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# HIRUDINIA

# Hirudinia (Class): Parasitic Leeches

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Phylum Annelida

  Class Hirudinia

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# Chapter 62

# Hirudinia (Class): Parasitic Leeches

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### Leeches as Parasites

Some of the most charismatic and well-known leeches are blood-feeding species that rely on vertebrates, yet some species feed on the hemolymph of invertebrates, while others are strictly predatory, while scavengers in the leech world are rare (Siddall et al., 2011). In this section, only the leeches that feed on vertebrate blood will be covered (for other species, see Govedich and Moser, 2015).

Leeches are considered temporary, mostly ectoparasites of vertebrates, feeding only for short periods of time, from a few minutes or hours as in the case of *Hirudo medicinalis* or species of *Haementeria,* to days or weeks in the case of species of family Praobdellidae (*Limnobdella, Tyrannobdella, Praobdella*, or *Limnatis*) that feed from the nasal passages of mammals, including humans (Sawyer, 1986; Phillips et al., 2010). Some leeches, such as those of the genus *Placobdella* are semi-permanent parasites mainly of freshwater turtles, but some species feed on salamanders or birds (Bolek and Janovy, 2005; McCallum et al., 2011; Oceguera-Figueroa et al., 2010). Species of the genus *Theromyzon* are also semi-permanent parasites of the nasal passages of aquatic birds, such as waterfowl. One of the most extreme cases of parasitism in leeches is represented by species of the genus *Ozobranchus*, which are permanent parasites of both marine and freshwater turtles, spending their whole life attached to their host and even lay their eggs onto the body surface of their hosts (Sawyer, 1986; Nakano et al., 2017). Notably, *Placobdelloides jaegerskioeldi* is only known from the rectal tissues of African hippopotamuses (Oosthuizen and Davies, 2011). Most blood-feeding leeches are generalists in terms of the number of species of hosts that can be parasitized, and many instances of blood-feeding species supplementing their diet with fish or amphibian eggs have been documented (Light et al., 2005; Romano and Di Cerbo, 2007).

### General Morphology

Several morphological characteristics distinguish Hirudinida from other annelids, including their possession of a fixed number of 34 **somites** superficially subdivided into **annuli**, a reduced or fully absent coelom, the absence of chaeta in adult stages, and the presence of 2 **suckers**, 1 at the most anterior part of the body with the mouth laying inside (**oral** or **anterior sucker**) and 1 at the most posterior part of the body (**anal** or **posterior sucker**) (Govedich and Moser, 2015; Sawyer, 1986).

Leeches are, in general, elongated with parallel body sides, without regionalized body parts, and are slightly dorsoventrally flattened (that is, *Hirudo* and *Macrobdella* species); however, this general pattern is somewhat variable (see Figures 1–3). Some fish parasites (such as those in the family Piscicolidae) are circular in cross-section and may have distinct body regions such as the slender anterior **trachelosome** and the posterior, wider **urosome**. Species of Glossiphoniformes are, in general, foliaceous and dorsoventrally flattened. At least 2 groups of parasitic leeches, *Branchellion* and *Ozobranchus,* have developed lateral projections of the body walls forming membranous branchiae (Sawyer, 1986; also see Figure 4).

The most conspicuous morphological characteristic of leeches, in addition to the annulated body, is the presence of suckers located at the anterior and posterior ends of the body. Suckers are rather large and muscularized organs mainly used for locomotion and attachment to their host and prey (Sawyer, 1986). In general, the posterior sucker is larger than the anterior and, in some species, like the members of the family Praobdellidae, the former can be considerably wider than the width of the main body (Phillips et al., 2010). In general, 2 main types of feeding apparatuses are recognizable for blood-feeding leeches: The **proboscis** and **jaws**. The proboscis is an eversible muscular organ used to penetrate the skin of the leech prey, whereas the jaw is armed with sclerotized denticles that pierce the skin.

### Reproduction

Leeches are hermaphroditic worms that perform cross-fertilization during copulation; some species have developed complex reproductive systems with a penis and vagina, such as the species of *Hirudo* and *Macrobdella*, whereas others have a simpler reproductive system with testisacs and ovisacs

Figure 1. Dorsal view of *Macrobdella decora* (family Macrobdellidae; collected from Buckingham, Gatineau, British Columbia, Canada) representing the morphological variation within the subclass Hirudinea. Source: C. Grenier, 2015. License: CC0.

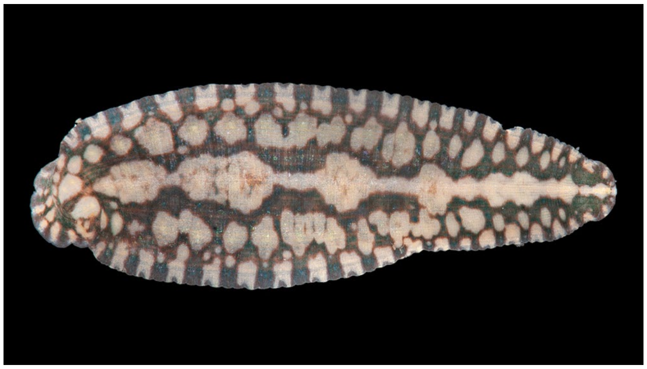

Figure 2. Dorsal view of *Placobdella parasitica* (family Glossiphoniidae; collected from Ingleside, Maryland, United States) representing the morphological variation within the subclass Hirudinea. Source: SERC Fisheries Conservation Laboratory, 2022. License: CC BY-NC.

Figure 3. Dorsal view of *Haementeria officinalis* (family Glossiphoniidae) representing the morphological variation within the subclass Hirudinea. Source: E. Caballero y Caballero and C. Loyola. License: CC BY-NC-SA 4.0.

connecting to their respective gonopores through relatively simple tubes, such as the species *Placobdella* and *Haementeria*. Fertilization is internal. In species with complex reproductive systems, the penis is inserted into the vagina to discharge the spermatozoids. In species with simple reproductive systems, the sperm transfer occurs through the implantation of spermatophores on the epidermis of the recipient leech (Salas-Montiel et al., 2017). Eggs are produced and enveloped by a proteinaceous membrane secreted by the clitellum (glandular area of the reproductive somites). In most of the species, this membrane hardens and forms a protective cocoon or case where the eggs develop; all the members of Glossiphoniformes keep the eggs within a thin and flexible membrane attached to the ventral surface where the eggs develop into young leeches that remain attached to their parent, representing an uncommon case of parental care within the Annelida (Sawyer, 1986). Their ontogeny is direct, without larval stages (Sket and Trontelj, 2008).

## Leeches as Vectors and Hosts

Leeches, like many blood-feeding invertebrates, may transmit bacteria or other microorganisms between hosts during the feeding process. PCR-based (Polymerase Chain Reaction-based) techniques have been used to detect bacterial communities in the digestive tract of leeches with relevant findings of *Bartonella* spp. in *Haemadipsa rjukjuana* from Korea, representing a human health concern (Kang et al., 2016). Recently, an unidentified blood-feeding leech has been implicated in the transmission of *Rickettsia* to humans (Slesak et al., 2015); however, the detailed mechanisms of the transmission patterns and frequencies need to be investigated in more detail. Leeches are occasionally vectors of *Trypanosoma* spp. and hemogregarines, particularly among fish, frogs, and turtles (Siddall and Desser, 1991; 1992).

Marine leeches of the genus *Ozobranchus*, which are permanent parasites of marine turtles, have been discussed as possible vectors of the chelonid fibropapilloma-associated herpesvirus (CCFPHV) due to the presence of relatively large loads of this virus in their body (Greenblatt et al., 2014). However, more experiments are needed to finally determine the role of leeches as vectors in these systems.

Leeches have also been recorded as intermediate hosts of cestodes (Regel, 2010), digeneans (McCarthy, 1990), and nematodes (Riggs and Ulmer, 1983). Macrophagous and blood-feeding leeches, such as *Haemopis* spp. and *Macrob-*

*della* spp., respectively, are definitive hosts for digeneans of the genus *Alloglossidium* that reach their adult stage in the leech intestine (Schmidt and Chaloupka, 1969; Beckerdite et al., 1974).

Recently, blood-feeding leeches (*Haemadipsa* spp.) have been successfully used to screen mammal diversity in Vietnam and southern Asia (Bangladesh, Cambodia, and China). PCR-amplification of the DNA (ingested DNA or iDNA) stored in the blood meal inside the crop of the leeches collected in the field revealed the presence of a wide diversity of mammal blood, such that a broad scope of host preference can be inferred for the leeches. In total, mammals of 6 orders (Artiodactyla, Carnivora, Chiroptera, Lagomorpha, Primates, and Scandentia) and 4 species of Aves were detected using this method. Amplifiable mitochondrial DNA was recovered from the gut content up to 140 days after blood ingestion; making leeches a promising candidate to uncover hidden vertebrate diversity (Schell et al., 2012; 2015; Tessler et al., 2018b).

Proboscis-bearing leeches that feed exclusively on vertebrate blood, such as species of *Placobdella*, *Placobdelloides*, and *Haementeria,* as well as species of Oceanobdelliformes (of the genera *Ozobranchus*, *Piscicola*, *Pontobdella*, *Branchellion*, and *Myzobdella,* among others) have established extreme symbiotic associations with bacteria, mainly Proteobacteria. Leeches of these groups house bacteria in specialized cells (bacteriocytes) that form specialized organs (bacteriomes) connected to the digestive system. It has been suggested that bacteria might complement the diet of these monophagous blood-feeding leeches, given the lack of, or low proportion of, vitamin B in vertebrate blood (Perkins et al., 2005: Kvist et al., 2011; Manzano et al., 2015). Associations between nutrient-supplying bacteria and their diet-restricted eukaryotic hosts have been heavily studied in various insect groups but poorly studied outside Arthropoda (see, for example, Aksoy, 1995; Douglas, 1998). Through genomic analyses of symbiotic bacteria, it has been demonstrated that the symbiont of the leech *Haementeria officinalis* has a much-reduced genome in terms of size, with high A + T content, and a reduced set of metabolic capabilities, all of which are a common characteristics of ancient obligate endosymbionts of arthropods. The genome of the *H. officinalis*-symbiotic bacterium*, Providencia siddalli*, has retained many pathways related to the biosynthesis of vitamin B, pointing towards a role in supplementing the blood-restricted diet of its host (Manzano-Marín et al., 2015).

Figure 4. General view of a leech, *Ozobranchus branchiatus* (family Ozobranchidae), displaying lateral branchiae. Source: Adapted from Lagunas-Calvo et al., 2021. License: CC BY-NC-SA 4.0.

## Zoogeography

Most leeches inhabit freshwater habitat, but there are marine, brackish, and terrestrial species, too. They are distributed worldwide, and their patterns of distribution broadly correspond with the biogeographic regions described based on other zoological groups, with some recognizable transitional zones and areas of endemism (Ringuelet, 1985, Sawyer 1986; Sket and Trontelj, 2008). Each biogeographic region is characterized by species flocks or genera; in the Nearctic, parasitic leeches are represented by the genera *Macrobdella*, *Philobdella*, and *Placobdella,* whereas in the Neotropics, parasitic leeches include *Mesobdella gemmata*, *Haementeria*, and *Oxyptychus*. In the transitional zone between these 2 areas (Mesoamerica), leeches from both areas co-occur, including *Macrobdella*, *Placobdella*, *Haementeria*, and endemics, such as *Limnobdella* and *Pintobdella* (Moser et al., 2016; Ringuelet, 1985; Oceguera-Figueroa and León-Règagnon, 2014). Palearctic parasitic leech fauna is characterized by species of *Hirudo*; however, other blood-feeding leeches are distributed in the region, such as those of the genus *Limnatis* and a single species of the otherwise Nearctic genus

*Placobdella* [*Placobdella costata* (Müller, 1846)] (Trontelj and Utevsky, 2005; Siddall et al., 2005). The leech fauna in the Afro-Tropical region is characterized by *Parapraobdella, Placobdelloides, Aliolimnatis*, and *Oosthuizobdella* (Sawyer, 1986; Phillips et al., 2011). The leech fauna of the Indian region is characterized by species in the genera *Haemadipsa*, *Hirudinaria*, and *Poecilobdella* (Sawyer, 1986), whereas the leech fauna in the East Asia region (Sino-Japanese region) is characterized by species of *Batracobdella, Hirudinaria, Hirudo nipponia, Poecilobdella*, and *Dinobdella* (Lai and Chen, 2010; Sawyer, 1986). Australia and New Zealand have a characteristic leech fauna, mainly represented by species of the genus *Chtonobdella* (Tessler et al., 2016), and other enigmatic leeches, such as *Ornithobdella edentula* found on nests of the New Zealand penguins *Eudyptes robustus* or the leech *Euranophila central,* a parasite of the frog *Litoria gilleni* from central Australia (Sawyer, 1986).

Some species display wide geographic distributions. For example, *Theromyzon* is a cosmopolitan genus (excluding Antarctica). This unusually broad distribution is probably related to the biology of their waterfowl hosts. Marine leeches such as those in the genera *Ozobranchus*, *Pontobdella*, and *Branchellion* display a broad geographic distribution attributable to the dispersal abilities of their hosts across the oceanic basins (Sawyer, 1986).

## Introduction to Hirudinea Classification

Jean Baptiste Lamarck coined the term Hirudinea in 1818 and the taxon was originally conceived of as a class within Annelida, or segmented worms, along with Polychaeta and Oligochaeta (Govedich and Moser, 2014). After 200 years of investigation, including the discovery of numerous species and groups, as well as the development of methods to better infer the phylogenetic relationships within this taxon, several changes have been proposed. These investigations have helped to reconcile taxonomic names and classification with the phylogenetics (Figure 5). It is now fully accepted that Oligochaeta is paraphyletic due to the inclusion of Hirudinea and, together, Oligochaeta, Hirudinea, and 2 small groups of leech-like worms (Branchiobdellida and Acanthobdellida) form the class Clitellata. Furthermore, phylogenetic studies have recovered Polychaeta as paraphyletic due the inclusion of Clitellata (Zrzavý et al., 2009; Struck et al., 2011; Kvist and Siddall, 2013; Weigert et al., 2014; Aguado et al., 2014). In further complicating the current conception of Annelida, Sipuncula (peanut worms), Siboglinidae, including pogonophores and vestimentiferans (deep-sea beard worms), and Myzostomida (which are parasitic on echinoderms) are now also considered to be annelids, although their morphological characteristics depart from the most common conditions of typical annelids and, interestingly, their phylogenetic position within the phylum is still unsettled (Aguado et al., 2014).

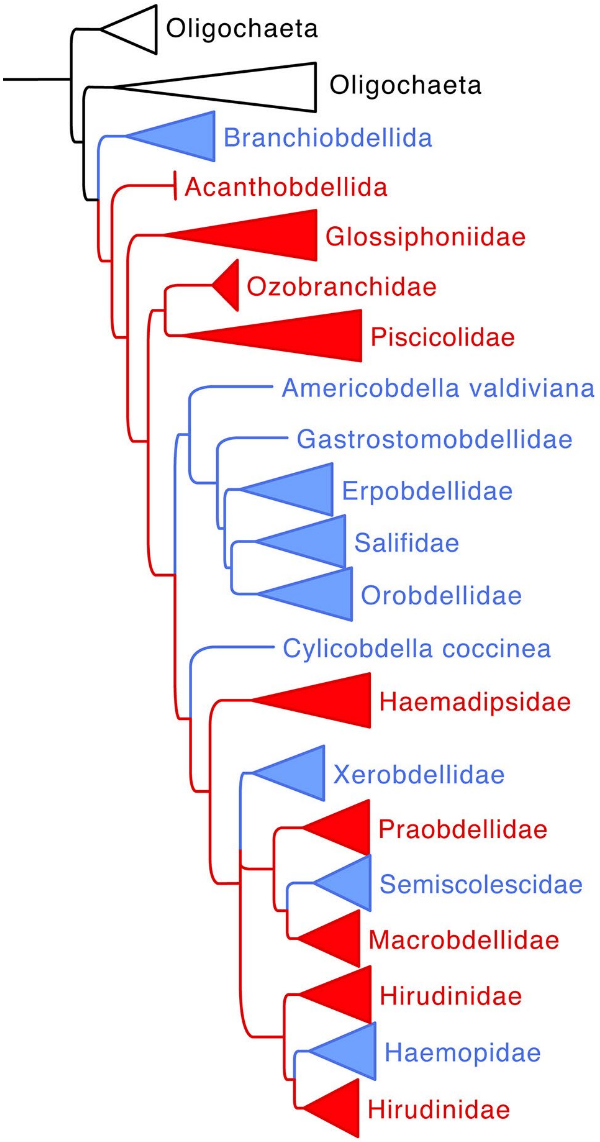

Figure 5. Composite phylogenetic diagram of the subclass Hirudinea summarizing the current knowledge of the relationships of major groups. Blood-feeding lineages are shown in red, non-blood-feeding lineages in blue. Source: A. Oceguera-Figueroa and S. Kvist. License: CC BY-NC-SA 4.0.

Order Acanthobdellida (salmonid parasites) and order Branchiobdellida (crayfish worms) were considered leech-like organisms that were thought to have developed suckers independently as an adaptation to their parasitic lifestyle. However, recent phylogenetic studies based mainly on molecular data clearly support their affinities with subclass Hirudinida (Siddall et al., 2001; Tessler et al., 2018). Both groups, Acanthobdellida and Branchiobdellida, are less speciose in comparison to Hirudinida, with only 2 species (*Acanthodella* *peledina* and *Paracanthobdella livanowi*) and approximately 140 species, respectively (Gelder, 2009; Sawyer, 1986).

The number of species included in this group is still growing, with more than 680 species distributed worldwide (Sket and Trontelj, 2008).

### Classification and Phylogeny

Historical classification of subclass Hirudinida recognized 2 orders, separated on the basis of the presence or absence of an eversible proboscis: Rhynchobdellida was used for proboscis-bearing leeches and Arhynchobdellida was used for species that lack such a structure (Sawyer, 1986). Recent phylogenetic studies based on molecular data failed to recover Rhynchobdellida as a monophyletic group (Apakupakul et al., 1999; Trontelj et al., 1999) and, consequently, Tessler and colleagues (2018) suppressed Rhynchobdellida and recognized 5 groups at the ordinal rank for all leeches: **Oceanobdelliformes**, including the families **Piscicolidae** (fish leeches; marine, brackish and freshwater species) and **Ozobranchidae** (turtle leeches; mainly marine, few species freshwater and brackish); **Glossiphoniformes** (blood and hemolymph feeders, freshwater species), **Americobdelliformes** (macrophagous, semi-terrestrial), **Erpobdelliformes** (macrophagous, freshwater), and **Hirudiniformes** (hematophagous and macrophagous, freshwater species).

Based on phylogenetic hypotheses and the mapping of feeding preferences onto the tree, as well as on the evidence provided by the analyses of the peptides of the saliva of some leeches (Siddall et al., 2011; Kvist et al., 2016), it has been suggested that the last common ancestor of all leeches was a blood-feeder (that is, adapted to feed on the vertebrate blood) and this feeding preference switched to macrophagy (feeding on small invertebrates and dead animals) and to liquidosomatophagy (feeding on hemolymph) on at least 6 or 7 independent occasions.

### Leech Therapy: History of Medical Applications

The so-called medicinal leeches are without doubt the most charismatic and infamous members of the group. Medicinal leeches have been used for centuries ostensibly to correct imbalances of the traditionally recognized 4 humors, namely, blood, phlegm, black bile, and yellow bile (Singh, 2010; Whitaker et al., 2004), as well as a variety of other ailments including mental disorders, whooping cough, gout, tumors, epilepsy, headaches, arthritis, and obesity (Weinfeld et al., 2000; Porshinsky et al., 2011). Leeching, or hirudotherapy, became the most popular mode of bloodletting in the Old World during the 18th and 19th centuries, in particular through the application of the renowned European medicinal leech *Hirudo medicinalis*. In order to fulfill the heavy demand on the medicinal leech, local leech populations were over-harvested to the point of local extinction; as a consequence, in 1823, restrictions were implemented to manage the number of leeches being exported through Hannover, Germany and collecting seasons were instituted in Russia (Wells and Combes, 1987; Whitaker et al., 2004; Elliott and Kutschera, 2011).

Currently, surgeons use leeches to aid in the salvage of venous-congested extremities that result from an imbalance between arterial inflow and venous outflow following surgery; this includes digits (Brody et al., 1989), nipples (Güneren et al., 2000), ears (Cho and Ahn, 1999), lips (Walton et al., 1998), nasal tips (Mortenson et al., 1998), and penis (Pantuck et al., 1996). Medicinal leech therapy has enormous utility in removing stagnant blood and allowing veins to recover (Singh, 2010; Porshinsky et al, 2011) and *Hirudo medicinalis* was approved as a medical device by the United States Food and Drug Administration (US FDA) in 2004 (Rados, 2004).

Recent phylogenetic analyses have clearly demonstrated that medicinal leeches do not form a monophyletic group. Instead, and with a broad definition of the term medicinal leech, 6 different groups include species that have been used for medicinal purposes around the world: *Haementeria* spp. in South America and Mexico; *Limnobdella* spp. in Mexico; *Macrobdella*, *Philobdella*, and *Oxyptychus* in the New World; *Hirudo* spp. in the Palearctic; *Haemadipsa* spp. and *Hirudinaria* in Southeast Asia, *Chtonobdella* spp. in Australia; and *Aliolimnatis* spp. in Africa (Oceguera-Figueroa, 2012; Phillips and Siddall, 2005; 2009; Phillips et al., 2010; Tessler et al., 2018).

### Preparation of Specimens

Proper fixation of leeches for morphological and molecular studies is important and necessary to understand biodiversity. To avoid morphological distortion of the specimen, it is important to narcotize or relax specimens before fixation. The main method consists of gradually adding drops of 95–100% ethanol to the water-filled container until the leeches' movements and reactions to touching stop. This process can take up to 30 minutes, depending on the specimen's size and, subsequently, the mucus produced during this operation should be removed with paper towels. Once relaxed, leeches must be straightened and placed in a container between paper towels and covered with 95–100% ethanol for 24 hours or more, depending on the size of the specimens. For molecular analyses, tissues (commonly parts of the posterior suckers, in order to avoid contaminations by potential blood meals), should be placed directly in 96% ethanol and kept at 4 °C, or colder conditions, if possible. For permanent slide preparations, in particular for small leeches, specimens should be flattened between 2 glass slides immediately after narcotization. Staining should be carried out with a mixture of Mayer's paracarmine and Ehrlich's haematoxylin and mounted on slides with Canada balsam. For histological preparations, the use of 4% paraformaldehyde, 2.5% glutaraldehyde, or instead, Fleming's or Bouin's fixatives is recommended.

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