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PHYLOGENY OF THE LEECH FAMILY GLOSSIPHONIIDAE BASED ON MITOCHONDRIAL GENE SEQUENCES AND MORPHOLOGICAL DATA

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ABSTRACT: The phylogenetic relationships of the Glossiphoniidae (Rhynchobdellida) were investigated using morphological characters and the mitochondrial genes cytochrome *c* oxidase subunit I and nicotinamide adenine dinucleotide dehydrogenase subunit 1. Thirty-five taxa representing 10 of the 23 currently recognized glossiphoniid genera were sampled, including more than 70% of known North American species, as well as others from Europe, South America, Africa, and a species endemic to Lake Baikal. Outgroup taxa included species from the Piscicolidae and Ozobranchidae. Cladistic analysis resulted in 1 most-parsimonious tree. Subfamily distinctions, i.e., Haementeriinae, Theromyzinae, and Glossiphoniinae, that have been based on eye morphology and reproductive biology are not corroborated. Results also provide insights into several problematic genus-level classifications. For example, relationships of *Placobdella* and *Haementeria* are clarified and elimination of *Desserobdella* may be necessary. Bloodfeeding from vertebrates is seen to be a primitive characteristic that has been lost twice within the clade. The hypothesis that the biannulate leech, *Oligobdella biannulata,* represents an important transitional form is re-evaluated in a phylogenetic context.

The Glossiphoniidae is a diverse group of leeches, with representatives found in freshwater habitats on all continents except Antarctica. Glossiphoniid leeches are members of the Rhynchobdellida characterized by the presence of a proboscis. In freshwater systems, species that feed from vertebrates are out-numbered both in number of species and absolute abundance by leeches that prey on invertebrates (Klemm, 1972, 1977, 1982, 1991; Sawyer, 1986). Glossiphoniid species are characterized as the only annelids that brood their eggs, carrying their young under their dorsoventrally flattened bodies. This unique family of leeches is ecologically and economically important. For example, glossiphoniids serve as environmental stress indicators due to their relative abundance in certain freshwater habitats (Klemm, 1991; Grantham and Hann, 1994). Glossiphoniids that feed from the blood of vertebrates serve as definitive hosts and vectors of apicomplexan blood parasites of vertebrates (Barta and Desser, 1986, 1989; Siddall and Desser, 1990, 1991, 1992, 1993; Barta, 1991; Siddall and Burreson, 1994). Glossiphoniids that feed from the hemolymph of invertebrates, on the other hand, serve as hosts for helminths (Vojtek et al., 1967; Spelling and Young, 1986; McCarthy, 1990). In spite of this broad ecological and parasitological importance, identification and classification have been problematic.

Identifying glossiphoniid leeches has been difficult due to ambiguous, incomplete, and inaccurate descriptions, lack of appropriate observations, and use of obsolete nomenclature. Soós (1969) noted that specific and generic characteristics needed to be established for all glossiphoniids prior to proper identification. Previously, phylogenetic relationships within the glossiphoniids have not been addressed. This phylogenetic analysis of the Glossiphoniidae provides a foundation for an in-depth determination of species relationships.

MATERIALS AND METHODS

Taxa

Species included in this study were chosen to represent a broad range of glossiphoniid leeches, though there was an emphasis on North American taxa. The genera *Actinobdella, Adaotobdella, Baicoloclepsis, Batracobdella, Batracobdelloides, Gloiobdella, Maiabdella, Marvinmeyeria, Oosthuizobdella, Parabdella, Paraclepsis, Placobdelloides,* and *Tribothrynobdella* were not available. Outgroup taxa consisted of the rhynchobdellids *Calliobdella vivida* (Piscicolinae; York River Estuary, Gloucester Point, Virginia), *Myzobdella lugubris* (Platybdellinae; York River Estuary, Gloucester Point, Virginia), *Ozobranchus margoi* (Ozobranchidae; ex. *Caretta caretta,* Virginia Beach, Virginia), and *Piscicola geometra* (Piscicolinae; Étang de La Musse, Paimpont, France).

DNA extraction

DNA was extracted from leech caudal sucker material using the QIAamp Tissue Kit (Qiagen Inc., Valencia, California). Muscular caudal sucker tissue was used to avoid contamination with leech gut contents.

Mitochondrial DNA sequence amplification

Cytochrome *c* oxidase subunit I (CO-I) fragments were amplified using the universal primers LCO1490, 5′ GGTCAACAAATCATAAAGATATTGG 3′ and HCO2198, 5′ TAAACTTCAGGGTGACCAAAAAATCA 3′ (Folmer et al., 1994). Nicotinamide adenine dinucleotide dehydrogenase subunit 1 (ND-1) fragments were amplified using the primers LND300, 5′ TGGCAGAGTAGTGCATTAGG 3′ and HND1932, 5′ CCTCAGCAAAATCAAATGG 3′. Amplification reactions were carried out in a 50-μl volume with 1.25 units AmpliTaq DNA polymerase (Perkin-Elmer Corporation, Foster City, California), the manufacturer's buffer II, 2.5 mM magnesium chloride, 0.25 mM concentrations of each dNTP, 1 μM concentrations of each primer and using 35 cycles of 94 C (135 sec), 44 C (20 sec), 70 C (90 sec), and 72 C (7 min). Amplification products were purified according to the QIAquick PCR Purification Kit protocol (Qiagen Inc.).

DNA sequencing

Each cycle sequencing reaction employed 1 of the above primers, amplification product, and BigDye™ (Applied Biosystems, Perkin-Elmer Corporation) using 30 cycles of 96 C (70 sec), 44 C (5 sec), and 60 C (4 min). Amplification products were sequenced in both directions. Centri-sep columns (Princeton Separations, Adelphia, New Jersey) were used to remove any unincorporated dyes and primers following completion of sequencing reactions. Products were electrophoresed on a 4% polyacrylamide gel in an ABI Prism™ 377 automated DNA sequencer (Applied Biosystems).

DNA sequence alignment

Sequence Navigator (Applied Biosystems) was used to reconcile sequences for light and heavy strands. CO-I fragments of 665 base pairs (bp) (excluding primer regions) were aligned by eye across all of the taxa because there were no insertions or deletions. The 654-bp (excluding primer regions) ND-1 fragments were aligned according to inferred amino acid sequences.

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TABLE I. Matrix of characters and states for morphological data.

Taxon	Characters											
	1	2	3	4	5	6	7	8	9	10	11	12
Alboglossiphonia heteroclita	3	3	–	1	0	0	1	1	1	1	1	1
Calliobdella vivida	2	–	3	1	0	0	0	1	1	1	1	1
Desmobdella paranensis	1	1	3	–	0	0	0	1	1	1	1	1
Desserobdella phalera TN	2	3	3	1	2	0	2	2	2	2	2	1
D. phalera VA	2	3	3	1	2	0	2	2	2	2	2	1
Desserobdella picta	2	3	3	1	2	0	1	1	1	1	2	1
Glossiphonia complanata FR	3	3	4	1	2	2	0	1	2	1	1	1
G. complanata MI	3	3	4	1	2	2	0	1	2	1	1	1
G. complanata ON	3	3	4	1	2	2	0	1	2	1	1	1
G. complanata UK	3	3	4	1	2	2	0	1	2	1	1	1
Haementeria ghilianii	1	3	2	2	0	0	0	1	1	1	1	1
Haementeria gracilis	1	3	–	2	2	2	1	2	2	1	1	1
Haementeria molesta	1	1	3	2	0	1	1	1	2	2	1	1
Haementeria tuberculifera	1	3	–	2	0	0	0	2	2	2	1	1
Helobdella elongata	1	1	3	1	0	0	0	1	1	1	1	1
Helobdella fusca	1	1	3	1	1	1	1	1	1	1	1	1
Helobdella lineata	1	1	3	1	1	1	1	1	2	2	1	1
Helobdella papillata	1	1	3	1	0	0	0	2	2	2	1	1
Helobdella stagnalis OH	1	1	3	1	0	0	0	1	1	1	1	2
H. stagnalis UK	1	1	3	1	0	0	0	1	1	1	1	2
Helobdella transversa	1	1	3	1	0	0	0	1	1	1	1	1
Helobdella triserialis "papillata"	1	1	3	1	0	0	0	1	2	2	1	1
H. triserialis "triserialis"	1	1	3	1	0	1	1	1	2	2	1	1
Hemiclepsis marginata	3	3	–	–	2	2	1	1	2	2	1	1
Marsupiobdella africana	1	3	3	1	0	0	0	1	1	1	1	1
Myzobdella lugubris	1	–	2	1	0	0	0	1	1	1	1	1
Oligobdella biannulata	1	1	2	1	0	0	0	1	1	1	1	1
Ozobranchus margoi	1	1	1	1	0	0	0	1	1	1	1	1
Piscicola geometra	2	5	3	1	0	0	0	1	1	1	1	1
Placobdella montifera	2	3	3	1	2	0	2	1	2	2	2	1
Placobdella ornata	2	2	3	2	2	0	2	2	2	2	1	1
Placobdella papillifera	2	2	3	2	2	2	2	2	2	2	1	1
Placobdella parasitica	2	2	3	2	2	0	0	1	1	1	1	1
Placobdella pediculata	2	1	3	1	0	0	0	1	1	1	1	1
Placobdella translucens	2	3	3	1	2	0	2	1	1	1	2	1
Theromyzon bifarium	4	3	3	1	0	0	0	–	–	–	1	1
Theromyzon tessulatum	4	7	3	1	2	0	0	2	2	1	1	1
Theromyzon trizonare	4	5	3	1	0	0	0	2	2	2	1	1
Torix baicalensis	2	3	–	–	0	0	0	2	2	2	1	1

Morphological data

Morphological characters (Table I) included:

Character 1, pairs of eyes (Fig. 1): 1 (1), 2 (2), 3 (3), 4 (4).

Character 2, number of annuli separating the male and female gonopores: 1 (1), 1.5 (2), 2 (3), 2.5 (4), 3 (5), 3.5 (6), 4 (7). This trait was not homologized for the piscicolid leeches because the number of annuli per midbody somite differs from that of glossiphoniids. Except for *Oligobdella biannulata,* the glossiphoniids are triannulate. *Piscicola geometra,* and *M. lugubris* have 12–14 annuli per somite (Sawyer et al., 1975; Sawyer, 1986), and *C. vivida* has 14 annuli per somite (Sawyer et al., 1975). In *O. margoi* segments I–IV are undivided, V–XI and XII–XVII are biannulate, XII is undivided, and XVIII–XXVI are triannulate (Davies, 1978).

Character 3, pairs of testisacs: 4 (1), 5 (2), 6 (3), 9 (4).

Character 4, salivary cells (Fig. 2): diffuse (1), compact (2).

Character 5, dorsal marginal lines: absent (0), solid (1), interrupted (2).

Character 6, dorsal paramedial lines: absent (0), solid (1), interrupted (2).

Character 7, dorsal medial line: absent (0), solid (1), interrupted (2).

Character 8, dorsal marginal papillae: absent (1), present (2).

Character 9, dorsal paramedial papillae: absent (1), present (2).

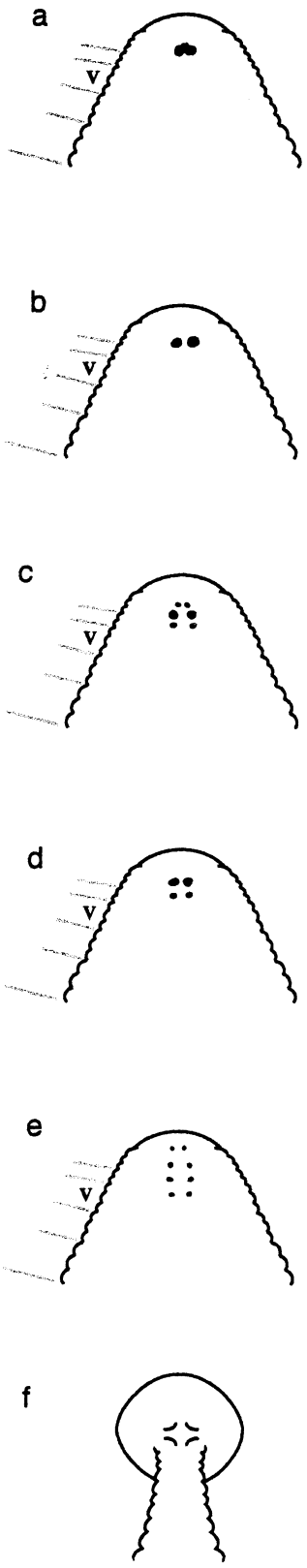


FIGURE 1. Arrangement of cephalic eyespots in *Placobdella* spp. (**a**), *Haementeria* spp. (**b**), *Glossiphonia* spp. (**c**), *Torix biacalensis* (**d**), *Theromyzon* spp. (**e**), and *Piscicola geometra* (**f**).

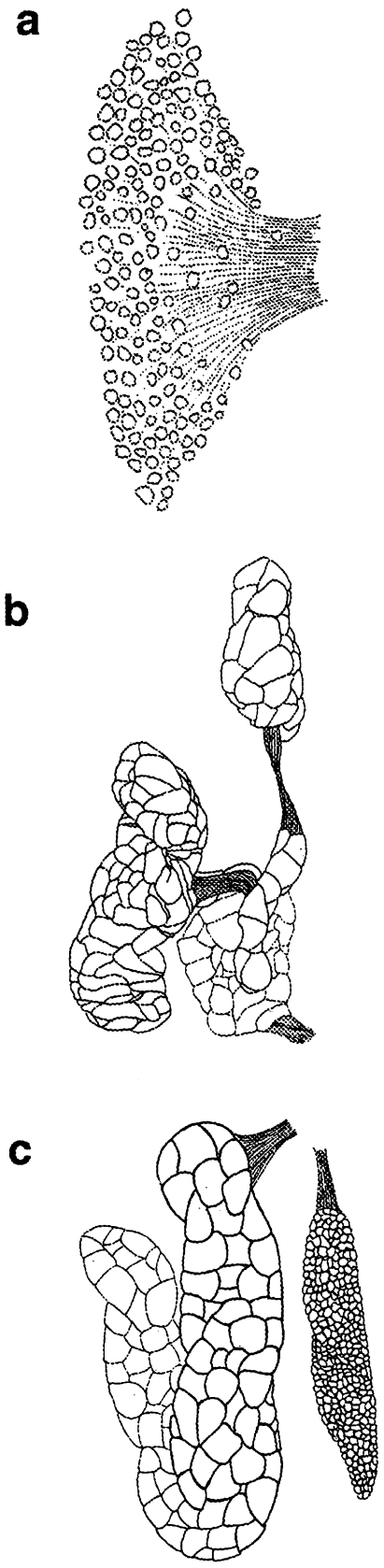


FIGURE 2. Diagram of diffuse salivary cell tissue (**a**), compact salivary cell tissue for the genus *Placobdella* (**b**), and compact salivary cell tissue for the genus *Haementeria* (**c**).

Character 10, dorsal medial papillae: absent (1), present (2).
Character 11, unpigmented neck ring: absent (1), present (2).
Character 12, scute: absent (1), present (2).
Other morphological characters, e.g., position of mouth pore, terminal versus subterminal, were excluded from the analyses because these fall on meristic grades that could not be coded as discrete states.

Phylogenetic analyses

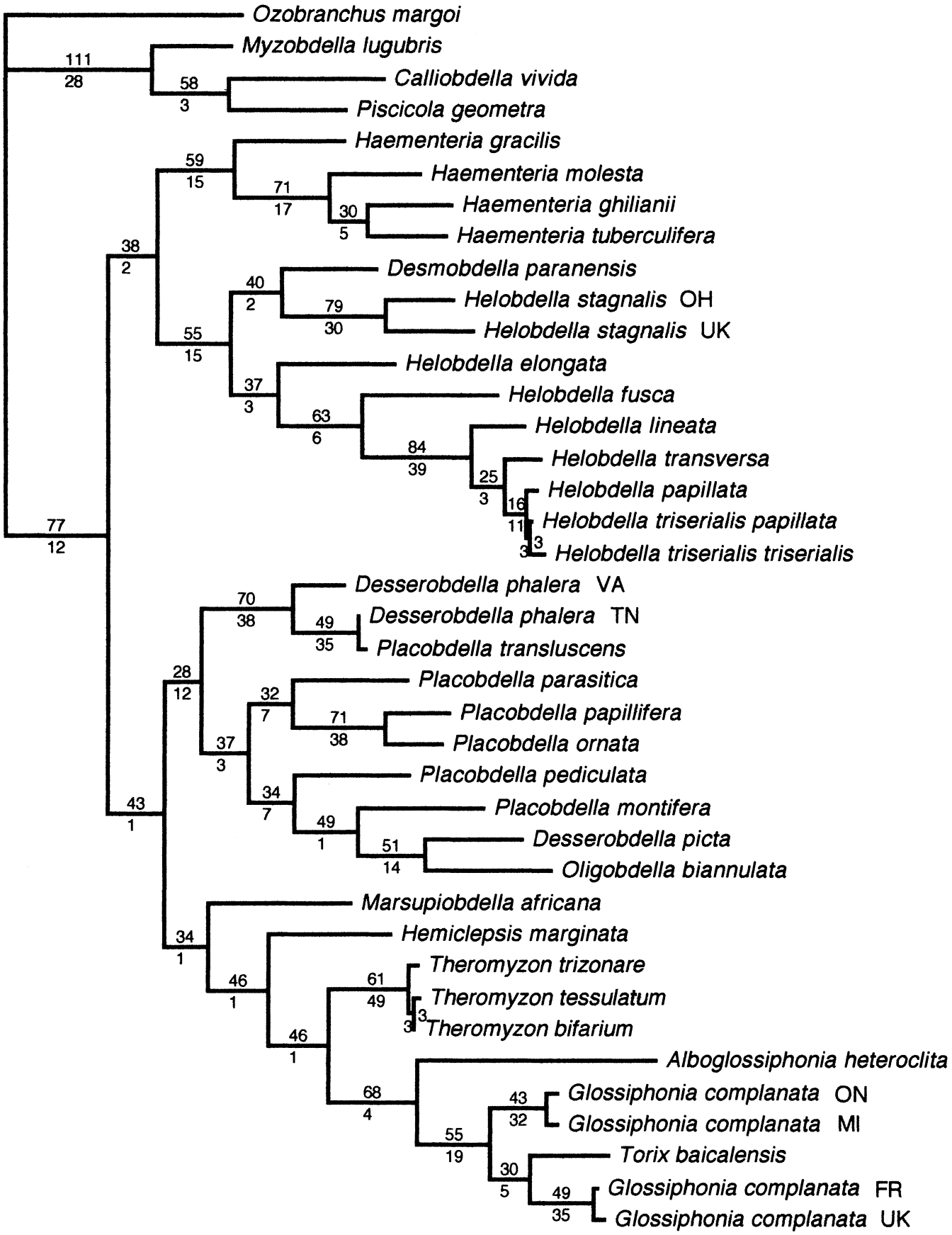
Phylogenetic analyses were performed using PAUP (Swofford, 1993). Heuristic searches used 20 replicates of random taxon addition and tree-bisection–reconnection branch swapping. All characters were left non-additive. Bremer support indices (Bremer, 1988) were obtained using AutoDecay (Eriksson and Wikström, 1996). Consistency and retention indices (Farris, 1989) were calculated with PAUP (Swofford, 1993).

RESULTS

Phylogenetic analyses using CO-I alone resulted in 4 equally parsimonious solutions that were 1,862 steps long with a consistency index (CI) of 0.29 and a retention index (RI) of 0.53. The strict consensus of these trees resolved 31 out of 36 possible clades. The strict consensus of 21 equally optimal trees that resulted from parsimony analysis of ND-1 alone (length = 1,958, CI = 0.38, and RI = 0.57) resolved 24 monophyletic groups for the included taxa.

The combined data set including 665 characters from CO-I, 654 characters from ND-1, and the 12 morphological characters yielded 1 completely resolved tree with a length of 3,995, a CI of 0.30, and an RI of 0.54 (Fig. 3). The basal nodes that split the glossiphoniids into 2 main groups had Bremer support indices (b) of 2 and 1. Except for *Theromyzon* and *Haementeria,* each genus with more than 1 included species was paraphyletic. *Theromyzon* (b = 49) is the genus with the least genetic differentiation. Two groups, *Haementeria* (b = 15) and some *Placobdella* species, have compact salivary cells, but these do not collectively form a clade. *Oligobdella biannulata* and *Desserobdella picta* grouped among species in *Placobdella* (b = 12). Eighty-one extra steps resulted from analyses constraining *Placobdella* species to be monophyletic. There is greater patristic separation between the Tennesseean (TN) *Desserobdella phalera* and the Virginian (VA) *D. phalera* than between *D. phalera* TN and *Placobdella translucens* from Michigan. *Torix baicalensis* falls out between the North American and European representatives of *Glossiphonia complanata.* Bremer support for the clade of *Glossiphonia* and *Torix* is 19, and 5 additional steps were required in analyses constraining for a monophyletic *Glossiphonia* clade. The positions of *Marsupiobdella africana* and *Hemiclepsis marginata* are supported weakly (b = 1). Most species of *Helobdella* form a group with the exception of *Helobdella stagnalis* standing as sister group to the South American *Desmobdella paranensis.* Bloodfeeding from vertebrates is a plesiomorphic trait and has been lost in both the helobdellid clade and in the clade consisting of *Glossiphonia, Alboglossiphonia,* and *Torix* (Fig. 4). The 4 *Haementeria* species and *Placobdella parasitica, Placobdella papillifera,* and *Placobdella ornata,* indepenently possess compact salivary glands and prefer reptilian hosts (Fig. 5).

The combination of outgroup taxa chosen for this analysis provided the best corroborated relationships of ingroup relationships. Removal of *P. geometra, M. lugubris, C. vivida,* or *O. margoi* resulted in a loss of resolution or the re-rooting of the ingroup, but relationships were otherwise unchanged.


Ozobranchus margoi
Myzobdella lugubris
Calliobdella vivida
Piscicola geometra
Haementeria gracilis
Haementeria molesta
Haementeria ghilianii
Haementeria tuberculifera
Desmobdella paranensis
Helobdella stagnalis OH
Helobdella stagnalis UK
Helobdella elongata
Helobdella fusca
Helobdella lineata
Helobdella transversa
Helobdella papillata
Helobdella triserialis papillata
Helobdella triserialis triserialis
Desserobdella phalera VA
Desserobdella phalera TN
Placobdella transluscens
Placobdella parasitica
Placobdella papillifera
Placobdella ornata
Placobdella pediculata
Placobdella montifera
Desserobdella picta
Oligobdella biannulata
Marsupiobdella africana
Hemiclepsis marginata
Theromyzon trizonare
Theromyzon tessulatum
Theromyzon bifarium
Alboglossiphonia heteroclita
Glossiphonia complanata ON
Glossiphonia complanata MI
Torix baicalensis
Glossiphonia complanata FR
Glossiphonia complanata UK

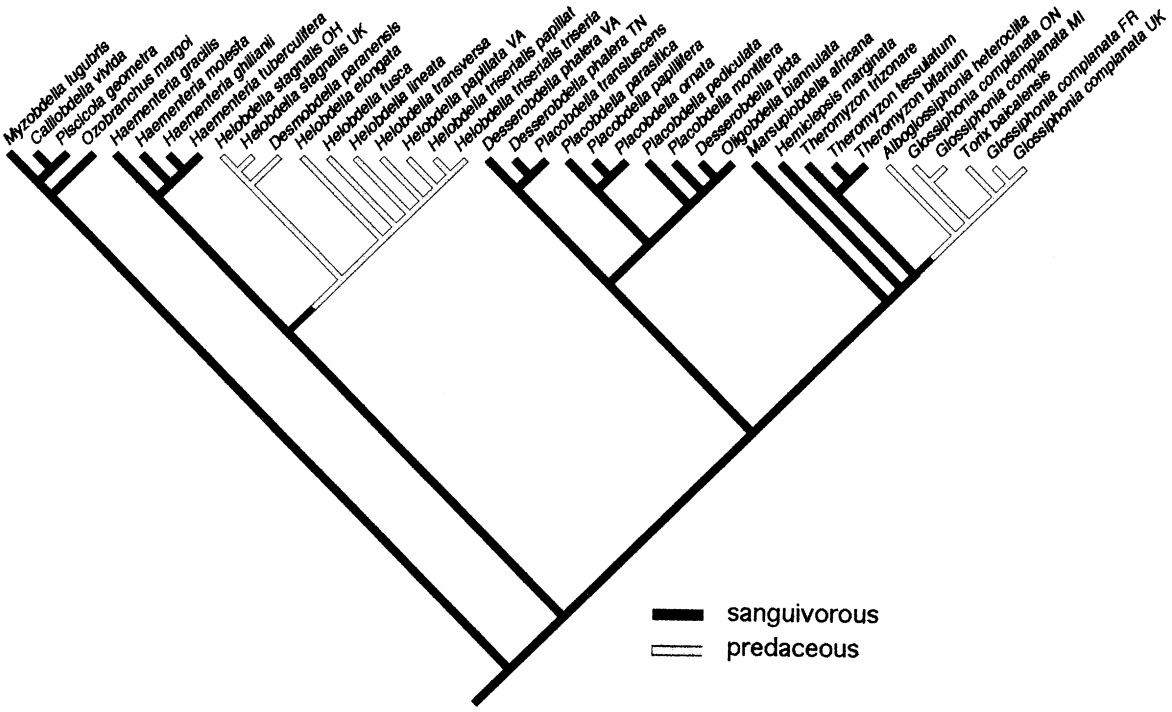


FIGURE 4. Optimization of bloodfeeding behavior on the most parsimonious tree.

DISCUSSION

Siddall and Burreson (1998) found that relationships within Glossiphoniidae were poorly supported using only 1 gene (CO-I) and 8 taxa. For an expanded taxonomic scope, the use of CO-I alone did not fully resolve relationships for this group. Neither, did the use of ND-1 alone. However, the combined information from both genes and from morphology (Fig. 3) resulted in a single topology. Whether or not one should combine multiple data sets in phylogenetics has been a matter of considerable debate (Eernisse and Kluge, 1993; Olmstead and Sweere, 1994; LaFay et al., 1995; Miyamoto and Fitch, 1995; Siddall, 1997). The combined, or "total evidence" approach has as its premise the assertion that although each data set may be biased by different constraints on CO-I versus ND-1 or morphology, relationships that are mutually corroborated should be due to some extrinsic phenomenon.

Glossiphoniidae presently is divided into 3 subfamilies: Theromyzinae, Glossiphoniinae, and Haementeriinae (Sawyer, 1986). Theromyzinae (genus *Theromyzon*) is characterized by 4 pairs of eyes and mating by male to female gonopore copulation. Members of the Glossiphoniinae (genera included in this study: *Desserobdella, Glossiphonia, Hemiclepsis, Placobdella,* and *Torix*) mate by hypodermic implantation of spermatophores, have cocoons attached directly onto a substrate (however, once the embryos hatch, they attach to the venter of the leech), and have multiple pairs of eyes (2–3 pairs). Haementeriinae (genera included in this study: *Desmobdella, Haementeria, Helobdella, Marsupiobdella, Oligobdella,* and *Alboglossiphonia*) also mate by hypodermic implantation of spermatophores, but cocoons appear to be attached directly to the venter. Representatives of this subfamily have 1–3 pairs of eyes.

The characterizations that Sawyer (1986) used for delimiting glossiphoniid subfamilies are not universal nor have all of the characteristics been observed for all species. There also is overlap of characters between subfamilies. Continual recognition of these subfamilies in light of phylogeny would require the acceptance of the paraphyletic groups Glossiphoniinae and Haementeriinae. The *Theromyzon* species sampled are monophyletic, but they fall out within a larger clade consisting of representatives from both the Glossiphoniinae and Haementeriinae.

The placobdellid group consists of *Placobdella, Desserobdella,* and *Oligobdella* species. All members of this clade, except *O. biannulata,* have 2 pairs of coalesced eyes wherein the posterior pair is larger than the anterior pair (Fig. 1). *Desserobdella phalera* failed to group with the type species of the genus (i.e., *D. picta*), rendering *Desserobdella* polyphyletic. *Desserobdella* was established by Barta and Sawyer (1990) with their redescription of *Clepsine picta* in "an attempt to more accurately reflect relationships of species which have, until now, been placed in the paraphyletic genus *Placobdella.*" *Desserobdella* differs only in the presence of diffuse salivary glands instead of the compact tissue of some *Placobdella* species (Barta and Sawyer, 1990). Jones and Woo (1990) placed *Desserobdella phalera* in the newly established *Desserobdella*

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FIGURE 3. Most-parsimonious tree resulting from cladistic analysis of combined CO-I, ND-1, and morphological data. Numbers above and below internodes are branch lengths and Bremer support values, respectively. Branch lengths are proportional to amount of change.

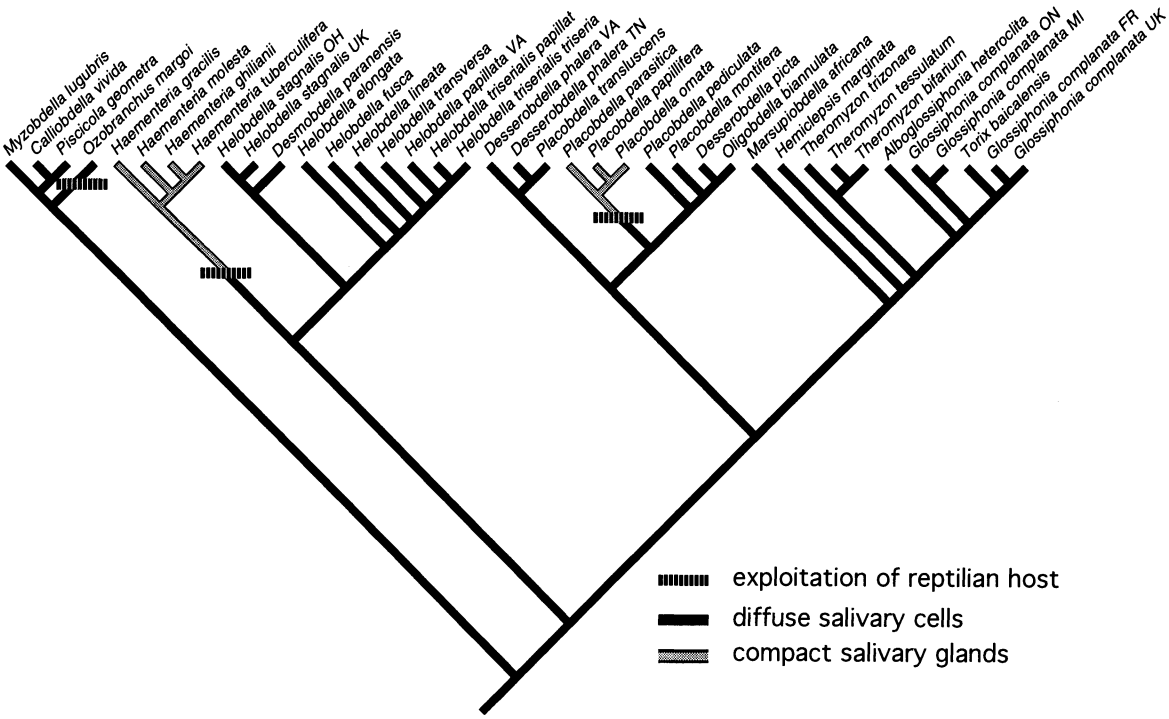

FIGURE 5. Salivary tissue type and exploitation of reptilian hosts as indicated on the most parsimonious tree.

genus in light of its also having diffuse salivary tissue. This trait proves to be plesiomorphic for all glossiphoniids, and there are no other morphological synapomorphies that would support a recent common ancestry for *D. picta* and *D. phalera.* Furthermore, not all *Placobdella* species have compact salivary glands. *Placobdella parasitica, P. papillifera,* and *P. ornata* are the only *Placobdella* species included here with compact salivary glands, and they do form a monophyletic group (Fig. 3). Each of these species also feeds on nonavian reptiles (Fig. 5).

The 2 representatives of *D. phalera* used in this study do not wholly resemble each other (Fig. 6). The representative from Tennessee possessed the characteristic small proportions, translucent body, and unpigmented neck ring typical of *D. phalera.* In contrast, the representative from Virginia, although not readily attributable to a species other than *D. phalera,* was more robust, opaque, and lacked the characteristic unpigmented neck ring. Furthermore, according to Klemm's (1982) criteria, *P. translucens* is indistinguishable from *D. phalera* differing only in degree of pigmentation and papillation. Phylogenetic analyses support a close relationship between the typical Tennessee *D. phalera* and *P. translucens* (Fig. 3).

Barta and Sawyer (1990) stated that "Although the definition of the genus *Placobdella* could have been broadened to encompass the characteristics of *Desserobdella* (*Clepsine*) *picta,* redefinition of the genus would have continued an unfortunate tradition of using the genus *Placobdella* as a repository for poorly known species (Soós, 1969)." Our results suggest that in order for the genus *Placobdella* to be monophyletic, 1 of 2 options can be pursued. One would be to expand *Placobdella* to include all taxa with the characteristic placobdellid eye morphology (Fig. 1). The second option would be to narrow the scope of *Placobdella* considerably, as previously suggested by Soós (1969) and Barta and Sawyer (1990), but with the attendant establishment of several monotypic genera.

Eye morphology has proven to be a problematic trait in differentiating between *Placobdella* and *Haementeria.* Although species in each of these genera are known to have compact salivary glands, new species often were placed without histological examination of the number of eyes. Autrum (1936; see also Elliott et al. [1979]) included *Placobdella* as a subgenus of *Haementeria.* Soós (1969), however, amended Autrum's classification and restored *Placobdella costata* as the type species of *Placobdella.* Hemmingway (1908) observed only 1 pair of eyes in *Placobdella pediculata* that would have placed it in *Haementeria.* Whitman (1892) noted histologically that *Clepsine* (*Placobdella*) *hollensis* had 2 pairs of eyes. In many original descriptions, the second reduced and coalesced pair of eyes in *Placobdella* species was missed and further confused the distinction between *Placobdella* and *Haementeria.* Like the closely related *Helobdella* species, *Haementeria* has only 1 pair of eyes (Fig. 1). *Placobdella* and *Haementeria* are phylogenetically distinct.

Insofar as *H. stagnalis* is the type species of *Helobdella,* this genus probably should include species of *Desmobdella* as well (Fig. 3). *Helobdella papillata, Helobdella lineata, Helobdella triserialis,* and to some extent *Helobdella fusca* are similar in appearance (Fig. 7). By way of example, the specimen here denoted *Helobdella triserialis papillata* possessed many of the traits characteristic of *H. triserialis* sensu stricto, but it also possessed black-tipped papillae, a trait that should distinguish *H. papillata* from other species (Klemm, 1972, 1982, 1991; Sawyer, 1986). Despite distinct descriptions of these 2 species, phylogenetic analysis supports a close relationship between *H. papillata* and *H. triserialis* (Fig. 3). Sawyer (1986) suggested

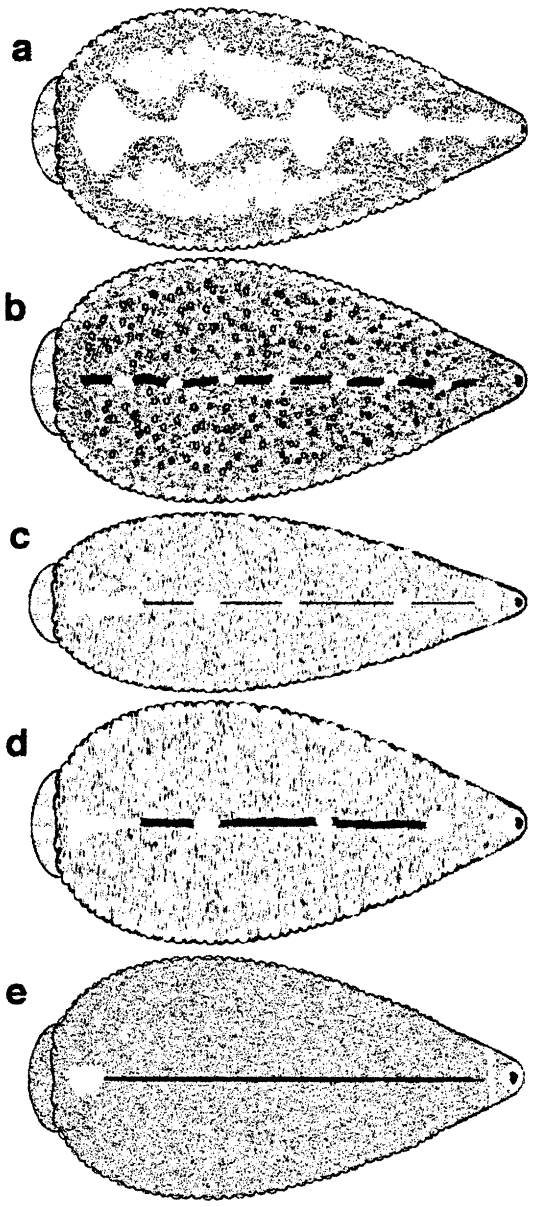

FIGURE 6. Dorsal pigmentation and papillae patterns in *Placobdella parasitica* (**a**), *P. ornata* (**b**), *Desserobdella phalera* TN (**c**), *D. phalera* VA (**d**), and *D. picta* (**e**).

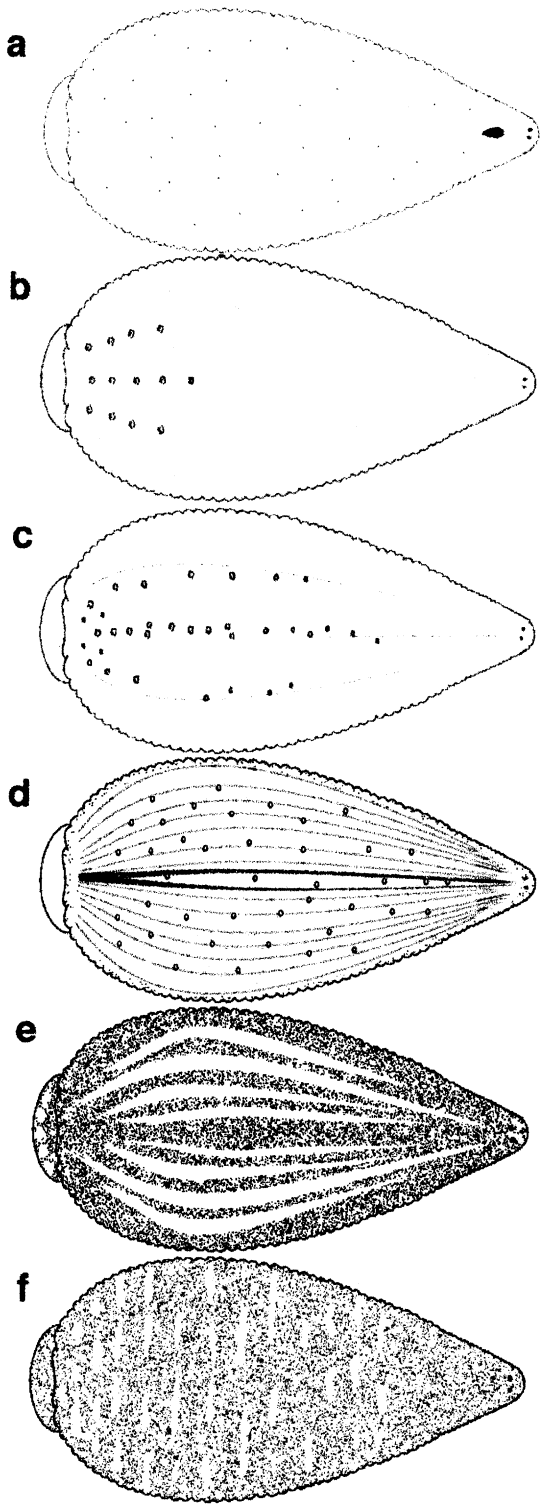

FIGURE 7. Dorsal pigmentation and papillae patterns in *Helobdella stagnalis* (**a**), *H. papillata* (**b**), *H. triserialis* (**c**), *H. lineata* (**d**), *H. fusca* (**e**), and *H. transversa* (**f**).

that *H. lineata* should be synonymized with *H. triserialis*. Phylogenetically, the more distinct *Helobdella transversa* clearly separates *H. lineata* from the *H. triserialis* species group. Only three extra steps would be necessary to form a monophyletic group of *H. lineata, H. triserialis,* and *H. papillata,* but it requires an additional 15 steps to exclude *H. papillata* from this clade. Others have synonymized *H. lineata* with *H. fusca* (Soós, 1969; Sawyer, 1986) because both possess longitudinal pigmentation (Moore 1906; Miller, 1929; Ringuelet, 1944; Soós, 1969; Klemm, 1972, 1982, 1985, 1991; Sawyer and Shelley, 1976; Sawyer, 1986; Fig. 7). Their positions on the cladogram suggest that this is a plesiomorphic similarity as opposed to evidence of a close relationship. It would require 41 extra steps for *H. lineata* and *H. fusca* to be a monophyletic group.

Leeches that bloodfeed from vertebrates have evolved precise mechanisms designed to cope successfully with problems presented by this specialized way of life (Sawyer, 1986). Most leeches in Arhynchobdellida and Rhynchobdellida are vertebrate bloodfeeders (Sawyer, 1986). However, there are fundamental differences, both morphologically and biochemically, between these 2 orders. Members of Arhynchobdellida have jaws armed with teeth that are used to bite their hosts. Coagulation of blood ingested by arhynchobdellids is prevented by a nonenzymatic polypeptide, for example hirudin, that inhibits the clotting enzyme thrombin. Members of Rhynchobdellida use a proboscis to bloodfeed from vertebrates and 1 species, *Haementeria ghilianii,* is known to secrete the fibrinogenolytic enzyme, hementin, to dissolve clots after they have been formed (Sawyer et al., 1991). Vertebrate bloodfeeding glossiphoniids play important roles in the transmission of hemogregarines and trypanosomes to aquatic vertebrates (Barta and Desser, 1986, 1989; Siddall and Desser, 1990, 1991, 1992, 1993; Barta, 1991; Siddall and Burreson, 1994). Interestingly, in most freshwater environments, the most common and widespread species are those that feed on invertebrates. These glossiphoniids can serve as intermediate and even definite hosts for helminths such as digenetic trematodes and nematodes (Vojtek et al., 1967; Spelling and Young, 1986; McCarthy, 1990). Sawyer (1986) suggested that bloodfeeding on vertebrates evolved in the ancestor of leeches and that this behavior was lost independently several times. Similar results also have been found by Siddall and Burreson (1995, 1996, 1998). Data from our analyses support these findings (Fig. 4). Bloodfeeding on vertebrates, in general, has been lost in the Glossiphoniidae at least twice, being absent not only in the apomorphic *Helobdella* clade but also independently in the clade consisting of *Glossiphonia, Alboglossiphonia,* and *Torix baicalensis.*

Glossiphonia complanata, Alboglossiphonia heteroclita, and *T. baicalensis* all are members of a larger clade that also includes *Hemiclepsis marginata* and *Theromyzon* species. Notably, all leeches in this group have multiple pairs of eyes, and 3 pairs of eyes appears to be the original condition (Fig. 1). There is an increase to 4 pairs of eyes for *Theromyzon* species, and *T. baicalensis,* endemic to Lake Baikal, was described as having 2 pair of eyes (Blanchard, 1849). Further examination of new specimens of *T. baicalensis* may justify its inclusion in *Glossiphonia.*

The observation that *T. baicalensis* falls out between the European and the North American representatives of *G. complanata* suggests that these 2 geographic isolates might be distinct species (Fig. 3). There are morphological differences, such as the separation of gonopores, and substantial genetic differences between geographic isolates. Although no other species disrupt the *H. stagnalis* clade, the genetic differences between the individuals from Ohio and from Britain are approximately the same as what was found between European and North American *G. complanata.* In CO-I, there are 53 nucleotide differences between the 2 specimens of *H. stagnalis,* whereas there are 65 and 64 nucleotide changes between the 2 localities for *G. complanata* (with only 5 differences among the 2 European specimens and only 8 differences among the 2 North American specimens). Differences in ND-1 are similar. Sixty-four nucleotide changes exist between the *H. stagnalis* specimens from Ohio and from Britain, whereas there are 53 and 56 nucleotide changes between *G. complanata* from Europe and North America (with only 7 changes among these new world representatives of *G. complanata* and 6 among those from Europe). Our data seem to imply that North American representatives of *G. complanata* and *H. stagnalis* are historically distinct from their European counterparts.

Oligobdella biannulata originally was described by Moore (1900) as the only glossiphoniid leech that is biannulate (all other glossiphoniids being triannulate). In addition, Moore (1900) conjectured on the significance of this feature in relation to the origins of the Hirudinea from a uniannulate oligochaete-like ancestor. Sawyer (1971) agreed, giving the impression that *O. biannulata* is primitive or is a "missing link" between Oligochaeta and Hirudinea. This hypothesis is no longer tenable. *Oligobdella biannulata* is well supported as a derived member of the placobdellid clade. Biannulation unequivocally is derived from triannulate leeches and is not a precursor to them.

This work does have an admitted North American focus on taxonomic sampling. This is not unusual from the point of view that Glossiphoniidae is particularly well represented in North America. However, glossiphoniids also are well represented in South America, and more intensive collections there are needed. Outstanding questions that await further data from new and old world taxa include the species status of *H. stagnalis* and *G. complanata* presently spread across many continents as well as further evaluation of the gain or loss of bloodfeeding in this group.

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