On the classification, evolution and biogeography of terrestrial haemadipsoid leeches (Hirudinida: Arhynchobdellida: Hirudiniformes)

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Abstract

A scourge of tropical and subtropical jungles, bloodfeeding terrestrial leeches of Haemadipsidae have long confused systematists and defied sensible biogeographic interpretation. The family Haemadipsidae usually includes problematic taxa that neither fit the typical IndoPacific distribution of the group, nor properly match diagnostic characters used to define the family. Historically, four additional families—Xerobdellidae, Diestecostomatidae Mesobdellidae and Nesophilaemonidae—have occasionally been recognized for New World and European representatives, though agreement on the composition of those families has not been consistent. Here, we expand the phylogenetic sampling of non-IndoPacific (among other) genera to include Meso American Diestecostoma species and Nesophilaemon skottsbergii from the Juan Fernandez Archipelago in order to critically assess prior hypotheses in a molecular phylogenetic analysis of arhynchobdellid leeches. The result, based on nuclear 18S rDNA and 28S rDNA and mitochondrial COI indicates that there are two distantly related lineages of bloodfeeding terrestrial leeches. The otherwise monophyletic family Haemadipsidae is found to exclude species of Xerobdella, Mesobdella and Diestecostoma. Xerobdellidae is formally resurrected to accommodate species of those three genera. Morphological characteristics corroborate the distinction of Haemadipsidae and Xerobdellidae on the basis of sexual and nephridial characters. Idiobdella seychellensis belongs in Haemadipsidae notwithstanding its lacking respiratory auricles. Nesophilaemon skottsbergii too is in Haemadipsidae notwithstanding its geographic proximity to the xerobdellid Mesobdella gemmata. The characters used to define haemadipsoid families are reevaluated. Feeding preferences and biogeographic patterns are also examined.

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Keywords: Haemadipsidae; Xerobdellidae; Phylogeny; Evolution; Terrestrial leeches; Biogeography

1. Introduction

Bloodfeeding terrestrial leeches have fascinated (and have been loathed by) “travelers, sportsman and missionaries, whose quest have carried them into the [leech infested] humid valleys and jungles” (Haeckel, 1883, p. 188) of the IndoPacific. Although these leeches are adapted to a terrestrial way of life, they are restricted to damp forests that are subject to seasonal rainfall (Sawyer, 1986). As such, the majority of bloodfeeding terrestrial leech species are distributed throughout tropical and subtropical Indo-Pacific landmasses. There are, however, 10 unusual and lesser-known terrestrial species found in isolated pockets of subtropical or temperate zones in South America, Meso America and Europe (Sawyer, 1986).

The classification of bloodfeeding terrestrial leeches has a tortuous history. The placement of the genera Xerobdella, Diestecostoma, Mesobdella and Nesophilaemon within Haemadipsidae (and among Hirudiniformes) has been especially problematic. Blanchard (1896) established Haemadipsinae (=Haemadipsidae sensu Soós, 1967) to distinguish bloodfeeding terrestrial leeches from their aquatic sanguivorous (i.e. bloodfeeding) and carnivorous counterparts in Hirudininae. Blanchard’s (1896, 1917) classification included those species that: (1) were terrestrial and
bloodfeeding in habit; (2) had a unique “haemadipsine” ocular arch with five pairs of eyespots and the 4th and 5th pair separated by two annuli. (Fig. 1a); (3) had lateral nephridia, with the last pair concealed beneath respiratory auricles (Fig. 1b); and (4) had a distribution on landmasses throughout the Indian Ocean and the Pacific Ocean. There were some geographic exceptions. Blanchard (1917) tentatively included the Chilean *Mesobdella gemmata* (E. Blanchard, 1849) and the European *Xerobdella lecomtei* (v. Frauenfeld, 1868) in Haemadipsidae, yet recognized that they deviated from other members of the family due to their isolated distributions, the eyespot arrangement, the ventral (rather than lateral) position of the nephridia and the lack of respiratory auricles. Not surprisingly, others proposed that these species were only distantly related to the true haemadipsids from the IndoPacific because they were geographically displaced and/or deviated from the morphological characters normally used to define members of Haemadipsidae (Moore, 1946; Richardson, 1969, 1971; Ringuelet, 1972).

In spite of being duognathous (two-jawed) and sharing characters with other duognathous IndoPacific haemadipsid leeches (e.g. lateral nephridia, respiratory auricles; Fig. 1b), Richardson (1971) proposed that *Nesophilaemon skottsbergii* (Johansson, 1924) shared a greater affinity to *Mesobdella gemmata*. This was in light of its proximity to the New World, approximately 700 km off the east coast of Chile in the Juan Fernandez Archipelago. Unlike *Nesophilaemon skottsbergii*, the remaining non-IndoPacific genera were trignathous (three-jawed) and lack respiratory auricles normally found in the IndoPacific leeches. Ultimately, debates and disagreement regarding the affinities of non-IndoPacific leeches to Haemadipsidae led to each taxon being placed into its own respective subfamily or family: Xerobdellinae (*sensu* Moore, 1946); Diestecostomatidae (Ringuelet, 1953); Mesobdellidae (Ringuelet, 1972) and Nesophilaemonidae (Ringuelet, 1982). In contrast, more recent treatments of bloodfeeding terrestrial leeches placed them all in a single family, Haemadipsidae, subdivided into the duognathous series and trignathous series (Sawyer, 1986; see also Table 1).

Table 1
Classification schemes for bloodfeeding terrestrial leeches: (A) adapted from Sawyer (1986) and (B) adapted from Ringuelet (1953a, 1982b), Borda and Siddall (2004a) and Richardson (1975a, 1978)

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<tr>
<th></th>
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<td>[<em>1 Genus</em>]</td>
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<tr>
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<td><em>Nesophilaemon</em></td>
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Listed genera are only those included in this study.

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**Fig. 1.** Diagnostic characters for haemadipsoid terrestrial leeches. (a) “Haemadipsine” ocular arch of *Nesophilaemon skottsbergii*, showing fourth and fifth eyespot pairs separated by two annuli (=segment); (b) lateral view of *Nesophilaemon skottsbergii* showing respiratory auricles and lateral nephridia; (c) ventral view of *Mesobdella gemmata* showing ventral nephridia and the medioventral pore (through the caudal sucker). a = annulus (annuli; pl.); cs = caudal sucker; es = eyespots; gp = gonopore; mvp = medioventral pore; n = nephridiopore; ra = respiratory auricles; s = somite.
1.1. Phylogenetic relationships of bloodfeeding terrestrial leeches

Trontelj et al. (1999) and Kutschera et al. (2007) showed that *Xerobdella lecomtei* was distantly related to the haemadipsid leeches based on nuclear 18S rDNA and COI, respectively. With an expanded taxonomic sampling of Arhynchobdellida, Borda and Siddall (2004a) corroborated previous work (i.e. Trontelj et al., 1999) by showing Haemadipsidae (*sensu* Sawyer, 1986) not to be monophyletic. Specifically, *Xerobdella lecomtei* and *Mesobdella gemmata* were found not to be sister to the monophyletic IndoPacific leeches (Trontelj et al., 1999; Borda and Siddall, 2004a). Borda and Siddall (2004a) and Kutschera et al. (2007) proposed returning the subfamily Xerobdellinae (Moore, 1946) to family status (*sensu* Harrant and Grasse, 1959) for the non-IndoPacific clade. However, without the inclusion of other non-IndoPacific genera (i.e. *Diestecostoma, Nesophilaemon*) a formal revision of the group was not possible.

In this study, we have expanded the taxonomic representation of Haemadipsidae (*sensu* Sawyer, 1986; Table 1A) and also include the type species of each non-IndoPacific putative family (*sensu* Moore, 1946; Harrant and Grasse, 1959; Ringuelet, 1953, 1972, 1982) in order to better understand their phylogenetic placement among hirudiniform leeches relative to traditional classification schemes (Moore, 1946; Sawyer, 1986; Caballero, 1956, 1959; Soós, 1966, 1967; Richardson, 1971, 1975, 1978; Ringuelet, 1972, 1982). In doing so, we reexamine the morphological characters used to define Haemadipsidae (Blanchard, 1896, 1917; Richardson, 1975), as well as examine the evolution of feeding preferences and the biogeographic patterns of haemadipsid terrestrial leeches in a phylogenetic framework.

2. Materials and methods

2.1. Taxa

Sampling localities and GenBank accession numbers are listed in Table 2. Species new to this study include: *Chetonobdella whitmani* (Lambert, 1899) from Australia, *Diestecostoma mexicana* (Moore, 1946), *Diestecostoma magna* (Moore, 1945), and *Diestecostoma trujillensis* (Ringuelet, 1976), all from Mexico, *Haemadipsa interrupta* (Moore, 1935) from Malaysia, *Idiobdella seychellensis* (Harding, 1913) from the Seychelles Archipelago, *Malagabdella fallax* (Blanchard, 1917) from Madagascar and *Nesophilaemon skottsbergii* (Johansson, 1924) from the Juan Fernandez Archipelago. Representatives from the

<table>
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(*) indicates type species for proposed families: Diestecostomatidae, Idiobdellidae, Mesobdellidae and Nesophilaemonidae.

a Apakupakul et al. (1999).
b Trontelj et al. (1999).
c Borda and Siddall (2004a).
d Siddall and Burreson (1998).
e Borda and Siddall (2004b).
major families of Hirudiniformes were included as outgroup taxa based on previous phylogenetic work (Borda and Siddall, 2004a). Terrestrial leeches were collected from exposed skin or from under rotting logs. Collection strategies involved walking along forest trails and searching through damp undergrowth, especially after rain, to attract leeches. Other methods included walking barefoot (i.e., third author) through the forest to increase skin exposure for leeches found in the undergrowth. After collection, leeches were relaxed using an ethanol gradient, starting with a ~10% ethanol solution and gradually adding 95%–100% ethanol until the leeches were relaxed (and dead). Leeches collected from the field were stored in 95–100% ethanol at −20 or at −4 °C until used for DNA extraction.

2.2. Morphology

Examination and dissection of Diestecostoma mexicana, Idiobdella seychellensis, Malagabdella fallax, Mesobdella gemmata (from Siddall and Borda, 2004), Nesophilaemon skottsbergii and Xerobdella lecomtei were done with a Nikon SMZ-U stereomicroscope. Photodocumentation of leeches was accomplished using a SPOT-RT (Diagnostic Instruments, Inc.) digital camera. Illustrations were facilitated in Adobe® Illustrator® 10 and Adobe® Photoshop® 7. Illustration of the reproductive systems for Haemadipsa sylvestris was adapted from Keegan et al. (1968).

2.3. Molecular techniques

Tissue from the caudal sucker was used in order to minimize the possibility of contamination from host/prey DNA found in the gastric and intestinal regions. The DNeasy Tissue Kit (QIAGEN Inc. Valencia, CA) was used or phenol–chloroform extractions were performed for tissue lysis and DNA purification. PCR amplification of nuclear 18S rDNA (18S) and 28S rDNA (28S), and mitochondrial cytochrome c oxidase subunit I (COI) gene fragments were accomplished using the primers listed in Table 3. Amplification reaction mixtures used Ready-To-Go™ PCR Beads (Amersham Pharmacia Biotech, Piscataway, NJ) with: 23 μl of RNase-free H2O, 0.5 μl of each 10 μM primer, and 1 μl DNA template (total volume, 25 μl). All amplification reactions were performed in an Eppendorf® Mastercycler®. Gene fragments were amplified using the following protocol: heated to 94 °C (1 min), followed by 35 cycles of 94 °C (30 s), 48–50 °C (30 s), and 68 or 72 °C (45 s) and a final extension at 68 or 72 °C (7 min). PCR amplification products were purified with AMPure™ (Agencourt Bioscience Corporation). Samples were cycle sequenced on an Eppendorf® Mastercycler® using 1 μl ABI Big Dye™ Terminator (v1.1 or v3.1), 1 μl Big Dye™ Extender Buffer (v. 1.1 or v. 3.1), 1 μl of 1 μM primer and 3 μl of cleaned PCR template (total 6 μl). Sequences were purified with CleanSeq™ (Agencourt Biocience Corporation) and analyzed with an ABI PRISM® 3730 sequencer. For alternative amplification and sequencing protocols see Oceguera-Figueroa et al. (2005).

Sequences were edited and reconciled using CodonCode Aligner (CodonCode Corporation). Alignment of the two nuclear gene fragments were accomplished using Clustal X software under a range of alignment parameters, gap cost:gap extension = 6:3; 10:5; 15:6.66 (default). Alignments for COI were done by eye, as there were no ambiguous insertions or deletion events. Individual rDNA

Table 3

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datasets were selected for inclusion in the combined analyses based on those alignment parameters that produced optimal trees.

2.4. Phylogenetic analyses

Maximum parsimony (MP) analyses of the combined 18S, 28S and COI data, as well as for each gene alone, were performed using PAUP* v. 4.06b10 (Swofford, 2000). Heuristic searches used 100 replicates of random taxon addition and tree-bisection-reconnection branch swapping. All characters were equally weighted and non-additive. Gaps were treated as missing data. TreeRot.v2c (Sorensen, 1999) was used to calculate Bremer support indices (b; Bremer, 1988). The retention (RI) and consistency (CI) indices were calculated in PAUP*. Parsimony jackknife (jac) values for combined analyses were obtained with 1000 heuristic pseudoreplicates, using random taxon addition and tree-bisection-reconnection branch swapping with 37% deletion (Farris, 1999).

Maximum likelihood (ML) analyses were performed for each gene, and across alignment parameters for nuclear genes, using PHYML v. 2.4.4.1 (Guindon and Gascuel, 2003). Modeltest 3.06 (Posada and Crandall, 1998) was used to select the appropriate model of evolution for each gene under the Akaike Information Criterion (AIC; Posada and Buckley, 2004). The general time reversible (GTR) model of evolution, with proportion of invariant sites (I) and gamma distribution (Γ) was selected for each gene. ML analyses were performed under GTR + I + Γ, with I and Γ fixed as specified by the AIC, and base frequencies were estimated from the data.

Combined data analyses using Bayesian Inference (BI) were performed in MrBayes v. 3.1.2 (Ronquist and Huelsenbeck, 2003). The data were partitioned in two ways: (1) partitioned by gene, for 18S and 28S, and by codon position for COI (five partition; 5p); and (2) partitioned by gene, 18S, 28S and COI (three partition; 3p). BI analyses assumed a decoupled GTR + I + Γ model for each data partition, based on the AIC (via ModelTest). The default prior distribution of parameters were used for Metropolis-Coupled Markov Chain Monte Carlo (MCMC) analyses, with one cold chain and three heated chains for 10 million generations and sampled every 1000th generation. The BI analyses burned-in almost instantly (<25,000 generations). Split frequencies of the standard deviation of simultaneous BI analyses were well below 0.01 and the R-statistic (Gelman and Rubin, 1992) for each parameter partition approached 1, ensuring convergence of chains, log likelihoods and model parameters. The burn-in was set to discard the first million generations, leaving 18,002 trees sampled total (from the two simultaneous runs) for estimation of posterior probabilities (pp). Bayes factors were used to assess the best partitioning scheme for the GTR + I + Γ model for combined data BI analyses, a posteriori (Nylander et al., 2004).

3. Results

3.1. Morphology of terrestrial leeches

With respect to the general classification of land leeches, the external and reproductive morphologies of select taxa were evaluated (Figs. 1 and 2). Species belonging to the genera Xerobdella, Diestecostoma and Mesobdella all possessed ventral paired nephridia, but lacked the 17th nephridial pair. The latter were “replaced” by a medioventral common pore at the base of the sucker (Fig. 1c). Annulation patterns of mid-body somites across these genera were variable: Xerobdella lecomtei was five-annulate, Mesobdella gemmata was three-annulate and Diestecostoma species were 10-annulate (D. mexicana, D. trujillensis) or 12-annulate (D. magna). In contrast to Mesobdella gemmata, which had only one annulus between the 4th and 5th pair of eyespots, Xerobdella lecomtei and Diestecostoma species each possessed a “haemadipsine” ocular arch (sensu Blanchard, 1917) (e.g. Fig. 1a).

All representatives of the non-IndoPacific genera that were examined shared the position of the male and female gonopores within somites XI (male) and XIII (female), regardless of number of annuli separating the gonopores. Xerobdella lecomtei also possessed an accessory pore in XII associated with the seminal receptacle. Both Mesobdella gemmata and Xerobdella lecomtei had micromorphic male and female apparati (Fig. 2a and b), with the male reproductive system (excluding testisacs) found almost entirely in XI, with partial extension into X, and the female systems in XII and XIII. The male system of Mesobdella gemmata included a pear-shaped penis sheath, with thin sperm ducts that inserted into sac-like epididymes, whereas the Xerobdella lecomtei possessed a spherical atrium, with defined ejaculatory bulbs that insert into small epididymes. The female reproductive system of Mesobdella gemmata consisted of a vaginal duct that inserted into a simple vaginal sac and a pair of globular ovisacs. In contrast, Xerobdella lecomtei possessed a short vaginal duct, with paired globular ovaries that inserted independently into the vaginal duct; vaginal sac absent. A spherical seminal receptacle was found anterior to the female reproductive system in XII. The reproductive systems of Diestecostoma mexicana was robust and elongated (Fig. 2c). The male system had a slender penis sheath that elongated posteriorly into XIV. At the base of the penis was a pair of ejaculatory bulbs that inserted into coiled epididymes that terminated into thin sperm ducts that traveled anteriorly beneath the penis sheath before turning posteriorly into gonopore at XI. The vaginal duct of Diestecostoma mexicana was also long, extending from XIII to XVI, and slightly widened into an elongated sac. At the base was a pair of fused ovisacs.

In contrast to the other non-IndoPacific species, the four-annulate Nesophilaemon skottsbergii possessed a lateral position of the paired nephridia, respiratory auricles (Fig. 1b) and had a “haemadipsine” ocular arch.
The male system of *Nesophilaemon skottsbergii* (Fig. 2d) consisted of a spherical atrium, with a pair of ejaculatory bulbs that inserted into thick sperm ducts extending posteriorly into XIX, before curving anteriorly. The female system includes a vaginal duct inserting into the gonopore at XII. The vaginal duct extended posteriorly, then coiled beneath the oviducal glandular sac that extended to XV, inserting into the anterior portion of the sac. Paired globular ovaries inserted independently, via oviducts, into a common oviduct that coiled along with the vaginal duct beneath the oviducal glandular sac. The reproductive systems of the five-annulate *Haemadipsa sylvestris* (Fig. 2e) and *Idiobdella seychellensis* (Fig. 2f) were similar in basic form in having the atrium present in XI, with sperm ducts that extended posteriorly and coiled at XV and XIII, respectively. The female systems included an elongated vaginal duct that either inserted directly (*Haemadipsa sylvestris*), or coiled beneath before inserting (*Idiobdella seychellensis*) into the oviducal glandular sac. The female gonopore was found in XII (*Haemadipsa sylvestris*) and XIII (*Idiobdella seychellensis*). Like *Nesophilaemon skottsbergii*, both haemadipsid species also possessed globular ovisacs that inserted independently, via oviducts, into a common oviduct before inserting into the oviducal glandular sac. Both the vaginal duct and the common oviduct of *Idiobdella seychellensis* coiled together beneath the sac. *Idiobdella seychellensis* lacked obvious respiratory auricles, unlike *Nesophilaemon skottsbergii*, and exhibited the lateral position of the nephridia and the “haemadipsine” ocular arch.

### 3.2. Molecular data and alignments

With respect to the alignment schemes for nuclear genes, the general trend was that as gap opening cost:gap extension cost parameters increased, tree length (MP) and log likelihood scores (ML) worsened. With the exception of 28S (15:6.66), the results of the analyses of 18S and 28S under different alignment schemes were merely nearest-neighbor interchange of each other. Based on the alignments that produced trees with the shortest number of steps and the highest likelihood scores, the alignment parameters 6:3 and 10:5 were selected for 18S and 28S, respectively, for all combined data analyses. For BI analyses, Bayes Factors favored the more parameter rich model over the 3p GTR + I + G model. The results from BI analyses are based on the topology and posterior probabilities of the 5p GTR + I + G model (Fig. 3).

The analyses of the combined 18S, 28S and COI data yielded identical topologies with all major clades strongly supported, regardless of optimality criteria used for analyses. The combined data analyses supported two lineages of “haemadipsoid” terrestrial leeches, which can be divided into an IndoPacific clade and a non-IndoPacific clade, which together were not each other’s closest relatives. Constraining the IndoPacific and non-IndoPacific clades to be monophyletic would require only an additional seven steps. The non-IndoPacific clade included *Mesobdella gemmata* as the sister taxon (jac = 100; b = 27; pp = 1.00) to *Xerobdella lecontei* + a monophyletic *Diestecostoma* (jac = 100; b = 27; pp = 1.00). *Diestecostoma trujillensis* was sister (jac = 100; b = 27; pp = 1.00) to *Diestecostoma mexicana* + *Diestecostoma magna* (jac = 86; b = 6; pp = 1.00). Together the non-IndoPacific clade was well supported as the sister group (jac = 90; b = 7; pp = 1.00) to members of the families Hirudinidae and Haemopidae (jac = 100; b = 24; pp = 1.00).

The southeastern Pacific *Nesophilaemon skottsbergii* was found nested within the duognathous IndoPacific clade (jac = 100; b = 23; pp = 1.00) and strongly supported as sister (jac = 100; b = 27; pp = 1.00) to Western Indian Ocean *Malagabdella fallax* + *Idiobdella seychellensis* (jac = 96; b = 6; pp = 0.99). Constraining *Nesophilaemon skottsbergii* to be monophyletic with *Mesobdella gemmata*, its geographically most proximal species, would require an additional 197 steps. The *Nesophilaemon* + *Malagabdel-la* + *Idiobdella* clade is sister to *Chtonobdella* species from Australia (jac = 100; b = 22; pp = 1.00). The monophyletic, trignathous *Haemadipsa* clade (jac = 99; b = 9; pp = 1.00) consisted of the Malaysian *Haemadipsa interrupta* + the Bornean *Haemadipsa sumatrana* (jac = 96; b = 7; pp = 0.98), which together were sister to *Haemadipsa sylvestris* from Vietnam. The IndoPacific (and south Pacific) leeches (jac = 100; b = 43; pp = 1.00) were reciprocally monophyletic (jac = 98; b = 10; pp = 1.00) with the non-IndoPacific clade + *hirudinid* clade (jac = 90; b = 7; pp = 1.00).

### 4. Discussion

Our results corroborate previous findings (Trontelj et al., 1999; Borda and Siddall, 2004a; Kutschera et al., 2007) and confirm what others (Richardson, 1969, 1971, 1975; Moore, 1946; Ringuelet, 1972, 1982) had suspected regarding the relationships of non-IndoPacific bloodfeeding terrestrial leeches. The presence of two haemadipsid terrestrial lineages among Hirudiniformes is well supported here and these results have shed light on the taxonomic status of Xerobdellidae, as well as on the placement of proposed associated genera. The expanded sampling of all
genera in question confirms that “haemadipsoid” leeches from Europe and the New World, to the exclusion of Nesophilaemon skottsbergii, are in fact only distantly related to the IndoPacific haemadipsid leeches. This group also appears to have greater affinities to the principally Old World and North American hirudinid leeches than with any other group (Trontelj et al., 1999; Borda and Siddall, 2004a; Kutschera et al., 2007). Borda and Siddall (2004a) proposed the resurrection of Xerobdellidae (sensu Moore, 1946 and Harrant and Grasse’), 1959) for bloodfeeding terrestrial leeches found in the New World (i.e. Mesobdella) and “displaced” in Europe. Our phylogenetic hypothesis confirms that Xerobdellidae should also include Diestecostoma species.

When Moore (1946) described Diestecostoma octanulata from Guatemala he addressed Caballero’s (1940) proposed relationship of Hygrobodella palaiei (=Diestecostoma mexicana) (Baird, 1969) to Haemadipsi-
for them” (Moore, 1946, p. 190), but acknowledged that a formal revision was premature without a better understanding of the South American leeches. Remarkably, Moore (1946) withdrew his initial conclusions in a footnote (in that same paper) after reviewing Ringuelet’s (1943) manuscript containing a description of the reproductive anatomy of Mesobdella gemmata as being “typically haemadipsine” in nature. From our phylogenetic results and from the examination of the reproductive morphologies it is clear that the apparent resemblance of Mesobdella gemmata, Diestecostoma species and Xerobdella lecomtei to haemadipsid leeches was superficial.

The taxonomic framework established by Blanchard (1896, 1917) was reinforced by others (Caballero, 1940, 1956, 1959; Ringuelet, 1944, 1953, 1954) and continued with Soós’s (1967) treatment of Haemadipsidae. Soós (1967) included in Haemadipsidae genera that were distributed throughout Asia (Haemadipsa), Madagascar (in the genus Chtonobdella), the Seychelles (Idiobdella), Australia (Philaemon, Chtonobdella), Papua New Guinea (Phytobdella), and the Pacific Islands (Phytobdella, including Nespophilaemon). Soós (1967) also included Mesobdella gemmata in the family. Soós (1966), however, also followed Moore’s (1946) recommendation in treating Diestecostoma species and Xerobdella species as a separate taxonomic group from haemadipsids in Diestecostomatidae (=Xerobdellidae, here), as proposed by Ringuelet (1953).

Richardson (1969, 1971) questioned the inclusion of Nespophilaemon skottsbergii (and Mesobdella gemmata) in Haemadipsidae (sensu Soós, 1967). He found that the reproductive morphology as illustrated and described by Ringuelet (1955) not only set Nespophilaemon apart from the four-annulate Australian genus Philaemon (as it was originally described), but also from haemadipsid leeches in general (Richardson, 1971). He concluded that Nespophilaemon skottsbergii (and Mesobdella gemmata) were haemadipsine only on the basis of annulation patterns and having a secondary adaptation for terrestrial and sandgivorous habits (Richardson, 1969). This resulted in their removal from Haemadipsidae and their placement in a new family, Mesobdellidae (Ringuelet, 1972).

Richardson (1975) later revised Soós’s (1967) classification of land leeches, to the exclusion of the non-IndoPacific genera (Mesobdella and Nespophilaemon), by raising Indo-Pacific haemadipsids to superfamily Haemadipsioidea and dividing the group on the basis of jaw and reproductive morphologies, as well as annulation patterns and geographies. Richardson (1975, 1978) restricted Haemadipsidae to the trignathous leeches from India, East Asia and Southeast Asia and erected two new families: Domanibdellidae, for the duognathous leeches found in Australia, Papua New Guinea, Madagascar and Oceana and another for those found in the Seychelles (Idiobdellidae).

IndoPacific Haemadipsidae (sensu stricto) was supported as a monophyletic group, however, our hypothesis was in conflict with Sawyer’s (1986) revision of Haemadipsidae (see Table 1A). Sawyer’s (1986) classification did not consider the zoogeography for haemadipsoid leeches, nor ignored Moore’s (1946) and Richardson’s (1975) taxonomic recommendations. Haemadipsidae was simply divided into two groups, the trignathous series (e.g. Diestecostoma, Haemadipsa, Mesobdella and Xerobdella) and the duognathous series (e.g. Chtonobdella, Idiobdella, Malagabdella, Nespophilaemon). Although he was aware that some of the genera he assigned to the trignathous series tended to be “islated groups which sometimes, but unnecessarily, [were] recognized as monotypic families in their own right” (Sawyer, 1986, p. 689), they were nevertheless included in Haemadipsidae. To Richardson’s (1975) credit, our phylogeny supports a monophyletic IndoPacific haemadipsid clade, divided into a monophyletic Asian trignathous clade (Haemadipsa) and a monophyletic nonAsian duognathous clade, resembling Richardson’s Haemadipsidae and Domanibdellidae, respectively. Our results indicate that Nespophilaemon skottsbergii is unequivocally a member of Haemadipsidae and associated with Richardson’s (1975) duognathous Domanibdellidae, based on morphology and molecular data. Puzzlingly, this southeastern Pacific species appears to be more closely allied to the isolated Malagabdella fallax and Idiobdella seychellensis from the western Indian Ocean than to duognathous Chtonobdella species from eastern Australia.

Insofar as each of the genera Mesobdella, Xerobdella and Diestecostoma are found to form a clade, only one family group name is required. Xerobdellinae (Moore, 1946), has precedence, in spite of Moore’s (1946) own ambivalence, and has inclusiveness insofar as he anticipated the group to include “the last two of these genera and possibly the first” (Moore, 1946, p. 190). Ringuelet (1953, 1976) clearly did not intend for Xerobdellinae (Moore, 1946) to be a subfamily of Diestecostomatidae. Ringuelet, 1953 insofar as Diestecostomatidae was intended to explicitly exclude Mesobdella (see also Soós, 1966). The name Xerobdellinae (Moore, 1946) also satisfies the International Code of Zoological Nomenclature descriptive requirements in which the family group name is to include species in genera that are the same in “the position of the nephridiopore, the first pair buccal, the last median beneath the sucker, and the remaining pairs ventral, in the absence of auricles, and the structure of the caudal sucker” (Moore, 1946, p. 190). Thus, we formally recognize Xerobdellidae (Moore, 1946), as valid, leaving each of Diestecostomatidae (Ringuelet, 1953) and Mesobdellidae (Ringuelet, 1972), as junior synonyms.

With respect to the IndoPacific haemadipsid leeches, although Richardson (1975) was correct to divide haemadipsids by zoogeographic region, the status of some groups at the family level (i.e. Domanibdellidae, Nespophilaemonidae and Idiobdellidae) will likely need revision. We recommend that they be recognized in Haemadipsidae sensu stricto, however, a broader sampling of IndoPacific species is warranted to properly address the phylogenetic and taxonomic status of species within this group.

4.1. Morphology of haemadipsoid leeches

Unique external morphological characters (e.g., annulation, ocular patterns and position of nephridia) and life history strategies have defined bloodfeeding terrestrial leeches apart from other groups of leeches (Blanchard, 1917; Hard- 
ed and Moore, 1927; Soós, 1966; Richardson, 1975; Sawyer, 1986); in particular, the presence of lateral nephridia and obvious respiratory auricles. However, despite lacking these precise diagnostic characteristics, the superficial similarities in general form and adaptation for terrestrial san-
guivory led workers to include non-auriculate, non-
IndoPacific haemadipsoid species in Haemadipsidae. The lateral position of nephridia in haemadipsid leeches has been speculated to aid in keeping the leech moist (Sawyer, 1986), particularly when actively feeding and during dry seasons. This does not explain why this would not be essen-
tial to other terrestrial leech groups (e.g. Cylicobdellidae, Xerobdellidae), which possess ventral nephridia.

The physiological function of the respiratory auricles is also not well understood, but is thought to act as a damp brush to form a watery seal along the margins of the caudal sucker (Sawyer, 1986). It is present in virtually all Haema-
dipsidae sensu stricto, although there are some exceptions (e.g., Idiobdella). Contrasting the respiratory auricles in Haemadipsidae is the opening of the 17th nephridial pair via a medioventral common pore in Xerobdellidae. This character is a synapomorphy for Xerobdellidae and appears to be a modification that might serve a similar function in purporting a watery seal along the margins of the caudal sucker, which seems necessary for life on land.

The reproductive systems of xerobdellid leeches not only drastically deviate in form from haemadipsids, they share little in common with each other. Xerobdella lecomtei and Mesobdella gemmata share the simple and micromorphic nature of the male and female systems (Fig. 2 a and b). An accessory female seminal receptacle is exclusive to Xerobdella species (Minelli, 1971, 1979) and is unusual for arhynchobdellid leeches (Sawyer, 1986). The micromor-
phic reproductive systems seen in Mesobdella gemmata and Xerobdella lecomtei, considerably contrasts with that of Diestecostoma mexicana. Based on the internal anatomy alone, it would be easy to believe that these species would not have a shared ancestry (contrary to Moore, 1946). However, the medioventral pore is unique to Xerobdell-
dae. The considerable internal morphological variation between the genera of Xerobdellidae might be best explained by long-term isolation from each other.

In contrast to Xerobdellidae, the reproductive systems of the haemadipsids in this study appear to share a basic form with a micromorphic male atrium, globular ovaries that insert independently into a common oviduct, and a long vaginal duct (either straight or coiled) that inserts into an oviducal glandular sac (Fig. 2 d–f). Richardson (1969, 1975) revised the classification of haemadipsids in recogni-
tion of the geographic distribution of species and of the systematic utility of the reproductive morphology of land leeches. In his evaluation, he may have underestimated the diversity of form in the reproductive systems of hae-
adipsids in light of his previous experience with the “monotony of the internal morphology in land leeches” (Richardson, 1978, p. 838). However, recent assessments of terrestrial leeches from Madagascar reveal a great diver-
sity of characteristics in the reproductive anatomy (see Borda, 2006, for an overview), which deviate from the basic form seen in other Haemadipsidae (e.g. Fig. 2d–f).

The availability of fresh samples of Nesophilaemon skottsbergii facilitated the reexamination of its reproductive morphologies. The only published description and illustration of the reproductive system of Nesophilaemon skottsbergii was in 1955 by Ringuelet. Thereafter, Richardson (1969) held that Nesophilaemon skottsbergii was closer to Mesobdella gemmata, despite the presence of a “haemadipsine” ocular arch, being four-annulate and being a duognathous leech. The duognathous condi-
tion of the jaws is known only in Haemadipsidae. Our dis-
sections reveal that Nesophilaemon skottsbergii is haemadipsid in all respects. The reproductive anatomy as illustrated by Ringuelet (1955) does not resemble fresh specimens whatsoever. Even among the haemadipsids, the reproductive anatomy of Nesophilaemon skottsbergii is dis-
tinct with its stout female oviducal glandular sac and long and thick sperm ducts, not typically seen in this group. As already mentioned, the reproductive anatomy of some Malagasy leeches appears to diverge from other Haemad-
ipsidae, therefore, it might be of no surprise that Nesophi-
aemon skottsbergii would be closely allied to this clade. The extent of the diversity of the reproductive anatomy across IndoPacific haemadipsid leeches is still needed to gain more insight.

4.2. Feeding habits of land leeches

Already a phylogenetic framework has clarified the diversity and evolution of life history strategies for leeches (e.g. Siddall and Burreson, 1996; Apakupakul et al., 1999; Trontelj et al., 1999; Kutscher and Wirtz, 2001; Utevsky and Trontelj, 2004; Borda and Siddall, 2004a,b; Williams and Burreson, 2006). In this framework, the preference for bloodfeeding is now understood to have been lost or have modified multiple times throughout the evolutionary history of leeches (Borda and Siddall, 2004a,b). Historically, haemadipsoid leeches have been associated with being uniformly sanguivorous. Our phylogenetic hypothe-
sis challenges this notion with the support of two distantly related terrestrial and bloodfeeding lineages, one of which includes species that have a more varied diet (Fig. 3).

When Frauenfeld (1868) described Xerobdella lecomtei from the Austrian Alps and placed it in Haemadipsidae, he did so based on its terrestrial habit and being parasitic on salamanders. Subsequent work indicated that Xerobdella lecomtei has a more diverse diet and actually feeds on invertebrates, including earthworms, gastropods and dip-
teran larvae (Reisinger, 1951; Moosbrugger and Reisinger, 1951; Moosbrugger and Reisinger,
1971; Sawyer, 1986; Kutschera et al., 2007). Its closest relatives, Diestecostoma species similarly have also been found on salamanders (e.g. Ambystoma species), as well as preying on oligochaetes (Caballero, 1940). Of all members of Xerobdellidae, species of Mesobdella are alone in being exclusively sanguivorous, readily observed feeding on humans (Siddall and Borda, 2004), but likely feeding on boreal frogs (Rinuelet, 1953). Haemadipsidae, on the other hand, are infamous for their voracious appetite for vertebrate blood. Idiobdella species appear to have lost this habit in favor of being a parasite of mollusks (Richardson, 1978) and other soft-bodied invertebrates (Harding, 1913). This may reflect the Seychelles Islands having initially been devoid of any vertebrates (Hume, 2004) prior to more recent introductions (e.g. Racey and Nicoll, 1984; Austin et al., 2003).

4.3. Biogeographic patterns

The diversification of Annelida has been attributed to the Cambrian explosion (Ayala et al., 1998; Rousset et al., 2007) with the oldest known fossils dating back to the Silurian (>400 Mya; Eriksson et al., 2004). Unequivocal fossils are not known for leeches (Siddall et al., 2006), as these soft-bodied annelids lack structures that would mineralize in order to leave their mark in the fossil record. However, the phylogenetic patterns observed in Fig. 3 appear to be consistent with geological events that might have contributed to the diversification of xerobdellid and haemadipsid leeches (Fig. 4).

The rarified endemicity and current distribution of Xerobdellidae may indicate a Pangaeana origin. The presence of Mesobdella gemmata in temperate regions of Valdivia (Chile) is consistent with the warm temperate zone of southwestern South America persisting since the Permian (>250 Mya; Rees et al., 2002). While, Diestecostoma species are distributed throughout northern South America and Meso America, with no records north of Mexico, any relationship with Xerobdella lecomtei would have been present through a Laurasian connection (~170 Mya; Scoteze, 2004) absent of any mechanism for intercontinental dispersal. That the distribution of the sister group of Xerobdellidae, the hirudinids, includes each of North America, Europe and Africa, further suggests a Pangaeana relationship.

The distribution of Haemadipsidae appears to have diversified via vacariant events related to the breakup of Gondwana. The relationships of duognathous leeches represented here from Australia, Madagascar, the granitic Seychelles Islands and the Juan Fernandez Archipelago, would need to have had an ancestor that was present before the Indo-Malagasy landmass began to separate from Gondwana (~150 Mya; Briggs, 2003), and after Africa separated from Madagascar (~165 Mya; Rabinowitz et al., 1983). The relationship of Idiobdella seychellensis and Malagabdella fallax reflects that their common

![Fig. 4. Biogeographic correlates to portions of the phylogenetic hypothesis for Haemadipsidae and Xerobdellidae. (a) Relationships among xerobdellid leeches appear to correspond to patterns expected for terrestrial taxa initially occupying Southern Pangaea, with eventual dispersal northward, then following the eventual split of Laurasia (~180 mya) and subsequently Laurentia and Eurasia (~100 mya). (b) Antarctic view of the earth, with a schematic representation of the relationships of Haemadipsidae (arrows).](image-url)
ancestor was at least present on the Indo-Malagasy landmass. After the separation from Madagascar (~85 Mya; Storey et al., 1995), the Indian landmass left behind remnants, which later became known as the Seychelle Islands (~65 Mya; Briggs, 2003) as India moved northward towards Asia (Scotese, 2004). This is confounded by the absence of a close relationship between these and Asian species. Although, the sampling of Asian Haemadipsa species is limited, the high diversity of Haemadipsa species throughout India, East Asia and Southeast Asia is well documented (Moore, 1924), possibly pointing towards an eastward diversification of the genus as a dispersal out of India, that is, after India’s collision into Asia (~50 mya; Briggs, 2003).

Among Haemadipsidae, Nesophilaemon skottsbergii has one of the most peculiar distributions and has been found only on Isla Mas Afuera (or Alejandro Selkirk Island). Ringuelet (1955) proposed that the presence of Nesophilaemon skottsbergii in the archipelago was best explained by long distance, trans-oceanic dispersal on Pterodroma species (Procellariiformes) from the Australasian region, given the endemic Pterodroma externa and Pterodroma longirostris on Isla Mas Afuera (Brooke, 1987). Though passive dispersal on birds is a reasonable hypothesis for Nesophilaemon skottsbergii—the volcanic Juan Fernandez Islands are young, with Isla Mas Afuera having formed ~1–2.4 mya (Baker et al., 1987)—an Australian origin is problematic given the close relationship between the East Pacific Nesophilaemon skottsbergii and species from the West Indian Ocean.

Few deep historical biogeographic studies exist for soft-bodied invertebrates that leave no fossil trace. Our robust phylogeny for the terrestrial bloodfeeding leeches, leaving perhaps as many questions unanswered as answered, beckons more extensive consideration of these notorious annelids.

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