

Phylogeny of the mayfly family Leptohyphidae (Insecta: Ephemeroptera) in South America

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Abstract. A cladistic analysis of the South American members of the Ephemeropteran family Leptohyphidae is presented. A matrix of 73 taxa and 124 morphological characters was analysed under two distinct weighting criteria (implied weighting, which weights characters as a whole, and self-weighted optimization, which differentially weights character state transformations). To assess the monophyly of the Leptohyphidae, representatives of Ephemerellidae, Ephemerithidae, Machadorythidae, Teloganodidae, Tricorythidae, Coryphoridae and Melanemerellidae were also included. Trees were rooted in Ephemerellidae. Conspicuous differences in consensus topology occur when transformation costs among character states are weighted (including asymmetries). The differences in the assessments of character reliability in the two weighting criteria used are discussed. In many cases, self-weighting, in allowing for asymmetries in transformation costs, considered many of the character state transformations as more reliable (= informative) than implied weights (which needlessly down-weighted the whole character). The results confirm the monophyly of Leptohyphidae and support its sister-group relationship with Coryphoridae. The shortest trees do not support the recently proposed division of Leptohyphidae into two subfamilies. Ephemerelloidea higher classification is discussed briefly.

Introduction

Mayflies (Ephemeroptera) are considered one of the oldest insect orders, with the earliest fossils coming from the Upper Carboniferous (Hubbard, 1990). The entire order comprises some 400 genera in 40 families (extant and fossil), with the number of species almost reaching 4000. The Neotropical region still lacks systematic collections from large areas of the Amazonian (Brazil, Bolivia, Peru, Ecuador, Colombia, Venezuela) and Orinoquian basins (Colombia and Venezuela), where numerous new taxa are expected to be discovered. Available phylogenetic analyses of Neotropical mayflies are restricted to species in particular genera or groups of genera in a few families (Leptophlebiidae: Pescador & Peters, 1980; Flowers & Domínguez, 1991; Domínguez, 1995, 1999; Domínguez *et al.*, 2001; Domínguez & Cuezco, 2002; Melanemerellidae: Molineri & Domínguez, 2003; Leptohyphidae: Molineri, 2002, 2004).

Leptohyphidae is a Pan-American family represented by *c.* 120 species, 67 of which are reported from South America, where the highest generic diversity occurs. This family, proposed by Peters & Peters (1993), is formed presently by

12 genera: *Allenhyphes* Hofmann and Sartori, *Amanahyphes* Salles & Molineri, *Haplohyphes* Allen, *Leptohyphes* Eaton, *Leptohyphodes* Ulmer, *Lumahyphes* Molineri, *Macunahyphes* Dias, Salles & Molineri, *Traverhyphes* Molineri, *Tricorythodes* Ulmer, *Tricorythopsis* Traver, *Vacupernius* Wiersema and McCafferty, and *Yaurina* Molineri. The speciose genus *Tricorythodes*, divided into several additional genera (*Ableptemetes*, *Asioplax*, *Epiphrades*, *Homoleptoxyphes*, *Tricorythyphes* and *Tricorythodes* s.s.) by Wiersema & McCafferty (2000, 2003) was represented by at least one representative of each group in the phylogeny presented here (the only exception is *Ableptemetes*). In revising the North and Central American species, these authors divided the family Leptoxyphidae into two subfamilies, Leptoxyphinae and Tricorythodinae (Wiersema & McCafferty, 2000).

Here, a weighted approach was chosen to analyse cladistically all the South American species of Leptoxyphidae, with datasets including characters from eggs, nymphs and adults. Two different weighting criteria, implied weighting (IW; Goloboff, 1993) and self-weighting (SW; Goloboff, 1997), were used to assess these relationships. In these methods, the weights are not given arbitrarily before

Table 1. List of species used to score the studied character states for the 'outgroup' families.

Family	Species	Material
Ephemerellidae	<i>Ephemerella trilineata</i> Berner	U.S.A.
Teloganodidae	<i>Lithogloea harrisoni</i> Barnard	South Africa
Melanemerellidae	<i>Melanemerella brasiliiana</i> Ulmer	Brazil
Ephemerythidae	<i>Ephemerythus niger</i> Gillies	Guinea, Sierra Leona, Tanzania
Tricorythidae	<i>Tricorythus</i> spp.	Liberia, Ivory Coast
Machadorythidae	<i>Machadorythus maculatus</i> Kimmins	Guinea, Mali
Coryphoridae	<i>Coryphorus aquilus</i> Peters	Brazil, Colombia

analysis, but instead assigned during tree searches, based on character congruence.

The IW proposed by Goloboff (1993) resolves character conflicts in favour of those characters showing less homoplasy in the trees. SW is a further refinement of the previous criterion (Goloboff, 1997) that individually evaluates each character state transformation. This criterion allows (but does not require) the occurrence of asymmetries in the costs of transformation among the states of the same character. A classic example of such an asymmetry is wing loss/wing regain in pterygote insects [recently debated by Whiting *et al.* (2003), Trueman *et al.* (2004) and Whiting & Whiting (2004)], in which regains are generally thought of as impossible (or at least more expensive than losses). This weighting method attempts to evaluate whether transformations in one direction (e.g. loss) are more common than in the other (e.g. regain), instead of this being assumed a priori, and then that information is used to down-weight those transformations. This is especially desirable in the case of the reduction of relatively complex structures (in leptohyphids, for example, abdominal gills and maxillary palpi of the nymphs, wing venation of the adults), in which assuming asymmetries prior to the analysis may be difficult to justify.

Therefore, the aims of this paper were: (1) to propose for the first time a cladistic framework for the family Leptohyphidae; (2) to discuss and illustrate (by a cladistic analysis of

this group of mayflies) the differences between IW and SW; and (3) to compare the consequences (e.g. in terms of differences in the consensus topology) of permitting asymmetries in the costs of character state changes.

Materials and methods

Taxon selection

A data matrix (Supplementary Material S1) was constructed for all the South American species of Ephemerelloidea (nine leptohyphid genera, one coryphorid and one melanemerellid) and representative members of the proposed sister groups of Leptohyphidae: Tricorythidae, Ephemerythidae, Machadorythidae, Teloganodidae (Landa & Soldán, 1985; Peters & Peters, 1993; McCafferty & Wang, 2000). Adults and nymphs of *Amanahyphes* Salles & Molineri (2006) and the nymphal stage of *Macunahyphes australis* [in Dias *et al.* (2005)] were only recently discovered and could not be included in the present analysis. The trees were rooted in *Ephemerella* (Ephemerellidae). A list of the nonleptohyphid species used to score the data in the matrix (those used as 'outgroups', representing each of the above-mentioned families) can be seen in Table 1. From the

Table 2. Ingroup taxa (Leptohyphidae). Deposition of studied material (*housing types) and recent revisions.

Genus	Species	Depository	Recent revisions
<i>Tricorythopsis</i> Traver, 1958	<i>T. artigas</i> Traver, 1958	MCR*, FAMU*	Molineri (1999b, 2001a)
	<i>T. chiriguano</i> Molineri, 2001a	UMSA*, IFML*	
	<i>T. gibbus</i> (Allen, 1967)	FAMU*, IFML	Molineri (2001a)
	<i>T. minimus</i> (Allen, 1973)	FAMU*, IFML	Molineri (2001a)
	<i>T. sigillatus</i> Molineri, 1999b	NMNH*, IFML*	
	<i>T. undulatus</i> (Allen, 1967)	FAMU*, IFML	Molineri (2001a)
	<i>T. volsellus</i> Molineri, 1999b	NMNH*, IFML*	
	<i>T. yacutinga</i> Molineri, 2001a	IFML*	
<i>Haplohyphes</i> Allen, 1966	<i>H. aquilonius</i> Lugo-Ortiz & McCafferty, 1995	MEUV, IFML	Molineri (2003a)
	<i>H. baritu</i> Dominguez, 1984	IFML*	Molineri (2003a)
	<i>H. dominguezi</i> Molineri, 1999a	NMNH*	Molineri (2003a)
	<i>H. huallaga</i> Allen, 1966		Molineri (2003a)
	<i>H. mithras</i> (Traver, 1958)		Molineri (2003a)
	<i>H. yanahuicsa</i> Molineri, 2003a	UMSA*, IFML*	

Table 2. Continued.

Genus	Species	Depository	Recent revisions
<i>Leptohiphodes</i> Ulmer, 1920	<i>L. inanis</i> Ulmer, 1920	MZSP, IFML	Molineri (2005)
<i>Macunahyphes</i> Dias <i>et al.</i> , 2005	<i>M. australis</i> (Banks, 1913)	FAMU, IFML	Molineri (2002); Dias <i>et al.</i> (2005)
<i>Tricorythodes</i> Ulmer, 1920	<i>T. dimorphus</i> Allen, 1967	IFML	Wiersema & McCafferty (2000)
	<i>T. arequita</i> Traver, 1959	MCR*, FAMU*	Molineri (2002)
	<i>T. barbatus</i> Allen, 1967	CAS*, IFML	Molineri (2002)
	<i>T. bullus</i> Allen, 1967	FAMU*, IFML	Molineri (2002)
	<i>T. cristatus</i> Allen, 1967	FAMU*	Molineri (2002)
	<i>T. curiosus</i> (Lugo-Ortiz & McCafferty, 1995)	FAMU	Wiersema & McCafferty (2000); Molineri (2002)
	<i>T. hiemalis</i> Molineri, 2001b	IFML*	Molineri (2002)
	<i>T. mirca</i> Molineri, 2002	UMSA*, IFML*	
	<i>T. nicholsae</i> (Wang <i>et al.</i> , 1998)		Wiersema & McCafferty (2000); Molineri (2002)
	<i>T. ocellus</i> Allen & Roback, 1969	FAMU*, IFML	Molineri (2005)
	<i>T. popayanicus</i> Domínguez, 1982	IFML*	Molineri (2002)
	<i>T. quizeri</i> Molineri, 2002	UMSA*, IFML*	
	<i>T. santarita</i> Traver, 1959	FAMU*, IFML	Molineri (2002)
	<i>T. yura</i> Molineri, 2002	UMSA*, IFML*	
	<i>T. zunigae</i> Molineri, 2002	MEUV*, IFML*	
<i>Leptohiphes</i> Eaton, 1882	<i>L. carinus</i> Allen, 1973	FAMU*	Molineri (2003b)
	<i>L. cornutus</i> Allen, 1967	FAMU*	Molineri (2003b)
	<i>L. eximius</i> Eaton, 1882	MACN*, IFML	Molineri (2003b)
	<i>L. ecuador</i> Mayo, 1968	FAMU*	Molineri (2003b)
	<i>L. illiesi</i> Allen, 1967	FAMU*	Molineri (2003b)
	<i>L. invictus</i> Allen, 1973	FAMU*	Molineri (2003b)
	<i>L. jodiannae</i> Allen, 1967	FAMU*	Molineri (2003b)
	<i>L. liniti</i> Wang <i>et al.</i> , 1998		Molineri (2003b)
	<i>L. maculatus</i> Allen, 1967	FAMU*	Molineri (2003b)
	<i>L. peterseni</i> Ulmer, 1920		Molineri (2003b)
	<i>L. petersi</i> Allen, 1967	FAMU*	Molineri (2003b)
	<i>L. plaumanni</i> Allen, 1967	FAMU*	Molineri (2003b)
	<i>L. populus</i> Allen, 1973	FAMU*	Molineri (2003b)
	<i>L. setosus</i> Allen, 1967	FAMU*	Molineri (2003b)
	<i>L. tacajalo</i> Mayo, 1968	FAMU*	Molineri (2003b)
	<i>L. tuberculatus</i> Allen, 1967	FAMU*	Molineri (2003b)
<i>Yaurina</i> Molineri, 2001d	<i>Y. yapa</i> Molineri, 2001d	NMNH*	Molineri (2004)
	<i>Y. yuta</i> Molineri, 2001d	IFML*	Molineri (2004)
	<i>Y. mota</i> Molineri, 2001d	IFML*	Molineri (2004)
	<i>Y. ralla</i> (Allen, 1967)	FAMU*	Molineri (2004)
<i>Traverhyphes</i> Molineri, 2001c	<i>T. (T.) indicator</i> (Needham & Murphy, 1924)	IFML	Molineri (2001c, 2004)
	<i>T. (T.) pirai</i> Molineri, 2001c	IFML*	Molineri (2004)
	<i>T. (T.) chiquitano</i> Molineri, 2004	UMSA*, IFML*	
sg. <i>Byrsahyphes</i> Molineri, 2004	<i>T. (B.) nanus</i> (Allen, 1967)	IFML	Molineri (2004)
	<i>T. (B.) yuqui</i> Molineri, 2004	IFML*	
sg. <i>Mocoithyphes</i> Molineri, 2004	<i>T. (M.) yuati</i> Molineri, 2004	IFML*	
	<i>T. (M.) edmundsi</i> (Allen, 1973)	FAMU*	Molineri (2004)
<i>Allenhyphes</i> Hofmann & Sartori, 1999	<i>A. flinti</i> (Allen, 1973)	FAMU*, IFML	Molineri & Flowers (2001)
	<i>A. vesus</i> (Allen, 1978)		Molineri (2004)
	<i>A. spinosus</i> (Allen & Roback, 1969)	ANSP*	Molineri (2004)
	<i>A. asperulus</i> (Allen, 1967)	CAS*	Molineri (2004)
<i>Lumahyphes</i> Molineri	<i>L. guacra</i> Molineri (Molineri & Zuñiga, 2004)	IFML*	
	<i>L. yagua</i> Molineri & Zuñiga, 2004	MEUV*, IFML*	
	<i>L. pijcha</i> Molineri, 2004	UMSA*, IFML*	
	<i>L. sp. Mexico</i> (undescribed)	IFML	Molineri & Zuñiga (2004)

68 species of Leptohiphidae known to occur in South America, 65 were included in the analysis¹. The only species excluded were *Leptohiphes mollipes* Needham and Murphy, *L. nigripunctum* Traver and *Tricorythodes lichyi* Traver, which could not be scored due to unavailability of material and poor existing descriptions. Two leptohiphid species not recorded from South America were also included: (1) *Allenhyphes vescus* (Allen) (scored to assess the monophyly of *Allenhyphes*, represented in South America by only one reliable species, *A. flinti*); and (2) *Tricorythodes dimorphus* (Allen), which represents the only distinct lineage (from the Caribbean region, North and Central America) in *Tricorythodes* not recorded yet from South America (included to represent the morphological diversity of the family better). Therefore, the total number of taxa included in the matrix was 74. Lists of material studied are published elsewhere (Molineri, 1999a,b, 2001a,b,c,d, 2002, 2003a,b 2004; Molineri & Zuñiga, 2004).

Depositories and abbreviations

Instituto-Fundación Miguel Lillo, San Miguel de Tucumán, Tucumán, Argentina (IFML); Museo de Entomología de la Universidad del Valle, Cali, Colombia (MEUV); Universidad Mayor de San Andrés, Instituto de Limnología, La Paz, Bolivia (UMSA); Museo Argentino de Ciencias Naturales Bernardino Rivadavia, Buenos Aires, Argentina (MACN); Museu de Zoologia, Universidade de São Paulo, São Paulo, Brazil (MZSP); Museo de Ciencias de la República, Universidad de la República, Montevideo, Uruguay (MCR); Florida A&M University, Tallahassee, Florida, USA (FAMU); National Museum of Natural History, Washington DC, USA (NMNH); California Academy of Science, San Francisco, USA (CAS) y Academy of Natural Sciences of Philadelphia, Philadelphia, USA (ANSP).

Characters and coding

The matrix of 123 morphological characters included 54 nymphal, 58 adult and 11 egg characters. Seventy-eight characters are binary, and 45 are multistate; multistate characters were treated as nonadditive. Noncomparable and missing characters were both assigned a '?' entry. For characters and their coding, see Appendix 1.

Phylogenetic analyses

Searches were conducted using the programs TNT (Goloboff *et al.*, 2004) and SL (Goloboff, 1998). TNT was used under IW to search for most-parsimonious trees. Trees were

¹During the revision of this manuscript, a few South American leptohiphid taxa were newly described (Dias & Salles, 2005; Molineri & Zuñiga, 2006; Salles & Molineri, 2006), and were not included in the present analysis.

searched with the 'new technology search' (sectorial searches, tree drifting and tree fusing; Goloboff, 1999). As the possible number of shortest trees in preliminary searches exceeded 80 000, the consensus was calculated by stabilizing the number of nodes 50 times (with TBR (tree bisection and reconnection) as the collapsing rule; see Goloboff & Farris, 2001).

SL (Goloboff, 1998) works under the SW criterion of Goloboff (1997). Searches with SL were made using the parsimony ratchet (Nixon, 1999). This search strategy was implemented with the command 'nixwts'. The amount of characters reweighted at each iteration was 20%, and a set of 20 iterations and 20 replications was conducted. The trees obtained from this method were submitted to branch swapping (SPR: subtree pruning and regrafting). The number of trees to retain at each replication was set to 1 (command 'hold/1'). Constrained searches were also performed to compare the results under both criteria. All nodes present in the consensus from SW were constrained in TNT (under IW) and then searches were performed as explained for nonconstrained searches. Constrained searches are not implemented in SL.

Group support estimation

Group support was calculated using frequency differences (Goloboff *et al.*, 2003), an improvement of jackknifing (Farris *et al.*, 1996). This value results from the difference between the frequency of the group and the frequency of the most common contradictory group. A set of 500 replications of symmetric jackknifing was conducted; symmetric jackknifing implies that the probability of each character to be up- or down-weighted is the same ($P = 0.33$, and that of being unmodified is also 0.33); the purpose of symmetric jackknifing is to eliminate bias because of the differential weights [see Goloboff *et al.* (2003) for a discussion]. For each replication of jackknifing the following search was conducted under IW (in parentheses, under SW): ten random addition sequences with TBR (one random addition sequence with SPR), keeping ten trees per replication (one tree), saving ten trees (one tree) and collapsing the trees with rearrangements of TBR (SPR) giving the same fit. As can be noted, searches under SW are much less aggressive because this criterion is much more time consuming (and TBR is not implemented in SL).

Bremer supports (absolute and relative) were calculated (Bremer, 1988, 1994). Under IW, 10 000 most-parsimonious trees and 10 000 suboptimal trees up to 10 units of fit below the optimal trees were gathered (by TBR swapping from optimal trees). Suboptimal trees were gathered in consecutive stages, saving at each stage 1000 trees and making the suboptimal trees worse by 0.1, 0.2, 0.3, 0.5, 1, 2, 3, 5, 8 and 10 units of fit (this stepwise search allows a more accurate estimation of the supports). Under SW, only absolute Bremer support is available; suboptimal trees were also gathered in consecutive stages, each one keeping 1000 trees with larger distortion ($D = 100, 300, 600, 1000, 1500, 2000, 3000$).

Results

Phylogenetic hypotheses

The consensus estimated under IW (Fig. 1) shows a topology different from the one obtained under SW (Fig. 2). SW found 7950 trees of minimum distortion ($D = 52152$, 403 transformations). IW found as many as 80 000 fittest trees (weighted homoplasy 32.86, 396 transformations), overflowing the tree buffer. For that reason, a different

approach was taken to calculate the consensus under IW: instead of obtaining all possible shortest trees, the consensus was stabilized 50 times (see Goloboff, 1999). This resulted in the 40 nodes that can be seen in Fig. 1.

Both consensus trees show the same basal topology, with *Lithogloea* (Teloganodidae) and *Melanemerella* splitting successively. The 'African' group appears paraphyletic, with *Ephemerythus* splitting first, followed by *Tricorythus*–*Machadorythus*. The next taxon to diverge is *Coryphorus*, constituting the sister group of the Leptohiphidae

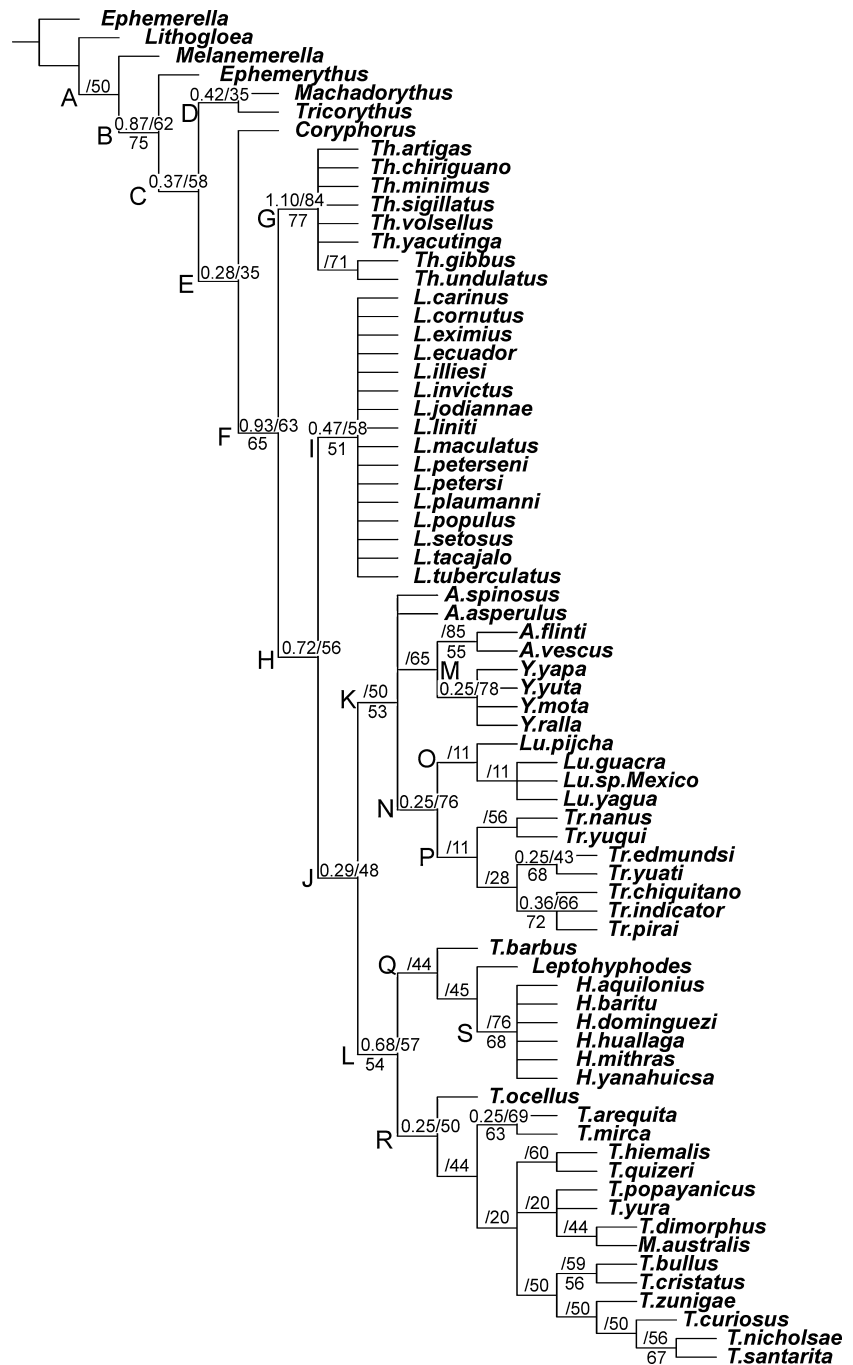


Fig. 1. Consensus stabilized 50 times under implied weighting. The numbers above the nodes indicate absolute (cut 0.25 fit) and relative Bremer support, below the nodes are frequency differences (cut 50). A = *Allenhyphes*; H = *Haplohyphes*; L = *Leptohiphes*; Lu = *Lumahyphes*; M = *Macunahyphes*; T = *Tricorythodes*; Th = *Tricorythopsis*; Tr = *Traverhyphes*; Y = *Yaurina*.

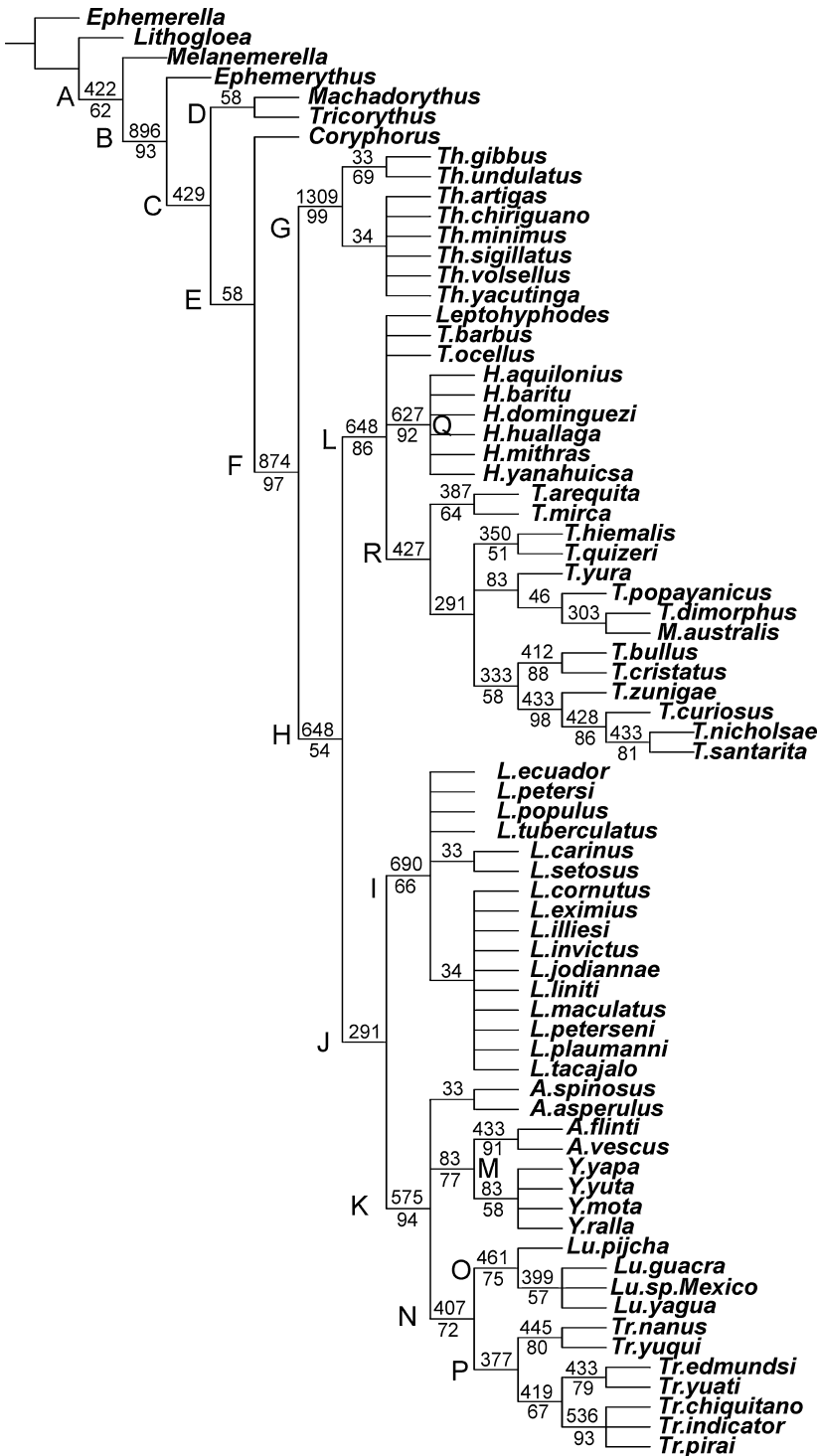


Fig. 2. Consensus from 7950 best trees under self-weighting. The numbers above the nodes indicate absolute support (as distortion), below the nodes are frequency differences (cut 50). A = *Allenhyphes*; H = *Haplohyphes*; L = *Leptoxyphes*; Lu = *Lumaxyphes*; M = *Macunaxyphes*; T = *Tricorythodes*; Th = *Tricorythopsis*; Tr = *Traverxyphes*; Y = *Yaurina*.

(the remaining taxa). Both IW and SW also agree in recovering four large groups of species in Leptoxyphidae: (1) *Tricorythopsis* (node G); (2) *Leptoxyphes* (node I); (3) *Allenhyphes*–*Traverxyphes* group (node K); (4) *Leptoxyphodes*–*Haploxyphes*–*Tricorythodes* group (node L). Both criteria

also place *Tricorythopsis* (node G) as the basal clade of Leptoxyphidae.

Differences in the results obtained from both searching criteria concern the way in which species groups 2 (*Leptoxyphes*), 3 (*Allenhyphes*–*Traverxyphes*) and 4

(*Leptohiphodes–Haplohyphes–Tricorythodes*) are related to each other. IW obtained the fittest trees with *Leptohiphodes* (group 2) as the sister of the rest (*Allenhyphes–Traverhyphes* + *Leptohiphodes–Haplohyphes–Tricorythodes*). *Allenhyphes–Traverhyphes* + *Leptohiphodes–Haplohyphes–Tricorythodes* share four character state changes (optimized under IW): presence of a longitudinal row of setae at the inner margin of maxillae (character 70, Fig. 9B), three lamellae on each abdominal gill III and IV (characters 92–93, Fig. 10H), and ventral inferior lamella of gill II perpendicular to the operculate lamella (character 102, Fig. 10E). SW recovered the *Leptohiphodes–Haplohyphes–Tricorythodes* group as sister of the other two (*Leptohiphodes* and *Allenhyphes–Traverhyphes*). *Leptohiphodes* + *Allenhyphes–Traverhyphes* share five character state changes (optimized under SW): hind wings present only in males (character 23), posterolateral spines present on abdominal segments VII–IX of the nymph (character 110), egg with chorionic plates separated by smooth chorion (character 116, Fig. 11A, B), egg with short adhesive filaments (character 118, Fig. 11B), and chorionic plates with a wide elevated margin (character 119, Fig. 11A, B).

Some differences in the internal resolution of each group also exist when comparing results from both criteria. The most remarkable are: (1) the group formed by *Tricorythodes barbatus*, *Leptohiphodes* and *Haplohyphes* (present under IW, Fig. 1) is absent from the SW consensus (Fig. 2); and (2) higher resolution at the specific level for the genera *Tricorythopsis* and *Leptohiphodes* under SW.

Character states defining major nodes

Important clades with some of their defining characters are listed below, with synapomorphies common to 7950 shortest trees obtained with SW listed in Supplementary Material S2.

Node B, representing the ancestor of the African Ephemerythidae, Tricorythidae and Machadorythidae, plus the Neotropical Coryphoridae and Leptohiphidae, is characterized (among other features) by: (a) base of vein ICu1 (character 17) fused with CuP at half from its base. This remarkable array of the cubital veins was often called ‘Tricorythid fork’ (and taken as synapomorphic for the African families listed above), but is also present in *Tricorythopsis* and some small *Tricorythodes* species; (b) costal projection of hind wings (character 24) large, extending well beyond costal margin. This is present in Ephemerythidae and most Leptohiphidae, but hind wings are absent in most of the remaining clades; (c) gill structure (pattern of lamellae division, character 97) single. The array of the numerous lamellae on each abdominal gill is single when all lamellae arise ventrally to the others (in contraposition to the bipinnated structure of other ephemereids).

Node E (Coryphoridae + Leptohiphidae) is defined by: (a) hypopharynx, with a subrectangular linguae (character 63); (b) labial palp (character 75) with reduced third and second segments.

Node F (Leptohiphidae): (a) compound eyes (character 2) lateral, not pedunculated; (b) size of male compound eyes (character 3) small, similar to female. All species of Leptohiphidae present these characteristics, except those with secondarily enlarged eyes in the male (*Leptohiphodes* and some Central American taxa); (c) male imaginal foretarsal claws (character 9): similar, both of the pair are blunt; (d) gill structure (character 96): smaller ventral lamellae developed only at base of dorsal lamella; (e) long adhesive filaments (character 118) present in the eggs.

Node H (Leptohiphidae without *Tricorythopsis*): (a) gonopore (character 40) associated with an acute and sclerotized structure (see evolution of penean structures in Fig. 12); (b) large membranous lobes present on penes (character 45); (c) three lamellae present on abdominal gill II (character 91).

Nodes L (*Leptohiphodes–Haplohyphes–Tricorythodes* group) and J (*Leptohiphodes* + *Allenhyphes–Traverhyphes* group) were defined previously (see Phylogenetic hypotheses).

Node K (*Allenhyphes–Traverhyphes* group): (a) absence of the basal row of spines on dorsum of nymphal femora II and III (character 87); (b) costal projection of hind wings (character 24) very large (0.53 or more of total wing length); (c) two longitudinal veins on male hind wings (character 28); (d) acute posteromedial projections on styliiger plate (character 31); (e) hind margin of styliiger plate (character 37) slightly concave; (f) gonopore (character 40) associated with a hollow spine.

Node R (nearly all *Tricorythodes* species): (a) sulcus (dorsal depression between mesonotal posterolateral protuberances) absent; (b) gonopore not associated with a spine; (c) membranous lobes of penes small. Most species of *Tricorythodes* had secondarily lost the apical spine of the penes and reduced the membranous lobes (Fig. 8D), although in some species (e.g. *T. ocellus*) these structures remain recognizable.

Constrained searches

Constraining all the nodes present in the consensus of the 7950 trees obtained under SW and then searching trees under IW obtained trees of fit 87.14, a weighted homoplasy of 32.86 and 396 transformations. SW trees optimized under IW had a fit of 86.65–86.84, a weighted homoplasy of 33.16–33.35 and 398 transformations. IW trees optimized under SW showed distortions from 52 323 to 52 587 and 402–404 transformations.

Group support

Under IW, the three measures of support (absolute and relative Bremer supports and frequency differences) were high (Fig. 1) for nodes B (including *Ephemerythus*, *Machadorythus* + *Tricorythus*, *Coryphorus* + Leptohiphidae), F (Leptohiphidae) and G (*Tricorythopsis*). Other groups also showed high support values, but not in the three measures simultaneously (Fig. 1).

Under SW (Fig. 2), the two calculated measures (absolute Bremer and frequency differences) largely supported nodes B, F, G, I (*Leptoxyphes* spp.), K (*Allenhyphes-Traverhyphes* group), L (*Tricorythodes* + *Leptoxyphodes* + *Haploxyphes*) and Q (*Haploxyphes* spp.). Frequency differences were high for many groups not well supported by absolute Bremer values (Fig. 2).

Discussion

Optimality criteria

The SW criterion implemented in *SL* takes into account possible asymmetries in transformation costs. These asymmetries, if they exist, are estimated from the data as a result of the frequency of each direction of transformation, instead of being assumed a priori. This means that, in any given tree, if losses are more frequent than regains, the cost of the losses will be set lower than the cost of the regains. This optimality criterion is particularly sensitive to characters that show a frequent reduction or loss. In many cases, SW, in allowing for asymmetries in transformation costs, considered many of the character state transformations as more reliable (= informative) than IW (which needlessly down-weighted the whole character). The results under the SW (Fig. 2) criterion are preferred here as leptoxyphids show many sets of character states interpretable as reductions, among others: fore wing venation (mainly in *Cu* area), hind wing (complete absence in some genera, number and complexity of veins), adult female caudal filaments, nymphal gills (number of gill-bearing abdominal segments, number of lamellae in each gill), nymphal maxillary palpi (size and number of segments) and nymphal posterolateral abdominal spines (number and development).

The results from constrained searches indicate a small difference in length among trees from SW and IW. As expected, those nodes that differ between both criteria do not show high support values.

Comparing results from IW and SW

Before comparing results from IW and SW, note that each criterion weights in a different manner. Given a single tree, IW weights entire characters, so one character may be good or not, depending on its homoplasy on the tree. SW, instead, weights individual state changes, so a character can have different weights in a single tree (i.e. if the character presents more than one possible reconstruction, each reconstruction implies a different set of transformation costs). Furthermore, one direction of the state change can be down-weighted in relation to the other. For example, in a binary character the transformation $0 \rightarrow 1$ can have the maximum possible weight if it only appears once, whereas the transformation $1 \rightarrow 0$, if more common (i.e. homoplastic), will show a lower value (the more the steps, the less the weight).

What happens with characters 70, 92, 93 and 102 (those defining *Allenhyphes-Traverhyphes* + *Leptoxyphodes-*

Haploxyphes-Tricorythodes under IW) under SW optimization? Why does SW reduce the weights of these characters while increasing those defining a different group (*Leptoxyphes* + *Allenhyphes-Traverhyphes*): characters 23, 110, 116, 118 (119)? Three of the four state changes (those of characters 70, 92 and 102) that would define the node *Allenhyphes-Traverhyphes* + *Leptoxyphodes-Haploxyphes-Tricorythodes* show relatively low weights under SW because they are much more common than the alternative state changes inside those characters (the transformations of character 93 are not easily comparable between IW and SW). For example, the change 2 (four lamellae on gill III) \rightarrow 3 (three lamellae on gill III) in character 92 appears four times in most SW trees, but the other possible state changes ($0 \rightarrow 2$, $0 \rightarrow 3$, $2 \rightarrow 0$, $3 \rightarrow 1$, $3 \rightarrow 2$) appear just once. Thus, SW down-weights only the common change ($2 \rightarrow 3$), but not the others. Something similar happens with characters 70 and 102; the transformations that would define the group *Allenhyphes-Traverhyphes* + *Leptoxyphodes-Haploxyphes-Tricorythodes* are more common than the others, and thus are not considered reliable by SW (bold in Supplementary Material S2).

Conversely, state changes defining the clade *Leptoxyphes* + *Allenhyphes-Traverhyphes* in SW (characters 23, 110, 116, 118, 119) occur once (except character 116), so they are down-weighted mildly (or not at all; see Supplementary Material S3). All state changes, weights and distortions (given by *SL*) for the characters involved in the relationships of *Leptoxyphes*, *Allenhyphes-Traverhyphes* and *Leptoxyphodes-Haploxyphes-Tricorythodes* are provided in Supplementary Material S3 (note the inclusion of characters 70, 92, 93 and 102 for comparison with trees obtained under IW).

Character 119 also constitutes an interesting example about how weights are assigned by each criterion. Character 119 does not show a good fit on the IW trees (adjusted homoplasy = 0.50, when perfect fit is 0.00), nor does it have a low distortion on SW trees (distortion index = 0.46, when minimum distortion is 1.00). Nevertheless if character 119 is not analysed as an entire entity, but is instead partitioned in its possible state changes, then the change of interest (defining the group *Allenhyphes-Traverhyphes* + *Leptoxyphodes-Haploxyphes-Tricorythodes*) has maximum weight ($02 > 1$, weight = 100), unlike the others ($0 > 2$ or $2 > 0$, weight = 66).

In conclusion, under SW, three of four synapomorphies recovered by IW are poor (weight = 66–85), whereas four of five synapomorphies recovered under SW are good (weight = 100). Thus, the trees of minimum distortion (Fig. 2) show the group formed by these five synapomorphies.

Higher classification

McCafferty & Wang (2000) proposed a phylogeny of the Ephemeroidea, including some of the groups treated here (Fig. 3A). These authors did not include individual or representative species from each taxon, but constructed hypothetical ancestors for each 'family group'. The most dramatic difference with the present study is in the absence of *Melanemerella* and the simplification of the diversity of

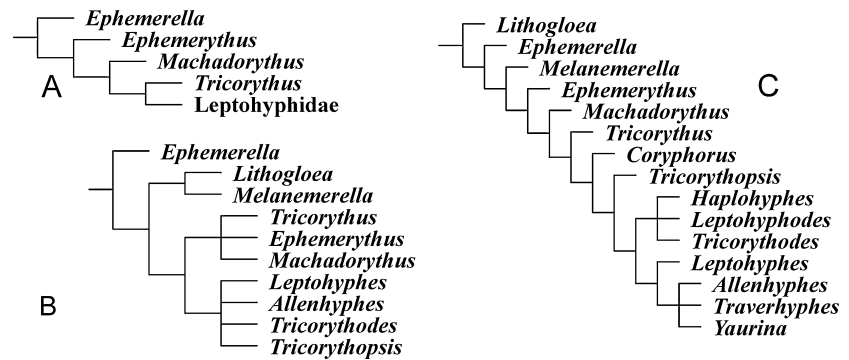


Fig. 3. Previous phylogenetic hypotheses (only taxa studied here included). A, McCafferty & Wang (2000); B, Kluge (2004); C, Molineri & Domínguez (2003).

Leptohiphidae + *Coryphorus* (in just one terminal) in the phylogeny of McCafferty & Wang (2000). In spite of their different approach, the more basal nodes of McCafferty & Wang (2000) are comparable with those in the present study, and the branching pattern of the taxa shared by both studies is similar. Molineri & Domínguez (2003) presented a phylogenetic analysis including *Melanemerella*, among other ephemeroideids, obtaining a pattern similar to that obtained here (Fig. 3C).

Kluge (2004) proposed a phylogeny for the Ephemeroidea, constructing some hypothetical terminals from various taxa. Those taxa shared with the analysis presented here are shown in Fig. 3B. Kluge's cladogram shows a monophyletic group formed by *Ephemerythus*, *Machadorythus* and *Tricorythus* (plus related African genera) as sister clade of Leptohiphidae. This author did not include *Coryphorus* in the phylogenetic diagram, treating this taxon as insertae sedis.

Subfamilies

Wiersema & McCafferty (2000) divided Leptohiphidae into two subfamilies: Leptohiphinae (*Allenhiphes*, *Haplohiphes*, *Leptohiphes*, *Leptohiphodes* and *Vacupernius*) and Tricorythodinae (*Coryphorus*, *Tricorythopsis*, *Tricorythodes*, *Asioplax*, *Epiphrales*, *Homoleptohiphes*, *Tricorythipes*). These authors stated that their classification was cladistically based, but did not include a matrix, a list of characters, nor a phylogenetic branching diagram. The subfamilies Leptohiphinae and Tricorythodinae, as defined by Wiersema & McCafferty (2000), were not recovered in the present study (Fig. 4). The genera *Coryphorus* and *Tricorythopsis* (assigned to Tricorythodinae) and *Leptohiphodes* and *Haplohiphes* (assigned to Leptohiphinae), present some of the characters hypothesized as synapomorphies of the subfamilies in Wiersema & McCafferty's (2000) scenario, but not others. This is even more evident when we consider that these authors had assigned nymphs and adults of the same species to different subfamilies: the adult of *Tricorythopsis artigas* Traver was assigned to Tricorythodinae and the nymph (originally described as *Leptohiphes tinctus* Allen, but a synonym of *T. artigas*; Molineri, 2001a) was assigned to Leptohiphinae. The same

was true for the nymphs *L. viriosus* Allen and *L. minimus* Allen [synonyms of *Tricorythopsis minimus* (Allen); Molineri, 2001a], transferred by Wiersema & McCafferty (2000) to *Allenhiphes* ('Leptohiphinae'), when they are actually *Tricorythopsis* ('Tricorythodinae').

These results strongly support the subdivision of Leptohiphidae into four groups: (1) *Tricorythopsis* [eight species studied here and four described recently by Dias & Salles (2005) and Molineri & Zuñiga (2006)]; (2) *Leptohiphes* [16 species included here and three described recently by Molineri & Zuñiga (2006)]; (3) the *Allenhiphes*–*Traverhiphes* group (19 species included here), including the genera *Allenhiphes*, *Lumahyphes*, *Traverhiphes* and *Yaurina* (*Vacupernius* not studied here); (4) the group formed by *Leptohiphodes*, *Haplohiphes* and *Tricorythodes* (23 species included).

As these four groups show high support values and are defined by numerous characteristics in the adult and nymphal stages, they could be treated as formal taxonomic entities (e.g. subfamilies). Alternatively, if the SW criterion is followed (as preferred here), three 'subfamilies' would be supported: one including *Tricorythopsis* species, another joining *Leptohiphes* with the *Allenhiphes*–*Traverhiphes* group and a third one for the genera *Leptohiphodes*, *Haplohiphes* and *Tricorythodes*. However, I prefer not to formally define these groups until other developing studies concerning the phylogeny of the Central and North American Leptohiphidae (D. Baumgardner, pers. comm.) can be integrated with the South American portion to provide a more stable classification of the family.

Conclusions

1. The monophyly of Leptohiphidae is highly supported. Its sister group is the monospecific taxon Coryphoridae. Both are more closely related to the Afro-Oriental groups (Ephemerythidae, Tricorythidae, etc.) than to other South American Ephemeroidea (*Melanemerellidae*).
2. The recently proposed subfamilies of Leptohiphidae, Leptohiphinae and Tricorythodinae (Wiersema & McCafferty, 2000), are not monophyletic groups. This is so regardless of the weighting method used (and is true also of analyses under equal weights).

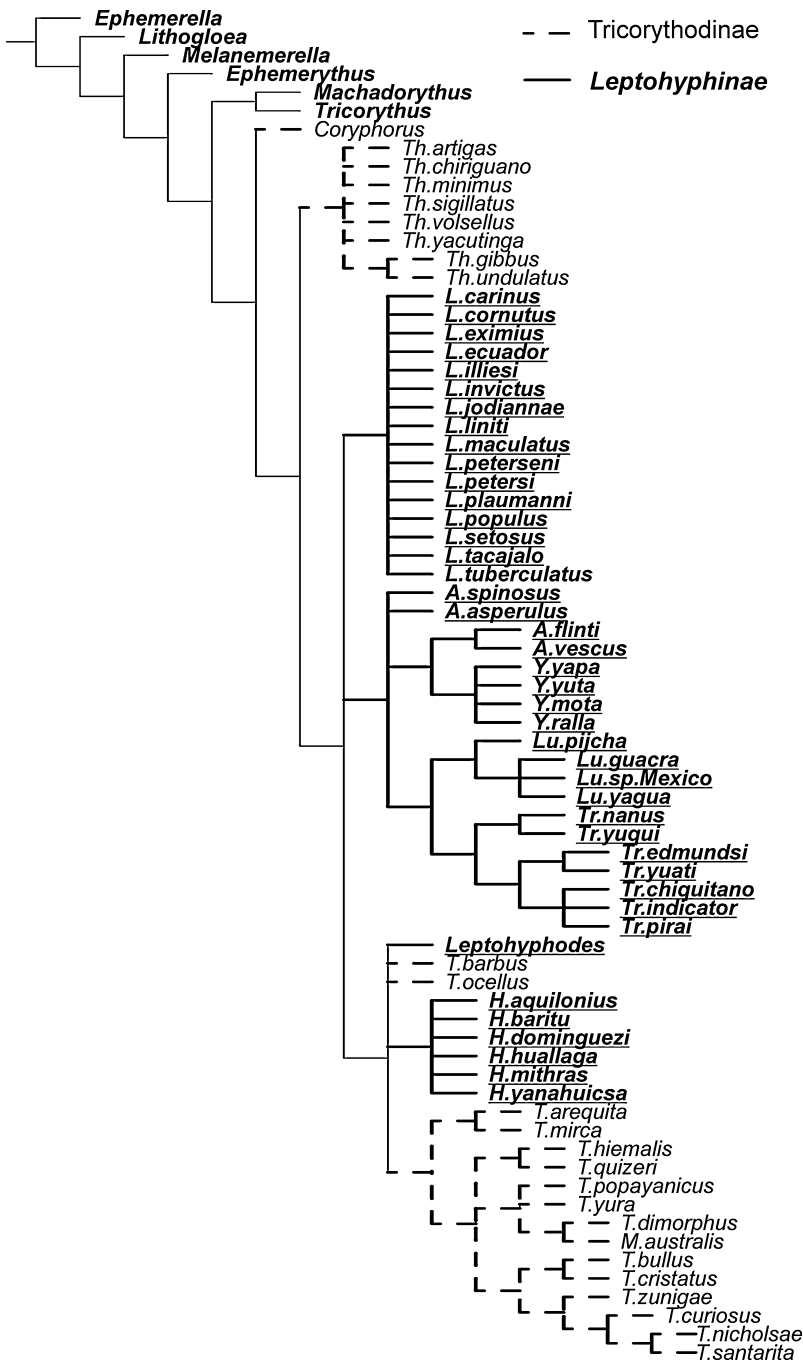


Fig. 4. Consensus from trees obtained under both criteria (implied and self-weighting). Superimposed are the proposed subfamilies of Leptohiphidae (Wiersema & McCafferty, 2000). Dashed lines, Tricorythodinae; entire thick line, Leptohiphinae. A = *Allenhyphes*; H = *Haplohyphes*; L = *Leptohiphes*; Lu = *Lumahyphes*; M = *Macunahyphes*; T = *Tricorythodes*; Th = *Tricorythopsis*; Tr = *Traverhyphes*; Y = *Yaurina*.

3. SW detects more accurately homoplastic changes than IW by not reducing the weight of entire characters in those cases where only one of the possible state changes is homoplastic.

Supplementary material

The data matrix (S1), character state changes (S2) and synapomorphy list (S3) are available at: <http://www.blackwellsynergy.com> under the DOI reference doi: 10.1111/j.1365-3113.2006.00357.x.

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Appendix 1. Characters and character states

Adults

1. *Small sublateral tubercles on hind margin of head*: (0) absent; (1) present (Fig. 5A).
2. *Position of compound eyes*: (0) laterodorsal; (1) lateral, pedunculated; (2) lateral, not pedunculated.
3. *Size of male compound eyes*: (0) small, similar to female (Fig. 5A); (1) large (Fig. 5B, C).
4. *Upper and lower division of male compound eyes*: (0) present (Fig. 5B, C); (1) absent (Fig. 5A).
5. *Mesopleurae, sutures on lateropostnotum*: (0) superior suture and inferior suture not forming a straight line; (1) superior suture and inferior suture forming a straight line. Character proposed by Kluge (1992) to distinguish *Leptohiphes* (with state 0) from *Tricorythodes* (with state 1).
6. *Internal and external parapsidal sutures*: (0) running independently until fore mesonotal transverse invagination; (1) fused before or at fore mesonotal transverse

- invagination. Character proposed by Wiersema & McCafferty (2000) to separate subfamilies Leptohiphinae (state 0) and Tricorythodinae (state 1)
7. *Sulcus (dorsal depression between postero-scutal protuberances)*: (0) present; (1) absent. Character proposed by Wiersema & McCafferty (2000), same as previous character.
 8. *Membranous filaments of mesoscutellum*: (0) absent or not extending beyond tip (Fig. 5F); (1) short, not reaching abdominal segment II; (2) long, reaching abdominal segment II (Fig. 5G).
 9. *Male foretarsal claw (imago)*: (0) dissimilar (one hooked and one blunt; Fig. 5D); (1) similar (both blunt; Fig. 5E).
 10. *Femoral forelegs (imago)*: (0) present; (1) absent.
 11. *Ventrodiscal extension of tibiae*: (0) absent; (1) present slightly marked; (2) present long. Wiersema & McCafferty (2000) proposed state 2 as a synapomorphy of Tricorythodinae.
 12. *Number of tarsal segments*: (0) 4; (1) 5. Character proposed by Wiersema & McCafferty (2000), explanation as in character 6.
 13. *Black macula on apex of tibiae*: (0) absent; (1) present.

14. *Fore wings with fringed hind margin (imago)*: (0) absent; (1) present (Fig. 6A–C).
15. *Sexual dimorphism in fore wing shape*: (0) absent; (1) present. State 0 refers to the species showing similar wings in both sexes (broadest at the middle, CuA lobe not enlarged). The alternative state 1 refers to those species showing male fore wings with an expanded CuA lobe (thus wings are broadest at base), whereas females present wings broadest at the middle.
16. *Ratio length of fore and hind wings (male)*: (0) 0.25 or more; (1) 0.20 or less.
17. *Base of vein ICu1*: (0) fused with (Fig. 6B) or clearly directed to (Fig. 6A) base of CuP; (1) free or fused with CuA or CuP by crossveins (Fig. 6C); (2) fused with CuP at half from base (Tricorythid fork, Fig. 6D).
18. *Base of vein CuP*: (0) present (paralleling CuA); (1) absent (ending at vein A).
19. *MA (Median Anterior Vein) fork*: (0) present; (1) absent, MA1 and MA2 joined by a crossvein.
20. *IMP (Intercalary Median Posterior Vein) fused with CuA, with MP2 appearing as an intercalary*: (0) absent; (1) present.

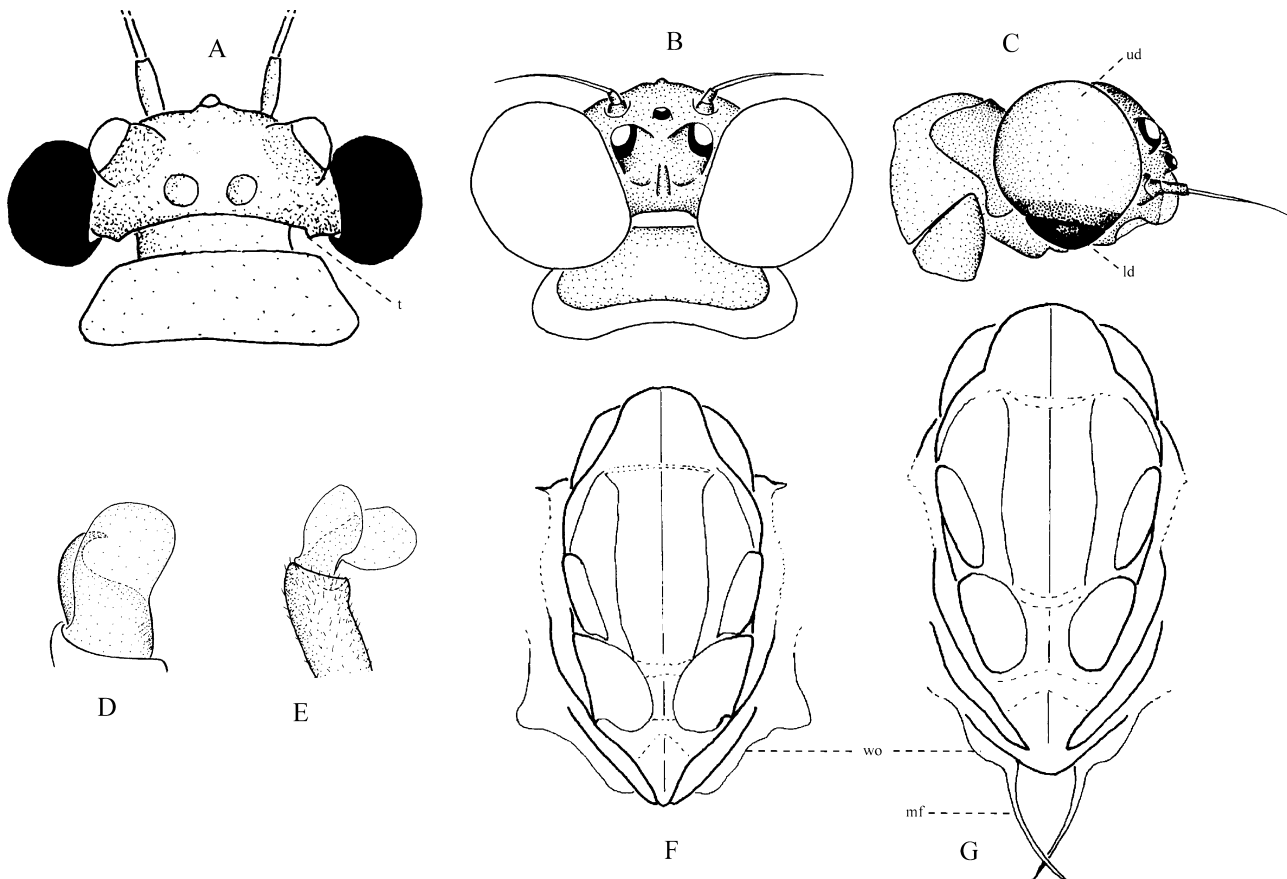


Fig. 5. Characters of the male imago. Head: A, *Traverhypthes*, d.v.; B, *Leptohiphodes*, d.v.; C, same, l.v. Fore tarsal claws: D, Coryphoridae; E, Leptohiphidae. Mesonotum: F, *Haplohypthes*; G, *Leptohiphes*. dv = dorsal view; ld = lower division of compound eye; lv = lateral view; mf = membranous filaments of mesoscutellum; t = tubercle; up = upper division of eye; wo = wing outgrowth.

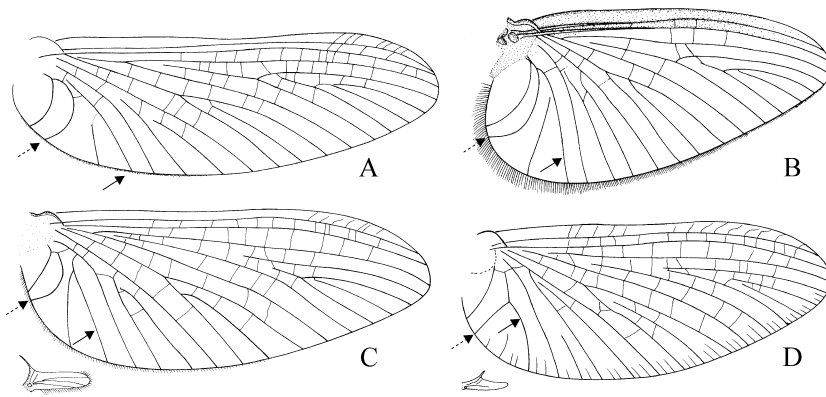


Fig. 6. Characters of the male imago. Wings: A, *Leptohyphodes*; B, *Tricorythodes*; C, *Haplohyphes*; D, *Ephemerythus*. Dashed arrow = longitudinal vein CuP; thicker arrow = intercalary vein ICu1.

21. Number of intercalary veins between CuA and CuP: (0) 3–4; (1) 2; (2) 0.
22. Marginal intercalaries: (0) mostly attached; (1) mostly detached (Fig. 6D); (2) mostly lost in at least one sex; (3) absent (Fig. 6A–C).
23. Hind wings (Fig. 6C, D): (0) present in both sexes; (1) present in male, absent in female; (2) absent in both sexes.
24. Costal projection of hind wings (male): (0) short and blunt (<0.2 of wing length); (1) large (0.3–0.46); (2) very large (0.53 or more).
25. Base of costal projection (basal angle): (0) rounded; (1) straight.
26. Hind wing margin (imago): (0) not fringed (Fig. 6D); (1) fringed on hind margin only; (2) fringed on hind margin and apex of fore margin (Fig. 6C).
27. Location of costal projection: (0) at one-half or one-third from base (Fig. 6D); (1) at base (Fig. 6C).
28. Number of longitudinal veins on male hind wings: (0) > 8; (1) 3; (2) 2; (3) 1.
29. Hind wing crossveins: (0) present; (1) absent.
30. Posterolateral projections of styliiger plate (external): (0) absent; (1) present.
31. Acute posteromedial projections of styliiger plate: (0) absent; (1) present.
32. Forceps: (0) two-segmented (Fig. 8A–C); (1) three-segmented (Figs 7A, 8D).
33. Length of first forceps segment: (0) long (first and second segments subequal in length); (1) medium (first segment one-third to three-quarters the length of second segment); (2) short (first segment one-fifth to one-quarter the length of second segment).
34. Basal swelling of first forceps segment: (0) absent; (1) present.
35. Basal swelling of second forceps segment: (0) absent (Figs 7A, 8A–C); (1) present (Fig. 8D).
36. Blunt posteromedial projections of styliiger plate: (0) absent; (1) present (Fig. 8D).
37. Form of hind margin of styliiger plate: (0) strongly projected medially; (1) slightly convex; (2) slightly concave.
38. Styliiger plate posteriorly projected forming a columnar base for each forceps: (0) absent; (1) present (Fig. 8B, C).
39. Penes with a basal sclerotized ring: (0) absent; (1) present (Fig. 7D).
40. Gonopore: (0) free, not associated with a spine (Fig. 8B); (1) associated with an acute and sclerotized structure (Figs 7A–C, 8A, C); (2) associated with a hollow spine (Fig. 7D).
41. Penes spine curvature in lateral view: (0) absent; (1) present.
42. Penes spine curvature in dorsal view: (0) absent; (1) present (Fig. 7D).

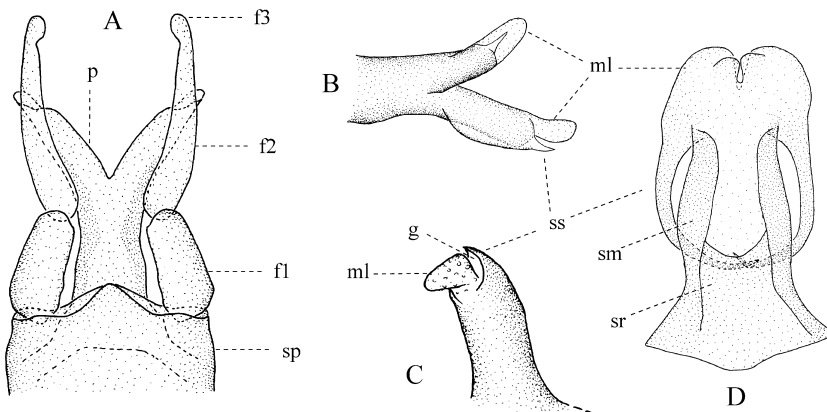


Fig. 7. Characters of the male imago. Genitalia: A, *Leptohyphes*, ventral view; Penes: B, *Leptohyphes*, ventrolateral view; C, *Leptohyphes*, posterior view; D, *Luma-hyphes*, ventral view f1, f2, f3 = forceps segments 1, 2 and 3; g = gonopore; ml = membranous lobe; sm = sclerotized margin; sp = styliiger plate; sr = sclerotized ring.

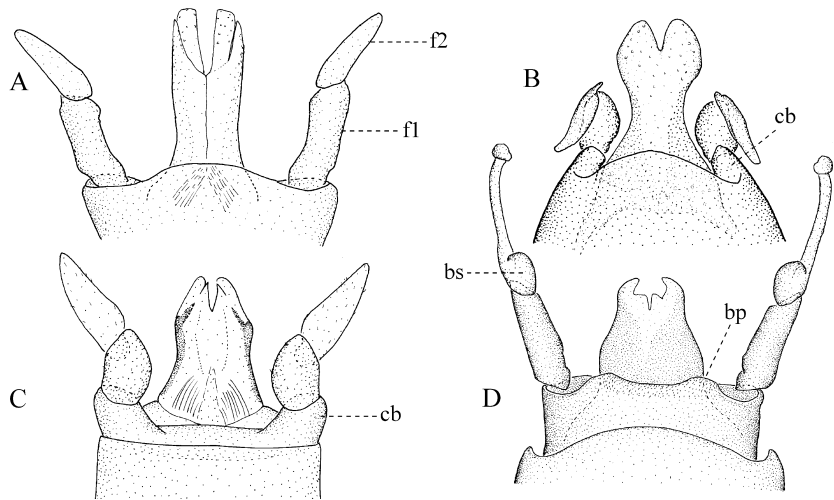


Fig. 8. Characters of the male imago. Genitalia: A, *Haplohyphes*; B, *Tricorythopsis*; C, *Leptohiphodes*; D, *Tricorythodes*. bs = basal swelling of forceps segment 2; bp = blunt posteromedial projection of styliger; cb = columnar base of styliger; f1, f2 = forceps segments 1 and 2.

43. *Insertion of penean spines*: (0) apical or subapical; (1) basal.
44. *Position of the penean spines*: (0) dorsal; (1) lateral (Fig. 7D); (2) ventral (Fig. 7A).
45. *Membranous lobes of penes*: (0) absent; (1) present, small; (2) present, large.
46. *Additional pair of smaller membranous lobes*: (0) absent; (1) present.
47. *Lateral margins of penes sclerotized* (Fig. 7D): (0) absent; (1) present (Fig. 7D).
48. *Dorsal membranous extensions of penes*: (0) absent; (1) present, double; (2) present, single. Molineri (2004) described this character in detail, presenting some scanning electron micrographs.
49. *Penes width*: (0) wider at base; (1) similar width along their length; (2) wider at apex.
50. *Fusion of penes*: (0) partial, divided on apical half (Fig. 7A); (1) total (may be divided on apical third; Figs 7D, 8A–D).
51. *Posterolateral margin of penes*: (0) rounded; (1) with a lateral notch.
52. *Angle between penean arms*: (0) 180°; (1) 45–90°; (2) < 30°.
53. *Lateral groove of penes*: (0) absent; (1) present.
54. *Small internal setae in deep penean cleft* (*Allenhyphes* kind): (0) absent; (1) present.
55. *Dorsal accessory structures of penes*: (0) absent; (1) present, single; (2) present, bifid; (3) present, double.
56. *Female gonopore with a black macula*: (0) absent; (1) present.
57. *Male terminal filament with a ventral spine at base*: (0) absent; (1) present.
58. *Length of female cerci*: (0) longer than fore wings; (1) subequal to or shorter than fore wings; (2) rudimentary.
- Nymph*
59. *General shape of body*: (0) slender (thorax relatively elongated, abdomen cylindrical and long); (1) robust
- (thorax relatively wider, abdomen shorter and triangular); (2) discoidal (body distinctly flattened, abdomen very short).
60. *Frontal projection*: (0) present; (1) absent.
61. *Genal projections*: (0) absent; (1) present, bare; (2) present, with spines
62. *Anteromedian emargination of labrum*: (0) shallow (Fig. 9F); (1) deep (Fig. 9G).
63. *Hypopharynx, form of linguae*: (0) trapezoid or triangular; (1) subrectangular.
64. *Hypopharynx, fore margin of linguae*: (0) acute; (1) blunt; (2) concave.
65. *Maxillary palpi, form of apical segment*: (0) oblong, basally wider; (1) other form.
66. *Segments of maxillary palpi*: (0) three-segmented (Fig. 9A); (1) two-segmented (Fig. 9B); (2) one-segmented; (3) absent.
67. *Size of maxillary palp*: (0) large (almost reaching apex of maxillae; Fig. 9A); (1) small (Fig. 9B).
68. *Maxillae, number of long and curved setae on distal brush of galea*: (0) <20; (1) >40 (Fig. 9A, B).
69. *Maxillae, setae at base of inner margin, group or transverse row*: (0) present (Fig. 9A); (1) absent (Fig. 9B).
70. *Maxillae, setae at base of inner margin, longitudinal row*: (0) absent (Fig. 9A); (1) present (Fig. 9B). This and the previous character are not treated as different states of a single character because some taxa (e.g. *Haplohyphes*) show both features at a time.
71. *Maxillae, suture between galea and lacinia*: (0) complete (Fig. 9A); (1) incomplete (Fig. 9B); (2) absent.
72. *General form of maxillae*: (0) *Leptohiphodes* kind (Fig. 9A–C); (1) *Haplohyphes* kind (Fig. 9D); (2) *Tricorythodes* kind (Fig. 9E).
73. *Maxillae, ratio stipes/galea-lacinia length*: (0) subequal or stipes shorter; (1) stipes longer than galea-lacinia.
74. *Labium, glossae and paraglossae*: (0) fused as in *Tricorythidae*; (1) not fused; (2) fused as in *Coryphorus*.

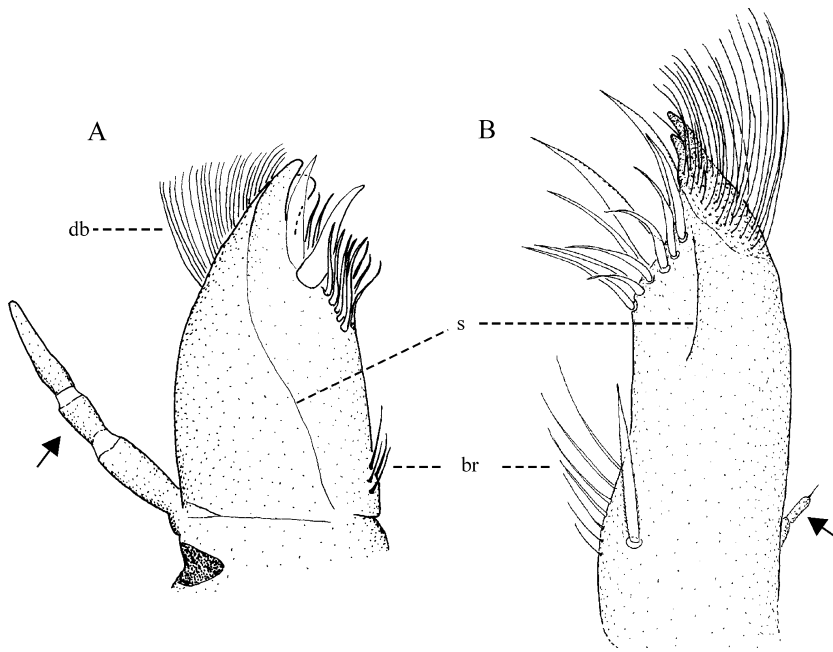


Fig. 9. Characters of nymphs. Maxilla: A, *Leptohyphes* sp.; B, *Traverhyphes* sp.; C, *Leptohyphes* sp.; D, *Haplohyphes* sp.; E, *Tricorythus* sp. Labrum: F, *Yaurina*; G, *Leptohyphodes*. Arrows = palp. br = setae at base of inner margin; db = distal brush of curved setae; s = suture between galea and lacinia.

75. *Labial palp*: (0) third segment reduced; (1) third and second segments reduced.
76. *Labium, submentum*: (0) rounded; (1) not rounded.
77. *Labium, prementum, basal and apical width*: (0) similar or wider at apex; (1) wider at base.
78. *Anterolateral projections of pronotum*: (0) present; (1) absent.
79. *Mature wingpads*: (0) blackish; (1) whitish.
80. *Tubercles on head and thorax*: (0) absent; (1) present.
81. *Form of femoral spines*: (0) short, length less than two times width; (1) median, length three to six times width; (2) long, setae-like.
82. *Row of stout spines at leading edge of fore femora*: (0) absent; (1) present. The leading edge of the fore femur may show varied setation, in most genera it is bare, but some taxa show long and thin setae, or stronger spine-like setae (but similar in aspect to the dorsal spine-like setae). These cases were scored here as state 0. State 1, present only in *Haplohyphes* species, refers to a row of short spine-like setae that differ markedly from the other setae present in the leg (which are hair-like).
83. *Transverse row on fore femora*: (0) subdistal; (1) submedian; (2) subbasal.
84. *Femora width*: (0) not wider than half length; (1) almost as wide as long.
85. *Longitudinal ridge on femora II and III*: (0) present; (1) absent.
86. *Distal denticles on tarsal claws*: (0) double row; (1) single row; (2) one, asymmetric; (3) absent.
87. *Basal row of spines/setae on dorsum of femora II and III*: (0) present; (1) absent.
88. *Dorsal tubercles on abdomen (at least in one abdominal segment)*: (0) absent; (1) present.
89. *Ventral lamellae of gills*: (0) bifid with numerous flaps; (1) not clearly bifid, generally without flaps.
90. *Form of ventral lamellae of gills*: (0) ovoid; (1) subtriangular.
91. *Number of lamellae on gill II*: (0) 5 or more; (1) 4; (2) 3; (3) 2; (4) 1.
92. *Number of lamellae on gill III*: (0) 10 or more (Fig. 10 B); (1) 5 (Fig. 10F); (2) 4 (Fig. 10D); (3) 3 (Fig. 10H).
93. *Number of lamellae on gill IV*: (0) 8 or more; (1) 5; (2) 4; (3) 3.
94. *Number of lamellae on gill V*: (0) 8 or more; (1) 4; (2) 3; (3) 2; (4) 1.
95. *Number of lamellae on gill VI*: (0) 5 or more; (1) 2; (2) 1; (3) 0 (gill absent).
96. *Gill structure (position of smaller lamellae)*: (0) along entire margin of main lamella; (1) only at base.
97. *Gill structure (pattern of lamellae division)*: (0) bipinnated (dorsal and ventral); (1) single (ventral or lateral).
98. *Form of gill II*: (0) subcuadrate; (1) subtriangular; (2) ovoid.
99. *Pigments on gill II*: (0) uniform, extensive (unpigmented patches may be present); (1) maculated, extensive; (2) only at base (less than 25% of gill surface).
100. *Ridges on gill II*: (0) absent or 1 slightly marked; (1) 1; (2) 2.
101. *'Basal spine' of gill II*: (0) present (Fig. 10A); (1) absent. Allen (1967, 1978) gave the name 'basal spine' to a colourless outgrowth of the ventral region of gill II (Baumgardner & McCafferty, 2000). Clearly this feature is not a spine (but probably a reduced ventral lamella). Nevertheless, the name is conserved because of its very common usage in the literature.
102. *Gill II, ventral inferior lamellae*: (0) parallel to dorsal lamellae (Fig. 10A); (1) perpendicular to dorsal lamellae (Fig. 10C, E, G); (2) reduced (Fig. 10I).

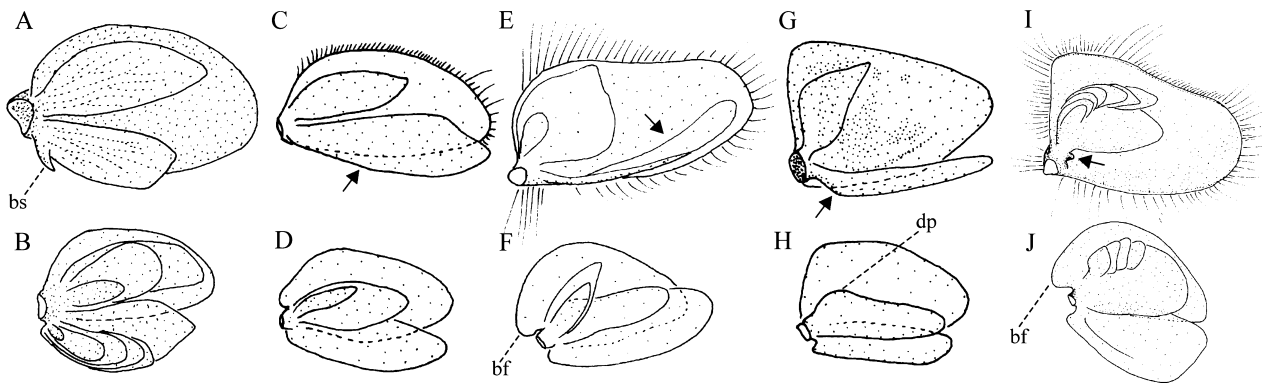


Fig. 10. Characters of nymphs. Abdominal gills II and III: A, B, *Leptohiphes cornutus*; C, D, *Traverhyphes*; E, F, *Haplohiphes*; G, H, *Tricorythodes*; I, J, *Leptohiphodes*. Arrows = inferior ventral lamellae. bf = basal flap of dorsal lamellae; bs = basal spine; dp = dorsal projection of ventral lamellae.

103. Gill II with a transverse weak line: (0) absent; (1) present.

104. Depigmented macula at posterolateral margin of gill II: (0) absent; (1) present.

105. Gill II: (0) normal, nonoperculate; (1) operculate (entirely covering remaining gills).

106. Basal flap of dorsal lamellae (gills III–V): (0) present (Fig. 10F, J); (1) absent (Fig. 10B, D, H).

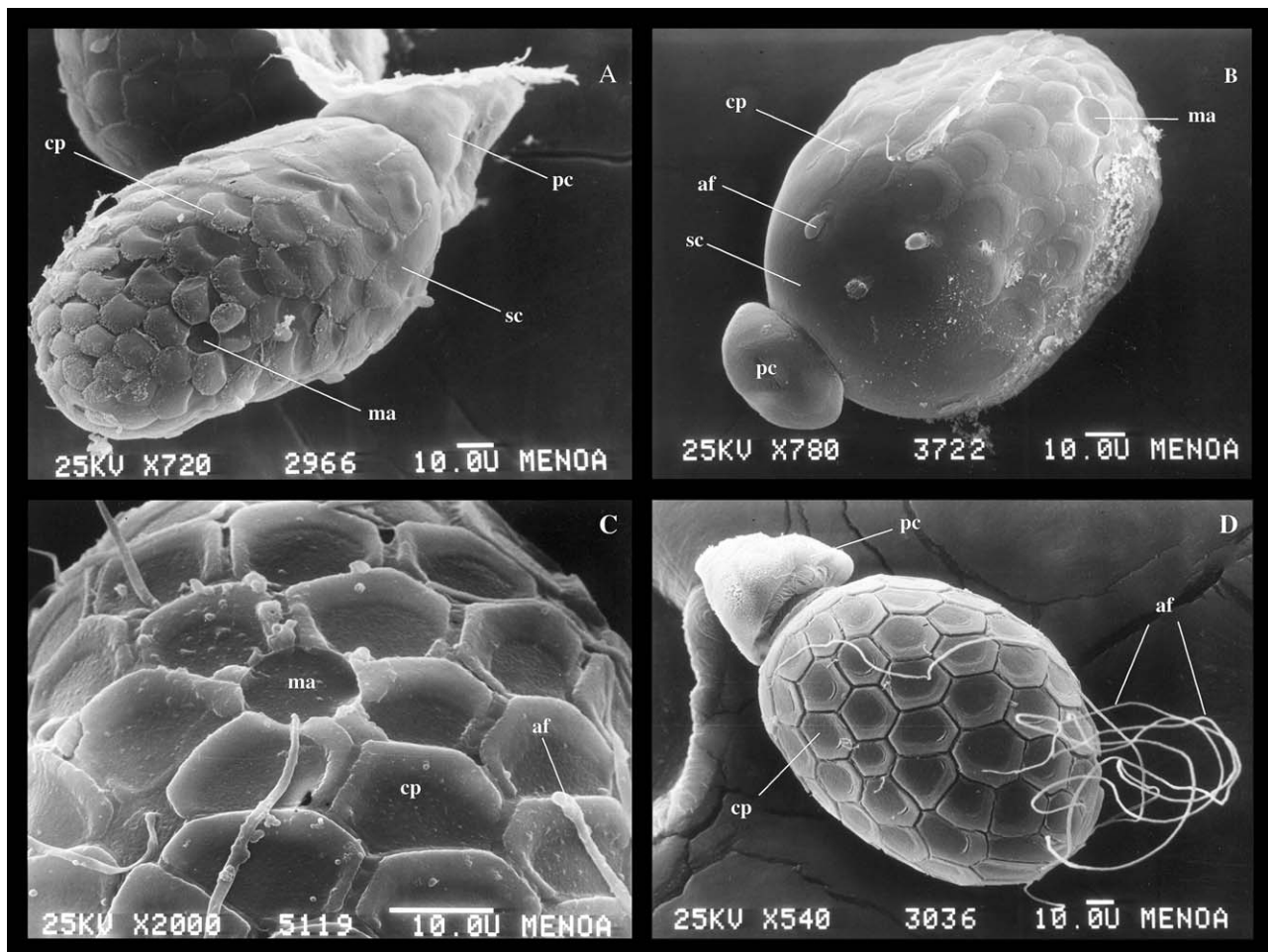


Fig. 11. Characters of eggs. A, *Traverhyphes indicator*; B, *Leptohiphes* sp.; C, *Haplohiphes baritu*, detail of micropylar area; D, *Tricorythodes hiemalis*. af = adhesive filaments; cp = chorionic plates; ma = micropylar area; pc = polar cap; sc = smooth chorion.

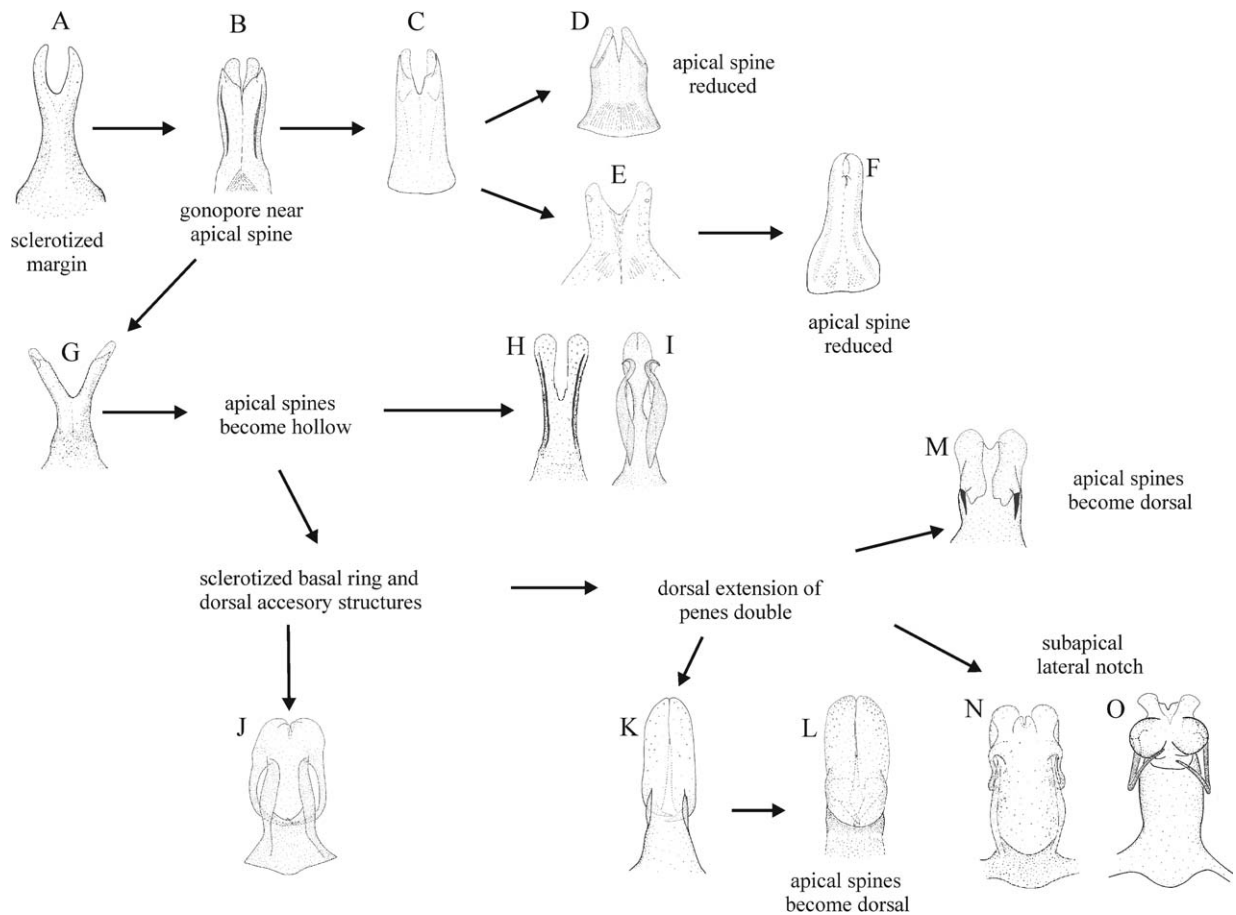


Fig. 12. Evolution of penean structures in Leptohiphidae. Imaginal penes, unless counter indicated: A, *Trichorythopsis*; B, *Haplohyphes* and *Leptohiphodes* (subimago); C, *Haplohyphes*; D, *Leptohiphodes*; E, *Tricorythodes ocellus*; F, *Tricorythodes quizeri*; G, *Leptohiphodes*; H, *Allenhyphes*; I, *Yaurina*; J, *Lumahyphes*; K, *Traverhyphes* (*T.*) *chiquitano*; L, *Traverhyphes* (*T.*) *indicator*; M, *Traverhyphes* (*Byrsahyphes*) *nanus*; N, *Traverhyphes* (*Mocohyphes*) *edmundsi*; O, *Traverhyphes* (*M.*) *yuati*.

107. *Dorsal projection of ventral lamellae (gills III–V)*: (0) present (Fig. 10H); (1) absent.
108. *Row of setae on abdominal tergum VII*: (0) absent; (1) present.
109. *Hind margin of abdominal terga III–V with small spicules*: (0) present; (1) absent. Character proposed by Wiersema & McCafferty (2000), explanation as in character 6.
110. *Posterolateral spines on abdominal segments*: (0) II or III–IX; (1) VI–IX; (2) VII–IX; (3) VII–VIII; (4) VII; (5) absent.
111. *Lateral flanges on abdominal segments*: (0) absent; (1) III–VI; (2) III–VII; (3) III–VIII; (4) III–IX.
112. *Dark annuli on caudal filament (with sexual dimorphism)*: (0) present; (1) absent.
113. *Number of polar caps*: (0) 1 (Fig. 11A, B, D); (1) 2.
114. *Sperm guide*: (0) absent; (1) present.
115. *Position of micropyle (or micropylar area)*: (0) not restringed; (1) polar; (2) equatorial.
116. *Chorionic plates*: (0) contiguous (Fig. 11C, D); (1) separated by smooth chorion (Fig. 11A, B); (2) as a hexagonal netting; (3) absent.
117. *Normal knob-terminated coiled threads*: (0) present; (1) absent.
118. *Adhesive filaments*: (0) absent; (1) short (Fig. 11B); (2) median (Fig. 11C); (3) long (Fig. 11D).
119. *Chorionic plates (those closest to capped pole)*: (0) uniform height; (1) wide elevated margin (Fig. 11A, B); (2) thin elevated margin (Fig. 11C, D).
120. *Small groove below knob-terminated coiled threads*: (0) absent; (1) present.
121. *Micropylar area (smooth region, as large as a chorionic plate)*: (0) present (Fig. 11A–C); (1) absent.
122. *Number of micropyles or micropylar areas*: (0) 3 or more; (1) 1, rarely 2.
123. *Knob-terminated coiled threads (or adhesive filaments)*: (0) inserted inside a groove; (1) inserted outside a groove.

Eggs