
    Char. 3: 0 --> 1

Node 177 :
  All trees:
    Char. 0: 0 --> 1
    Char. 11: 0 --> 1
    Char. 42: 2 --> 0
    Char. 47: 0 --> 1
    Char. 64: 0 --> 1
    Char. 75: 1 --> 0
    Char. 99: 1 --> 0

Node 178 :
  All trees:
    Char. 121: 1 --> 0
    Char. 122: 1 --> 0
  Some trees:
    Char. 6: 0 --> 1
    Char. 35: 01 --> 2
    Char. 39: 1 --> 2
    Char. 63: 12 --> 2
    Char. 94: 01 --> 1
    Char. 97: 0 --> 1
    Char. 101: 0 --> 2
    Char. 110: 0 --> 1
    Char. 117: 01 --> 0

Node 179 :
  All trees:
    Char. 0: 1 --> 0
    Char. 22: 1 --> 0
    Char. 53: 0 --> 1

Node 180 :
  All trees:
    Char. 13: 0 --> 1
    Char. 60: 0 --> 1
```

GENERAL CONCLUSIONS

Internal relationships in Ephemeroptera remain uncertain, and there is no consensus for most clades. There are few comprehensive phylogenies based on tested evidence for the order, and there is no classification for the order based on a tested homology hypothesis. Although homology hypotheses for morphological characters are relatively well established, we believe there is room for further study with morphological and molecular data. Morphological data for the elaboration of phylogenetic hypotheses and consequently for their classification can be used for Ephemeroptera as long as modern methods based on scientific criteria are applied.

In the last 30 years, 3 main groups have been concerned with the phylogeny of the higher groups of mayflies: McCafferty and coworkers, Kluge and Ogden and coworkers. In light of this, we believe it is important that more scientists devote themselves to studying the phylogeny of the group and do so based on methods that can be replicated so that we have a greater number of phylogenetic hypotheses that can be tested for robustness. These increased efforts will help us to get a better overview of the evolution of the group, the relationships between families and between major groups, and in this way to establish a sound modern classification of the order.

In our cladistic analysis of the morphological matrix, we found two very different topologies using different K values; a similar one for K values of 8, 9, 11, and 12, and a very different one for a K value of 10. The consensus of all analyzes was unresolved for deep relationships within Ephemeroptera. Monophyly is supported for 7 suprafamilial groups proposed by Kluge (2004): Caenotergaliae, Ephemerella/Fg1, Eusetisura, Fimbriatotergaliae, Fossoriae, Heptagennota, and Posteritorna. Three other groups attributed to McCafferty were also recovered: Carapacea (= Posteritorna), Ephemerelloidea (= Ephemerella/Fg1), and Caenoidea (= Caenotergaliae). Anteritorna, Branchitergaliae, and Tridentiseta were not recovered as monophyletic in any of the analyzes with IW. Five families were not found to be monophyletic in any of our analyzes: Ephemerellidae, Ephemeridae, Nesameletidae, Potamanthidae, and Teloganodidae.