Phylogeny and revision of the leech genus *Helobdella* (Glossiphoniidae) based on mitochondrial gene sequences and morphological data and a special consideration of the *triserialis* complex

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The relationships, as well as identification of species, within *Helobdella* (Glossiphoniidae) were explored through phylogenetic analysis and through an overview of the historical systematics of the genus. The phylogeny was determined using morphological data and the mitochondrial gene sequences of cytochrome c oxidase subunit I and nicotinamide adenine dinucleotide dehydrogenase subunit I. A broad representation of 15 ingroup species was sampled, including 10 individuals from South America. Outgroup taxa included five species of *Haementeria*. Cladistic analysis of all available data resulted in one most parsimonious tree. Results shed light on genetic divergence of members classified as the same species, including those that are not monophyletic. Historically, external morphological characters have played a significant role in contributing to the confusion in the classification of *H. triserialis*, *H. papillata*, *H. lineata* and *H. fusca* in North America. Re-evaluation of Verril's *Clepsine papillifera* var. b and var. d in a phylogenetic context provides a solution. Additionally, the genera *Adaetobdella*, *Acritobdella*, *Dacnobdella* and *Gloiobdella* created by Ringuelet are returned to *Helobdella* based on overlapping morphological characters.

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Introduction

Species in the genus *Helobdella* are generally small, dorsoventrally flattened leeches whose ancestors appear to have given up blood-feeding in favour of predation on aquatic invertebrates. The phylogenetic position of this genus in the family Glossiphoniidae has been investigated previously (Light & Siddall 1999) and *Helobdella stagnalis* was included in a broad sample from this family relative to other families of leeches (Siddall & Burreson 1998; Apakupakul *et al*. 1999). Although these prior analyses considered species of *Helobdella* only from the Northern Hemisphere, most of the morphological diversity of this genus occurs in South America (Weber 1915; Ringuelet 1944; Sawyer 1986). Ringuelet (1978) has already subdivided *Helobdella* into *Adaetobdella*, *Acritobdella*, *Dacnobdella*, and *Gloiobdella* on the basis of combinations of internal and external morphological character variation. It is likely that one or more of these genera are not monophyletic because there is some overlap in the character combinations that define them. Moreover, because Ringuelet (1978) did not specify what would be left as defining characteristics of *Helobdella*, it is possible that recognition of any of these genera would render *Helobdella* paraphyletic.

The taxonomy of North American species of *Helobdella* has led to substantial confusion regarding the identity of leeches in the species *H. triserialis*, *H. lineata*, *H. papillata*, and *H. fusca*. *H. triserialis* was described originally from Chile (Blanchard 1849). *H. lineata* and *H. papillata* at first were varieties of Verrill's (1872, 1874) *Clepsine papillifera* — a conglomerate species that later (Moore 1952) was understood to also comprise *Placobdella papillifera* and *Placobdella montifera*. Verrill's (1872, 1874) leeches eventually became confused with Castle's (1900) *Glossiphonia fusca*, all of which were synonymized with *H. triserialis* by Ringuelet (1943). An important distinction made by Moore (1952) in recognizing an earlier error of his own making (Moore 1906) could have prevented this confusion but it remains unrectified in recent systematic accounts of North American taxa (e.g. Klemm 1982; Sawyer 1972, 1986). Taxonomic names for individuals

used have initially followed the latter and contemporary usage but are rectified later in light of the phylogenetic results.

With several newly acquired taxa from South America that together span the morphological diversity of Ringuelet's (1978) genera, and including South and North American representatives of *H. triserialis* with allied taxa, here we re-examine the relationships of the genus *Helobdella* with morphological characters and two mitochondrial gene sequences.

Materials and methods

Taxa

Helobdella species included in this study represent a broad global distribution. These glossiphoniid leeches were mainly sampled from North and South America, but also included one representative of *H. stagnalis* from Europe and *H. papillornata* from Australia. The outgroup taxa were chosen based on a prior phylogenetic analysis (Light & Siddall 1999) which found *Haementeria* to be most closely related to *Helobdella*. These included the following South American species: *Haementeria lutzi*, *Ha. gracilis*, *Ha. molesta*, *Ha. ghilianii*, and *Ha. tuberculifera*. *Helobdella* and *Haementeria* share the synapomorphy of one pair of cephalic eyespots. GenBank accession numbers and sampling localities of taxa are included in Table 1.

In light of apparent confusion relating to the systematics of the *triserialis*-group we photodocumented the individual leeches used for DNA isolation with a SPOT-RT 3-chip digital camera (Diagnostic Instruments, Inc.) attached to a Nikon SMZ-U stereomicroscope.

DNA extraction and purification

Leeches were stored in 100% ethanol at −20 °C or at ambient temperature until use for DNA extraction. Tissue from the caudal sucker was removed and utilized for DNA extraction. The caudal sucker is specifically used in order to minimize the possibility of contamination from host/prey DNA found in the gastric regions. DNeasy Tissue Kit (QIAGEN Inc. Valencia, California) was used for tissue lysis and DNA purification.

Mitochondrial DNA sequence amplification

PCR amplification and sequences of two mitochondrial gene regions were used for molecular phylogenetic analysis. The universal primers, LCO1490, 5′-GGTCAACAAATCATAAAGATATTGG-3′ and HCO2198, 5′-TAAACTTCAGGGTGACCAAAAAATCA-3′, were used to amplify cytochrome c oxidase subunit I (CO-I) fragments of 665 base pair (bp) length. Nicotinamide adenine dinucleotide dehydrogenase subunit I (ND-I) fragments (654 bp) were amplified using the primer pairs, LND300, 5′-TGGCAGAGTAGTGCATTAGG-3′ and HND1932 5′-CCTCAGCAAAATCAAATGG-3′ (Light & Siddall 1999). Amplification reactions for CO-I and for ND-I contained 1.25 units of AmpliTaq DNA polymerase (Perkin-Elmer Corporation, Foster City, California), 10X II Buffer, 2.5 mM magnesium chloride, 0.25 mM of each dNTP (1 mM total), 10 μM of each primer, and template for a 25-μL total volume. Alternatively, Ready-To-Go PCR Beads (Amersham Pharmacia Biotech, Piscataway, NJ) were used, for which each 25 μL reaction contains 1.5 units DNA polymerase, 10 mM Tris-HCI (pH 10), 50 mM potassium chloride, 1.5 mM magnesium chloride, 200 μM of each dNTP, stabilizers, 10 μM of primer pair mix, template and water. In a GeneAmp PCR System 9700 (P E Applied Biosystems), reaction mixtures were heated to 94 °C for 5 min, followed by 15 cycles of 94 °C (45 s), 46 °C (45 s), and 72 °C (45 s), then 25 cycles of 94 °C (20 s), 45 °C (20 s) and 72 °C (30 s) and a final extension at 72 °C (6 min). The QIAquick PCR Purification Kit protocol (QIAGEN, Inc.) was employed to purify amplification products.

DNA Sequencing

Amplification products were sequenced in both directions. Each sequencing reaction mixture, including 4 μL BigDye (Applied Biosystems, Perkin-Elmer Corporation), 2 μL of 1 μM primer (single primer for each direction), and 5 μL of DNA template, ran for 40 cycles of 96 °C (10 s), 50 °C (10 s) and 60 °C (4 min). Sequences were purified by running each reaction through Centri-sep columns loaded with G-50 Sephadex to remove primers and unincorporated dyes. Products were electrophoresed in an ABI Prism 3700 sequencer (Applied Biosystems).

DNA sequence alignment

Sequences of complimentary strands were edited and reconciled using Sequence Navigator (Applied Biosystems). Alignment of CO-I fragments was done by eye across all taxa because there were no insertions or deletions. ND-I fragments were aligned according to inferred amino acid sequences.

Morphological data

The following morphological characters were included (Table 2):

1. *Presence of nuchal glands or a chitinoid scute on somite VIII:* (0) absent (1) present (Fig. 1A) (2) glands only.
2. *Position of eyes (somite number):* (1) II (2) III (3) IV
3. *Cephalic lobe:* (0) absent (1) present
4. *Subdivided annuli:* (0) absent (1) present
5. *Number of annuli separating gonopores:* (1) 1 (2) 2
6. *Presence of dorsal papillae:* (0) absent (1) present
7. *Postcaeca:* (0) absent (1) present in somite XIX only (Fig. 1B) (2) extend from XIX through XIV.

Table 1 Taxa and localities for leeches used in the phylogenetic analysis of *Helobdella*.

Taxon	Locality	GenBank accession no. CO-I	ND-I
Haementaria ghilianii	BioPharm		
	French Guiana	AF329035	AF329058
Haementaria gracilis	Arroyo Aspinas		
	Uruguay	AF329034	AF329057
Haementaria lutzi	Rio Pastaza		
	Ecuador	AF329033	AF329056
Haementaria molesta	Arroyo Aspinas		
	Uruguay	AF329469	AF329055
Haementaria tuberculifera	Arroyo Aspinas		
	Uruguay	AF329036	AF329059
Desmobdella paranensis	Arroyo Aspinas		
	Uruguay	AF329037	AF329060
Gloiobdella elongata	Silver Lake		
	Michigan	AF329045	AF329068
Gloiobdella michaelseni	Lago Calafquen		
	Chile	AF536824	AF536825
Helobdella bolivianita	Laguna Volcan		
	Bolivia	AF329053	AF329076
Helobdella fusca	Wild Goose Lake		
	Michigan	AF329038	AF329061
Helobdella lineata	Douglas Lake		
	Michigan	AF329039	AF329062
Helobdella lineata VA	Gloucester		
	Virginia	n/a	AF329078
Helobdella nununununojensis Pusupunku	Ulla Ulla		
	Bolivia	AF329047	AF329070
Helobdella nununununojensis Tojoloque	Madidi		
	Bolivia	AF329048	AF329071
Helobdella papillata	Gloucester		
	Virginia	AF329046	AF329069
Helobdella ringueleti	Madidi		
	Bolivia	AF329051	AF329074
Helobdella robusta	California	AF178680	AF178680
Helobdella sorojchi speckled	Madidi		
	Bolivia	AF329050	AF329073
Helobdella sorojchi striped	Madidi		
	Bolivia	AF329049	AF329072
Helobdella stagnalis OH	Columbus		
	Ohio	AF329040	AF329063
Helobdella stagnalis UK	Cotswolds		
	England	AF329041	AF329064
Helobdella trasversa	Cheboygan State Pk		
	Michigan	AF329044	AF329067
Helobdella triserialis	Laguna Volcan		
	Bolivia	AF329054	AF329077
Helobdella triserialis black-tipped	Round Lake		
	Michigan	AF329043	AF329066
Helobdella triserialis colourless	Lake Huron		
	Michigan	AF329042	AF329065
Helobdella papillornata	Magill Creek		
	Brisbane Australia	AF329052	AF329075

8. *Gastric crop:* (1) presence of lateral caeca or sacs (Fig. 1C) (2) lacking caeca altogether.
9. *Distribution of salivary cells:* (1) diffuse (2) compact glands (Fig. 1D)

Phylogenetic analyses

Phylogenetic analyses were performed using PAUP* (Swofford 2000). Heuristic searches used 20 replicates of random taxon addition and tree-bisection-reconnection branch swapping.

Table 2 Morphological data matrix of characters and states.

Characters	1	2	3	4	5	6	7	8	9
Haementaria ghilianii	0	2	0	0	2	1	2	1	2
Haementaria gracilis	0	2	0	0	2	1	2	1	2
Haementaria lutzi	0	2	0	0	2	1	2	1	2
Haementaria molesta	0	2	0	0	2	1	2	1	2
Haementaria tuberculifera	0	2	0	0	2	1	2	1	2
Desmobdella paranensis	0	2	0	0	1	0	2	1	1
Gloiobdella elongata	0	3	1	0	1	0	0	2	1
Gloiobdella michaelseni	0	3	1	0	1	0	0	2	1
Helobdella bolivianita	1	2	0	1	1	0	2	1	2
Helobdella fusca mottled	0	2	0	0	1	0	2	1	1
Helobdella lineata	0	2	0	0	1	0	2	1	1
Helobdella lineata VA	0	2	0	0	1	1	2	1	1
Helobdella robusta	0	2	0	0	1	1	2	1	1
Helobdella nunununununojensis	0	1	0	0	1	0	1	2	2
Helobdella papillata	0	2	0	0	1	1	2	1	1
Helobdella ringueleti	2	2	0	1	1	0	2	2	1
Helobdella sorojchi	0	1	0	1	1	0	2	1	2
Helobdella stagnalis OH	1	2	1	0	1	0	2	1	1
Helobdella stagnalis UK	1	2	1	0	1	0	2	1	1
Helobdella trasversa	0	2	0	0	1	0	2	1	1
Helobdella triserialis black-tipped	0	2	0	0	1	1	2	1	1
Helobdella triserialis colourless	0	2	0	0	1	1	2	1	1
Helobdella triserialis Bolivia	0	2	0	0	1	1	2	1	1
Helobdella papillornata	0	2	0	0	1	1	2	1	1

All characters were left nonadditive. Bremer support (b) indices (Bremer 1988) were obtained using TreeRot (Sorenson 1999) and parsimony jackknife (jac) values with 100 replicates and branch swapping with XAC (Farris 1999). Retention indices were calculated with PAUP* (Swofford 2000).

Results

Phylogenetic analysis of morphological characters resulted in 17 equally parsimonious trees, each 17 steps long with a retention index (RI) of 0.90. The only groups agreed on by these trees were ingroup monophyly (*H. bolvianita* + *H. stagnalis*) (*H. sorojchi* + *H. nunununununojensis*) and (*H. ringueleti* + *Gloiobdella* spp.). Separate analysis of CO-I resolved 94 trees of length 1215 with an RI of 0.53, while ND-I resolved three trees with 1026 steps and an RI of 0.65.

All *Haementeria* spp. grouped together based on morphology and ND-I, but unexpectedly, this genus was polyphyletic for CO-I. Consistent monophyletic relationships were observed for CO-I and ND-I between *H. lineata*, *H. lineata* VA, *H. robusta*, *H. transversa*, *H. papillata* and *H. triserialis* vars. black-tipped and colourless, as well as *H. ringueleti* with *H. bolivianita*. *Desmobdella paranensis*, *H. nunununununojensis* and *H. sorojchi* form a clade both with CO-I and with ND-I. The position of *D. paranensis* changes, grouping either with *H. sorojchi* for the CO-I data, or with *H. nunununununojensis* and *H. elongata* for the ND-I data.

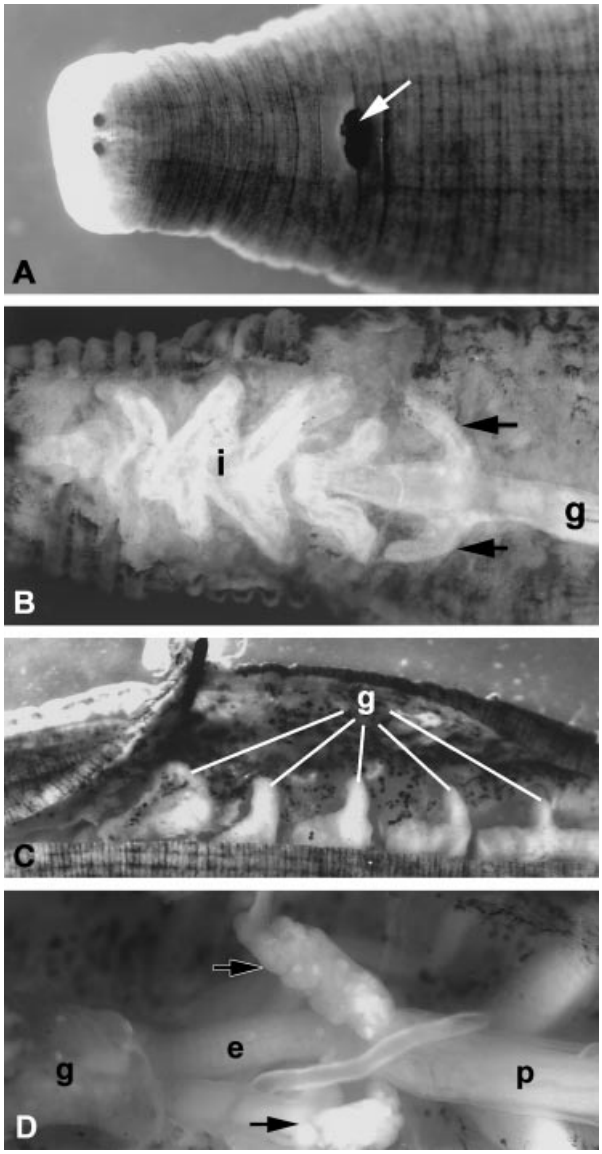

Fig. 1 A–D. Morphological character states for some species of *Helobdella*. —A. Nuchal scute (arrow) on somite VIII of *H. bolivianita*. —B. Gastric tube (g) with short postcaeca (arrows) anterior to the intestinal caeca (i) of *H. nunununununojensis*. —C. Typical glossiphoniid digitiform gastric caeca (g) in *H. bolivianita*. —D. Compact salivary glands (arrows) situated between the oesophagus (e) and proboscis (p) of *H. bolivianita*.

Combining all available data (nine morphological characters, 665 characters for CO-I and 654 characters for ND-I) resulted in one tree with a length of 2321, and RI of 0.57 (Fig. 2). The ingroup (*Helobdella* spp.) was strongly supported (b = 24, jac = 100%). There is a basal split separating two principal groups of leeches: the *stagnalis*-group (b = 3) and the

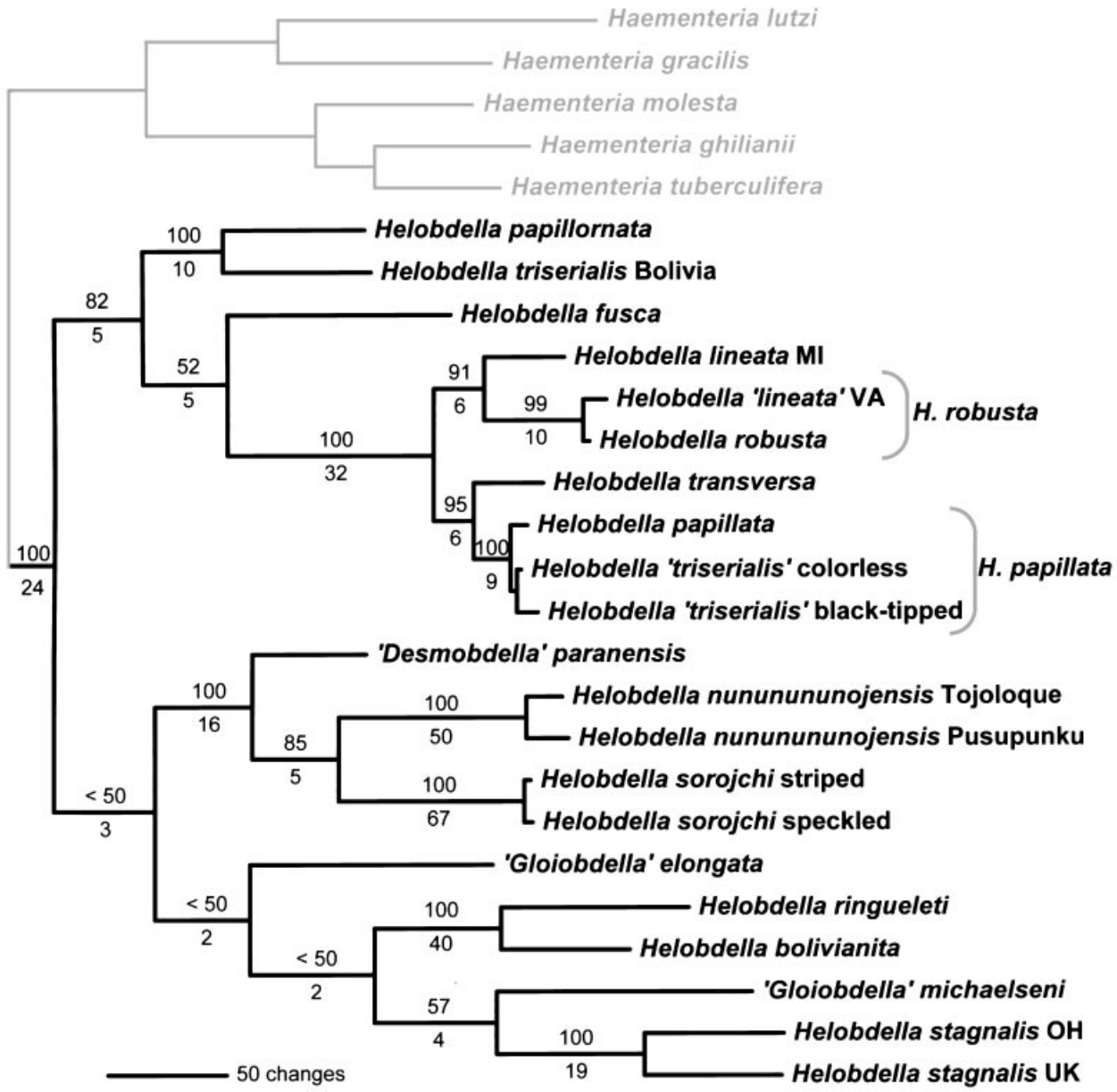

Fig. 2 Single most parsimonious tree found from phylogenetic analysis of the combined morphological, CO-I and ND-I datasets (Length is 2321 with an RI of 0.57). Numbers above internodes are parsimony jackknife support values. Numbers below internodes are Bremer support values. Names indicated on the cladogram are those applied a priori. Branches are drawn proportional to amount of change. Given the results and reinvestigation of the taxonomic history of the group: *Desmobdella* and *Gloiobdella* are now junior synonyms of *Helobdella*; *H. lineata* VA is actually *H. robusta*, *H. triserialis* black-tipped and *H. triserialis* colourless are now *H. papillata*.

triserialis-group (b = 5). Although the latter is distinguished by having some taxa with papillae, this trait is plesiomorphic (present in *Haementeria* spp.) and also is lost twice in the group.

The *stagnalis* clade exhibits the most changes in the included morphological characters. Generally, *Helobdella* are thought of as having diffuse salivary tissue, however, compact salivary cells are present in *H. nununununojensis* and in *H. sorojchi*, as well as in *H. bolivianita* which does not group with the former two. The relative position of the Bolivian species *H. ringueleti* and *H. bolivianita* (b = 40) with *Gloiobdella* species and the North American and European *H. stagnalis* isolates was weakly supported (b = 2, jac < 50%). With the exception of *G. michaelseni*, these have the widely recognizable

Table 3 Genetic divergence of sister taxa of *Helobdella*.

	p Distance (%)		Nucleotide difference (bp)	
	CO-I	ND-I	CO-I	ND-I
H. sorojchi var. striped vs. *H. sorojchi* var. speckled	0.5	0.6	3	4
H. nununununojensis Tojoloque vs. *H. nununununojensis* Pusu Punku	2.7	2.7	16	17
H. lineata VA vs. H. robusta	—	2.1	—	13
H. papillata vs. *H. triserialis* var. colourless vs. *H. triserialis* var. black-tipped	< 1.4	< 1.6	< 9	< 10
H. triserialis vs. *H. papillornata*	16	19	101	118
H. stagnalis OH vs. *H. stagnalis* UK	8	10.3	53	64

nuchal glands in somite VIII. The plesiomorphic gastric morphotype (presence of digitiform caeca) is lost four times in *H. nununununojensis*, *G. elongata*, *G. michaelseni* and *H. ringueleti*. Subdivided annuli are present in *H. ringueleti* and *H. bolivianita*, as well as in the unrelated *H. sorojchi*.

Table 3 lists various sister taxa with measures of genetic divergence expressed as absolute nucleotide differences and percentages (or p) distances.

Photodocumentation of individual leeches from the *triserialis*-clade used in this study are in Fig. 3 including *H. papillornata* (Fig. 3A), *H. triserialis* from Bolivia (Fig. 3B,C), *H. papillata* (Fig. 3D), *H. lineata* (Fig. 3E,F), *H. transversa* (Fig. 3G) and *H. fusca* (Fig. 3H).

Discussion

With the exception of backbone relationships in the *stagnalis* clade, the results from the combination of morphological characters, CO-I and ND-I yield a robust hypothesis for the included species of *Helobdella*. Remarkably a clade with low support (b = 2, jac < 50%) is a group that contains taxa defined by a morphological character that few would doubt. The presence of nuchal glands on somite VIII is perhaps the best recognized character within the genus *Helobdella*. This is a characteristic of the type species *H. stagnalis* as well as *H. ringueleti* and *H. bolivianita* included here. There are an additional 11 species, all of which are South American, that possess a chitinoid scute or the associated nuchal glands (reviewed in Siddall 2001a) and previously there has been little reason to doubt their monophyly. That the scuteless *G. michaelseni* falls out within this clade casts doubt even on this characteristic as being unequivocally meaningful of any phylogenetic relationship. The remaining morphological characters, all of which have implications for Ringuelet's (1978) subdivision of the genus, exhibit either convergence or reversals or some combination thereof in various parts of the tree.

Taxonomic revision

Most leech systematists familiar with taxa in the Nearctic or the Old World take it for granted that species of *Helobdella* exhibit a fairly uniform set of characteristics which includes a single pair of ocelli, typical glossiphoniid gastric caeca, salivary tissue arrangement that is diffuse in the parenchyma and typical glossiphoniid annulation patterns. In South America, however, this generalization does not readily obtain. With the description of *Desmobdella paranensis*, Oka (1930) had already placed one *Helobdella*-ally in its own genus. Ringuelet (1978), subdivided the genus in recognition of the greater morphological diversity he had observed for its member taxa. Four overlapping morphological characters were used in the recognition of four new genera. *Adaetobdella*, *Acritobdella* and *Dacnobdella* were in part defined by the presence of 'glandular' compact salivary cells. These then were distinguished on the basis of whether or not they had subdivided annuli (*Acritobdella*), nuchal glands (*Dacnobdella*) or neither (*Adaetobdella*). A priori then, it would be impossible for these characters all to delimit monophyletic groups with unique unreversed synapomorphies. In addition, Ringuelet (1978) established *Gloiobdella* for those species that were known to lack the typical digitiform caeca of most glossiphoniids and had a simple gastric tube instead. All of these characters were used in the construction of this phylogenetic analysis. Moreover, we added the differences known for presence and extent of the sixth pair of caeca (postcaeca or diverticula) because species of *Gloiobdella* typically have no postcaeca or they exist in somite XIX only (Blanchard 1900; Moore 1911; Cordero 1937; Ringuelet 1942a,b, 1944, 1959). Sawyer (1986), recognized *Gloiobdella* as a valid genus but subsumed *Acritobdella* and *Dacnobdella* in *Adaetobdella*, recognizing only the presence and absence of compact salivary glands as a reasonable distinction for the latter from *Helobdella* proper (and incidentally obviating the consistency of the nuchal scute). Herein, *H. sorojchi* has the characters of *Acritobdella*; *H. bolivianita* with compact salivary cells and a scute would have to fall within *Dacnobdella*; *H. nununununojensis* would belong in *Gloiobdella* in that it lacks gastric caeca and has very short postcaeca. However, recognition of any of these genera would render the genus *Helobdella* paraphyletic. Moreover, gastric caeca are lost four times (for *G. elongata*, *G. michaelseni*, *H. ringueleti*, and *H. nununununojensis*), postcaeca are reduced three times (for *G. elongata*, *G. michaelseni*, and *H. nununununojensis*), and subdivision of annuli is apparent for *H. bolivianita*, *H. ringueleti*, and *H. sorojchi*.

In light of the foregoing we formally return species of *Adaetobdella*, *Dacnobdella*, *Desmobdella* and *Gloiobdella* to the

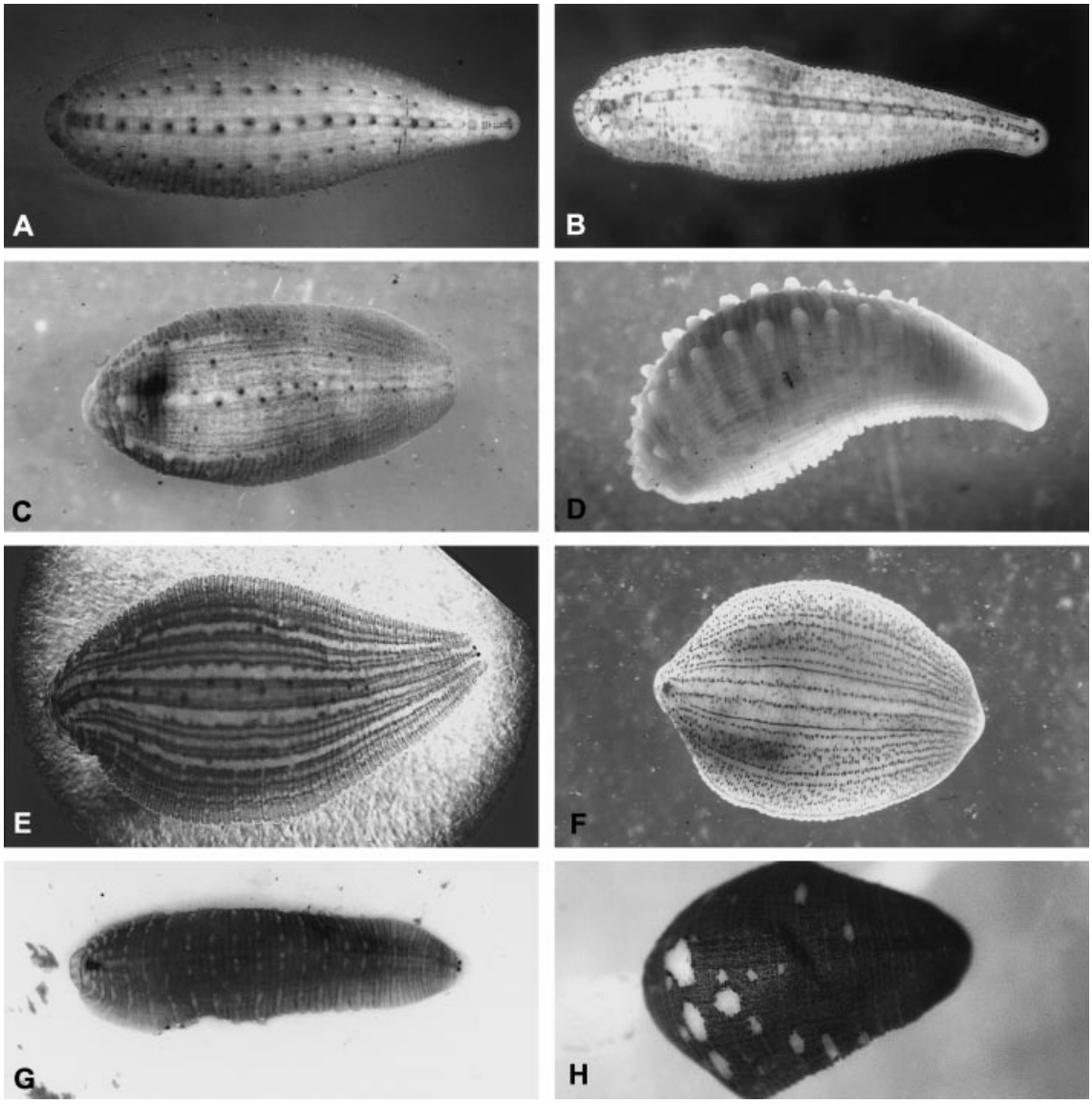

Fig. 3 A–H. Photographs of the individual leeches used that placed in the *triserialis* group. —A. *H. papillornata* from Australia. —B. *H. triserialis* (*sensu stricto*) from Bolivia. —C. *H. triserialis* black-tipped from Michigan (now *H. papillata*). This was listed as *H. triserialis triserialis* in Light & Siddall (1999). —D. *H. papillata* from Virginia. —E. *H. lineata* VA from Virginia (now *H. robusta*). —F. *H. lineata* from Michigan. —G. *H. transversa* from Michigan. —H. *H. fusca* (mottled form) from Michigan.

genus *Helobdella* with the description: **Gonopores separated by one annulus, one pair of cephalic eyespots; neither oesophageal organs nor mycetomes are present; none known to be sanguivorous. With characters of the family Glossiphoniidae**. This includes:
Helobdella chaquensis (Ringuelet, 1978) comb. n.
Helobdella cryptica (Ringuelet, 1978) comb. n.
Helobdella elongata Castle, 1900
Helobdella longicollis Weber, 1915
Helobdella malvinensis (Ringuelet, 1978) comb. n.
Helobdella michaelseni Blanchard, 1900
Helobdella paranensis (Oka, 1930) comb. n.
Helobdella obscura Ringuelet, 1942
Helobdella similis Ringuelet, 1942, and
Helobdella xenoica (Ringuelet, 1975) comb. n.

Intraspecific issues

Multiple representatives of species that prove to be monophyletic include *H. nununununojensis*, *H. sorojchi*, and *H. stagnalis*. Siddall (2001b) discovered three new species of this

genus from the Bolivian Andes. The description of *H. sorojchi* encompassed both striped and speckled morphotypes found together in Qanchis Qocha. Because their internal anatomy was not distinguishable, Siddall (2001b) argued they should be placed in the same species. Polymorphism in the external coloration of glossiphoniid leeches is well known (Ringuelet 1943; Klemm 1982; Sawyer 1986; Light & Siddall 1999). The molecular data used here confirm this presumption insofar as the two morphotypes of *H. sorojchi* are each other's closest relatives and differ by only a few nucleotides. The Andes appear to exhibit high endemism for species of *Helobdella* (Weber 1916; Siddall 2001b) and, with the exception of *H. nunununununojensis*, each species of leech found in the Apolobamba range was found only in a single valley (Siddall 2001b). Contra the external polymorphism of some leeches, there may also be cryptic species not distinguishable by their external morphological characters. Though they differ by more than the *H. sorojchi* individuals, the two representatives of *H. nunununununojensis* appear to be monophyletic and a single species despite being in nonadjacent valleys.

The representatives of *H. stagnalis* from Ohio and England are not morphologically distinguishable (Moore 1952; Sawyer 1986). Neither is the external morphology of the Bolivian *H. triserialis* from *H. papillornata* in Australia. However, the genetic separation between each of these pairs (~10% and nearly 20%) is on par with that seen between *H. bolivianita* and *H. ringueleti* and far exceeds that between *H. transversa* and *H. papillata* (5%) though no one would confuse any of these for each other. Although there are no morphological characters available to distinguish the European and North American isolates of *H. stagnalis*, should multiple representatives from both continents prove to be monophyletic and phylogenetically distinct, resurrection of Verrill's (1872) *H. elegans* for the North American isolates would be required. Unfortunately this may then require a molecular definition of the species distinctions much as there has been for other closely allied taxa. It is not clear if the Australian *H. papillornata* that looks like *H. triserialis* has evolved in isolation from those in South America as the genetic distances suggest. The type locality for *H. triserialis* is in Chile (Blanchard 1849), but the taxon is widespread and polymorphic (Ringuelet 1943). It is quite possible that *H. papillornata* resulted from a recent introduction, but from a genetic stock that is distinct from *H. triserialis* found in Bolivia.

The Helobdella triserialis *complex*

The New World *triserialis*-clade clearly is problematic. From our tree alone (Fig. 2) it is apparent that *H. triserialis* from South America does not even group close to *H. triserialis* from North America. What is even more distressing is that in North America, *H. triserialis*, *H. lineata* and *H. fusca* have been used interchangeably over the last century. The type locality for *H. triserialis* is in Chile (Blanchard 1849) though it is widely known across South America in various forms (reviewed in Ringuelet 1943). Those leeches in North America that currently are recognized as *H. triserialis* were first included broadly in Verrill's (1872) *Clepsine papillifera* for which he listed varieties a through d (Verrill 1872, 1874). Verrill's (1872, 1874) description of *C. papillifera* var. b was of a leech with three rows of dark-tipped papillae, while that of *C. papillifera* var. d *lineata* was of a leech that had 'about 12 longitudinal stripes of deep brown … back nearly smooth with only a few minute and but slightly raised papillae' (Verrill 1874: 683).

Moore (1901) suggested that Verrill's (1874) *C. papillifera* var. d *lineata*, was in all essential respects the same as Blanchard's (1849) *Glossiphonia triserialis*. However, he later seems to have changed his mind. Believing incorrectly that the epithet '*lineata*' was preoccupied by *Hirudo lineata*, Moore (1906) brought Verrill's (1872, 1874) *C. papillifera* var. d *lineata* into synonymy with Castle's (1900) *Glossiphonia fusca*. This was later reinforced by Moore (1920) and followed strictly by others (e.g. Ryerson 1915; Moore 1924; Miller 1929; Meyer 1937; Mathers 1948). However, the description of *G. fusca* is quite different. Castle (1900) describes a uniformly coffee-brown leech with an irregular pattern of seven longitudinal rows of clear areas, 'in the region of somites XXII–XXVI, the median row … is suddenly replaced by a continuous clear band' and with 'skin slightly rougher owing to the stronger development of papillae'.

Over 40 years later, Moore (1952) acknowledged his error (Moore 1906) in believing that what is now known as *Dina lineata* preoccupied *Helobdella lineata*. He then recognized *H. papillata* for those leeches he had described as '*G. fusca* strongly papillated type' (Moore 1906) and which were defined by Verrill (1872, 1874) as 'var. b' with a 'single median row [of papillae] anteriorly, which becomes double posteriorly where there is also a row on each side' and in which the tips of these papillae typically are dark brown (Verrill 1872, 1874). Moore (1952) also fully recognized *Helobdella lineata* for Verrill's (1874) *C. papillifera* var. d *lineata* for those longitudinally striated leeches lacking pronounced papillation. It seems that few paid attention to this distinction.

Ringuelet (1943) was following Moore (1906) in equating Verrill's (1874) *H. lineata* with Castle's (1900) *H. fusca*. However, he then followed Moore's (1906) failure to distinguish between Verrill's (1874) var. b and var. d when synonymizing all of the foregoing under the previously only South American name *H. triserialis* (Blanchard 1849) doing so in light of there being three rows of dark-tipped papillae. In North America, Sawyer (1967) was the first to recognize Ringuelet's synonymy and used *H. triserialis* instead of *H. lineata*. Klemm (1972) recognized each of *H. fusca*, *H. lineata* and *H. papillata* without distinguishing them

morphologically. Sawyer (1972) reverted to *H. lineata* for leeches with three rows of black tipped papillae, used *H. papillata* for those with very large papillae, and recognized *H. fusca* for those leeches with no papillae and with either three pairs of white stripes or a mottled appearance but always with the defining median clear anal patch described by Castle (1900). Klemm (1976) also used the specific epithet *lineata* for those North American leeches with the three rows of black tipped papillae and *papillata* for those with three rows of large white papillae lacking pigment. Klemm (1982) then applied *H. triserialis* to the former and also recognized the mottled form of *H. fusca*. Sawyer (1986) returned again to *H. triserialis* for the leeches he had previously included in *H. lineata* (Sawyer 1972) and seems to have agreed with Klemm (1976) in using *H. papillata* for those lacking pigment.

Moore (1952) is the authority for *H. papillata* and he clearly applied this name to Verrill's (1872, 1874) *C. papillifera* var. b which possesses three irregular rows of papillae irrespective of pigmentation. These are in the Peabody Museum with handwriting by Verrill that reads '*Clepsine papillifera* var. b. (Spring Station near Jacumba Mts., S. Cal. August 20, 75 5) Dr E. Palmer.' Although these might easily be confused (Ringuelet 1943; Sawyer 1967, 1986; Klemm 1982) with Blanchard's (1849) *H. triserialis* because of the papillation, they should not have been confused (Moore 1906; Ringuelet 1943; Sawyer 1967, 1972; Klemm 1976) with Verrill's (1874) *H. lineata* which was reported as being smooth and having barely discernible papillae. The holotype is a single specimen in the Peabody Museum with handwriting by Verrill reading '*C. papillifera* var. *lineata*. L. Raymond Neb. T.M. Prudden.'

The specimen used here for '*H. triserialis* black-tipped' (Fig. 3C) precisely matches Verrill's (1872, 1874) var. b and thus Moore's (1952) *H. papillata*. The specimen used here for '*H. triserialis* colourless' is identical to the former save for its lack of pigmentation. The specimen used here for *H. papillata* matches Sawyer's (1972; and see Klemm 1976, 1982) description for those with very large papillae (Fig. 3D). Our phylogenetic analysis (Fig. 2) reveals that all three of these are each others' closest relatives with marginal genetic distinction. In other words, Moore's (1952) application of *H. papillata* for those leeches with three irregular rows of papillae described by Verrill (1872, 1872) as var. b should all be included in *H. papillata* irrespective of the size of those papillae or pigmentation thereof.

The specimen used here for *H. lineata* (Fig. 3F) matches Verrill's (1874) *C. papillifera* var. d *lineata*, and Moore's (1952) *H. lineata*. That is, it has 12–14 longitudinal rows of brown pigmentation that are interrupted 'by fine transverse lines of whitish' (Verrill 1874) and though there are papillae, they are very fine, visible only out of fluid under reflected light.

Our *H. lineata* VA (Fig. 3E), though properly identified according to current keys, is clearly distinct from the other *H. lineata* obtained from Michigan. The former was designated *H. lineata* in light of the dorsal uninterrupted longitudinal brown pigmentation (*sensu* Verrill 1874). However, Verrill's (1874; never fully repeated in current keys) description of an even number would preclude a mid-dorsal line for bilaterally symmetrical pigment patterns (cf. Fig. 3F). In contrast, this specimen closely matches the description of *H. robusta* with fine dark papillae, uninterrupted longitudinal brown pigmentation, including a mid-dorsal region, and with two paramedial pairs of uninterrupted clear stripes (Shankland *et al*. 1992). Not surprisingly, this individual and the GenBank sequences for *H. robusta* are sister taxa (Fig. 2) notwithstanding that they are from opposite sides of the continent.

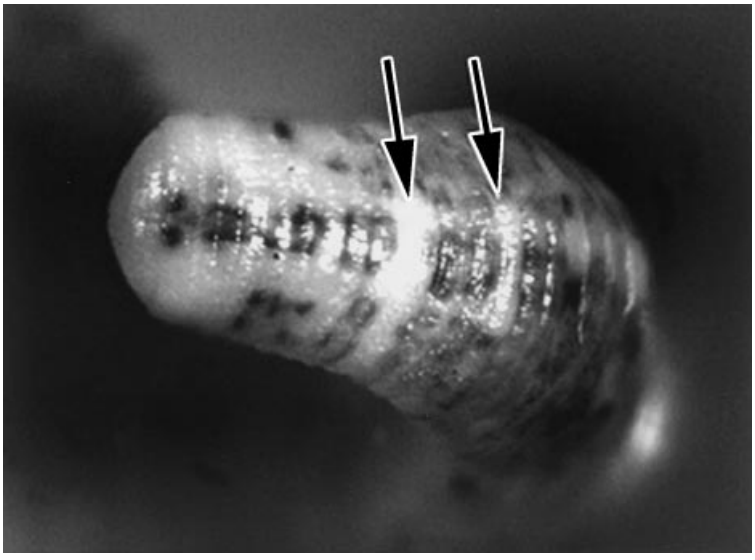

Fig. 4 Cephalic region of *H. triserialis* (*sensu stricto*) from South America exhibiting the transverse interruption (arrows) of longitudinal brown pigment patterns unlike North American representatives of *H. papillata* (previously also known as *H. triserialis*).

Although our representative for *H. fusca* (Fig. 3H) matches the mottled form noted by Sawyer (1972; see also Klemm 1982), we are not completely convinced that this matches Castle's (1900) description. Castle (1900) did note the unpigmented anal patch, but also indicated that there were seven regular arrays of clear spaces more anteriorly, and indicated a papillated appearance. Comparison with Castle's type material (MCZ 1811) is futile as these are now cleared, stained with carmine and mounted flat under coverslips. Discovery of other helobdellids with the anal patch but more regular dorsal patterns may reveal multiple species for this polymorphic leech.

Helobdella triserialis sensu stricto (from South America) still bears considerable resemblance to what is now recognized as the North American *H. papillata* (compare A and B with C and D in Fig. 3), but there is one distinguishing feature. Anterior to the genital somites and near the head, *H. triserialis* (*sensu stricto*) and *H. papillornata* exhibit transverse interruptions in the longitudinal pigmentation in a manner not seen for any North American leech (Fig. 4). This pattern is also evident in Ringuelet's (1943) figs 3 and 4 for *H. triserialis*. Separating the various North American species may still prove difficult which is why we have included photographs here. Should there be any confusion regarding an unusual form, we hope that application of the molecular protocols outlined here and placement with either CO-I or ND-I will serve to identify the leech by association.

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