# Review of the evolution of life history strategies and phylogeny of the Hirudinida (Annelida: Oligochaeta)

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With 4 figures and 1 table

Several hypotheses have been proposed for the phylogenetic relationships and evolution of life history strategies of leeches using a limited sampling of taxa and families. In this review we reinvestigate the evolution of life history strategies, including feeding preferences, habitat preferences and cocoon deposition based on a reanalysis of the phylogenetic relationships of leeches with the inclusion of representatives of all families of the Hirudinida. From the resulting hypotheses, it can be inferred that leeches have a common origin in an ectoparasite of vertebrate blood, with several convergent losses of sanguivory for a carnivorous way of life. The evolution of medically important anticoagulants across leech groups provides support for a sanguivorous ancestor, as does the presence of anticoagulants in some non-bloodfeeding species. There also may have been a secondary return to sanguivory for the Hirudiniformes coincident with the development of armed jaws. Leeches appear to have evolved from a freshwater ancestor, with a movement into marine environments in the ancestor of the piscicolid leeches and onto land in the ancestor of hirudiniform leeches. The latter is further corroborated by mode of cocoon deposition in the group.

## 1 Introduction
## 1.1 An origin for the Hirudinida

The origin of leeches and their phylogenetic placement within the Clitellata has been controversial and a topic of considerable debate among annelid systematists. The phylogenetic affinities between leeches and branchiobdellidans were proposed as early as 1823, when Odier described branchiobdellidans, better known as the crayfish worms, under the umbrella of leeches. Branchiobdellidans are leech-like freshwater ectosymbionts of crustaceans, in particular, crayfish (Crustacea: Astacoidea; Hobbs et al. 1967). Some species are grazers of the detritus or epizoic flora found on the surfaces of theirs hosts, while others are parasitic in habit, inhabiting the gill chambers and feeding on dermal tissue, apparently ingesting haemolymph (Holt 1965, Sawyer 1986). In Livanow's (1906, 1931) descriptions of *Acanthobdella peledina*, he considered this ectosymbiont of salmonid fish to be reminiscent of an ancient hirudinean. Acanthob-

dellidans have a morphology that appears transitional between oligochaetes and leeches, including the possession of setae restricted to the cephalic region, an oligochaete-like seminal funnel, yet they possess leech-like fused male gonopores, oblique musculature and a caudal sucker for attachment to their host.

Leeches, and leech-like worms, are a group of specialized clitellate annelids. Clitellates are identified based on the possession of a clitellum – a swollen gland located one-third of the way down on the body and associated with cocoon secretion and deposition; it is most prominent in the common earthworm (e.g. Oligochaeta: Lumbricidae). Clitellates are also hermaphrodites. Unlike the typical earthworm, which has a variable number of body somites (segments), leeches, branchiobdellidans and acanthobdellidans are characterized by a fixed number of somites (i.e. 34, 15 and 29, respectively). Additional morphological characters distinguish them from other clitellates: the possession of a caudal (posterior) muscular sucker - leeches are distinct in also possessing a well developed muscular oral (anterior) sucker - that is used to aid in the attachment to their hosts and for locomotion on land or in water, a reduction of coelomic space, the reduced (acanthobdellidans) or complete loss (leeches and branchiobdellidans) of setae, and adaptation to an ectocommensalistic or ectoparasitic way of life.

Before the late 1990's, hypotheses on the evolution of leeches were limited to the subjective interpretation and identification of plesiomorphic morphological characters and corresponding homologies. The cladistic analyses of morphological data supported leeches, branchiobdellidans and *Acanthobdella peledina* having a common origin (Brinkhurst & Gelder 1989, Purschke et al. 1993, Brinkhurst 1994, Siddall & Burreson 1995) and the subsequent inclusion of molecular data further corroborated this hypothesis (Siddall & Burreson 1998, Apakupakul et al. 1999, Trontelj et al. 1999). Others, however, explained these relationships based on the convergent adaptation to an ectocommensalistic lifestyle and not due to shared ancestry (Holt 1989, Brinkhust & Gelder 1989, Purschke et al. 1993, Brinkhurst 1994 and 1999). The lack of exact correspondence of several morphological characters was central to this debate. Siddall et al. (2001) reexamined the higher-level relationships within the Clitellata using an expanded taxonomic sampling, with over 100 annelids and using molecular data alone (i.e. nuclear 18S rDNA and mitochondrial cytochrome *c* oxidase subunit I gene sequences). These results supported, once again, the notion that leeches, branchiobdellidans and *Acanthobdella peledina* are a monophyletic group, each in their own respective clades and together are a derived group of oligochaetes, with lumbriculids (Lumbriculida) as their sister taxon. As such, Siddall et al. (2001) proposed that each (i.e. leeches, branchiobdellidans and *Acanthobdella peledina*) be considered orders of equal ranking: Hirudinida,

Branchiobdellida and Acanthobdellida, respectively and that Clitellata be synonymized with Oligochaeta.

Phylogenetic assessments based on morphology, behavior and molecular data have provided a rich framework for understanding the evolutionary history of leeches (Apathy 1888, Wendrowsky 1928, Livanow 1931, Autrum 1939, Ringuelet 1954, Mann 1961, Sawyer 1986, Siddall & Burreson 1995, 1996 and 1998, Apakupakul et al. 1999, Light & Siddall 1999, Nesemann & Neubert 1999, Trontelj et al. 1999, Kutschera & Wirtz 1986 and 2001, Borda & Siddall 2004, Utevsky & Trontelj 2004). For the purposes of this study, the molecular data from Borda & Siddall (2004) were reanalyzed with the inclusion of additional representatives from the families Salifidae, Haemopidae and Hirudinidae. Members of related outgroup taxa from the orders Acanthobdellida, Branchiobdellida and Lumbriculida (Siddall et al. 2001, Martin et al. 2000) were also included.

## 1.2 Classification

There are approximately 650 described species of leeches worldwide (Sawyer 1986), an underestimation with many more species yet to be discovered. Several monographs have described the diversity of leeches of the world, including descriptions of the Neotropical and Subantarctic taxa (Weber 1915, Cordero 1937, Ringuelet 1944, 1976 and 1985, Caballero 1956, Soos 1966, Siddall 2001, Siddall & Borda 2004), North American leeches (Klemm 1982), terrestrial leeches and other Asian leeches (Blanchard 1917, Moore 1924b, Harding & Moore 1927, Keegan et al. 1968, Soos 1967, Richardson 1978) and many more. The most comprehensive monograph to date is Roy Sawyer's (1986) 3-volume *Leech Biology and Behaviour*. At the time it was, and for the most part still is, the most up to date classification for leeches, based on traditional methods in taxonomy and classification.

The advent of molecular data acquisition (used alone or in combination with behavioral and morphological data) for phylogenetic work brought new light into leech systematics in the late 1990's. Several papers collectively established the framework for the higher-level evolutionary relationships of leeches (Siddall & Burreson 1995 and 1998, Apakupakul et al. 1999, Trontelj et al. 1999, Borda & Siddall 2004) and the inclusion of a broader taxonomic sampling of leech species within groups has also been instrumental in understanding the interfamilial, intergeneric and intrageneric relationships (Govedich et al. 1998, Light & Siddall 1999, Trontelj et al. 1996, Trontelj & Sket 2000, Siddall 2002, Siddall & Borda 2003, Utevsky & Trontelj 2004, Pfeiffer et al. 2004).

Leeches have been classified into two major groups: the Rhynchobdellida Blanchard, 1894 and the Arhynchobdellida Blanchard, 1894 (see Sawyer 1986).

A proposed revision for the classification of the Hirudinida is listed in table 1; also listed are the taxonomic names that will be considered here.

## 1.3 "Rhynchobdellida"

Rhynchobdellid leeches are characterized by the possession of a muscular proboscis that is used to penetrate deep vascularized tissue for bloodfeeding (e.g. *Haementeria ghilianii*) or sucking the coelomic fluid of their invertebrate prey (e.g. *Glossiphonia complanata*). There are three rhynchobdellid families, the dorso-ventrally flattened Glossiphoniidae Vaillant, 1890, the freshwater and marine fish leeches in the Piscicolidae Johnston, 1865 and the turtle leeches of the Ozobranchidae Pinto, 1921.

Sawyer (1986) divided the Glossiphoniidae into subfamilies: Glossiphoniinae, Haementeriinae, and Theromyzinae based on characters related to mode of reproduction, cocoon deposition and parental care (see *Cocoons and Parental Care*, below). Except for the Theromyzinae, the subfamilies of the Glossiphoniidae were rendered paraphyletic in an analysis by Light & Siddall (1999). Theromyzinae (i.e. *Theromyzon*) is monophyletic, but was found nested within a clade containing members from the other two subfamilies. Moreover, reproductive behaviors and mode of parental care overlap across glossiphoniid taxa and were considered not to be consistent characters for delimiting groups. Without reevaluation of the Glossiphoniidae with an expanded taxon sampling, glossiphoniid leeches remain classified under a single family.

The family Piscicolidae is divided into the Platybdellinae Epshtein, 1970, Pontobdellinae Llewellyn, 1966, and Piscicolinae Caballero, 1940. The family is roughly divided by the specialization of coelomic space, the presence or absence of pulsatile vesicles and reproductive morphologies. Unlike the Glossiphoniidae, results from a recent phylogenetic investigation of the Piscicolidae (Utevsky & Trontelj 2004) suggest that the traditional groupings into subfamilies are valid, based on morphology and mitochondrial DNA sequence data.

Lastly, the Ozobranchidae, which are parasitic on sea turtles, appears to be an anomaly in terms of its phylogenetic placement within the Rhynchobdellida. The inclusion of the Ozobranchidae in previous phylogenetic analyses find the Ozobranchidae as sister to the Piscicolidae (Siddall & Burreson 1998, Apakupakul et al. 1999) or as sister to the Glossiphoniidae (Utevsky & Trontelj 2004). The inclusion of only a single species (i.e. *Ozobranchus margoi*) as the sole representative for the family and limited molecular data (i.e. 18S rDNA, mt COI, mt NADHI) underscores the need for addition taxonomic representation for the family, as well as the exploration of new genes.

Phylogenetic assessments including members of the Rhynchobdellida have indicated that the group is not monophyletic (Apakupakul et al. 1999, Trontelj

et al. 1999, Borda & Siddall 2004). Save for the presence of a proboscis, the Glossiphoniidae and Piscicolidae appear to have independent origins (Apakupakul et al. 1999, Trontelj et al. 1999, Borda & Siddall 2004). The term rhynchobdellid leech will still be used here in reference to leeches that possess a proboscis, but continued use of Rhynchobdellida would imply accepting the paraphyletic assemblage for the group.

## 1.4 Arhynchobdellida

Arhynchobdellid leeches lack a proboscis and are characterized by a pharynx that has been modified into muscular "jaws" for feeding. The monophyletic Arhynchobdellida Blanchard, 1894 are further subdivided into the Erpobdelliformes Caballero, 1952 and the diverse Hirudiniformes Caballero, 1952 (Siddall & Burreson 1995, 1996 and 1998, Apakupakul et al. 1999, Trontelj et al. 1999, Borda & Siddall 2004, and others). The former are strictly carnivorous and traditionally include the families Salifidae Johansson, 1910 and Erpobdellidae Blanchard, 1894. The Erpobdellidae are widely distributed throughout the Northern Hemisphere/Palaearctic region and the Salifidae throughout the Southern Hemisphere, except South America (Sawyer 1986); both are found in freshwater systems.

Traditionally, hirudiniform leeches were divided into five families: Americobdellidae Caballero, 1956, Cylicobdellidae Ringuelet, 1972, Haemadipsidae Blanchard, 1893, Haemopidae Richardson, 1969, and Hirudinidae Whitman, 1886 (see Sawyer 1986). The first two families and the Haemopidae are predaceous carnivores, and the remaining sanguivorous hirudiniformes were principally divided based on habitat preference (i.e. the semi-aquatic Hirudinidae and the terrestrial Haemadipsidae). Hirudiniform leeches are found in all continents, except Antarctica, in freshwater and terrestrial communities. Some of the families are strictly endemic to certain regions, such as Cylicobdellidae known only from South America, Americobdellidae found in Chile and the Haemadipsidae from the IndoPacific.

Phylogenetic work including members of the Arhynchobdellida found that the majority of the traditional families in the Hirudiniformes were not true monophyletic assemblages, namely the families Hirudinidae, Haemopidae and Haemadipsidae (Siddall & Burreson 1995, Apakupakul et al. 1999, Trontelj et al. 1999, Borda & Siddal 2004). Apakupakul et al. (1999) found that the Hirudinidae was paraphyletic and could be roughly divided into a New World and an Old World Hirudinidae. The inclusion of more hirudinid taxa validated the monophyly of one of Richardson's (1969) subfamilies, Macrobdellinae. Borda & Siddall (2004) proposed to raise this group to family level for the North and South American "medicinal" leeches (e.g. *Macrobdella* species, *Oxyptychus*

species). Other members of the Hirudinidae also did not group together. For example, the Eurasian medicinal leeches were monophyletic, but the African *Limnatis nilotica* was found not to group either with the Macrobdellidae or with the Hirudinidae. Rather it was found as sister to the Macrobdellidae and the South American "haemopids" An expanded sampling of hirudinid taxa is still necessary to have a better understanding of the relationships of the Hirudinidae of the world.

As with the Hirudinidae, the Haemopidae also was paraphyletic (Borda & Siddall 2004) and divided geographically into a clade with species found in the North America and the Palaearctic, which was sister to the Hirudinidae and a second clade, including *Semiscolex similis* and *Patagoniobdella* species from South America, sister to the Macrobdellidae. Borda & Siddall (2004) proposed the resurrection of Blanchard's (1896; Scriban and Autrum 1934) family Semiscolecidae for the South American species.

Trontelj et al. (1999) and Borda & Siddall (2004) showed that the terrestrial sanguivores of the Haemadipsidae appear to have multiple origins, with an Indopacific clade and a second terrestrial lineage including New World and European terrestrial species (e.g. *Xerobdella lecomtei, Mesobdella gemmata*). One obvious morphological feature that sets the IndoPacific haemadipsids apart from the others is the presence of the respiratory auricle – a phalange-like flap over the opening of last nephripore found on the dorso-lateral surface of the caudal sucker; a synapomorphy for the group (Siddall & Burreson 1995, Apakupakul et al. 1999, Borda & Siddall 2004). This character is absent in non-IndoPacific species. Borda & Siddall (2004) suggested raising and resurrecting the subfamily Xerobdellinae proposed by Moore (1946) to family level to include the New World and European terrestrial species. Without the inclusion of other nonauriculate terrestrial species (e.g. *Mesobdella* species from southern South America, *Diestcostoma* species From Mexico, *Nesophilaemon* species from the Juan Fernandez Island, Chile and *Xerobdella* species from southern European mountains and *Idiobdella* species from the Seychelles) the monophyly and phylogeny of this family cannot be assessed at this time.

The phylogenetic placements of Americobdellidae and Cylicobdellidae had long been open to question due to the lack of representative taxa in previous molecular phylogenetic work, as well as their having a mixture of morphological characters transitional between groups of leeches. Morphologically, the monotypic Americobdellidae (i.e. *Americobdella valdiviana*) stands alone as a basal arhynchobdellid, in its own lineage (Siddall & Burreson 1995 and 1996). Although, *A. valdiviana* was traditionally classified a hirudiniform leech, Borda & Siddall (2004) found *A. valdiviana* to be most closely related to the Erpobdelliformes and not a hirudiniform at all. The position of Cylicobdelli-

dae as a hirudiniform and the basal most group was supported by the results from Borda & Siddall (2004).

In this contribution we reexamine the evolution of life history strategies of the Hirudinida, including habitat preference, feeding preference, cocoon deposition and parental care, in light of the current understanding of the relationships of leeches.

## 2 Materials and Methods

Sequences for protein-coding genes (18S rDNA, 28S rDNA, mt 12S rDNA and mt COI) used in this study were obtained and reported in previous phylogenetic work (Borda & Siddall 2004 and references therein; Apakupakul et al. 1999), unless otherwise specified. The following were included as outgroup taxa: *Lumbriculus variegatus* (mt COI – Price and Saunders, unpublished; 18S rDNA – Siddall et al. 2001), *Eclipidrilus frigidus* (18SrDNA, mt COI – Siddall et al. 2001), *Cambarincola gracilis, Branchiobdella pentadonta, Branchiobdella parasitica* (18SrDNA, mt COI – Gelder & Siddall 2001), *Acanthobdella peledina* (18SrDNA, mt COI – Siddall et al. 2001; 18SrDNA – Trontelj et al. 2001); all sequences are available on GenBank. Taxa new to this study include: *Whitmania laevis* from Taiwan (AY786447; AY786454; AY786467), *Haemopis terrestris* from Ohio (AY786446; AY786459; AY786452; AY786465), *Hirudo medicinalis* from France (AY786451; AY786458; AY786464), as well as members of the family Salifidae: *Linta be* Westergren & Siddall, 2004 from Madagascar (AY786453; AY786460; AY786466), *Barbronia gwalagwalaensis* Westergren & Siddall, 2004 from South Africa (AY786449; AY786455; AY786462), *Barbronia weberi formosana* from Kauai, Hawaii (AY786448; AY786456; AY786461) and an undescribed *Barbronia* species collected from Kruger National Park, South Africa (AY786450; AY786457; AY786463). *Ozobranchus margoi* also was included (Ozobranchidae; 18S rDNA – Apakupakul et al. 1999 and mt COI – Siddall & Burreson 1998). A 121 bp region of the 18S rDNA sequence for *O. margoi* was deleted due to variation that appeared to be inconsistent with other leech taxa when aligned.

DNA extraction, PCR amplification and sequencing for all genes were performed as described elsewhere (Borda & Siddall 2004, Apakupakul et al. 1999). Sequences were reconciled and edited using Sequence Navigator (Applied Biosystems). Alignments for the two nuclear sequences and mt 12S sequences were accomplished using Clustal W in MacVector™ 7.0 (Symantec Corporation) software package under the following alignment parameters: gap cost = 6; open gap = 3 (6:3) or gap cost = 10; open gap = 3 (10:3) and for COI using alignment parameters gap cost = 20 and open gap = 1 to ensure that gaps were

not inserted. The alignment that produced the shortest number of steps, regardless of number of trees, was favored for this study.

Parsimony analyses were performed using PAUP\* 4.06b10 (Swofford 2000) using the heuristic search option. The analysis 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 (Sorrenson 1999) was used to calculate Bremer support indices (b; Bremer 1988). The parsimony jacknife (jac; Farris 1999) values for all analyses were obtained with 1000 heuristic pseudoreplicates, using simple addition sequence and tree-bisection-reconnection branch swapping with 37 % deletion.

## 3  Results and Discussion
### 3.1  Phylogeny

The phylogenetic analyses of two nuclear genes (18S rDNA, 28S rDNA) and two mitochondrial genes (12S rDNA, COI) combined (3118 characters total, of which 1190 were parsimony-informative) for 67 taxa resulted in two equally parsimonious trees with a tree length of 8167 steps (CI=0.329; RI=0.642; Fig. 1), under alignment parameter set 6:3. The analysis of the data under alignment parameter set 10:3 produced a single tree that was 190 steps longer (L=8357; CI=0.325; RI=0.641). Therefore, the hypotheses in this study are based upon the results from alignment parameter set 6:3. The consensus of the two trees (not shown) supported the monophyly of Acanthobdellida, Branchiobdellida (jac = 100), and the Hirudinida (jac = 99) in 100 % of parsimony jackknife pseudoreplicates, with the Branchiobdellida sister to the Hirudinida but poorly supported (jac=55). The phylogenetic position of *Acanthobdella peledina* and branchiobdellidans with respect to leeches has varied in previous phylogenetic studies (Siddall & Burreson 1995, 1996 and 1998, Apakupakul et al. 1999, Trontelj et al. 1999, Siddall et al. 2001). The topology also weakly suggests that the Piscolidae+Ozobranchidae (jac = 100) are the most basal lineage of the Hirudinida. This position also appears to be poorly supported. This grouping was not supported above 50 % in the jackknife analysis. The two parsimony trees were also incongruent in the positions of the Haemadipsidae and the clade including *Limnatis nilotica*+Semiscolecidae+Macrobdellidae within the Hirudiniformes (see Fig. 1).

The monophyly of all families of Hirudinida (Tab. 1) has been discussed in detail elsewhere (Siddall & Burreson 1995 and 1998, Light & Siddall 1999, Apakupakul et al. 1999, Borda & Siddall 2004) and is corroborated here. As previously mentioned, Borda & Siddall (2004) and Siddall & Borda (2004) proposed the revision of removing Americobdellidae from the Hirudiniformes and placing the family with the Erpobdelliformes in light of an apparent closer

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| Larvenmerkmale / Art / Merkmal | Submentum | | | Frontoclypeus | | | | Kopf | | | Prosternite | | | Parietalia | | Kiemen |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ch. lepida | X | | | X | | | X | X | | | | | | X | | |
| H. angustipennis | | X | | X | | | X | | X | | | | X | X | | |
| H. botosaneanui | | X -> | <- X | X | | | X | | X | | | X | | | X | |
| H. bulbifera | X | | | | | | X | | X | | | X | | X | | |
| H. bulgaromanorum | | | X | | | X | | X | | | | X | | X | | |
| H. contubernalis | | X | | V | | X | | | X | | X | | | X | | |
| H. dinarica | | X | | | | | X | | X | | | X | | X -> | <- X | |
| H. exocellata | | X | | | | X | | | X | | | X | | X | | |
| H. fulvipes | X | | | | | | X | | X | | | X | | X -> | <- X | |
| H. guttata | X -> | <- X | | X | | | X | | | X | X | | | X | | |
| H. incognita | | | X | X | | | X | | X | | | X | | X | | |
| H. instabilis | X | | | | | | X | | X | | | X | | X | | |
| H. modesta | | X | | | | X | | | X | | | X | | X | | |
| H. ornatula | | | X | V | | X | | | X | | X | | | X | | |
| H. pellucidula | | X | | V | | | X | | X | | | X | | X | | |
| H. saxonica | X | | | X | | | X | | | X | | X -> | <- X | X -> | <- X | |
| H. silfvenii | X | | | X | | | X | | | X | | X | | X | | |
| H. siltalai | X | | | | | | X | | X | | | X | | X | | X |
| H. tenuis | X | | | X | | | X | | X | | | X | | | X | |
| H. tobiasi | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| D. felix | X | | | | X | X | | | X | | X | | | X | | X |
| Erläuterungen: X = zutreffendes Merkmal; V = variabel; U = unbekannt; -> <- = intermediär | | | | | | | | | | | | | | | | |

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— 50 changes
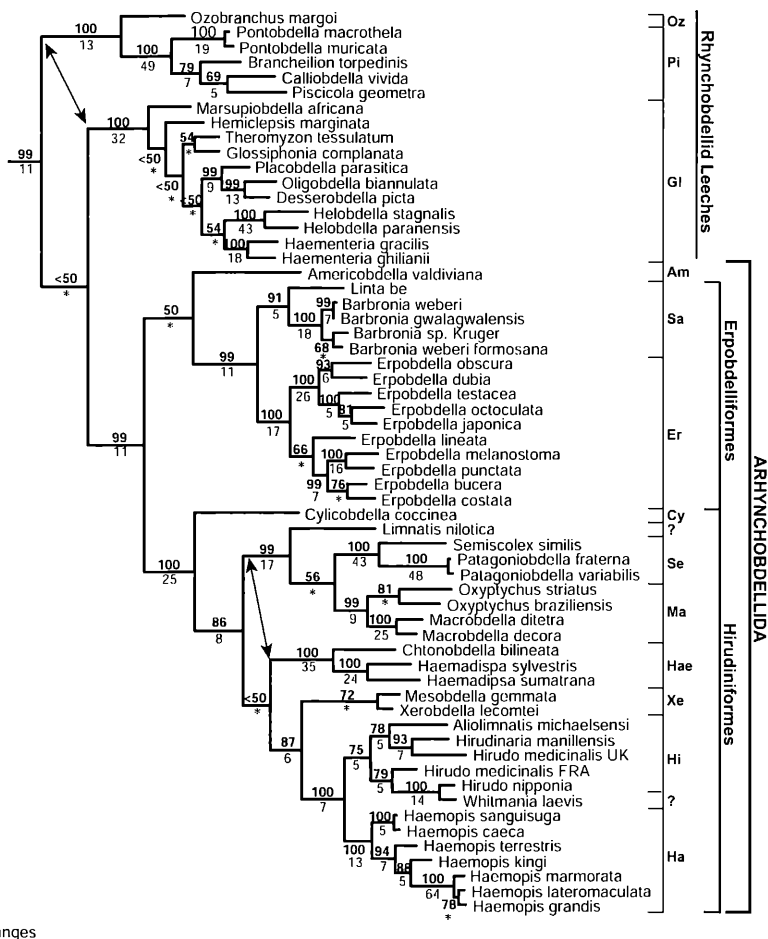
**Fig. 1: One of two most parsimonious trees resulting from the analysis of combined 18S rDNA, 28S rDNA, 12S rDNA and cytochrome *c* oxidase subunit I sequence data. The topology is based on the alignment parameters 6:3, which produced the shortest trees. Arrows at the nodes indicate positions that are considered to be equivocal. Jackknife values and Bremer support indices are located above (bold) and below the node, respectively. The asterisks (*) below the node indicate Bremer support values under 5**

**Tab. 1: Higher-level classification scheme of the Hirudinida adapted from Sawyer (1986), Siddall (2002) and Borda & Siddall (2004). Families, number of genera in each family and genera included in this study are listed**

**PHYLUM** Annelida

    **CLASS** Oligochaeta (= Clitellata)

        **ORDER** Hirudinida

            "Rhychobdellida"

| | |
|---|---|
| **FAMILY** | Glossiphoniidae (~13 genera) |
| | *Glossiphonia* |
| | *Helobdella* |
| | *Theromyzon* |
| | *Haementeria* |
| | *Desserobdella* |
| | *Placobdella* |
| | Ozobranchidae (2 genera) |
| | *Ozobranchus* |
| | Piscicolidae (~21 genera) |
| | *Piscicola* |
| | *Stribarobdella* |
| | *Pontobdella* |

          Arhynchobdellida

            Hirudiniformes (5 families)

| | |
|---|---|
| **FAMILY** | Cylicobdellidae* (6 genera) |
| | *Cylicobdella* |
| | Haemopidae* (4 genera) |
| | *Haemopis* |
| | *Whitmania* |
| | Hirudinidae* (19 genera) |
| | *Aliolimnatis* |
| | *Hirudinaria* |
| | *Hirudo* |
| | Macrobdellidae (3 genera) |
| | *Macrobdella* |
| | Oxyptychus |
| | Haemadipsidae* (17 genera) |
| | *Chtonobdella* |
| | *Haemadipsa* |
| | Semiscolecidae (2 genera) |
| | *Patagoniobdella* |
| | *Semiscolex* |
| | Xerobdellidae* (2 genera) |
| | *Mesobdella* |
| | *Xerobdella* |
| | *Limnatis*** |

           Erpobdelliformes (3 families)

| | |
|---|---|
| **FAMILY** | Americobdellidae*** (1 genus) |
| | *Americobdella* |
| | Erpobdellidae* (3 genera) |
| | *Erpobdella* |
| | Salifidae* (4 genera) |
| | *Barbronia* |
| | *Linta* |

*Families of traditional classification (Sawyer 1986)
**Results from Borda & Siddall (2004) found *Limnatis nilotica* (formerly part of the Hirudinidae) to be the sister taxon to Semiscolecidae and Macrobdellidae, therefore, without a better understanding of the closest relatives to *Limnatis* through further study with additional hirudinid taxa a new family cannot be designated
***The family Americbodellidae is tentatively placed in the Erpobdelliformes as proposed by Borda & Siddall (2004) until further study is possible with an expanded taxon sampling of the Arhynchobdellida. The taxonomic placement of Amerciobdellidae is questionable in light of very low support found here for the grouping with the Erpobdelliformes

relationship with the other erpobdelliform families in their phylogeny. In this study, however, *Americobdella valdiviana* is only weakly supported as an erpobdelliform (jac = 50).

Previous work had only included a single representative for the erpobdelliform family Salifidae (Trontelj et al. 1999, Borda & Siddall 2004). The inclusion of newly described species as representatives of the family (Westergren & Siddall 2004) opens the opportunity for further study. *Linta be* was described as the first ever recorded aquatic species from Madagascar. Interestingly, *Linta be* was found basal (jac = 91) to a monophyletic *Barbronia* clade (jac = 100) (see also Westergren & Siddall 2004). The Austrian *Barbronia weberi* was found to be sister to the South African *B. gwalagwalensis* (jac = 99) and *Barbronia* sp., also from South Africa, was sister to the Hawaiian *B. weberi formosana* (jac = 68). The revision of the latter may be necessary, as it appears that *B. weberi formosana* is a valid species, rather than a variant of *B. weberi*. *Haemopis terrestris* collected from Ohio was basal in a monophyletic clade with the remaining North American *Haemopis* species (jac = 94). Lastly, Borda & Siddall (2004) found the genus *Hirudo* to be paraphyletic with two species as representatives (*H. medicinalis* and *H. nipponia*). However, DNA extraction and sequences of *H. medicinalis* included in previous phylogenetic work (Siddall & Burreson 1998, Apakupakul et al. 1999, Borda & Siddall 2004) were collected from specimens bred in captivity (e.g. BioPharm, UK). The inclusion of a specimen of *H. medicinalis* collected from Rennes, France, in the analysis reveals a possible divergence between captive bred individuals and individuals that are found in the wild. Morphologically, both specimens fit the descriptions of *H. medicinalis*, but show considerable variation in the mitochondrial COI gene. The independent analyses of 18S and 28S recover *H. medicinalis* as monophyletic, but COI suggests that they are not. *H. medicinalis* from France was found in a monophyletic group (jac = 79) with *Hirudo nipponia*, which surprisingly was found to be sister to *Whitmania laevis* from Taiwan (jac = 100), previously thought to be a haemopid.

## 3.2 Feeding habit

Although leeches are notorious for their bloodfeeding habits, sanguivorous species are far outnumbered by their predatory and carnivorous counterparts (Sawyer 1986). For example, there are over 40 species in the non-bloodfeeding genus *Helobdella* (Siddall & Borda 2003), approximately 40 species in the genus *Erpobdella* (Siddall 2002), and countless other carnivorous taxa (e.g. *Barbronia, Cylicobdella, Glossiphonia, Haemopis, Semiscolex*). An optimized reconstruction of the feeding habits of leeches is shown in figure 2.
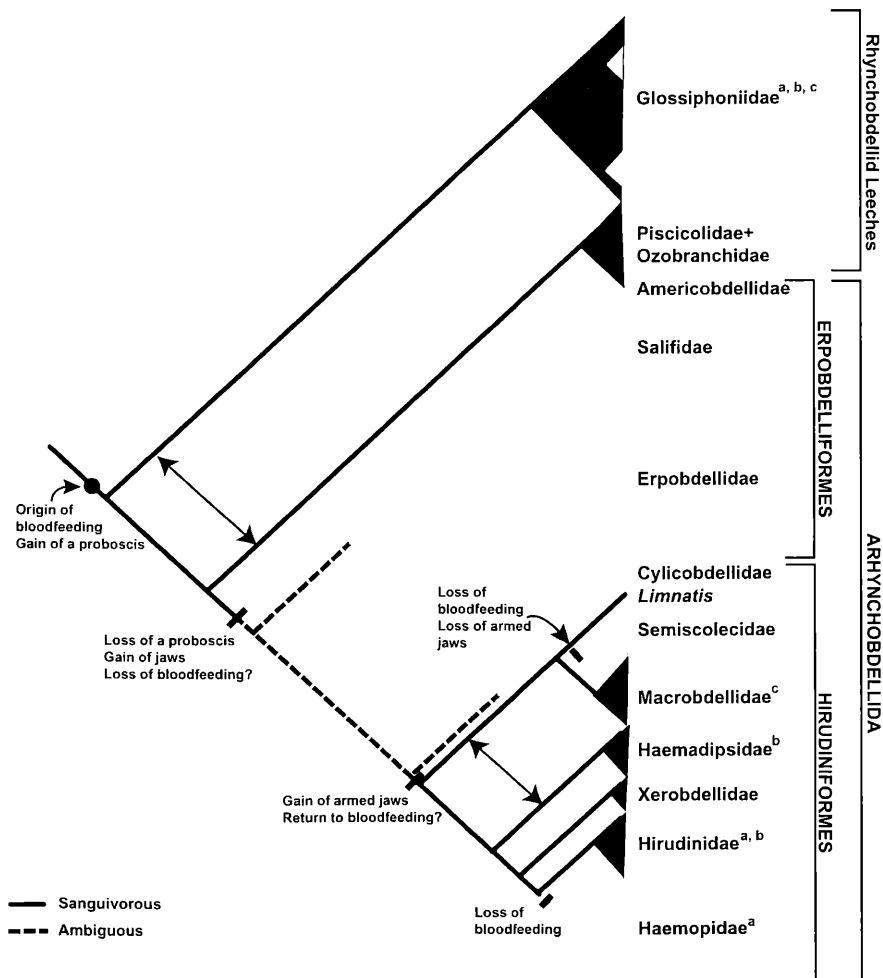
**Fig. 2: The optimized reconstruction of the evolution of feeding preferences in the Hirudinida. Arrows indicate equivocal position of taxonomic groups. Known anticoagulants present in groups of leeches: a) inhibitor of factor Xa; b) thrombin inhibitors; c) inhibitor of glycoprotein IIb/IIIa (adapted from Siddall 2003); black = sangivorous, grey = carnivorous, dashed black = ambiguous**

The positions of branchiobdellidans and *Acanthobdella peledina* as the most closely related groups to leeches provide a gradation of feeding behaviors towards strict sanguivory (see Fig. 1). Parasitic branchiobdellidans and *Acanthobdella peledina* feed on the dermal tissue of their hosts and as a consequence ingest blood (Holt 1965 and 1989, Sawyer 1986). The related branchiobdellidans and *Acanthobdella* either are ectocommensalistic or ectoparasites of crustaceans and salmonid fish, respectively, and similarly, the Piscicolidae, Ozobranchidae and some glossiphoniid species (e.g. *Placobdella parasitica, P. ornata*) are ectoparasites of blood specific to fish (in the former) or turtles (in the latter). Morphologically, the possession of a proboscis appears to be pleisiomorphic, as it is present in the basal most groups (i.e. Glossiphoniidae, Ozobranchidae, Piscicolidae), which is corroborated by presence of a rudimentary proboscis in *Acanthobdella peledina* (Sawyer 1986, Siddall & Burreson 1995, Trontelj et al. 1999).

If this hypothesis accurately represents the evolution of leeches, there is an indication of a common origin in a sanguivorous ancestry, with multiple independent losses of the behavior throughout the evolutionary history of leeches (Siddall & Burreson 1996, Apakupakul et al. 1999, Light & Siddall 1999, Trontelj et al. 1999, Borda & Siddall 2004). Within the Glossiphoniidae there have been at least two convergent losses of bloodfeeding, for example in *Helobdella* species and *Glossiphonia* species (Light & Siddall 1999; Fig. 2). Losses of sanguivory also are well represented among arhynchobdellid taxa, with over half of the families having abandoned bloodfeeding for a predatory and carnivorous lifestyle (Borda & Siddall 2004; Fig 2).

The biomedical interests and identification of coagulation inhibitors in a wide range of species have uncovered that anticoagulants are not restricted to sanguivorous species, but also are found in some non-bloodfeeding species (Siddall 2003; see Fig. 2). This not only supports a shared ancestry in a sanguivore, but also the retention of pleisiotypic characteristics from the leech ancestor in derived groups, regardless of feeding preference. *Hirudin*, a potent thrombin inhibitor, which remains concentrated in the wound for hours after the leech has stopped feeding, was the first anticoagulant to be isolated from *H. medicinalis*. Thrombin inhibitors have also been isolated from haemadipsids (i.e. *haemadin*) and from *Theromyzon* species (i.e. *theromin*) (Sawyer 1986). The giant Amazonian leech, *Haementeria ghilianii* produces another medically important platelet inhibitor (Sawyer 1986, Baskova et al. 1987, Munro et al. 1992a and 1992b, Vindigni et al. 1994, Yang et al. 1997, Hong et al. 1999). Protease in-

hibitors (anti-factor Xa) are shared by multiple sanguivorous species (*Thero-myzon* species, Haementeria species, Hirudinidae) and in the carnivorous genus *Whitmania* (Siddall 2003).

The morphological characteristics and feeding preferences of the arhyncho-bdellid ancestor remains unresolved based on the phylogeny. It is possible that the arhynchobdellid ancestor did not possess a proboscis nor armed jaws, but instead had a pharynx that was modified into rudimentary jaws as seen in the Americobdellidae and Cylicobdellidae. If there were two origins of bloodfeed-ing in leeches, the evolution of the armed jaws of sanguivorous hirudiniform species may have been coincident with the reacquisition of a bloodfeeding habit. The absence of sanguivory is apparent in the majority of the basal-most groups of Arhynchobdellida (i.e. Americobdellidae, Erpodelliformes and Cyli-cobdellidae). Complicating this picture, members of the Semiscolecidae and some species of the Haemopidae (e.g. *Haemopis caeca*) have independently lost a bloodfeeding habit. As such, there are two possible scenarios based on the cur-rent phylogenetic hypothesis (Fig. 2): either the arhynchobdellid ancestor was a bloodfeeder and there have been at least four losses of bloodfeeding within the group or the arhynchobdellid ancestor had abandoned sanguivory and there was a secondary return to bloodfeeding in the Hirudiniformes. Either hypothe-sis requires subsequent losses of bloodfeeding in groups such as the Semiscoleci-dae and Haemopidae.

## 3.3 Habitat preference

Leeches are better known for being aquatic in habit as is exemplified by the prevalence of freshwater taxa in the tree (Fig. 3). All rhynchobdellid leeches are aquatic, as are members of the Erpobdelliformes. While some hirudiniform leeches (i.e. Hirudinidae; Haemopidae; Macrobdellidae; Semiscolecidae) spend most of their lives in water, they nonetheless exhibit behaviors, such as foraging and cocoon deposition, on land. Furthermore, within the Hi-rudiniformes, species in the families Xerobdellidae and Haemadipsidae are spe-cifically adapted to a terrestrial lifestyle, albeit a damp one. The reconstruction of the evolution of habitat preferences depicted in Fig. 3 indicates that the an-cestral leech was adapted to a freshwater environment (see also Siddall & Bur-reson 1986). The shared ancestry of leeches with the freshwater Acanthobdel-lida, Branchiobdellida and Lumbriculida further corroborates this notion. With the exception of some Piscicolidae (and Ozobranchidae), which are found in environments ranging from freshwater to marine (Sawyer 1986, Siddall & Bur-reson 1996, Utevsky & Trontelj 2004), all aquatic leeches are restricted to fresh-water systems.
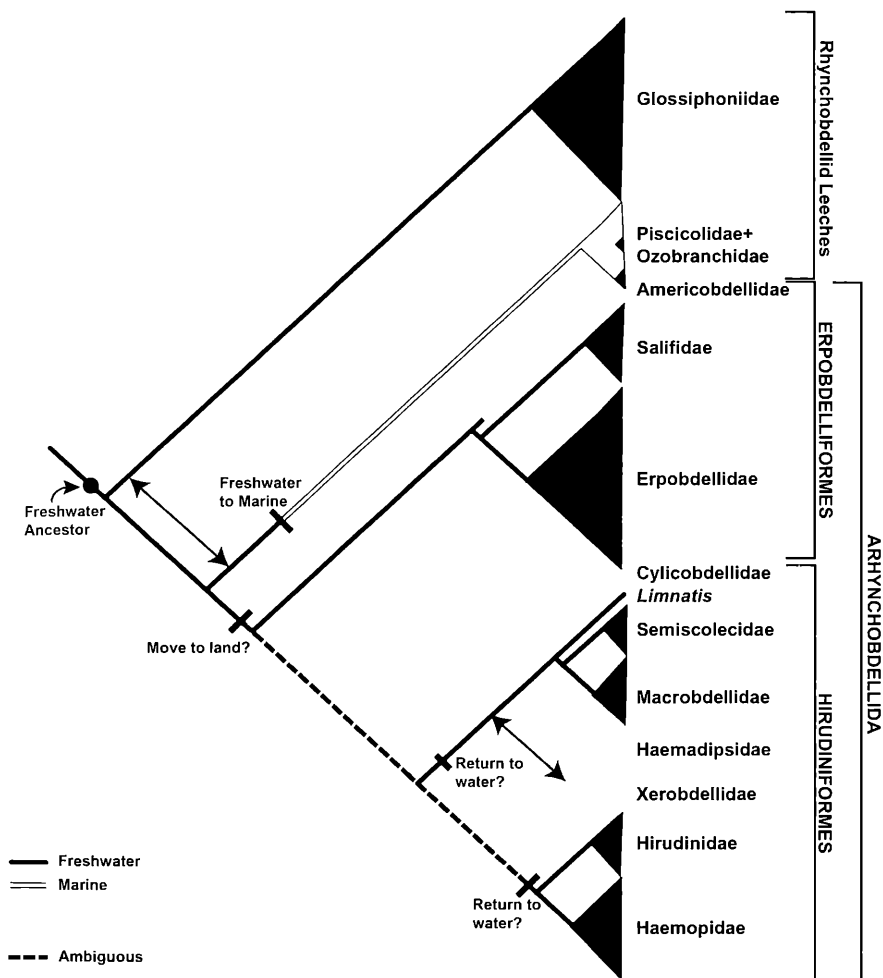
**Fig. 3: The optimization of the evolution of habitat preference. Arrows indicate equivocal position of taxonomic groups. black = freshwater; white = marine; gray = terrestrial; dashed gray = amphibious; dashed black = ambiguous**

However, somewhere in the ancestry of the Arhynchobdellida, there must have been a movement out of the water and onto land. Clues to the origin of terrestrialism may lie with *Americobdella valdiviana,* a leech that several have suggested may represent a relictual taxon (Ringuelet 1954, Siddall & Burreson 1996, Borda & Siddall 2004, Siddall & Borda 2004). This robust leech resembles a hirudinid externally, but internally has reproductive morphologies that deviate from most arhynchobdellid leeches. *Americobdella valdiviana* is the only

arhynchobdellid leech retaining the pleisiomorphic intergonadal conducting tissue, a feature known in the piscicolid leeches and *Acanthobdella peledina* (Sawyer 1986, Siddall & Burreson 1995). *Americobdella* seems to be more amphibious than terrestrial in habit, with reports of the species found in habitats ranging from damp earth to ditches and streams (Moore 1924a). It is possible that *Americobdella* spends most of its life in the damp soil, as it is a predator of terrestrial earthworms (Moore 1924a, Ringuelet 1985, Siddall & Borda 2004), retreating to water to incubate or to digest its meal. Our own collections of *A. valdiviana* from Valdivia, Chile resulted in specimens collected both from damp soil in a forest and from under a rock of a shallow stream (Siddall & Borda 2004). The analysis weakly supports *Americobdella* as an erpobdelliform leech (jac = 50), such that the ancestral state (aquatic or terrestrial) for the Arhynchobdellida cannot be definitively deduced. Nevertheless, terrestrialism appears be the original state for hirudiniform leeches. Cylicobdellids are terrestrial predators of oligochaetes and place as the basal most hirudiniform lineage. The evolution of the remaining groups have radiated into strictly terrestrial species (Haemadipsidae and Xerobdellidae) or those of semi-aquatic and amphibious habit (Hirudinidae, Haemopidae, Macrobdellidae and Semiscolecidae). Regardless of habitat preference, all hirudiniform leeches share a common mode of cocoon deposition (see below), which corroborates terrestrialism as the ancestral state of the group.

## 3.4 Cocoons and parental care

The diversity of habitat preferences in leeches also is reflected in behaviors associated with parental care. Ultimately the type of parental care exhibited by leeches determines the fate of the cocoon and offspring after deposition. For most leeches, cocoons are secreted from the clitellum, which is slipped off the head, and is encased in a protective cover that is resistant to environmental fluctuations and is abandoned, with no parental care exhibited by the leech (Sawyer 1986, Siddall & Burreson 1996, Apakupakul et al. 1999, Kutschera & Wirtz 1986 and 2001). Piscicolids and erpobdelliform leeches deposit hardened proteinaceous cocoons onto smooth substrate. Members of the Hirudiniformes leave the freshwater environment to deposit and abandon their cocoons on moist shorelines of lakes, ponds and streams. Members of the Glossiphoniidae produce and deposit a fragile membranous sac that is invariably guarded by the parent. The leech remains covering the soft cocoon until the embryos emerge and after the hatchings will attach to the venter of the parent (Kutschera and Wirtz 1986 and 2001, Sawyer 1986). In the case of sanguivorous species hatchlings remain on the venter until they are carried to their first blood meal (Kutschera & Wirtz 1986 and 2001, Sawyer 1986).
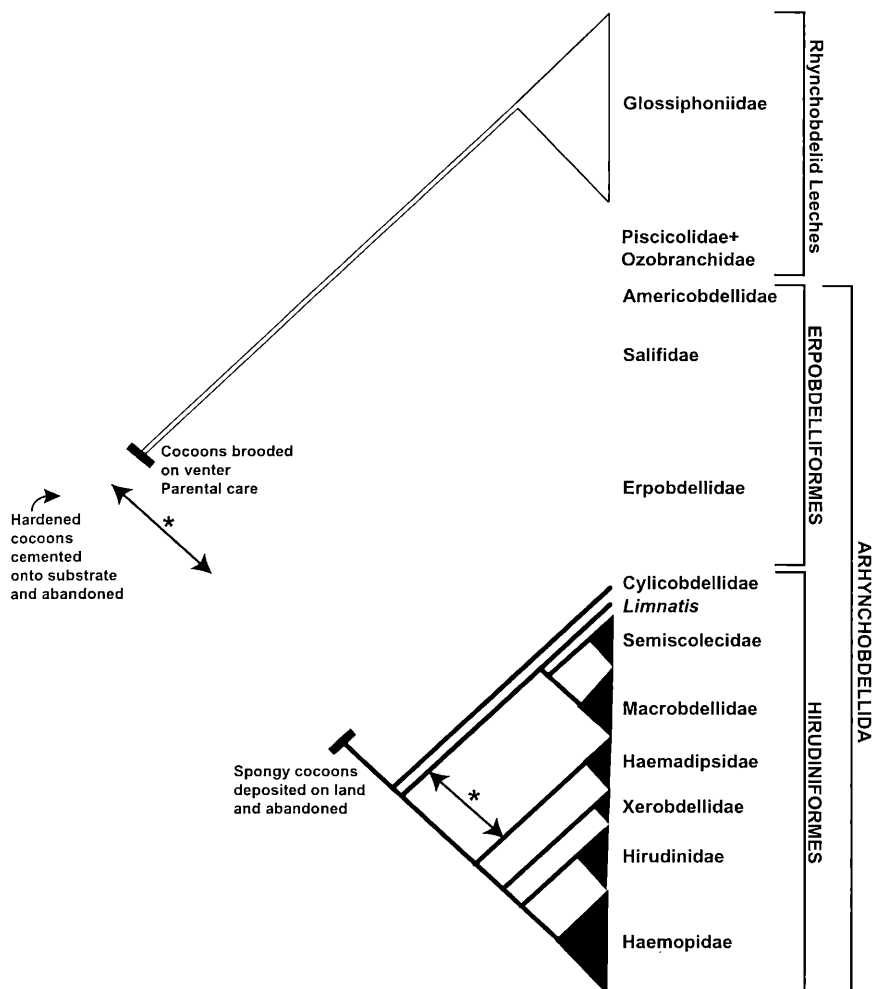
**Fig. 4: The optimization of the evolution of cocoon deposition in the Hirudinida. Black = cocoon deposition on land (no parental care), white = cocoon brooded on venter of leech (parental care), gray = cocoon cemented to substrate in aquatic habitats (no parental care), black = cocoons deposited on land (no parental care), dashed gray = unknown**

According to the current phylogenetic estimate (Fig. 4), and corroborated in other works (Siddall & Burreson 1996, Apakupakul et al. 1999, Kutschera & Wirtz 2001), deposition of a hardened protective cocoon onto a smooth substrate appears to be the ancestral condition in the Hirudinida. This behavior is apparent in the Piscicolidae and the Erpobdelliformes and is exhibited in the re-

lated *Acanthobdella peledina* and branchiobdellidans (Fig. 4; Sawyer 1986, Siddall & Burreson 1995 and 1996, Kutschera & Wirtz 1986 and 2001), all of which are accomplished in their aquatic environments. The abandonment of cocoons after deposition is a behavior that is retained in the Hirudiniformes, but the development of a protective casing that prevents desiccation is unique and associated to the modes of terrestrialism in the hirudiniform ancestor. The secretion of a membranous sac and exhibition of parental care is unique to glossiphoniids. The reproductive behaviors of *Americobdella valdiviana* are unknown. Such knowledge could provide clues for the missing link between rhynchobdellid leeches and the Arhynchobdellida.

## Acknowledgments

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