



Combined molecular and morphological phylogeny of Ephemerellinae (Ephemerellidae: Ephemeroptera), with remarks about classification

T. HEATH OGDEN^{1,2,5}, JONATHAN T. OSBORNE², LUKE M. JACOBUS^{3,4}, & MICHAEL F. WHITING²

¹Department of Biological Sciences, Idaho State University

²Department of Integrative Biology, Brigham Young University

³Department of Entomology, Purdue University

⁴Department of Biology, Indiana University

⁵Corresponding author. E-mail: ogdet@isu.edu

Abstract

This study represents the first combined molecular and morphological analysis for the mayfly family Ephemerellidae (Ephemeroptera), with a focus on the relationships of genera and species groups of the subfamily Ephemerellinae. The phylogeny was constructed based on DNA sequence data from 3 nuclear (18S rDNA, 28S rDNA, histone H3) and 2 mitochondrial (12S rDNA, 16S rDNA) genes, and 23 morphological characters. Taxon sampling for Ephemerellidae included exemplars from all 25 extant genus groups and additional representatives from those genera with the highest diversity. Ephemerellidae appears to consist of three major clades. *Ephemerella*, the largest genus of Ephemerellidae, and *Serratella* were not supported as monophyletic, and each had representatives in two of the three major clades. However, the genera *Drunella* and *Cincticostella* were supported as monophyletic. Lineages strongly supported as monophyletic include a grouping of the Timpanoginae genera *Timpanoga*, *Dannella*, *Dentatella* and *Eurylophella*, and groupings of the Ephemerellinae genera *Torleya*, *Hyrtanella* and *Crinitella* and the genera *Kangella*, *Uracanthella* and *Teloganopsis*. The placement of the Timpanoginae genus *Attenella* fell within Ephemerellinae, based on molecular and combined data, but it grouped with other Timpanoginae based on morphological data alone. Further study and analysis of Ephemerellidae morphology is needed, and classification should be revised, if it is to reflect phylogenetic relationships.

Key words: Ephemeroptera; Mayflies; Molecular phylogeny; Pannota; Ephemerellidae

Introduction

The systematics of the mayfly family Ephemerellidae (Insecta: Ephemeroptera) has a long and complex history. Klapálek (1909) first recognized Ephemerellidae as a family group, but the concept dates back to Eaton's (Eaton, 1883–1888) section VI of *Ephemerella* Walsh. Allen (1965, 1980, 1984) and Edmunds et al. (1963) revised the composition of the family. During the last two decades, Ephemerellidae has been refined further as part of an effort to have taxonomic classifications that reflect phylogenetic hypotheses (McCafferty, 1991). The families Austremerellidae, Melanemerellidae, Philolimniidae, Teloganellidae, Teloganodidae and Vietnamellidae each contain genera that have been removed from Ephemerellidae as part of this effort (McCafferty & Wang, 1997; McCafferty & Wang, 2000; Jacobus & McCafferty, 2006).

Currently, the family Ephemerellidae is classified as part of the infraorder Pannota of the suborder Furcatergalia. Based on current classifications (Hong, 1979; McCafferty, 2000; McCafferty, Jacobus, & Wang, 2003; McCafferty & Wang, 2000; Sartori, 2004) the family Ephemerellidae includes the following genera: *Attenella* Edmunds, *Caudatella* Edmunds, *Caurinella* Allen, *Cincticostella* Allen, *Clephemera* Lin [fossil], *Crinitella* Allen and Edmunds, *Dannella* Edmunds, *Dentatella* Allen, *Drunella* Needham, *Ephacera* Paclt,

Ephemerella Walsh, *Eurylophella* Tiensuu, *Hyrtanella* Allen and Edmunds, *Kangella* Sartori, *Serratella* Edmunds, *Teloganopsis* Ulmer, *Timpanoga* Needham, *Torleya* Lestage, *Turfanerella* Demoulin [fossil], and *Uracanthella* Belov. Five subgenera are recognized for the genus *Drunella*: *Drunella* s.s., *Eatonella* Needham, *Myllonella* Allen, *Tribrochella* Allen, and *Unirhachella* Allen. Two subgenera (*Cincticostella* s.s. and *Rhionella* Allen) are recognized under the genus *Cincticostella* (Allen, 1980), and *Ephemerella* contains the subgenera *Ephemerella* s.s., *Amurella* Kluge and *Notacanthella* Kluge (Kluge 2004). These genus groups represent approximately 300 nominal species and are classified into two subfamilies: Ephemerellinae and Timpanoginae (Brittain & Sartori, 2003; McCafferty, 2000).

McCafferty (2000) revised the classification of Timpanoginae to include the tribes Attenellini (*Attenella*), Timpanogini (*Danella* + *Timpanoga*) and Eurylophellini (*Dentatella* + *Eurylophella*). All other genera comprise the subfamily Ephemerellinae. Ephemerellinae contains the monogeneric tribe Hyrtanellini Allen and the large, diverse tribe Ephemerellini s.s. (Jacobus and Sartori 2004). The taxonomic placement of the two fossil genera, *Clephemera* and *Turfanerella*, is uncertain (McCafferty, 1990; Kluge, 2004), and placement in Ephemerellidae is questionable.

Most studies of Ephemerellidae have been geographically restricted, and include, for example, reviews of North America (Allen & Edmunds, 1962, 1963, 1965), Korea (Yoon & Bae, 1988; Yoon & Kim, 1981), Taiwan (Kang & Yang, 1995), China (You & Gui, 1995), Europe (Jacob, 1993; Studemann, Landholt, & Tomka, 1995), and Japan (Ishiwata, 2000, 2001, 2003). A regional, piecemeal, approach to the systematics of this group has inherent problems, such as those indicated by (Edmunds, 1959; McCafferty, 1991; Studemann & Landholt, 1997). Relationships within Ephemerellinae have been unclear due to this geographic approach. As a result of the poor systematics, the “genera show unstable and fluctuating characters and often mosaic-like occurrence of characters as well” (Landa et al., 1982). Furthermore, a preponderance of hypothetically plesiomorphic morphological characters (McCafferty & Wang, 2000), poorly delimited species and generic boundaries (Jacobus & McCafferty, 2003a, 2003b), and the apparently arbitrary assignment of some species to genera (Studemann & Landholt, 1997; Thomas, Masselot, & Brulin, 1999) also have hampered the formulation of phylogenetic hypotheses. This latter problem of assigning species to genera is illustrated well by the taxonomic history of the species, *Uracanthella punctisetae* (Matsumura), which has been associated with five different generic groups, depending on the time and place it was studied. The synonymy of this widespread Asian species (Tong and Dudgeon, 2000; Ishiwata, 2001; Beketov and Kluge, 2003) includes binomial combinations with the genera *Drunella*, *Ephemerella*, *Serratella* and *Uracanthella* (Ishiwata, 2001). Zaika (2000) listed the species as belonging to *Torleya*, but as a subgenus of *Ephemerella*.

More exploration has focused on relationships of species groups and genus groups of Timpanoginae (Allen, 1977; Kluge, 2004; McCafferty, 1977, 1978, 2000; McCafferty & Wang, 1994) than Ephemerellinae, due in part to these groups being more geographically restricted, more easily recognized and in most cases, less diverse. Kluge (2004) discussed relationships of some of the generic groups of the subfamily Ephemerellinae; Studemann and Landolt (1997) explored egg morphologies; and Landa et al. (1982) detailed the internal anatomies of some genera. Otherwise, this subfamily has not been studied comprehensively on a global scale.

The purpose of this paper is to provide the first global investigation of phylogenetic relationships of the subfamily Ephemerellinae, based on newly generated molecular data and the existing published morphological characters utilized for distinguishing genera. Modifications of existing classification schemes are beyond the scope of the current study and will be addressed elsewhere. At this time, we address specifically: (1) What are the phylogenetic relationships between the major lineages of extant Ephemerellinae? (2) Does the current classification reflect these relationships? (3) Is the current suite of morphological characteristics used for identifying genera useful for recognizing phylogenetic relationships?

Material and methods

Taxon Sampling (Table 1 and 2)

Taxonomic sampling consisted of 33 exemplars from the global Ephemerellidae fauna. We included five species from Timpanoginae representing each of the five extant genera (*Attenella*, *Timpanoga*, *Dannella*, *Dentatella*, and *Eurylophella*) to test the monophyly of Ephemerellinae. All 13 extant genera of Ephemerellinae were represented by exemplar species. Additionally, we included exemplar species representing each of the subgenera of the genera *Cincticostella*, *Drunella* and *Ephemerella*. We attempted to utilize type species of nominal genera and subgenera whenever fresh material was available; otherwise, we used presumably closely related species from the same biogeographic region as the type species. We note that the *Ephemerella* subgenus *Amurella*, which has an eastern Palearctic type species, is represented by its eastern Nearctic representative, *Ephemerella septentrionalis* (McDunnough), per Kluge (2004). The genus *Eurylophella*, which has a western Palearctic type species, is represented by an eastern Nearctic species, *Eurylophella verisimilis* (McDunnough). We also included additional species, representing morphological diversity within the larger genera *Drunella*, *Ephemerella*, and *Serratella*.

The morphological characters that we studied were selected from among those traditionally used to differentiate between major species groups (Allen, 1980; Jacobus & McCafferty, 2004a, 2006; Jacobus & Sartori, 2004; Kluge, 2004), and these were coded into a morphology matrix (Table 1). The specimens from which tissue was extracted for molecular analyses were used to code morphological characters, whenever possible; however, additional conspecific specimens often were used to verify character states from other metamorphic stages and some character states that were obliterated by the removal of tissue. Occasionally, a literature source was consulted; if so, this is indicated below. Our matrix was built using MacClade (Maddison & Maddison, 2005), and it includes the following characters from the egg (E), larva (L) and male adult (A) stages. Characters with multiple states were coded as unordered. Some character states were not applicable to all taxa studied. These included the relative development of gills 3 and dorsal abdominal spines. The relative development of gills 4 was coded only for those taxa for which gills 4 are the most anterior gills. If a character state could not be coded, a dash (-) was inserted into the data matrix.

List of characters

- 1 (E). Number of polar caps [zero=0; one=1; two=2].
- 2 (L). Presence [1] or absence [0] of occipital spines.
- 3 (L). Maxilla with [1] or without [0] flattened, setose crown.
- 4 (L). Maxilla with [1] or without [0] distal bladellike structure.
- 5 (L). Maxillary palp robust [0], reduced [1] or absent [2].
- 6 (L). Forefemur enlarged [1] or not enlarged [0].
- 7 (L). Claws with [1] or without [0] pallisade of denticles.
- 8 (L). Dorsal thoracic spines present [1] or absent [0].
- 9 (L). Prothorax greatly expanded and projected anteriorly [1] or not developed in this way [0].
- 10 (L). Mesothoracic notum with [1] or without [0] sharp anterolateral spines.
- 11 (L). Abdominal friction disc present [1] or absent [0].
- 12 (L). Abdomen with dorsal spine(s) absent [0], single [1] or paired [2].
- 13 (L). Paired abdominal spines, if present, large and stout [1], or weakly developed [0].
- 14 (L). Gills 1 present [0] or absent [1].
- 15 (L). Gills 3 present [0] or absent [1].
- 16 (L). Gills 3, when present, imbricate [0] or operculate [1].
- 17 (L). Gills 4 imbricate [0], semi-operculate [1] or operculate [2] (when gills 3 absent).
- 18 (L). Gills 6 ventral lamella cleft present [0] or absent [1].
- 19 (L). Caudal filaments with [1] or without [0] hairlike lateral setae.

20 (A). Genital forceps segment 3 elongate [1] or ovoid [0].

21 (A). Penes with [1] or without [0] stout setae.

22 (A). Penes with dorsal projection absent [0], present laterally [1] or present along proximal ridge of gonopore [2].

23 (A). Penes lobes elongate and with deep medial cleft [1] or with another morphology [0].

TABLE 1. Morphological Matrix.

Genus	Species	Characters																						
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
<i>Attenella</i>	<i>margarita</i>	1	0	0	0	0	0	0	1	0	0	0	2	0	0	1	-	0	0	1	1	0	0	0
<i>Caudatella</i>	<i>hystrix</i>	1	1	0	0	0	0	0	1	0	0	0	2	1	1	0	0	-	1	0	0	0	0	0
<i>Caurinella</i>	<i>idahoensis</i>	1	0	0	0	1	0	0	0	0	0	0	0	-	1	0	0	-	1	0	0	0	0	0
<i>Cincticostella</i>	<i>elongatula</i>	1	0	1	1	1	0	0	0	1	0	0	2	0	1	0	0	-	1	1	0	0	0	0
<i>Cincticostella</i> (<i>Rhionella</i>)	<i>insolta</i>	?	0	1	1	0	0	0	0	1	0	0	2	0	1	0	0	-	1	1	0	?	?	?
<i>Crinitella</i>	<i>coheri</i>	1	0	0	0	1	0	1	0	0	0	0	0	-	1	0	0	-	0	1	0	0	1	0
<i>Dannella</i>	<i>provonshai</i>	2	0	0	0	1	0	0	0	0	0	0	0	-	0	1	-	1	0	1	0	0	0	0
<i>Dentatella</i>	<i>coxalis</i>	0	0	0	0	2	0	0	0	0	0	0	2	0	0	1	-	2	0	0	0	0	0	0
<i>Drunella</i>	<i>ishiyamana</i>	1	0	0	0	0	1	0	0	0	0	0	2	0	1	0	0	-	1	1	1	0	0	0
<i>Drunella</i>	<i>pelosa</i>	1	1	0	0	0	0	0	0	0	0	1	2	0	1	0	0	-	1	0	1	0	0	0
<i>Drunella</i>	<i>spinifera</i>	1	1	0	0	0	1	0	1	0	0	0	2	1	1	0	0	-	1	0	1	0	0	0
<i>Drunella</i> (<i>Eatonella</i>)	<i>doddsi</i>	1	0	0	0	0	1	0	0	0	0	1	2	0	1	0	0	-	1	0	1	0	0	0
<i>Drunella</i> (<i>Myllonella</i>)	<i>coloradensis</i>	1	1	0	0	0	1	0	0	0	0	0	2	0	1	0	0	-	1	0	1	0	0	0
<i>Drunella</i> (<i>Tribrochella</i>)	<i>trispina</i>	1	1	0	0	0	1	0	0	0	0	0	2	0	1	0	0	-	1	1	1	0	0	0
<i>Drunella</i> (<i>Unirhachella</i>)	<i>tuberculata</i>	1	1	0	0	0	1	0	1	0	0	0	2	0	1	0	0	-	1	1	1	0	0	0
<i>Ephacera</i>	<i>longicaudata</i>	1	0	0	1	0	0	0	1	0	1	0	2	0	1	0	0	-	1	0	0	0	0	0
<i>Ephemerella</i>	<i>atagosana</i>	1	0	0	0	0	0	0	0	0	0	0	2	0	1	0	0	-	1	1	0	1	0	0
<i>Ephemerella</i>	<i>berneri</i>	1	0	0	0	0	0	0	0	0	0	0	2	1	1	0	0	-	1	1	0	0	0	1
<i>Ephemerella</i>	<i>cornutus</i>	1	1	0	0	1	0	0	1	0	0	0	2	1	1	0	0	-	0	0	0	1	1	0
<i>Ephemerella</i>	<i>excrucians</i>	1	0	0	0	0	0	0	0	0	0	0	2	0	1	0	0	-	1	1	0	1	0	0
<i>Ephemerella</i>	<i>maculata</i>	1	0	0	0	0	0	0	0	0	0	0	0	-	1	0	0	-	1	1	0	0	0	0
<i>Ephemerella</i>	<i>needhami</i>	1	0	0	0	0	0	0	0	0	0	0	2	0	1	0	0	-	1	1	0	0	0	1
<i>Ephemerella</i>	<i>septentrionalis</i>	1	1	0	0	0	0	0	0	0	0	0	1	-	1	0	0	-	0	0	0	1	0	1
<i>Ephemerella</i> (<i>Notacanthella</i>)	sp.	1	1	0	0	0	0	0	1	0	1	0	2	1	1	0	0	-	1	1	?	?	?	?
<i>Eurylophella</i>	<i>verisimilis</i>	0	1	0	0	2	0	0	0	0	0	0	2	0	0	1	-	2	0	1	0	0	0	0
<i>Hyrtanella</i>	<i>pascalae</i>	1	1	0	0	1	0	1	0	0	0	0	1,2	0	1	0	1	-	0	1	1	0	?	0
<i>Kangella</i>	<i>brocha</i>	1	0	1	0	2	0	0	0	0	0	0	0	-	1	0	0	-	0	0	?	?	?	?
<i>Serratella</i>	<i>serrata</i>	1	1	0	0	1	0	0	1	0	0	0	2	0	1	0	0	-	0	0	0	1	1	0
<i>Serratella</i>	<i>teresa</i>	1	0	0	0	1	0	0	0	0	0	0	2	0	1	0	0	-	1	0	0	0	0	0
<i>Teloganopsis</i>	<i>media</i>	1	0	1	0	2	0	0	0	0	0	0	0	-	1	0	0	-	0	0	0	0	2	0
<i>Timpanoga</i>	<i>hecuba</i>	1	0	0	0	0	0	0	0	0	0	0	0,2	0	0	1	-	1	0	1	0	0	0	0
<i>Torleya</i>	<i>major</i>	1	0	0	0	1	0	0	0	0	0	0	0	-	1	0	1	-	0	0	1	0	2	0
<i>Uracanthella</i>	<i>punctisetae</i>	1	0	1	0	2	0	0	0	0	0	0	0	-	1	0	0	-	0	0	0	1	2	0

*Subgenera in parentheses

Molecular data

(table 2)

TABLE 2. Taxon list and Genbank accession numbers. Subgenera are indicated in parentheses, following the genus. NA = data was not generated

Genus	Species	12S rDNA	16S rDNA	18S rDNA	28S rDNA	histone H3
<i>Attenella</i>	<i>margarita</i>	FJ442997	FJ443027	FJ443048	FJ443075	NA
<i>Caudatella</i>	<i>hystrix</i>	FJ442998	AY749787	AY749866	AY749962	AY749719
<i>Caurinella</i>	<i>idahoensis</i>	FJ442999	FJ443028	FJ443049	FJ443076	FJ443101
<i>Cincticostella</i>	<i>elongatula</i>	FJ443000	NA	FJ443050	FJ443077	FJ443102
<i>Cincticostella (Rhionella)</i>	<i>insolta</i>	NA	FJ443029	FJ443051	FJ443078	FJ443103
<i>Crinitella</i>	<i>coheri</i>	NA	NA	FJ443052	NA	FJ443104
<i>Dannella</i>	<i>provonshai</i>	FJ443001	FJ443030	FJ443053	FJ443079	FJ443105
<i>Dentatella</i>	<i>coxalis</i>	FJ443002	FJ443031	FJ443054	FJ443080	FJ443106
<i>Drunella</i>	<i>ishiyamana</i>	FJ443007	FJ443035	FJ443057	FJ443083	FJ443109
<i>Drunella</i>	<i>pelosa</i>	FJ443005	FJ443033	FJ443055	FJ443081	FJ443107
<i>Drunella</i>	<i>spinifera</i>	FJ443006	FJ443034	FJ443056	FJ443082	FJ443108
<i>Drunella (Eatonella)</i>	<i>doddsi</i>	FJ443004	AY749756	AY749836	AY749915, AY749916	AY749698
<i>Drunella (Myllonella)</i>	<i>coloradensis</i>	FJ443003	FJ443032	AY338694	AY338651	AY338618
<i>Drunella (Tribrochella)</i>	<i>trispina</i>	FJ443008	FJ443036	FJ443058	FJ443084	NA
<i>Drunella (Unirhachella)</i>	<i>tuberculata</i>	FJ443009	FJ443037	FJ443059	FJ443085	FJ443110
<i>Ephacerella</i>	<i>longicaudata</i>	FJ443010	FJ443038	FJ443060	FJ443086	FJ443111
<i>Ephemerella</i>	<i>atagosana</i>	FJ443011	FJ443039	FJ443061	FJ443087	FJ443112
<i>Ephemerella</i>	<i>berneri</i>	FJ443012	FJ443040	FJ443062	FJ443088	FJ443113
<i>Ephemerella</i>	<i>cornutus</i>	FJ443013	FJ443041	FJ443063	FJ443089	NA
<i>Ephemerella</i>	<i>excrucians</i>	NA	NA	AY749838	AY749918, AY749919,	AY749700
<i>Ephemerella</i>	<i>maculata</i>	FJ443014	NA	FJ443064	FJ443090	FJ443114
<i>Ephemerella</i>	<i>needhami</i>	FJ443015	FJ443042	FJ443065	FJ443091	FJ443115
<i>Ephemerella</i>	<i>septentrionalis</i>	FJ443016	NA	FJ443066	FJ443092	NA
<i>Ephemerella (Notacanthella)</i>	<i>sp.</i>	FJ443020	FJ443044	FJ443068	NA	NA
<i>Eurylophella</i>	<i>verisimilis</i>	FJ443017	FJ443043	FJ443067	FJ443093	FJ443116
<i>Hyrtanella</i>	<i>pascalae</i>	FJ443018	AY749815	AY749891	AY750012	AY749742
<i>Kangella</i>	<i>brocha</i>	FJ443019	NA	NA	FJ443094	FJ443117
<i>Serratella</i>	<i>serrata</i>	FJ443021	FJ443045	FJ443069	FJ443095	FJ443118
<i>Serratella</i>	<i>teresa</i>	FJ443022	NA	FJ443070	FJ443096	FJ443119
<i>Teloganopsis</i>	<i>media</i>	FJ443023	FJ443046	FJ443071	FJ443097	FJ443120
<i>Timpanoga</i>	<i>hecuba</i>	FJ443024	NA	FJ443072	FJ443098	NA
<i>Torleya</i>	<i>major</i>	FJ443025	FJ443047	FJ443073	FJ443099	FJ443121
<i>Uracanthella</i>	<i>punctisetae</i>	FJ443026	NA	FJ443074	FJ443100	FJ443122

*Subgenera in parentheses

For each exemplar, muscle tissue was dissected, incubated, and DNA was extracted following the Qiagen DNeasy protocol for animal tissue (Valencia, CA). Genomic DNA vouchers and specimen vouchers were

deposited at the Insect Genomics Collection (IGC), M.L. Bean Museum, Brigham Young University. Templates and controls were amplified in a Perkin-Elmer 9700 thermocycler using primers modified for insects. Five genes were targeted for amplification and sequencing: 18S rDNA (18S), 28S rDNA (28S), 16S rDNA (16S), 12S rDNA (12S), and histone H3 protein coding for the nucleosome (H3). Primer sequences for 18S and 28S are given elsewhere (Ogden & Whiting, 2003; Ogden & Whiting, 2005; Wheeler, Whiting, Wheeler, & Carpenter, 2001). Product yield, specificity, and potential contamination were monitored via agarose gel electrophoresis. The successful amplicons were purified and cycle-sequenced using ABI Prism Big Dye® Terminator version 3.0 chemistry. The sequencing reactions were column purified and analyzed with the ABI 3100 automated sequencer. In nearly all cases, DNA was sequenced from complementary strands, with sufficient overlap for the larger genes to ensure accuracy of the results. Manual correction of chromatography data was facilitated by the program Sequencher® 4.0 (Genecodes, 1999). Genbank accession numbers are given in Table 2. Specimens vouchers are deposited in the Insect Genomics Collection (IGC) of Brigham Young University. Collection data for the specimens used in the analysis is available online at <http://whitinglab.byu.edu/Ephemeroptera/datasets.htm>.

Materials examined

The materials examined for morphological data are deposited with the following institutional collections: The Natural History Museum, London, England [BMNH]; Brigham Young University, Provo, Utah [BYU]; California Academy of Sciences, San Francisco, California [CAS]; C. P. Gillette Museum of Arthropod Diversity, Colorado State University, Ft. Collins, Colorado [CSUC]; Hokkaido University, Sapporo, Japan [EIHU]; Florida A&M University, Tallahassee, Florida [FAMU]; Iowa State University, Ames, Iowa [ISUI]; Musée de Zoologie, Lausanne, Switzerland [MZL]; Purdue University Entomological Research Collection, West Lafayette, Indiana [PERC]; the Royal Ontario Museum, Toronto, Ontario, Canada [ROME]; Seoul Women's University, Seoul, Korea [SWU]; and Virginia Polytechnic Institute and State University, Blacksburg, Virginia [VPIC]. Some material was collected as part of the All Taxa Biodiversity Inventory of Great Smoky Mountains National Park (Sharkey, 2001) and as part of biotic inventories of Thailand (Panrong, Buathong, & Sites, 2002; Sites, Wang, Perkam, & Hubbard, 2001).

Attenella margarita (Needham): USA, Montana, Odell Cr, 1-VIII-1981, 21 male adults [PERC]; New Mexico, Rio Costilla at 7S boundary, 17-VIII-1989, GZ Jacobi, one larva [PERC]; Wyoming, Teton Co, Snake R at Moose, Grand Teton National Park, 18-VIII-1985, GF Edmunds, Jr., three larvae (eggs dissected) [PERC].

Caudatella hystrix (Traver): USA, Idaho, Lemhi Co, Spring Cr 1.5 mi NE Shoup, Salmon NF, 2/10-VII-1964, CR Whitt, IR Thornton, subimago [PERC]. Montana, Ravalli Co, E Fk Bitterroot R, 10 mi above jct with W Fk Bitterroot R, 24-VI-1965, JR Grierson, one larva [PERC]. Washington, Pierce Co, Mount Rainier NP, springfed stream, Westside Rd, 1.2 mi N of Hwy 706, 16-VI-2004, emerged 21-VI, Kondratieff, Schmidt, three male adults, one female adult, associated larval exuviae [PERC].

Caurinella idahoensis Allen: USA, Idaho, Idaho Co, Bridge Cr at Hoodoo Lake Rd (FR360), 46°21'53"N, 114°38'11"W (WGS84), 1708m elev, 29-VII-2002, WP McCafferty, LM Jacobus, three male adults, two female adults, one male subimago, two female subimagos, associated exuviae (alates emerged 9-VIII through 17-VIII), four larvae [PERC]; same data, one male adult, one set larval exuviae [CSUC]. Literature consulted: Jacobus and McCafferty (2004a: Fig. 1).

Cincticostella elongatula (McLachlan): Japan, Ibaraki, Tomobe, Taira-cho, 9-II-2002, T Fujitani, seven larvae [PERC]; Osaka, Kaizuka, Sobura, 15-IV-2001, T Fujitani, one male adult [PERC]. Japan (no other data), Pryer, one male adult, two female adults [BMNH]. Literature consulted: Ishiwata (2003: Figs. 3 and 4).

Cincticostella insolta (Allen): Thailand, Mae Hong Son Prov, Namtok Maw Pang, 19°22'N, 98°22'E, 850m, 14-X-2002, CMU team, one larva [ISUI]. Egg and male adult unknown.

Crinitella coheri (Allen and Edmunds): Nepal, Palung, 5850', 17-IV-1957, EI Coher, two larvae (holotype; paratype, mouthparts on slide) [PERC]; Thailand, Mae Hong Son, River Nam Lang, Soppong, 3–27/IV/2003, Braasch, four larvae, three male adults, one female subimago [PERC]; Mae Hong Son Prov, Namtok Mae Surin NP, Nam Mae Surin, above falls, 18°56'N, 98°04'E, 1220 m, 15-X-2002, GW Courtney, one larva [ISUI]. Literature consulted: Jacobus and Sartori (2004: Fig. 20).

Dannella provonshai (McCafferty): USA, Tennessee, Blount Co, Great Smoky Mountains National Park, Forge Cr at Parsons Branch Rd, 18-V-2001, CD&RP Randolph, LM Jacobus, two larvae [PERC] Literature consulted: McCafferty (1977).

Dentatella coxalis (McDunnough): Canada, Ontario, Lake Huron at Howdenvale, 29-V-1974, one larva [PERC]; Quebec, Riviere du Loup, 10/12-X-1999, three larvae (*D. danutae* holotype and paratypes). Literature consulted: Burian (Burian, 2002); McCafferty (2000).

Drunella (Myllonella) coloradensis (Dodds): USA, Utah, Salt Lake Co, Mill Cr., Mill Creek Canyon, WL Peters, K Terry, three male adults [PERC]; Montana, Glacier NP, Avalanche Cr at Avalanche Campground, 10-IX-1958, RW Baumann, two male subimagos, four larvae [PERC]. Literature consulted: Studemann and Landolt (1997: Figs 52 and 53).

Drunella (Eatonella) doddsii (Needham): USA, Montana, Missoula Co, Lolo Cr, 0.6 mi NE Lolo Pass, 46°38'37"N, 114°34'44"W (WGS84), 28-VII-2002, WP McCafferty, LM Jacobus, two larvae [PERC]; Utah, Utah Co, Aspen Grove Cr, VI-1965, GF Edmunds, one male adult [PERC]. Literature consulted: Studemann and Landolt (1997: Fig. 54), Allen and Edmunds (1962: Figs. 28–33).

Drunella ishiyamana Matsumura: Japan, Ishiyama, 7-VIII-1903, one male adult (*D. ishiyamana* type) [EIHU]; Japan, Nara, Kawakami, Unokawa, Nakai Stream, 8-VI-2002, T Fujitani, seven larvae [PERC]; Japan, Tochigi Pref., Yudaki Falls, 7km north of Lake Chuzenji, 12-VII-2002, Terry & Jarvis, DNA holotype EP204, larvae [BYU]. Literature consulted: Okazaki (1984: Fig. 26); identified as *D. cryptomeria*.

Drunella pelosa (Mayo): USA, Idaho, Lemhi Co, Salmon R below jct Panther Cr, ca 8.5 mi W of Shoup, Salmon NF, 6–16-VII-1964, one male adult [PERC]; California, Los Angeles Co, Elizabeth Lake, 16-V-1951, one larva (mouthparts on slide) [PERC]. Egg unknown.

Drunella (Drunella) spinifera (Needham): USA, Montana, Missoula Co, Lolo Cr, 0.6 mi NE of Lolo Pass, 46°38'37"N, 114°34'44"W (WGS84), 28-VII-2002, WP McCafferty, LM Jacobus, 13 larvae [PERC]; Washington, Pierce Co, Hillside stream, 1.5 mi N Hwy 706 Westside Rd, Mt Rainier NP, 14-VII-2003, Kondratieff & Schmidt, one male adult (reared), associated larval exuviae [CSUC]. Egg unknown.

Drunella (Tribrochella) trispina (Ueno): Japan, Nara, Higashi-Yoshino, Omata, Omata Stream, Kuramae Bridge, 5-V-2002, T Fujitani, three larvae [PERC]. Literature consulted: Okazaki (1984: Fig. 50). Male adult unknown [adult described as *trispina* by Ueno (1931) is actually that of *sachalinensis* (Ishiwata, 2001)].

Drunella (Unirhachella) tuberculata (Morgan): USA, North Carolina, Swain Co, Great Smoky Mountains NP, Kanati Fork, 0.3 km upstream from Newfound Gap Rd (US441), Kanati Fork Trailhead at 35°35'14"N, 83°21'48"W (NAD27), 26-IX-2002, LM&PD Jacobus, one larva [PERC]; South Carolina, Oconee Co, E Fk Chattooga R, Rt 107, 1 km S of North Carolina state line, 35°N, 83°04'W, 3-X-1997, S Spichiger, one male adult (genitalia on slide), associated exuviae (reared) [PERC]. Literature consulted: Jacobus and McCafferty (2004b).

Ephacerella longicaudata (Ueno): Korea, Gyeonggi-do Namyangju-si Kwangrung (St), 16-I-1983, larvae [SWU—EPH-3193]; same locale, but 15-IV-1984, one male adult [SWU—EPH-3192]. Literature consulted: Okazaki (1984: Figs. 34 and 35) [data omitted: images poor].

Ephemerella atagosana Imanishi: Japan, Honshu, Nara, Higashi-yoshino, Omata, Omata Stream, Kuramae Bridge, 5-V-2001, T Fujitani, one larva [PERC]; Tokyo, Ange R, Hachiouji-shi, 30-IV-1985, S Ishiwata, one male adult [PERC]. Korea, Jeollabuk-do, Muju, Deokyusan (Mt), 2-V-1983, YJ Bae, one female adult

(reared; eggs dissected) [SWU-EPH-1606]; Donggureung, Namyangju, 10-V-1994, YJ Bae, one male adult, associated exuviae [SWU-EPH-1614].

Ephemerella bernerii Allen and Edmunds: USA, Virginia, Henry Co, Smith R at Rt674, 26-IV-1980, emerged 7-V-1980, Kondratieff, one male adult, two female adults, associated exuviae (eggs dissected) [VPIC].

Ephemerella cornutus Gose: Japan, Nara, Kawakami, Unokawa, Nakai-keikoku, 5-V-2002, T Fujitani, one larva [PERC]; Nara, Kawakami, Unokawa, Nakai Stream, 8-VI-2002, T Fujitani, four male adults [PERC].

Ephemerella maculata Traver: USA, California, Napa Co, Hopper Cr, 2-V-2000, larvae (eggs dissected) [PERC]; San Luis Obispo Co, blacklight at Tassajara Cr, 7 mi. north of San Luis Obispo, 6-VI-1971, JD Pinto, one male adult [PERC]. Literature consulted: Smith (1935: Fig. 28) described the egg as having no polar cap; examination of eggs dissected from larvae (Fig. 7) reveals that his observation was incorrect.

Ephemerella needhami McDunnough: USA, Indiana, Elkhart Co, Elkhart R, 1 mi southeast of Millersburg at bridge on gravel road, 21-V-1972, WP McCafferty, AV Provonsha, one male adult, associated exuviae [PERC]; Bartholomew Co, White Cr, private farm crossing south of Co Rd 930 South, 39°03'02"N, 85°58'01"W, 19-IV-2005, LM Jacobus, JM Webb, JM Hwang, MW Jacobus, one larva [PERC]; Michigan, Arenac Co, Omer, Rifle R at public access site, 44°02'41"N, 83°51'20"W, 24-VI-2002, LM Jacobus, BL Hass, subimago (eggs dissected), associated exuviae [PERC].

Ephemerella (Amurella) septentrionalis McDunnough: USA, Massachusetts, Amherst, 3-VI-1939, JR Traver, eight male adults, eight female adults [PERC]; Tennessee, Blount Co, Abrams Cr at Cades Cove Loop Rd, Great Smoky Mountains NP, stream bank (flowing water), 35°35'36"N, 83°50'42"W (NAD27), 16-V-2003, LM&BLH Jacobus, one larva [PERC]. Literature consulted: Smith (1935).

Ephemerella (Ephemerella) sp. (cf. *dorothea infrequens* McDunnough/*excrucians* Walsh): USA, Utah, Utah Co, South Fork Provo R, Hwy 35 above Woodland, 27-V-2000, TH Ogden, one larva [BYU].

Ephemerella (Notacanthella) sp.: Vietnam, Nghe An, West of Con Cuong, Khe Moi River Forestry Camp, Khe Moi River, 27-X-1994, DC Currie, ROM946108, two larvae [ROME].

Eurylophella verisimilis (McDunnough): USA, Tennessee, Blount Co, Great Smoky Mountains National Park, Forge Cr at Parsons Branch Rd, 18-V-2001, CD&RP Randolph, LM Jacobus, one larva [PERC]; Cades Cove Ranger Station, 13/21-VI-2001, CD&RP Randolph, LM Jacobus, four male adults, four female adults [PERC].

Hyrtanella pascalae Jacobus and Sartori: Island of Borneo, Indonesia, East Kalimantan, Riv Seturan, Loc Seturan, Aff cours principal, 3°00'05"N, 116°30'48"E, 28/III/2001, P Derleth, B Feldmeyer, three larvae (paratypes) [PERC]. Literature consulted: Jacobus and Sartori (2004).

Kangella brocha (Kang and Yang): Taiwan, Hsinchu Hsien, Wufeng, 1991-10-24(I), SC Kang, HC Chang, one larva (paratype) [MZL]. Literature consulted: Kang and Yang (1995; Figs 18 and 19).

Serratella serrata (Morgan): USA, North Carolina, Macon Co, Cullasaja R at Rt 1672, 7-VII-1989, Kondratieff, 21 male adults, six female adults (eggs extracted from one), three male subimagos, subimago exuviae [VPIC]; Virginia, Montgomery Co, Little R along Little Camp Rd, 37°00'07"N, 80°24'59"W, 15-VI-2003, JM Webb, LM Jacobus, three larvae [PERC].

Serratella teresa (Traver): USA, California, Napa Co, Garnett Cr, 1-V-2000, larvae (dissected for eggs) [PERC]; San Mateo Co, San Gregorio Cr, 17-VI-1950, WC Day, three male adults, two female adults, one larva [CAS].

Teloganopsis media Ulmer: Malaysia (east), Sabah, Liwagu R at Liwagu Cave, southeast of Kinabalu NP Headquarters, 1525 m, S7, 14-15-VIII-1972, WL & JG Peters, one larva [FAMU]; Liwagu R at bridge, Ranau, 335 m, S4e, 11-16-VIII-1972, GF & CG Edmunds, four male adults, two male subimagos, associated subimago exuviae [PERC]; Sungai Moyog, 3 mi E of Penampung, 27-IX-1978, GF & CH Edmunds, three male subimagos, three female subimagos (eggs dissected) [PERC].

Timpanoga hecuba (Eaton): USA, Montana, Powell Co, Nevada Cr at SR141, 28-VII-2002, WP McCafferty, LM Jacobus, three larvae [PERC]; Ravalli Co, Bitterroot R, 25-VIII-1964, GF Edmunds, Jr., one male adult, one set subimago exuviae, one set larval exuviae [PERC]. Literature consulted: Smith (1935).

Torleya major (Klapálek): Germany, Stream Fulda, D-36110 Schlitz/Pfordt, 16-IV-2002, R Lieske, ten larvae (some gills removed) [PERC]; Simmerbach (a confluence of the Nahe River, a left hand tributary of the River Rhine), 3-V-2003 (reared to 18-V-2003), A Haybach, three male adults, two female adults (all reared), five subimago exuviae [PERC]. Literature consulted: Studemann et al. (1995: Figs. 30-32); Studemann and Landolt (1997: Figs. 20 and 21).

Uracanthella punctisetae (Matsumura): Japan, Shizuoka, Shimizu, Kakita Stream, Kakitagawa Bridge, 23-IV-2001, one larva [PERC]. North Korea, Myohyang Mts, Str Hyangson, 28-V-1986, five male adults, one female adult [PERC]. Literature consulted: Okazaki (1984: Fig. 36).

Phylogenetic analyses

Sequences were initially assembled in Sequencher® 4.0 (Genecodes, 1999). The protein coding H3 gene was manually aligned with reference to the amino acid sequence. Multiple sequence alignment was performed on the ribosomal genes in MUSCLE (Edgar, 2004a, 2004b) using default settings. Some taxa had missing data, as indicated (Table 2), in one or more of the genes that were submitted to MUSCLE for alignment.

The aligned molecular data were combined with the morphological data and were analyzed under different approaches. First, partitioned Bayesian estimation analysis was performed in MrBayes 3 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003) using mixed models, in order to include the morphological partition. 1,500,000 generations were sampled every 1000 generations. The first 1,000,000 generations were excluded (as the burn in). Two separate runs were performed. Posterior probabilities were taken from the trees that resulted, post burn in, from the two MrBayes runs. Furthermore, the combined dataset was analyzed in PAUP* 4.0b10 (Swofford, 2002) under maximum parsimony, with gaps treated as missing and as a fifth state character. Parsimony settings consisted of multiple random additions (100 for combined datasets and 10,000 random additions for morphological dataset) with TBR swapping. Strict and 50% consensus trees were examined. Bootstrap and Bremer values were calculated for the topology resulting from the combined dataset parsimony analysis with gaps treated as missing. The Bootstrap analyses consisted of 1000 replicates with 20 random additions per replicate. Bremer values were computed via Treerot (Sorenson, 1999) and PAUP*. The subfamily Timpanoginae were used as outgroup taxa for rooting purposes. Additionally, a few exploratory analyses were done with species from families closely related to the Ephemerellidae to ensure that rooting using only Timpanoginae taxa would not be an issue, and in all cases, Ephemerellinae was confirmed to be monophyletic.

Additionally, analyses were carried out in PAUP* under maximum parsimony, with gaps treated as missing, for subset data partitions such as: mitochondrial data, nuclear data, and each partition individually. The original dataset file and results will be available at the website <http://whitinglab.byu.edu/Ephemeroptera/datasets.htm>.

The molecular dataset resulting from the MUSCLE alignment was also analyzed with MODELTEST (Posada & Crandall, 1998) to identify the most “justified” model for likelihood settings. The model selected (GTR+G+I) and the following parameters were implemented in the PAUP block: Lset Base=(0.2295 0.2449 0.2896) Nst=6 Rmat=(0.6376 2.1573 1.6472 0.7677 3.5978) Rates=gamma Shape=0.4507 Pinvar=0.4058. A maximum likelihood search was then performed in PAUP*.

Results

The MUSCLE alignments for each gene consisted of 274 characters for 12S, 478 characters for 16S, 1,882 characters for 18S, and 2,395 characters for 28S. The aligned characters for the ribosomal genes (5,029 total), the H3 protein coding gene (367 nucleotides long), and the morphological data (23 characters) combined for a total of 5,419 characters. 1,067 of these characters were parsimony informative. The partitioned Bayesian analysis of the combined data resulted in a fairly resolved topology (Figure 1). Congruence with some of the other analyses performed is represented on this tree by the use of squares colored in gray if the node is supported with the other approaches. Four additional topologies are given in Figure 2. For maximum parsimony (MP), the combined data analysis resulted in 1 most parsimonious tree (Fig. 2a), with a length of 5437. The maximum likelihood (ML) analysis of the molecular data resulted in one most likely topology (score = 31976.70842) (Fig. 2b). Maximum parsimony analysis of molecular data resulted in 6 most parsimonious trees with a length of 5357; the majority consensus of these trees is given as Figure 2c. The morphological data analysis resulted in many most parsimonious trees (our settings retained 4,895) with a length of 63; the majority consensus of these trees is given as Figure 2d.

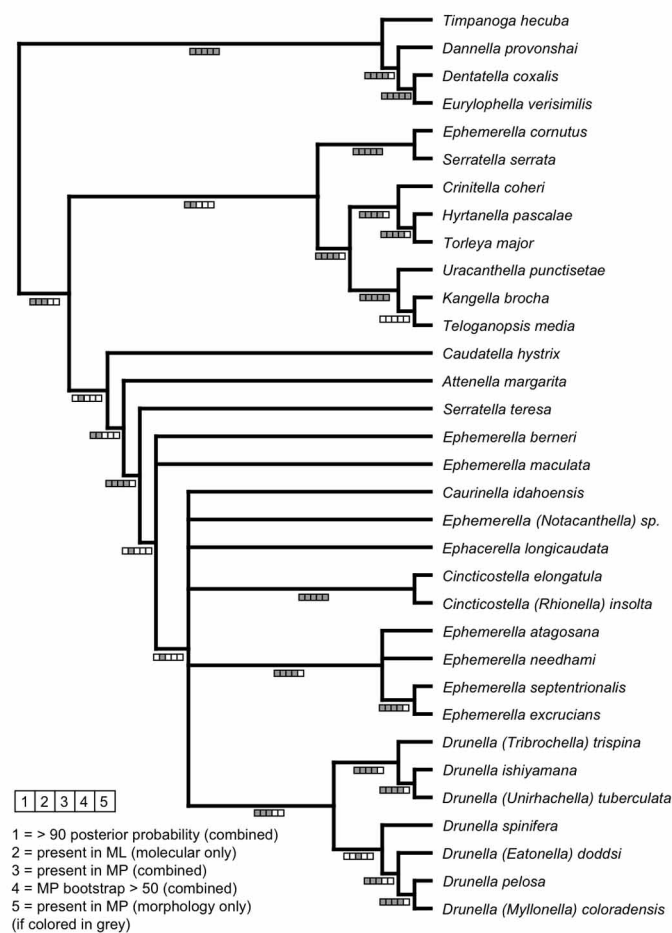


FIGURE 1. Combined data (molecular and morphology) mixed model Bayesian analysis topology. The tree represents the 50% consensus of all of the Bayesian topologies that were selected outside of the “burn in”. For each node the five squares represent sensitivity of the data to different approaches of analysis. The first square is colored gray when the Bayesian posterior probability was greater than 90. The second square is grayed when the node is supported in the maximum likelihood (ML) tree. The third square is grayed when the node is supported in the maximum parsimony (MP) tree. The fourth square is grayed to represent a greater than 50% bootstrap value. The fifth square is grayed when the node is supported in the maximum parsimony analysis of the morphological data.

Discussion

Although we were not specifically testing the monophyly of Timpanoginae in this study, molecular evidence suggests that this group may not be monophyletic as previously hypothesized (Kluge, 2004; McCafferty, 1977, 2000; McCafferty & Wang, 1994). Based on our molecular evidence, *Attenella* is supported as nesting within Ephemerellinae, in contrast to the hypothesis that *Attenella* is sister to a clade containing the four other Timpanoginae genera (Kluge, 2004; McCafferty & Wang, 1994). Moreover, the relationships within this subfamily differ in that *Dannella* was recovered as sister to (*Dentatella* + *Eurylophella*) and not as sister to *Timpanoga*. However, the monophyletic grouping of *Timpanoga*, *Dannella*, *Dentatella* and *Eurylophella* is consistent with the recent phylogenetic hypotheses reviewed above. Because of the inclusion of *Attenella*, the subfamily Ephemerellinae was not recovered as monophyletic in any analysis containing molecular data.

The branching order along the backbone of our trees and of some of the major clades is not very well supported in general, and thus some relationships remain difficult to assess. However, a few major clades were recovered with strong support. These include the clade of *Timpanoga*, *Dannella*, *Dentatella* and *Eurylophella* mentioned above, which was recovered in all five depicted analyses; within this clade, the grouping of *Dentatella* and *Eurylophella* was recovered consistently. Furthermore, the lineage containing ((*Torleya* + *Hyrta-nella*) + *Crinitella*) and (*Kangella* + *Uracanthella* + *Teloganopsis*) was strongly supported as monophyletic with high congruence across the different analyses, and *Ephemerella cornutus* grouped with *Serratella serrata* consistently. This clade is similar to Kluge's (2004) *Torleya/g1* grouping. *Uracanthella*, *Teloganopsis* and *Kangella* form a robustly supported clade present in all 5 depicted analyses. Larvae of these genera lack paired spines on abdominal terga and have dense fields of setae at the apex of the maxilla.

A large clade containing *S. teresa* and corresponding. Roughly to Kluge's (2004) *Ephemerella/fg4* grouping is present in four of the depicted analyses (>90 Bayesian, ML, MP, MP >50% bootstrap) but not supported by the MP morphology analysis (see legend in figure 1). With the exception of *Ephemerella septentrionalis*, each taxon has the ventral lamellae of gills 6 with the lateral lobes "fused together, so its ventral lobe is integral, not bifurcate" (Kluge, 2004).

The sampled species of the genus *Drunella* were supported as a monophyletic group (>90 Bayesian, ML, MP analyses). Each of these *Drunella* species has male adults with elongate genital forceps segment 3 and larvae with the forefemur enlarged and/or marginally denticulate and with a felt of long setae on the abdominal sterna (Jacobus & McCafferty, 2004b). The subgenus *Drunella*, following the concept of Allen (1980), was not supported as monophyletic in any analysis. These results corroborate the decision of Jacobus and McCafferty (2004b) not to recognize Allen's (1980) subgeneric groups. No analysis indicated that *Cincticostella* is not monophyletic.

The genera *Serratella* and *Ephemerella* were not supported as monophyletic in any analysis, which was not unexpected, because *Ephemerella* has been essentially the default genus for newly described species (e.g., Kluge, Zhou, Jacobus, & McCafferty, 2004), and both genera are poorly defined. Also, some authors have chosen not to recognize the genus *Serratella* (e.g., Ishiwata, 2001). However, some *Ephemerella* species were supported as constituting a monophyletic clade by all but the exclusively morphological analysis.

Conclusions

This study is the first combined data phylogenetic analysis of the family Ephemerellidae, which appears to consist of three major clades (Figs. 1; 2a,b). The current classification of the family does not reflect the relationships indicated by our research. Timpanoginae was found to be nonmonophyletic because *Attenella* nested well within the subfamily Ephemerellinae. Within Timpanoginae (excluding *Attenella*), *Dannella* is highly supported as sister to (*Eurylophella* + *Dentatella*). The subfamily Ephemerellinae was not supported as monophyletic only due to the placement of *Attenella*. The large tribe Ephemerellini was not recovered as mono-

phyletic, due to the placement of *Hyrtanella*, the type genus of the monogeneric tribe Hyrtanellini, within one of its major branches. Our results suggest a broader concept of Hyrtanellini and a more restricted concept of Ephemerellini may be necessary. Of the large, diverse genera for which we included multiple exemplars, the genera *Drunella* and *Cincticostella* were recovered as monophyletic based on our sampling, while *Ephemerella* and *Serratella* were nonmonophyletic.

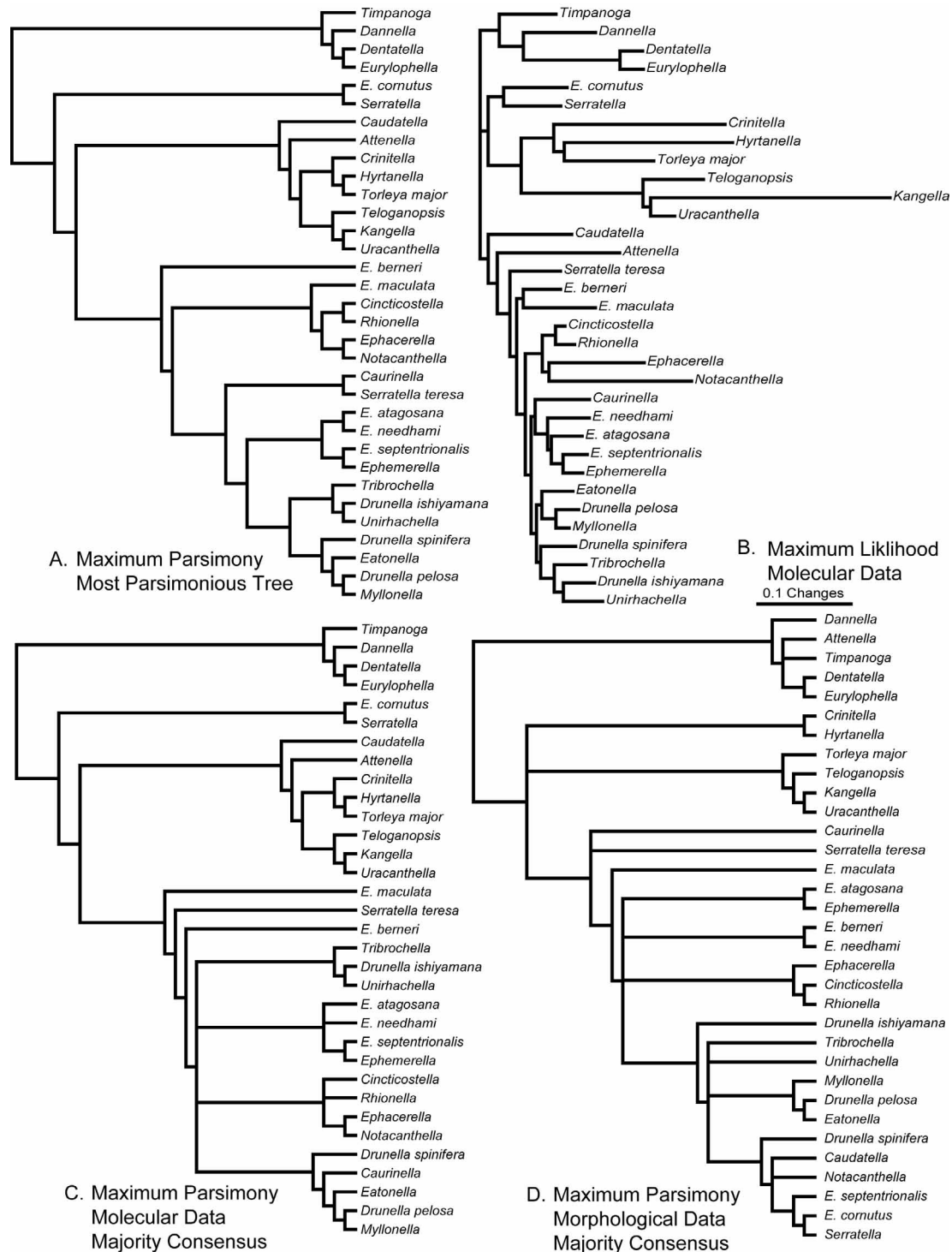


FIGURE 2. Four additional trees resulting from analyses. A. Most Parsimonious tree from maximum parsimony analysis of combined data (one most parsimonious topology; Length = 5437). B. Most likely tree from maximum likelihood analysis of molecular data (Score = 31976.70842). C. Majority-rule consensus of most parsimonious trees from maximum parsimony analysis of molecular data (6 most parsimonious trees found; Length = 5357). D. Majority-rule consensus of most parsimonious trees from maximum parsimony analysis of morphological data (Length = 63).

The contribution of the diagnostic morphological characters to the combined analysis appears to be limited, as, for example, only five nodes of our Bayesian tree were consistent with those recovered from analysis of only the morphological data. In general, the morphological characters currently used to diagnose genera do not allow for a reflection of monophyletic groupings, as evidenced by the nature of *Ephemerella* and *Serratella* and the placements of *Attenella* and *Ephemerella (Amurella) septentrionalis* in our tree. *Attenella* has been considered part of the Timpanoginae, rather than Ephemerellinae, and Kluge (2004) included *E. septentrionalis* in his *Torleya/g1* group, rather than *Ephemerella/fg4*, where it falls here.

The placement of *Attenella* in our molecular and combined data trees was surprising, given that it has been considered sister to the Timpanoginae genera *Timpanoga*, *Eurylophella*, *Dannella* and *Dentatella* based on each of these genera lacking lamellate gills on abdominal segment 3.

Considering that the branching order of major clades is weakly supported by our molecular data, further study and analysis of Ephemerellidae morphology is needed. A more complete sampling of taxa and morphological characters, especially new characters for differentiating species groups, may offer additional evidence, that when combined with molecular data, will be important for resolving the phylogeny of Ephemerellidae. The supraspecific classification of Ephemerellinae clearly requires further evaluation and possible revision, especially if it is to reflect monophyletic groupings of species and genera.

Acknowledgments

We thank M. Sartori, J-L. Gattolliat, Sartori Lab, W. P. McCafferty, McCafferty Lab, A. Haybach, FAMU group, J. Peters, T. Fujitani, B. O'Donnell, G. Courtney, D. Lenat, Y. J. Bae, D. Braasch, D. Goodger, S. Hiner, S. Ishiwata, N. Kluge, B. Kondratieff, W. Pulawski, S. Spichiger, J. Voshell, K. Yoshizawa and R. Wisseman for providing specimens and other advise on the project; Whiting Lab for valuable discussion and assistance. G. Hass helped build a rearing apparatus for Michigan field studies. Examination of some specimens via Scanning Electron Microscopy was made possible by Chia-Ping Huang, Debra Sherman and Lu Sun (West Lafayette, Indiana). DNA sequences are deposited in GenBank under accession numbers. Discover Life In America, Inc. and the U. S. National Park Service provided funding and facilities for some associated fieldwork. This work was supported in part by NSF grants 0206505 (MFW and THO), and DEB-0120718 (MFW). Furthermore, this material is based upon work supported under a National Science Foundation Graduate Research Fellowship and USEPA fellowship 91601701-0 to LMJ.

References

- Allen, R.K. (1965) A review of the subfamilies of Ephemerellidae (Ephemeroptera). *Journal of Kansas Entomological Society*, 38, 262–266.
- Allen, R.K. (1977) A review of *Ephemerella* (*Dannella*) and the description of a new species (Ephemeroptera: Ephemerellidae). *The Pan-Pacific Entomologist*, 53, 215–217.
- Allen, R.K. (1980) Geographic distribution and reclassification of the subfamily Ephemerellinae (Ephemeroptera: Ephemerellidae). In: J. F. a. K. E. M. Flannagan (Ed), *Advances in Ephemeroptera Biology*, Plenum, New York, pp. 71–91.
- Allen, R.K. (1984) A new classification of the subfamily Ephemerellinae and the description of a new genus. *The Pan-Pacific Entomologist*, 60, 245–247.
- Allen, R.K. & Edmunds, G.F. (1962) A revision of the genus *Ephemerella* (Ephemeroptera: Ephemerellidae). V. The subgenus *Drunella* in North America. *Miscellaneous Publications of the Entomological Society of America*, 4, 145–179.
- Allen, R.K. & Edmunds, G.F. (1963) A revision of the genus *Ephemerella* (Ephemeroptera: Ephemerellidae). VI. The subgenus *Serratella* in North America. *Annals Entomological Society of America*, 56, 583–600.
- Allen, R.K. & Edmunds, G.F. (1965) A revision of the genus *Ephemerella* (Ephemeroptera, Ephemerellidae). VIII. The subgenus *Ephemerella* in North America. *Miscellaneous Publications of the Entomological Society of America*, 4,

- Brittain, J.E. & Sartori, M. (2003) Ephemeroptera (Mayflies). In: V. H. Resh & R. T. Cardé (Eds), *Encyclopedia of Insects*. Academic Press, San Diego, pp. 373–380.
- Burian, S.K. (2002) Taxonomy of *Eurylophella coxalis* (McDunnough) with notes on larval habitat and behavior (Ephemeroptera:Ephemerellidae). *Journal of the North American Benthological Society*, 21, 602–615.
- Eaton, A.E. (1883–1888) A revisional monograph of recent Ephemeridae or mayflies. *Transactions of the Linnean Society of London*, 3, 1–352.
- Edgar, R.C. (2004a) MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics*, 5, 1–19.
- Edgar, R.C. (2004b) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, 32, 1792–1797.
- Edmunds, G.F. (1959) Subgeneric groups within the mayfly genus *Ephemerella* (Ephemeroptera: Ephemerellidae). *Annals Entomological Society of America*, 52, 543–547.
- Edmunds, G.F., Allen, R.K. & Peters, W.L. (1963) An annotated key to the nymphs of the families and subfamilies of mayflies (Ephemeroptera). *University of Utah Biological Series*, 13, 1–49.
- Genecodes (1999) Sequencher. In: Genecodes Co., Ann Arbor, MI.
- Hong, Y.-C. (1979) On Eocene Philolimnias gen.nov. (Ephemeroptera, Insecta) in amber from Fushung Coalfield, Liaoning Province. *Scientia Sinica*, 22, 331–339.
- Huelsenbeck, J.P. & Ronquist, F.R. (2001) MRBAYES: Bayesian inference of phylogeny. *Bioinformatics*, 17, 754–755.
- Ishiwata, S.-I. (2000) Notes on mayflies in Kanagawa Prefecture. *Natural History Report of Kanagawa*, 21, 73–82.
- Ishiwata, S.-I. (2001) A checklist of Japanese Ephemeroptera. In: Y. J. Bae (Ed), *The 21st century and aquatic entomology in East Asia. Proceedings of the 1st symposium of aquatic entomologists in East Asia*. Korean Soc. Aquatic Entom., Seoul, pp. 55–84.
- Ishiwata, S.-I. (2003) A revision of the genus *Cincticostella* from Japan. *Species Diversity*.
- Jacob, U. (1993) Zur Systematik und Verbreitung der europäischen Ephemerellidae (Ephemeroptera). *Verhandlungen der Westdeutschen Entomologischen Tagung, 1992*, 101–110.
- Jacobus, L.M. & McCafferty, W.P. (2003a) Contribution to the biology and systematics of *Ephemerella alleni* (Ephemeroptera: Ephemerellidae). *The Pan-Pacific Entomologist*, 79, 207–211.
- Jacobus, L.M. & McCafferty, W.P. (2003b) Revisionary contributions to North American *Ephemerella* and *Serratella* (Ephemeroptera: Ephemerellidae). *Journal of the New York Entomological Society*, 111, 174–193.
- Jacobus, L.M. & McCafferty, W.P. (2004a) Contribution to the morphology and descriptive biology of *Caurinella ida-hoensis* (Ephemeroptera: Ephemerellidae). *Western North American Naturalist*, 64, 101–108.
- Jacobus, L.M. & McCafferty, W.P. (2004b) Revisionary contributions to the genus *Drunella* (Ephemeroptera : Ephemerellidae). *Journal of the New York Entomological Society*, 112, 127–147.
- Jacobus, L.M. & McCafferty, W.P. (2006) Reevaluation of the phylogeny of the Ephemeroptera infraorder Pannota (Furcatergalia), with adjustments to higher classification. *Transactions of the American Entomological Society*, 132, 81–90.
- Jacobus, L.M. & Sartori, M. (2004) Review of the genus *Hyrtanella* (Ephemeroptera: Ephemerellidae). *Zootaxa*, 785, 1–12.
- Kang, S.C. & Yang, C.T. (1995) Ephemerellidae of Taiwan (Insecta, Ephemeroptera). *Bulletin of National Museum of Natural Science*, 5, 95–116.
- Klapálek, F. (1909) Ephemeridae, Eintagsfliegen. In: A. Brauer (Ed), *Die Süßwasserfauna Deutschlands*, pp. 1–32.
- Kluge, N., Zhou, C.-F., Jacobus, L.M. & McCafferty, W.P. (2004) A new southeast Asian mayfly species (Ephemeroptera: Ephemerellidae: Ephemerellinae). *Journal of the New York Entomological Society*, 112, 148–152.
- Kluge, N.J. (2004) *The phylogenetic system of Ephemeroptera*. Dordrecht, The Netherlands: Kluwer Academic.
- Maddison, D.R. & Maddison, W.P. (2005) MacClade 4: Analysis of phylogeny and character evolution. In: Sinauer Associates, Sunderland, Massachusetts.
- McCafferty, W.P. (1977) Biosystematics of *Dannella* and related subgenera of *Ephemerella* (Ephemeroptera: Ephemerellidae). *Annals of the Entomological Society of America*, 70, 881–889.
- McCafferty, W.P. (1978) A natural subgeneric classification of *Ephemerella bartoni* and related species (Ephemeroptera: Ephemerellidae). *The Great Lakes Entomologist*, 11, 137–138.
- McCafferty, W.P. (1991) Toward a phylogenetic classification of the Ephemeroptera (Insecta): a commentary on systematics. *Annals of the Entomological Society of America*, 84, 343–360.
- McCafferty, W.P. (2000) A hierarchical classification of the Timpanoginae (Ephemeroptera: Ephemerellidae) and description of a new species from Quebec. *Annals de Limnologie*, 36, 157–161.
- McCafferty, W.P., Jacobus, L.M. & Wang, T.-Q. (2003) Reconfirmation of the genus *Dentatella* Allen (Ephemeroptera: Ephemerellidae). *Proceedings of the Entomological Society of Washington*, 105, 768–788.
- McCafferty, W.P. & Wang, T.-Q. (1994) Phylogenetics and the classification of the Timpanoga complex (Ephemeroptera: Ephemerellidae). *Journal of the North American Benthological Society*, 13, 569–579.

- McCafferty, W.P. & Wang, T.-Q. (1997) Phylogenetic systematics of the family Teloganodidae (Ephemeroptera: Pannota). *Annals of the Cape Provincial Museums*, 19, 387–437.
- McCafferty, W.P. & Wang, T.Q. (2000) Phylogenetic systematics of the major lineages of pannota mayflies (Ephemeroptera: Pannota). *Transactions of the American Entomological Society*, 126, 9–101.
- Ogden, T.H. & Whiting, M. (2003) The Problem with “the Paleoptera Problem”: Sense and Sensitivity. *Cladistics*, 19, 432–442.
- Ogden, T.H. & Whiting, M.F. (2005) Phylogeny of Ephemeroptera (mayflies) based on molecular evidence. *Molecular Phylogenetics and Evolution*, 37, 625–643.
- Okazaki, H. (1984) Observation of the eggs of Japanese mayflies by scanning electron microscope. *Biology of Inland Waters*, 3, 19–27.
- Panrong, S., Buathong, M. & Sites, R.W. (2002) New records of Behningiidae, Potamanthidae, and Prosopistomatidae (Ephemeroptera) from Thailand. *ScienceAsia*, 28, 407–409.
- Posada, D. & Crandall, K.A. (1998) Modeltest: Testing the model of DNA substitution. *Bioinformatics*, 14, 817–818.
- Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19, 1572–1574.
- Sartori, M. (2004) Kangella nom. Nov. (Ephemeroptera, Ephemerellidae), replacement name pro Eburella Kang & Yang, 1995 nec Monné & Martins, 1973 (Coleoptera, Cerambycidae). *Aquatic Insects*, 26, 75–76.
- Sharkey, M.J. (2001) The All Taxa Biological Inventory of the Great Smoky Mountains National Park. *The Florida Entomologist*, 84, 556–564.
- Sites, R.W., Wang, T.-Q., Perkam, S. & Hubbard, M.D. (2001) The mayfly genera (Ephemeroptera) of southern Thailand. *The Natural History Bulletin of the Siam Society*, 49, 243–268.
- Smith, O.R. (1935) The eggs and egg-laying habits of North American mayflies. In: J. G. Needham, J. R. Traver and Y.-C. Hsu (Ed), *The biology of mayflies* Comstock, Ithica, New York, pp. 67–89.
- Sorenson, M.D. (1999) TreeRot. In. Boston University, Boston, MA.
- Studemann, D. & Landholt, P. (1997) Eggs of Ephemerellidae (Ephemeroptera). In: P. a. M. S. Landolt (Ed), *Ephemeroptera & Plecoptera: Biology-Ecology-Systematics*. Mauron + Tinguely & Lachat SA, Fribourg, pp. 362–371.
- Studemann, D., Landholt, P. & Tomka, I. (1995) Eggs of European Ephemerellidae (Ephemeroptera). In: L. D. a. J. H. C. Corkum (Ed), *Current directions in research on Ephemeroptera*. Canadian Scholars’ Press, Toronto, pp. 407–422.
- Swofford, D.L. (2002) PAUP* Phylogenetic analysis using parsimony (*and other methods). In. Sinauer Associates, Sunderland, MA.
- Thomas, A., Masselot, G. & Brulin, M. (1999) Mayflies of France: bibliographical complement to the catalog of recorded and potential species (Thomas & Masselot, 1996)[Ephemeroptera]. *Ephemera*, 1.
- Ueno, M. (1931) Contributions to the knowledge of Japanese Ephemeroptera. *Annotationes Zoologicae Japonenses*, 13, 189–231.
- Wheeler, W.C., Whiting, M.F., Wheeler, Q.D. & Carpenter, J.C. (2001) The phylogeny of the extant hexapod orders. *Cladistics*, 17, 113–169.
- Yoon, I.B. & Bae, Y.J. (1988) The classification of the Ephemerellidae (Ephemeroptera) in Korea. *Entomological Research Bulletin, Korean Entomological Institute, Seoul*, 14, 23–44.
- Yoon, I.B. & Kim, M.L. (1981) A taxonomic study on the larvae of Ephemerellidae (Ephemeroptera) in Korea. *Entomological Research Bulletin, Korean Entomological Institute, Seoul*, 8, 33–59.
- You, D.-S. & Gui, H. (1995) *Economic Insect Fauna of China, Fasc. 48. Ephemeroptera*. Beijing: Science Press.
- Zaïka, V.V. (2000) *An atlas guide to the aquatic invertebrates of Tuva and West Mongolia, Part 1: Mayflies—Insecta, Ectognatha, Ephemeroptera*. Kyzyl.