34 Phylogeny of *Neochoroterpes* (Ephemeroptera: Leptophlebiidae)

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The phylogeny of Neochoroterpes *was inferred by cladistic analysis of 13 characters. Character polarity was determined by outgroup rooting using* Choroterpes inornata *and a hypothetical leptophlebiid ancestor. Character types included morphological, electrophoretic, behavioural and developmental characters. The resulting cladogram shows that* N. kossi *branched earliest,* N. oklahoma *next and that* N. nanita *and* N. orientalis *are sister species. Support for the generic status of* Neochoroterpes *is indicated.*

Introduction

Neochoroterpes was described by Allen (1974). Three species from southwestern North America were described. Allen (1974) designated *Neochoroterpes* as the third subgenus of *Choroterpes* Eaton and noted the mosaic nature of character distribution among the three *Choroterpes* subgenera, *Choroterpes* s.str. Peters and Edmunds, *Euthraulus* Barnard and *Neochoroterpes*. Henry (1993) revised *Neochoroterpes*, elevated the taxon to the generic level and recognized four species, *N. kossi, N. oklahoma, N. nanita* and *N. orientalis*.

Several character types were used to construct the cladogram of *Neochoroterpes*. Larval and imagine morphological characters as well as electrophoretic, behavioural and developmental characters were used. All clades and species are defined by at least one morphological character state. Electrophoretic, behavioural or developmental characters support the morphological character distributions.

The objective of this study was to infer the phylogeny of *Neochoroterpes* by use of rigorous cladistic analysis.

Materials and Methods

Material examined included imago and larval specimens of *Choroterpes inornata* and the four species of *Neochoroterpes, N. kossi, N. oklahoma, N. nanita,* and *N. orientalis*. Discussions of species concepts, descriptions and sample localities of *Neochoroterpes* were in Henry (1993). Specimens studied were from across the range of the species.

The cladistic study was based on 13 characters (Table 1). The characters were either used in previous leptophlebiid studies (Peters and Edmunds 1964, 1970; Allen 1974; Peters 1980; Savage and Peters 1982; Flowers and Dominguez 1991) or were new. In addition to larval and imagine exoskeletal characters, electrophoretic, behavioural and developmental characters were included. The electrophoretic character (11) was obtained by standard starch gel electrophoresis (Selander et al. 1971) with specific enzyme staining (Shaw and Prasad 1970). The developmental character (12) and the behavioural character (13) were obtained from rearing studies and field observations.

Character state polarity was determined by reference to a hypothetical leptophlebiid ancestor. The ancestor was based on conclusions of character polarities established in previous leptophlebiid studies (Peters and Edmunds 1970; Peters 1980; Savage and Peters 1982; Flowers and Dominguez 1991). Where character polarity was ambiguous, the character was left unordered (1,11).

The data set (Table 2) was analyzed using the computer program PAUP (Swofford 1985). Plesiomorphic states were coded 1. Missing data were coded 9. Outgroup rooting was used. The branch-and-bound (BANDB) and unweighted character options were used in all analyses. The options (TREEOUT=4, APOLIST, CHGLIST and BLRANGE) generated data for both character state mapping and the existence of polychotomies.

Results and Discussion

The results are summarized by the distribution of apomorphies in Fig. 1. The branching sequence of *Neochoroterpes* is supported by six character distributions (apomorphies 1, 3, 4, 6, 10, 12). The eight remaining apomorphies are autapomorphies.

Neochoroterpes is defined by three synapomorphies (3, 4, 6). Synapomorphies 3 and 4 occur in other, distantly related leptophlebiid lineages. Character 3 is the pattern of dorsal labral setae. Savage and Peters (1982) considered the scattered pattern a pleisiomorphy of Leptophlebiidae that was retained by Leptophlebiinae lineages, and the row pattern was considered an Atalophlebiinae apomorphy. The presence of the scattered pattern in both the atalophlebiin *Neochoroterpes* lineage

Table 1. Character and character states used in the cladistic analysis of *Neochoroterpes*. States coded as 1 are plesiomorphic.

Character No.	Character State and Description
	Larvae
	Gills
1	1 - Gill 1 without lateral branch
	2 - Gill 1 with long lateral branch, distinctly tracheated
	3 - Gill 1 with lateral branch reduced, untracheated
2	1 - Terminal projections of gills 2-7 with thin, delicate terminal processes
	2 - Terminal projections of gills 2-7 thick, heavy terminal processes
	Labrum
3	1 - Dorsal labral setae in two rows
	2 - Dorsal labral setae scattered
	Mandibles
4	1 - Outer margins of mandibles with setae centred
	2 - Outer margins of mandibles with setae in distal half
	Labium
5	1 - Normal setae on segments 2 and 3 of labial and maxillary palpi
	2 - Abundance of long, fine setae on segments 2 and 3 of labial and maxillary palpi
	Imagines (male or female)
	Forewing
6	1 - MP1 connected to MP2 in fore wings
	2 - MP1-MP2 of fore wings disconnected
	Hindwing
7	1 - Hind wing large; forewing 4-6 times longer
	2 - Hind wing small; forewing 7-9 times longer
8	1 - Costal projection in basal half of wing
	2 - Costal projection in distal half

Table 1. (continued)

Male Imago

9 1 - Penes same length or longer than bulbous base of forceps
2 - Penes shorter than bulbous base of forceps, triangular

10 1 - Penes with distinct longitudinal fold
2 - Penes without distinct longitudinal fold

Electrophoresis (larvae)

11 1 - Fixed slow MDH-1 electromorph
2 - Fixed fast MDH-1 electromorph

Developmental

12 1 - With female adult stage
2 - Without female adult stage

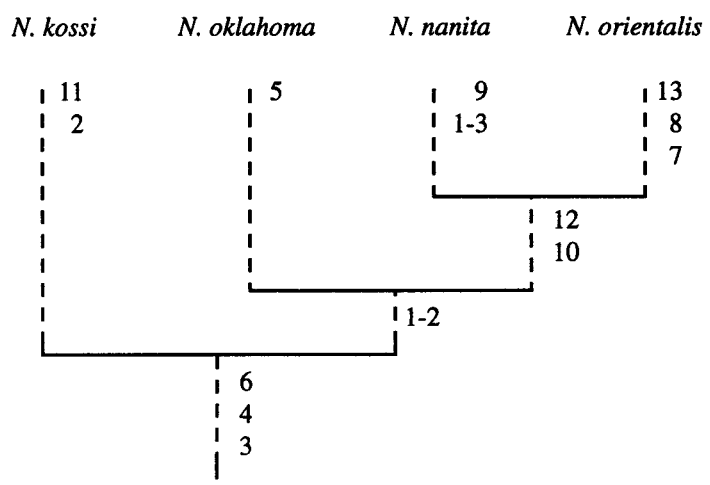
Behavioural

13 1 - Mating flight in morning
2 - Mating flight other than morning

Table 2. Matrix of character state distributions. Pleisiomorphic states are coded 1. Unknown states are coded 9.

Taxon	Character No. 1111 1234567890123
Hypothetical ancestor	1111111111911
C. inornata	1111111119911
N. kossi	1222121111111
N. oklahoma	2122221111211
N. nanita	3122121122221
N. orientalis	2122122212922

Figure 1. **Cladogram of *Neochoroterpes*. Numbers represent apomorphies. Characters and character states correspond with numbers in Table 1.**



and the distantly related leptophlebiin lineages is herein considered the result of independent, convergent evolutionary events in distantly related lineages and a synapomorphy at the level of *Neochoroterpes*.

Character 4 is the location of setae on the outer mandibular margin. The distal location is present in *Neochoroterpes* as well as in *Meheathraulus*. Presence of the character state in these two genera is herein considered the result of independent, convergent evolutionary events and a *Neochoroterpes* apomorphy.

Within *Neochoroterpes*, *N. kossi* branches first and is defined by two autapomorphies, 2 and 11. Autapomorphy 2 is the thick terminal gill filaments on gills 2-7. Autapomorphy 11 is an electrophoretic character. Polarity of this character was inferred by unordering the character. Character states are unknown for *N. orientalis* and *C. inornata* and the state was designated as unknown for the hypothetical ancestor.

The derived clade of *N. oklahoma* + *N. nanita* + *N. orientalis* is defined by a branched first gill (synapomorphy 1-2). This multistate character has two derived states; a long branch that receives a visible trachea (synapomorphy 1-2) and a small, untracheated branch (synapomorphy 1-3). The transformation series of this

character (1-1, 1-2, 1-3) was inferred by unordering the character under the principle of maximum parsimony.

The next species to branch off is *N. oklahoma*, which is defined by the abundance of long, fine setae on segments 2 and 3 of the labial and maxillary palpi (autapomorphy 5). The derived sister clade of *N. nanita* and *N. orientalis* is defined by two synapomorphies. Synapomorphy 10 is the absence of a longitudinal fold on the penes and synapomorphy 12 is the absence of the female imago. Edmunds and McCafferty (1988) provided evidence for the loss of the female adult stage as a derived character. Neither field collections nor laboratory rearings have as yet yielded a female imago of *N. nanita* or *N. orientalis*.

Neochoroterpes nanita is defined by two autapomorphies (1-3, 11). Autapomorphy 1-3 is inferred to be a reduction in both branch length and tracheation of the fork of gill 1. Character 9 is the length of the penes compared with the bulbous forceps base. The apomorphic state of penes shorter than the bulbous forceps base is present in *N. nanita*.

Neochoroterpes orientalis is defined by three autapomorphies (7, 8, 13). Autapomorphy 7 is the small size of the hind wing and autapomorphy 8 is the position of the costal projection in the distal half of the hindwing. Character 13 is a behaviour characteristic, the timing of the mating swarm. On two occasions I have been at a locality where larvae were ready to emerge. Although the timing would have been ideal for other *Neochoroterpes* species, no swarms were seen.

Edmunds et al. (1976:217) made reference to southwestern North American species of *Choroterpes* s.str. and *Neochoroterpes* as follows: "It appears that the phyletic and taxonomic relationship of the species of *Choroterpes* in Mexico and north to Texas and Arizona are far from resolved but are of considerable biogeographic interest." I believe this paper and the *Neochoroterpes* revision (Henry 1993) provide answers to the taxonomic and phyletic relationship of *Neochoroterpes*. Although the phyletic relationship of *Choroterpes* s.str., *Euthraulus* and *Neochoroterpes* remain unresolved, the *Neochoroterpes* lineage is well defined by three synapomorphies. This evidence supports Henry's (1993) recognition of the generic status of *Neochoroterpes*.

References

Allen, R.K. 1974. *Neochoroterpes*, a new subgenus of *Choroterpes* Eaton from North America (Ephemeroptera: Leptophlebiidae). *Can. Entomol.* 105: 161-168.

Edmunds, G.F., Jr. and W.P. McCafferty. 1988. The mayfly subimago. *Annu. Rev. Entomol.* 33: 509-29.

Edmunds, G.F., Jr., S.L. Jensen and L. Berner. 1976. *The Mayflies of North and Central America.* Minneapolis: University of Minnesota Press.

Flowers, R.W. and E. Dominguez. 1991. Preliminary cladistics of the *Hermanella* Complex (Ephemeroptera: Leptophlebiidae, Atalophlebiinae). P. 49-62 in J. Alba-Tercedor and A. Sanchez-Ortega (Eds.), *Overview and Strategies of Ephemeroptera and Plecoptera.* Gainesville: Sandhill Crane Press Inc.

Henry, B.C., Jr. 1993. A revision of *Neochoroterpes* (Ephemeroptera: Leptophlebiidae) new status. *Trans. Am. Entomol. Soc.* 119:317-333.

Peters, W.L. 1980. Phylogeny of the Leptophlebiidae (Ephemeroptera): an introduction. P. 33-41 in J.F. Flannagan and K.E. Marshall (Eds.), *Advances in Ephemeroptera Biology.* New York: Plenum.

Peters, W.L. and G.F. Edmunds, Jr. 1964. A revision of the generic classification of the Ethiopian Leptophlebiidae (Ephemeroptera). *Trans. R. Entomol. Soc. Lond.* 116: 225-253.

Peters, W.L. and G.F. Edmunds, Jr. 1970. Revision of the generic classification of the Eastern Hemisphere Leptophlebiidae (Ephemeroptera). *Pacif. Insects* 12: 157-240.

Savage, H.M. and W.L. Peters. 1982. Systematics of *Miroculus* and related genera from Northern South America (Ephemeroptera: Leptophlebiidae). *Trans. Am. Entomol. Soc.* 108: 491-600.

Selander, R.K., J.H. Smith, S.Y. Yang, W.E. Johnson and J.B. Gentry. 1971. Biochemical polymorphism and systematics in the genus *Peromyscus.* I. Variation in the old-field mouse (*Peromyscus polionotus*). *Studies in Genetics VI* 7103: 49-90. Austin: University of Texas Publ.

Shaw, C.R. and R. Prasad. 1970. Starch gel electrophoresis of enzymes - a compilation of recipes. *Biochem. Genetics* 4: 297-320.

Swofford, D.L. 1985. *PAUP, Phylogenetic Analysis Using Parsimony, Version 2.4.* Illinois Natural History Survey, Champaign, Illinois.