

CSIRO Publishing

Invertebrate Systematics

Continuing *Invertebrate Taxonomy*



Volume 16, 2002
© CSIRO 2002

All enquiries and manuscripts should be directed to:



Invertebrate Systematics
CSIRO Publishing
PO Box 1139 (150 Oxford St)
Collingwood, Vic. 3066, Australia

Telephone: +61 3 9662 7629
Fax: +61 3 9662 7611
Email: publishing.is@csiro.au

Published by CSIRO Publishing
for CSIRO and the Australian Academy of Science

www.publish.csiro.au/journals/is

Phylogeny of the leech family Erpobdellidae (Hirudinida : Oligochaeta)

Mark E. Siddall

Division of Invertebrate Zoology, American Museum of Natural History, Central Park West at 79th Street,
New York, New York 10024, USA. Email: siddall@amnh.org

Abstract. The phylogenetic relationships of the family Erpobdellidae, a group of non-sanguivorous leeches useful as bioindicators, were investigated with the combined use of morphological characters, mitochondrial cytochrome *c* oxidase subunit I, mitochondrial 12S rDNA and nuclear 18S rDNA. Analyses of separate data sets and the combined data provide strong support for the contention that generic distinctions in the family are not reflective of phylogeny. The resulting hypothesis indicates the number of pairs of labial eyes as the sole morphological predictor of phylogenetic relationship. The traditionally used degree of annular subdivision is the least consistent character. In the absence of readily definable morphological synapomorphies for the resulting clades, the genera *Dina*, *Mooreobdella*, *Nephelopsis* and *Trocheta* are formally synonymised under the genus *Erpobdella*, the type genus of the family.

Introduction

Erpobdellid leeches are macrophagous predators of aquatic invertebrates (Young and Ironmonger 1979; Toman and Dall 1997) having abandoned the blood feeding habits of their ancestors (Siddall and Bureson 1998; Apakupakul *et al.* 1999). Species in this group have long been investigated as model organisms both for ecological studies of invertebrate species interactions (Anholt 1986; Seaby *et al.* 1995; Zerbst-Boroffka 1999) and as indicator species for freshwater toxicology (Wicklum and Davies 1996; McNicol *et al.* 1997; Wicklum *et al.* 1997; Zaranko *et al.* 1997). Members of the Erpobdellidae (species of *Erpobdella*, *Dina*, *Mooreobdella*, *Trocheta* and *Nephelopsis* as well as the recently established genera *Motobdella* and *Croatobranthus*) are characterised by their possession of multiple testisacs per somite and are most common in North America and Europe where they form assemblages that are distinct from the erpobdelliform Salifidae (e.g. species of *Salifa* and *Barbronia*) more common in Africa and Asia (Soos 1966; El Shimy 1996).

Blanchard's (1892) establishment of the genus *Dina* is broadly understood to have failed to distinguish it from *Erpobdella* (see Moore 1912; Pawlowski 1955). As such, the generic status of erpobdellids has experienced considerable confusion particularly concerning the separation of *Erpobdella* and *Dina*. Most treatments currently consider only the number or subdivision of annuli per somite (e.g. Sawyer 1986; Neubert and Nesemann 1995; Trontelj and Sket 2000). In this framework, species of *Erpobdella* have the plesiomorphic five-annulate condition whereas

erpobdellids possessing a widened and subdivided fifth annulus are collectively placed in the genus *Dina*. Similarly, the more extensively subdivided eight-annulate taxa are considered to belong to *Trocheta* (e.g. Nesemann and Neubert 1994). Generic distinctions not based on this annulation paradigm include *Mooreobdella* species, which lack anteriorly directed portions of the sperm ducts (preatrial loops), and *Motobdella* species which possess paired postcaeca, as well as those in monotypic genera like *Nephelopsis obscura*, which is very large and has a pair of highly coiled cornua on the male atrium, and *Croatobranthus mestrovi*, a cave-dwelling leech with somatic appendages. Indeed, since Whitman (1886, 1892), annulation patterns have held an important position in leech systematics (e.g. Blanchard 1894; Moore 1900, 1939; Harant 1929; Mann 1953; Pawlowski 1955; Harant and Grassé 1959; Sawyer 1986). Johansson (1910*a*, 1910*b*) appears to have dissented from this typological view, having placed the type species of *Dina* (i.e. *Dina lineata*) in the genus *Herpobdella* [sic]. However, he later expressed his own confusion over the establishment of *Dina* (Johansson 1914) and continued thereafter to include the subgeneric term *Dina* in parentheses (e.g. Johansson 1929; compare also Bennike 1940 with Bennike 1943). Moore's position on the matter is equally obscure insofar as he initially seems to have relied on male sexual anatomy (Moore 1912) adding later that the 'most obvious distinction is not found in the enlargement and subdivision of [annulus] b6... but in the form of the male atrium' (Moore 1930:182). However, he then proceeded to place *Dina dubia* only on the basis of the subdivided fifth

annulus (Moore and Meyer 1951; but see Sawyer 1986). Similarly, Mann (1959) held that both the bursal and ovarian organisations were distinctive between *Erpobdella* and *Dina* notwithstanding that he too had earlier included the type species of *Dina* in the genus *Erpobdella* (see Mann 1952).

It is interesting that the generic level of distinction among erpobdellids has not been resolved despite this group being the subject of more phylogenetic analyses than any other group of leeches (e.g. Trontelj *et al.* 1996; Govedich *et al.* 1998; Trontelj and Sket 2000). In part this is due to prior analyses including only European taxa (e.g. Trontelj *et al.* 1996; Trontelj and Sket 2000) or only North American taxa (e.g. Govedich *et al.* 1998). Here, European and North American species in the family Erpobdellidae are included in a combined phylogenetic analysis using nuclear, mitochondrial and morphological data.

Materials and methods

Most of the sequence information and accession numbers used in this study have been reported in prior works (Siddall and Bureson 1998; Apakupakul *et al.* 1999; Trontelj *et al.* 1999; Trontelj and Sket 2000; Siddall *et al.* 2001; Sket *et al.* 2001). Sequences new to this study include mitochondrial cytochrome *c* oxidase subunit I for *Haemopsis sanguisuga* [AF462021] from Svart Arn, Sweden, and mitochondrial 12S rDNA for *Dina dubia* [AF462022] from Northern Michigan, *Dina japonica* [AF462023], *Erpobdella punctata* [AF462024], *Erpobdella testacea* [AF462025], *Mooreobdella buccera* [AF462026], *Mooreobdella melanostoma* [AF462027] and *Nephelopsis obscura* [AF462028].

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

The universal primers, LCO1490, 5'-GGTCAACAAATCATAAA-GATATTGG-3' and HCO2198, 5'-TAAACTTCAGGGTGACCAAAA-AAATCA-3' were used to amplify cytochrome *c* oxidase subunit I (CO-I) fragments of 665 base pair (bp) length. The 12S mitochondrial ribosomal gene was amplified with primers 12S-A 5'-AACIIGGATTA-GATACCC-3' and 12S-B 5'-GAGAGTGACGGGCGATGTGT-3'. Amplification reactions for CO-I and for 12S rDNA contained 1.25 units of AmpliTaq DNA polymerase (Perkin-Elmer Corporation, Foster City, CA), 10X II Buffer, 2.5 mM magnesium chloride, 0.25 mM of each dNTP (1 mM total), 10 μM of primer pair mix, 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 Taq DNA polymerase, 10 mM Tris-Hydrochloric acid (pH 10), 50 mM potassium chloride, 1.5 mM magnesium chloride, 200 mM of each dNTP, stabilizers, 10 μM of primer pair mix, template and water. In a GeneAmp PCR System 9700 (Applied Biosystems, Perkin-Elmer Corporation), reaction mixtures were heated to 94°C for 5 minutes, followed by 15 cycles at 94°C (45 sec), 46°C (45 sec) and 72°C (45 sec), then 25 cycles at 94°C (20 sec), 45°C (20 sec) and 72°C (30 sec) and a final extension at 72°C (6 min). The QIAquick PCR Purification Kit protocol (QIAGEN, Inc.) was employed to purify amplification products.

Amplification products were sequenced in both directions. Each sequencing reaction mixture, including 4 μL BigDye™ (Applied Biosystems, Perkin-Elmer Corporation), 2 μL 1 μM primer (single

primer for each direction) and 5 μL of DNA template, ran for 40 cycles at 96°C (10 sec), 50°C (10 sec) 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, Perkin-Elmer Corporation).

Sequences of complimentary strands were edited and reconciled using Sequence Navigator (Applied Biosystems, Perkin-Elmer Corporation). Alignment of CO-I fragments was done by eye across all taxa because there were no insertions or deletions. Alignment of 18S rDNA and 12S rDNA was accomplished with Clustal W (in MacVector, Oxford Molecular Group).

Morphological characters that could be scored across all of the included taxa were those that have traditionally been used to distinguish among various erpobdellid taxa:

1. Number of annuli between gonopores: (0) two; (1) two and a half; (2) four or more.
2. Number of labial eyes: (0) two (i.e. one pair); (1) four (i.e. two pairs).
3. Preatrial loops: (0) present; (1) absent.
4. Ovisacs: (0) elongate along nerve cord; (1) convoluted or folded back to gonopore.
5. Fifth annulus: (0) not subdivided; (1) subdivided.

All data and alignments are available at <http://research.amnh.org/~siddall/>.

Phylogenetic analyses were performed using PAUP* (Swofford 2000). Heuristic searches used 20 replicates of random taxon addition and tree-bisection-reconnection branch swapping. All characters were left non-additive and of equal weight. Bremer support (b) indices (Bremer 1988) were obtained using TreeRot (Sorenson 1999) and parsimony jackknife (jac) values were obtained with 200 replicates of five random additions of branch swapping with XAC (Farris 1999). Retention indices and bootstrap values were calculated with PAUP* (Swofford 2000).

Results

Mitochondrial cytochrome *c* oxidase subunit I exhibited approximately equal variation among the Erpobdellidae (between 13% and 17%), but only the monophyly of *Erpobdella punctata* with the genus *Mooreobdella* resulted from parsimony jackknife analysis of this gene alone. The nuclear 18S rDNA gene showed the least variation across the ingroup (<5% divergence) whereas the mitochondrial 12S rDNA showed substantial variation across the Erpobdellidae (between 5% and 27%). Both of these data partitions yielded well-supported groups with parsimony jackknifing that were congruent with those obtained from combining all data in analyses (below).

Parsimony analysis of the combined nuclear, mitochondrial and morphological data (2941 characters) yielded one optimal tree with 1350 steps and a retention index of 0.69 (Fig. 1). In that tree, the only non-monotypic erpobdellid genus that was monophyletic was *Trocheta*. There are two major clades in the ingroup, both of which include nominal species of *Erpobdella* and *Dina*. The North American and Asian *Dina* species group with the European *Erpobdella* species (and thus the type of that genus) whereas the North American *Erpobdella* and *Mooreobdella* species group with *Croatobranchnus mestrovi* and the European *Dina*

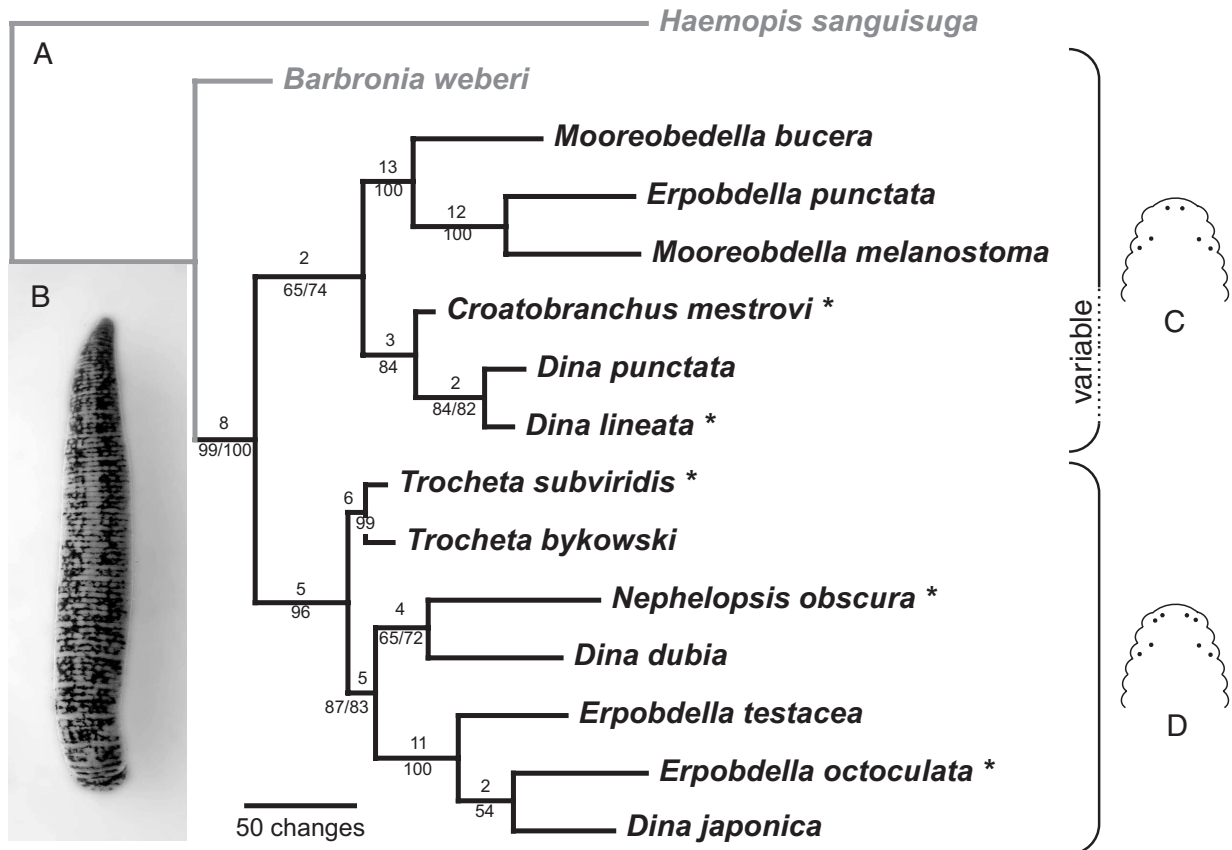


Fig. 1. Optimal tree (A) resulting from parsimony analysis of combined morphological and molecular data. Branch lengths are proportional to amount of change. Outgroup taxa are in grey. Numbers above nodes are Bremer support values. Numbers below nodes are parsimony jackknife values/bootstrapped values (or the agreement in both where only one number appears). Type species for each genus are indicated with asterisks. The type species for the family is *Erpobdella octoculata* (B). The only morphological character transformation with perfect consistency on this topology is the change from one pair of labial eyes (C) to two pairs of labial eyes (D).

species (and thus the type of that genus). The morphological character with the worst fit (retention index = 0.50) on the resulting hypothesis of phylogenetic relationships was the presence or absence of a subdivided 5th annulus. The character with the best fit to the tree was the number of labial eye spots (retention index = 1.00), transforming from two (i.e. one pair) to four (i.e. two pairs) once on the tree as a synapomorphy for the monophyletic group that contains, among other species, *Trocheta* spp. and *Erpobdella octoculata* (the type species of that genus). The character with the next-best fit (retention index = 0.83) was the apomorphic condition of having folded-back ovaries (occurring once as a synapomorphy for the clade containing *Dina punctata*, *Erpobdella punctata*, *Mooreobdella* spp. and *Croatobranchnus*; but reversing within that for *Dina lineata*, and changing convergently for *Nephelopsis obscura*).

Discussion

The results of the phylogenetic analysis, including 13 ingroup taxa from the family Erpobdellidae with a salifid

and a hirudinid as outgroups and combining data from mitochondrial CO-I, mitochondrial 12S rDNA, nuclear 18S rDNA and the five morphological characters, strongly corroborate the contention of Apakupakul *et al.* (1999) that erpobdellid systematics is in need of radical revision. Trontelj and Sket (2000) have already noted that the degree of subdivision of annuli is an unreliable character to distinguish species of *Dina* and *Trocheta*. Because *Trocheta krasense* grouped with *Dina punctata* and *Dina lineata*, they formally suppressed the subfamily Trochetinae (see Trontelj and Sket 2000) although they retained *Dina* and *Erpobdella*, notwithstanding their determination that this would render the former paraphyletic with respect to the European species they included. Inclusion of the North American erpobdellids here indicates that retention of all of these groups would be unwise because it would render *Mooreobdella* paraphyletic and *Dina* and *Erpobdella* polyphyletic.

Clearly annulation typology has not served the systematics of leeches very well. As subdivision of the fifth

annulus is the least consistent character on the tree, its use should simply be abandoned (see also Trontelj and Sket 2000). Similarly, though the lack of preatrial loops has traditionally been used to define the North American genus *Mooreobdella*, the resulting phylogeny supports the view that this has reversed in *Erpobdella punctata*, also North American.

In the face of such a disparity between phylogeny and systematics there are various options available for the future of erpobdellid leech taxonomy. One option is to retain subdivisions in spite of the lack of sufficient morphological characters to distinguish taxa phylogenetically (Trontelj and Sket 2000); that is, retain existing delimitations of *Dina*, *Trocheta*, *Erpobdella*, *Mooreobdella* and *Nephelopsis* on the basis of typological character delimitations (see for example, Cantino *et al.* 1999, Table II). This is unsatisfactory in that it does not allow the names applied to taxa to reflect any new understanding of relationships or of the conservation of morphological characters. A simple alternative to that aphylogenetic approach would be to synonymise all erpobdellid genera under the oldest generic name for the group (i.e. *Erpobdella*) on the grounds, for example, that the European *Erpobdella octoculata* and North American *Erpobdella punctata* belong to distinctly separate parts of the tree. A third alternative would be to find something predictive from character transformations and alter taxon names to reflect that new understanding. Clearly the type species of *Dina* and the type species of *Erpobdella* fall into separate clades (Fig. 1). However, it might be unwise, and is certainly impractical, to require generic determination on the basis of DNA sequence data and grouping in a phylogenetic tree. One striking feature of the hypothesis (Fig. 1) is the fact that *Erpobdella octoculata* falls into an exclusively eight-eyed clade (two pairs of labial eyes) and *Dina lineata* falls into a six-eyed clade (one pair of labial eyes, the only exception is the blind, cave-dwelling *Croatobranthus*). Redefining the limits of *Erpobdella* and *Dina* might be thought to be simple. *Erpobdella* species could be those with the typical erpobdellid multiple clusters of testisacs per somite and lack of pharyngeal stylets that possess the apomorphic condition of two labial doublets of eye spots where present (and then usually four more eyespots posteriorly in two pairs). *Dina* would then be redefined as those species with the typical erpobdellid multiple clusters of testisacs per somite and lack of pharyngeal stylets possessing the plesiomorphic condition of one labial pair of eye spots where present (and then usually four more eyespots posteriorly in two pairs). Because the *Trocheta*-like annulation pattern has already been shown to be convergently acquired, and in light of annulation in general not being consistent with phylogeny, it would be appropriate to simply recognise the octoculate *Trocheta* as species of *Erpobdella*. However, the *Dina* condition of possessing only two (one pair of) labial eyes is plesiomorphic for the group—

being present in *Salifa* and *Barbronia* (Nesemann 1995; El Shimy 1996)—and should not be relied on to predict a monophyletic group. Unfortunately no other morphological character is sufficiently consistent to define a monophyletic group. Moreover *Croatobranthus*, like other cave-dwelling leeches, are eyeless (Manoleli *et al.* 1998; Kerovec *et al.* 1999), which would preclude their generic determination at all. Thus, each of *Dina*, *Mooreobdella*, *Nephelopsis* and *Trocheta* are suppressed as subjective junior synonyms of *Erpobdella* Blainville, 1818. This does, however, raise the problem of homonymy for the resulting North American *Erpobdella punctata* (Leidy, 1870) and European (previously *Dina*) *Erpobdella punctata* (Johansson, 1927). Johansson (1927) described the latter originally as *Dina lineata punctata*, but Nesemann's (1990) action validated the species. Thus, a new name with obvious etymology, *Erpobdella johanssoni* is proposed here for *Dina punctata* (Johansson, 1927) Nesemann, 1990. The genus *Erpobdella* Blainville, 1818 then includes the following 37 nominal species:

- Erpobdella absoloni* (Johansson, 1913), comb. nov.
- Erpobdella anoculata* (Moore, 1898)
- Erpobdella apathyi* (Gedroyc, 1916)
- Erpobdella bucera* (Moore, 1953), comb. nov.
- Erpobdella bykowski* (Gedroyc, 1913), comb. nov.
- Erpobdella concolor* Annandale, 1913
- Erpobdella costalis* Sawyer & Shelley, 1976
- Erpobdella dubia* (Moore & Meyer, 1951)
- Erpobdella eturpshem* (Sket, 1989), comb. nov.
- Erpobdella fervida* (Verrill, 1874), comb. nov.
- Erpobdella japonica* (Pawlowski, 1952), comb. nov.
- Erpobdella johanssoni* (Johansson, 1927), nom. nov.
- Erpobdella krasense* (Sket, 1968)
- Erpobdella krilata* (Sket, 1989), comb. nov.
- Erpobdella lahontana* Hovingh & Klemm, 2000
- Erpobdella latestriata* (Neubert & Nesemann, 1995), comb. nov.
- Erpobdella lineata* (Müller, 1774)
- Erpobdella maoriana* (Mason, 1976), comb. nov.
- Erpobdella mauchi* Nesemann, 1995
- Erpobdella melanostoma* (Sawyer & Shelley, 1976), comb. nov.
- Erpobdella mestrovi* (Kerovec *et al.*, 1999), comb. nov.
- Erpobdella microstoma* (Moore, 1901), comb. nov.
- Erpobdella monostriata* (Gedroyc, 1916)
- Erpobdella nigricolis* (Brandes, 1900)
- Erpobdella obscura* (Verrill, 1872), comb. nov.
- Erpobdella octoculata* (Linnaeus, 1758). **Type species.**
- Erpobdella parva* (Moore, 1912), comb. nov.
- Erpobdella punctata* (Leidy, 1870)
- Erpobdella quaternaria* (Moore, 1930), comb. nov.
- Erpobdella ratschaensis* (Kobakhidze, 1958), comb. nov.
- Erpobdella stschegolewi* Lukin & Epshtein, 1950
- Erpobdella subviridis* (Dutrochet, 1817), comb. nov.

Erpobdella svilesta (Sket, 1989), comb. nov.
Erpobdella testacea (Savigny, 1820)
Erpobdella tetragon (Sawyer & Shelley, 1976), comb. nov.
Erpobdella triannulata Moore, 1908
Erpobdella xiangjiangensis (Yang, 1983), comb. nov.

The above approach of summary synonymisation might seem nihilistic to some. However, it does serve to clarify the taxonomic status of the monotypic genera. Admittedly, *Erpobdella obscura*, comb. nov. is an unusually large erpobdellid (Verrill 1872; Moore 1912). Beyond that though, its anatomy is unremarkable and there seems to be no additional reason to exclude it from the genus *Erpobdella*. In contrast, *Erpobdella mestrovi*, comb. nov. does possess a remarkably altered morphology with somatic appendages (Kerovec *et al.* 1999). Nonetheless, its inclusion in the genus *Erpobdella* underscores from whence this strange cave-dwelling adaptation arose—ultimately from a common ancestor with *Erpobdella lineata*, comb. nov. and *Erpobdella johanssoni*, comb. nov. in Europe.

It is possible that there are other valid erpobdellid genera. The genus *Motobdella* was recently established for two species with an otherwise unknown synapomorphy of paired postcaeca and the group is supported as monophyletic (Govedich *et al.* 1998). Their possession of the plesiomorphic single pair of labial eyes suggests that *Motobdella* may be sister to the remaining Erpobdellidae. Expansion of taxonomic samples to include these, *Fadejewobdella*, *Archaeobdella*, the Asian and African salifids as well as the unusual South American genera, *Americobdella* and *Lumbricobdella*, should serve to further clarify our understanding of the evolutionary history of these important non-blood-feeding leeches.

Acknowledgments

I greatly appreciate the scientific expertise and substantive comments of Liz Borda in the preparation of this work. This research was funded in part by the Louis and Dorothy Cullman Program in Molecular Systematic Studies and by the Ambrose Monell Foundation.

References

- Anholt, B. (1986). Prey selection by the predatory leech *Nepheleopsis obscura* in relation to three alternative models of foraging. *Canadian Journal of Zoology* **64**, 649–655.
- Apakupakul, K., Siddall, M. E., and Burreson, E. M. (1999). Higher level relationships of leeches (Annelida: Clitellata: Euhirudinea) based on morphology and gene sequences. *Molecular Phylogenetics and Evolution* **12**, 350–359.
- Bennike, S. A. B. (1940). On some Iranian freshwater Hirudinea. In 'Danish Scientific Investigations in Iran, Part II'. pp. 1–10. (Einar Munksgaard: Copenhagen.)
- Bennike, S. A. B. (1943). Contributions to the ecology and biology of Danish freshwater leeches (Hirudinea). *Folia Limnologica Scandinavica* **2**, 1–109.
- Blanchard, R. (1892). Sur la présence de la *Trocheta subviridis* en Ligurie et description de cette Hirudinée. *Atti della Società Linguistica di Scienze naturali e geografiche* **3**, 1–31.
- Blanchard, R. (1894). Hirudinées de l'Italie continentale et insulaire. *Bollettino dei Musei di zoologia e anatomia comparata dell'Università (Torino)* **9**, No. 192.
- Bremer, K. (1988). The limits of amino-acid sequence data in angiosperm phylogenetic reconstruction. *Evolution* **42**, 795–803.
- Cantino, D., Bryant, H. N., de Queiroz, K., Donoghue, M. J., Eriksson, T., Hillis, D. M., and Lee, M. S. Y. (1999). Species names in phylogenetic nomenclature. *Systematic Biology* **48**, 790–807.
- El Shimy, N. A. (1996). Revision of the genus *Barbronia* Johansson, 1918 (Hirudinea: Erpobdelliformes: Barbroniidae) in Egypt. *Zoology in the Middle East* **12**, 99–104.
- Farris, J. S. (1999). 'XAC, Program and Documentation.' (Swedish Natural History Museum: Stockholm, Sweden.)
- Govedich, F. R., Blinn, D. W., Keim, P., and Davies, R. W. (1998). Phylogenetic relationships of three genera of Erpobdellidae (Hirudinoidea), with description of a new genus, *Motobdella*, and species *Motobdella sedonensis*. *Canadian Journal of Zoology* **76**, 2164–2171.
- Harant, H. (1929). Essai sur les Hirudinées. *Archives de la Société des Sciences Montpellier* **10**, 1–76.
- Harant, H., and Grassé, P. P. (1959). Classe des annélides achètes ou hirudinées ou sangsues. In 'Traité de Zoologie'. (Ed. P. P. Grassé.) Vol. 5, part 1, pp. 471–593. (Mason: Paris.)
- Johansson, L. (1910a). Zur Kenntnis der Herpobdelliden Deutschlands. *Zoologischer Anzeiger* **35**, 705–714.
- Johansson, L. (1910b). Zur Kenntnis der Herpobdelliden Deutschlands. *Zoologischer Anzeiger* **36**, 367–379.
- Johansson, L. (1914). Ergebnisse einer von Prof. Franz Werner im Sommer 1910 mit Unterstützung aus dem Legate Wedl ausgeführten Forschungsreise nach Algerien, VIII. Hirudineen. *Aus den Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften in Wien, Mathematisch-Naturwissenschaftliche Klasse (Abt. 1)* **123**, 837–852.
- Johansson, L. (1927). Hirudineen aus dem nördlichen und östlichen Spanien, gesammelt von Dr. F. Haas in den Jahren 1914–1919. *Abhandlungen der Senckenbergischen Naturforschenden Gesellschaft* **39**, 217–231.
- Johansson, L. (1929). Hirudinea (Egel). In 'Die Tierwelt Deutschlands und der angrenzenden Meeresteile nach ihren Merkmalen und nach ihrer Lebensweise'. (Ed. F. Dahl). Tiel 15. pp. 133–155. (Fischer, Jena: Munich.)
- Kerovec, M., Kuëiniæ, M., and Jalziæ, B. (1999). *Croatobranchnus mestrovi* sp. n. -predstavnik nove endemske podzemne vrste pijavica. *Spelolog* **44/45**, 35–36.
- Mann, K. H. (1952). A revision of the British leeches of the family Erpobdellidae with an account of *Dina lineata* (O. F. Müller, 1774), a leech new to the British fauna. *Proceedings of the Zoological Society of London* **123**, 377–391.
- Mann, K. H. (1953). The segmentation of leeches. *Biological Reviews* **28**, 1–15.
- Mann, K. H. (1959). On *Trocheta bykowskii* Gedroyc, 1913, a leech new to the British fauna, with notes on the taxonomy and ecology of other Erpobdellidae. *Proceedings of the Zoological Society of London* **132**, 369–379.
- Manoleli, D. G., Klemm, D. J., and Sarbu, S. M. (1998). *Haemopsis caeca* (Annelida: Hirudinea: Arhynchobdellida: Haemopidae), a new species of troglobitic leech from a chemoautotrophically based groundwater ecosystem in Romania. *Proceedings of the Biological Society of Washington* **111**, 222–229.
- McNicol, D. K., Mallory, M. L., Mierle, G., Scheuhammer, A. M., and Wong, A. H. K. (1997). Leeches as indicators of dietary mercury exposure in non-piscivorous waterfowl in Central Ontario, Canada. *Environmental Pollution* **95**, 177–181.
- Moore, J. P. (1900). A description of *Microbdella biannulata* with especial regard to the constitution of the leech somite. *Proceedings of the Academy of Natural Sciences of Philadelphia* **52**, 50–73.

- Moore, J. P. (1912). Classification of leeches. In 'The Leeches of Minnesota'. (Ed. H. F. Nachtrieb.) pp. 63–150. (Geological and Natural History Survey of Minnesota: Minneapolis.)
- Moore, J. P. (1930). Leeches (Hirudinea) from China with descriptions of new species. *Proceedings of the Academy of Natural Sciences of Philadelphia* **81**, 267–295.
- Moore, J. P. (1939). Additions to our knowledge of African leeches. *Proceedings of the Academy of Natural Sciences of Philadelphia* **90**, 297–360.
- Moore, J. P., and Meyer, M. C. (1951). Leeches (Hirudinea) from Alaskan and adjacent waters. *Wasmann Journal of Biology* **9**, 11–77.
- Nesemann, H. (1990). Die semiaquatischen Egel mitteleuropäischer Fließgewässer (Hirudinea: Erpobdellidae, Hirudinidae). *Zeitschrift der Angewandte Zoologie* **77**, 219–252.
- Nesemann, H. (1995). On the morphology and taxonomy of the Asian leeches (Hirudinea: Erpobdellidae, Salifidae). *Acta Zoologica Academiae Scientiarum Hungaricae* **41**, 165–183.
- Nesemann, H., and Neubert, E. (1994). New data to the leeches of the subfamily Trochaetinae (Hirudinea, Erpobdellidae). *Miscellanea Zoologica Hungarica* **9**, 19–28.
- Neubert, E., and Nesemann, H. (1995). Contribution to the knowledge of the genus *Dina* Blanchard, 1892 (Hirudinea: Erpobdellidae). *Hydrobiologia* **315**, 89–94.
- Pawlowski, L. K. (1955). Révision des genres *Erpobdella* de Blainville et *Dina* R. Blanchard (Hirudinea). *Bulletin de la Société des Sciences et des Lettres de Łódź, Classe III de Sciences Mathématiques et Naturelles* **6**, 1–15.
- Sawyer, R. T. (1986). 'Leech Biology and Behaviour.' (Clarendon Press: Oxford.)
- Seaby, R. M. H., Martin, A. J., and Young, J. O. (1995). The reaction time of leech and triclad species to crushed prey and the significance of this for their coexistence in British lakes. *Freshwater Biology* **34**, 21–28.
- Siddall, M. E., and Burrenson, E. M. (1998). Phylogeny of leeches (Hirudinea) based on mitochondrial cytochrome *c* oxidase subunit I. *Molecular Phylogenetics and Evolution* **9**, 156–162.
- Siddall, M. E., Apakupakul, K., Burrenson, E. M., Coates, K. A., Erséus, C., Gelder, S. R., Källersjö, M., and Trapido-Rosenthal, H. (2001). Validating Livanow's hypothesis: molecular data agree that leeches, branchiobdellidans and *Acanthobdella peledina* form a monophyletic group of oligochaetes. *Molecular Phylogenetics and Evolution* **21**, 346–351.
- Sket, B., Dovc, P., Jalzic, B., Kerovec, M., Kucinic, M., and Trontelj, P. (2001). A cave leech (Hirudinea, Erpobdellidae) from Croatia with unique morphological features. *Zoologica Scripta* **30**, 223–229.
- Soos, A. (1966). Identification key to the leech (Hirudinoidea) genera of the world, with a catalogue of the species. III. Family: Erpobdellidae. *Acta Zoologica Academiae Scientiarum Hungaricae* **12**, 371–407.
- Sorenson, M. D. (1999). 'TreeRot. v2b.' (Department of Biology, Boston University: Boston, MA.)
- Swofford, D. L. (2000). 'PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4.' (Sinauer Associates: Sunderland, MA.)
- Toman, M. J., and Dall, P. C. (1997). The diet of *Erpobdella octoculata* (Hirudinea: Erpobdellidae) in two Danish lowland streams. *Archiv für Hydrobiologie* **140**, 549–563.
- Trontelj, P., and Sket, B. (2000). Molecular re-assessment of some phylogenetic, taxonomic and biogeographic relationships between the leech genera *Dina* and *Trocheta* (Hirudinea: Erpobdellidae). *Hydrobiologia* **438**, 227–235.
- Trontelj, P., Sket, B., Dovc, P., and Steinbrück, G. (1996). Phylogenetic relationships in European erpobdellid leeches (Hirudinea: Erpobdellidae) inferred from restriction-site data of the 18S ribosomal gene and ITS2 region. *Journal of Zoological Systematics and Evolutionary Research* **34**, 85–93.
- Trontelj, P., Sket, B., and Steinbrück, G. (1999). Molecular phylogeny of leeches: congruence of nuclear and mitochondrial rDNA data sets and the origin of bloodsucking. *Journal of Zoological Systematics and Evolutionary Research* **37**, 141–147.
- Verril, A. E. (1872). Descriptions of North American fresh water leeches. *American Journal of Science* **3**, 126–139.
- Whitman, C. O. (1886). The germ layers of *Clepsine*. *Zoologischer Anzeiger* **9**, 171–176.
- Whitman, C. O. (1892). The metamerism of *Clepsine*. In 'Festschrift zum 70. Geburtstag von Rudolf Leuckarts. Dem Verehrten Jubilar Dargebracht von Seinen Dankbaren Schülern'. pp. 385–395. (Engelmann: Leipzig.)
- Wicklum, D., and Davies, R. W. (1996). The effects of chronic cadmium stress on energy acquisition and allocation in a freshwater benthic invertebrate predator. *Aquatic Toxicology (Amsterdam)* **35**, 237–252.
- Wicklum, D., Smith, D. E. C., and Davies, R. W. (1997). Mortality, preference, avoidance, and activity of a predatory leech exposed to cadmium. *Archives of Environmental Contamination and Toxicology* **32**, 178–183.
- Young, J. O., and Ironmonger, J. W. (1979). The natural diet of *Erpobdella octoculata* (L.) (Hirudinea: Erpobdellidae) in British lakes. *Archiv für Hydrobiologie* **87**, 483–503.
- Zaranko, D. T., Griffiths, R. W., and Kaushik, N. K. (1997). Biomagnification of polychlorinated biphenyls through a riverine food web. *Environmental Toxicology and Chemistry* **16**, 1463–1471.
- Zerbst-Boroffka, I. (1999). Osmotic adaptation of the endemic fauna to the ancient freshwater Lake Baikal. *Naturwissenschaften* **86**, 330–333.

Manuscript received 20 April 2001; revised and accepted 11 September 2001.