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Relationship of leeches and blood parasites

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ABSTRACT

Leeches are a group of organisms belonging to the class Hirudinea within the phylum Annelida, with over 650 species that can live in marine, freshwater, and terrestrial environments. Some predatory species among them feed by consuming other Annelida species, various invertebrates, snails, and insect larvae. Most leeches, however, are ectoparasites that primarily feed on the blood of vertebrates and mammals, including fish, frogs, turtles, snakes, snails, and crustaceans. A small number of leech species are known as "medicinal leeches" and have been used for therapeutic purposes from the past to the present. It is known that leeches can transmit a large number of viral and bacterial agents during their blood-sucking feeding, and it has also been reported that they are vectors of many Trypanasoma species seen in fish and blood parasites such as various Haemogregarina and Haemococcidios. The presence of ricketsial agents such as *Bartonella* spp. and *Ehrlichia* spp. has also been shown in various molecular studies. Until now, the studies about leeches as vectors of blood parasites are not at a adequate level. Therefore, in this review, it is aimed to present the information and the results of some laboratory studies performed so far in order to shed light on the studies to be carried out on the subject.

Keywords: Hirudinea, leech, medical leech, ectoparasite, blood parasites

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Introduction

Leeches belong to the class Hirudinea within the phylum Annelida, which has more than 15,000 members. Within this class, there are over 650 species that can live in marine, freshwater, and terrestrial environments. Most members of this class are ectoparasites and typically feed by sucking blood. However, not all leeches are bloodsucking. Some species are predators, feeding on invertebrates, individuals from other classes of Annelida, snails, and insect larvae (Govedich, 2010; Godekmerdan, 2015 ; Sağlam, 2018). Bloodsuckers live as ectoparasites on fish, frogs, turtles, snails, crustaceans, vertebrates, and even humans (Singh, 2010; Küçük and Yaman, 2019).

Taxonomy

Leeches are a rich class in the Annelida phylum, Clitellata class and Hirudinea subclass, which includes a wide variety of species with many differences in both their lifestyle and diet. The subclass Hirudinea consists of the orders Hirudinida, Branchiobdellida (symbionts of crabs) and Acanthobdellida (leech-like parasites). The order Hirudinida is divided into 5 suborders. These are Americobdelliformes, Erpobdelliformes, Hirudiniformes, Glossiphoniformes and Oceanobdelliformes. Ozobranchidae family and Piscicolidae family, who are members of the suborder Oceanobdelliformes, live in sea and salty waters. Real leeches, which are the members of Hirudinidae family, are included in the sub-order Hirudiniformes. Medical leech species, that have been used as a treatment method from the past to the present, are also members

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Table 1. Taxonomy of leeches (Tessler et al., 2018).

Category	Subcategory	Description
Phylum	Annelida	
Class	Clitellata	
Subclass	Hirudinea	
Orders of Hirudinea	Hirudinida	
	Branchiobdellida	Symbiotic species
	Acanthobdellida	Leech-like parasites
Suborders of Hirudinida	Americobdelliformes	
	Erpobdelliformes	
	Glossiphoniformes	
	Oceanobdelliformes	
	Hirudiniformes	The Hirudinea family is included in this suborder.

of the Hirudinidae family (Borda and Sidall, 2004). Information about taxonomy is shown in Table 1.

Medical leeches are leeches whose ectoparasitism is used to as an advantage in traditional and modern medicine. Globally, 12 of the most well-known and reported leech species are classified as medicinal leeches. These species can be listed as follows: Turkish Medicinal Leech (*Hirudo sulukii*) (Saglam et al, 2016), Southern European Medicinal Leech (*Hirudo verbana* (Carena, 1820), European Medicinal Leech (*Hirudo medicinalis)* (Linnaeus, 1758), Caucasian Medicinal Leech (*Hirudo orientalis*) (Utevsky and Trontelj, 2005), North African Medicinal Leech (*Hirudo troctina*) (Johnson, 1816) Korean Medicinal Leech (*Hirudo nipponia)* (Whitman 1886), Chinese Medicinal Leech (*Hirudo tianjinensis*) (Wang et al, 2022), Far East Asian Medicinal Leech (*Hirudinaria javanica*) (Wahlberg, 1856), Malaysian Medicinal Leech (*Hirudinaria manillensis* (Lesson, 1842), North American Medicinal Leech (*Macrobdella decora*) (Say, 1824), Australian Medicinal Leech (*Richardsonianus australis*) (Bosisto, 1859) and New Zealand Medicinal Leech (*Richardsonianus mauianus*) (Benham, 1907) (Sağlam; 2004, Zaidi et al., 2011). Medicinal leech species that are found in our country are *Hirudo verbana*) and (*Hirudo sulukii*) (Sağlam, 2016).

History of treatment with leech

Leeches have been used for therapeutic purposes since ancient times. There are pictures showing the use of medical leeches on a tomb wall dating back to 1500 BC in Egypt (Porshinsky et al., 2011). Leech treatment is mentioned in Sanskrit inscriptions in 1300 BC. Its medical use was first recorded in 200 BC in Ionia, one of the ancient Anatolian civilisations, by Nicader of Colophon in a medical poem called Alexipharmaca (Whitaker et al., 2004). Galen, one of the famous physicians of the Roman period, emphasised that leeches restore balance according to the humoral theory by absorbing excess blood in the body. Ibn-i Sina (980-1037), known as Avicenna by Western civilisations, included leech treatment in his work Al-Kanun Fi't-Tıbb. It is known that leech treatment was used by physicians during the Ottoman period and there are many written works on the subject, especially from the 18th century. Leeches used for therapeutic purposes were first named as Hirudo medicinalis by Linnaeus in 1758 (Mory et al., 2000). In the 1800s, leeches were used very intensively in Europe. In 1884, Dr John B. Haycraft isolated the anticoagulant substance in leech saliva and named it as Hirudin (Haycraft, 1884).

In the 1980s, medical leech therapy has regained importance as a supportive modern treatment in order to provide blood supply and accelerate wound healing, especially in reconstructive surgery (in the replacement of severed limbs and flap surgery) (Okka, 2013). The US Food and Drug Administration (FDA) approved the use of leeches as 'medical devices' in the field of plastic and reconstructive surgery in 2004. With the efforts of the Ministry of Health of the Republic of Turkey, the 'Regulation on Traditional and Complementary Medicine Practices' was published in the Official Journal No. 29158 on 27.10.2014 and the leech application was standardised and accepted (Ayhan and Mollahaliloğlu, 2018).

Biology of leeches

Leeches belong to the Annelida group, which do not have a hard exoskeleton (chitin), but are covered with a thin and flexible cuticle, which allows them to be in close contact with water (Aloto and Ethicha, 2018). Their bodies are typically dorsoventrally flattened. Their colour and patterns, which are important in morphological determination, vary among species. Depending on the species, they are brown, dark green or black in colour and may have brown, orange or red stripes and patterns (Munshi et al., 2008). The morphological appearance of Hirudo verbana is shown in Figure 1.

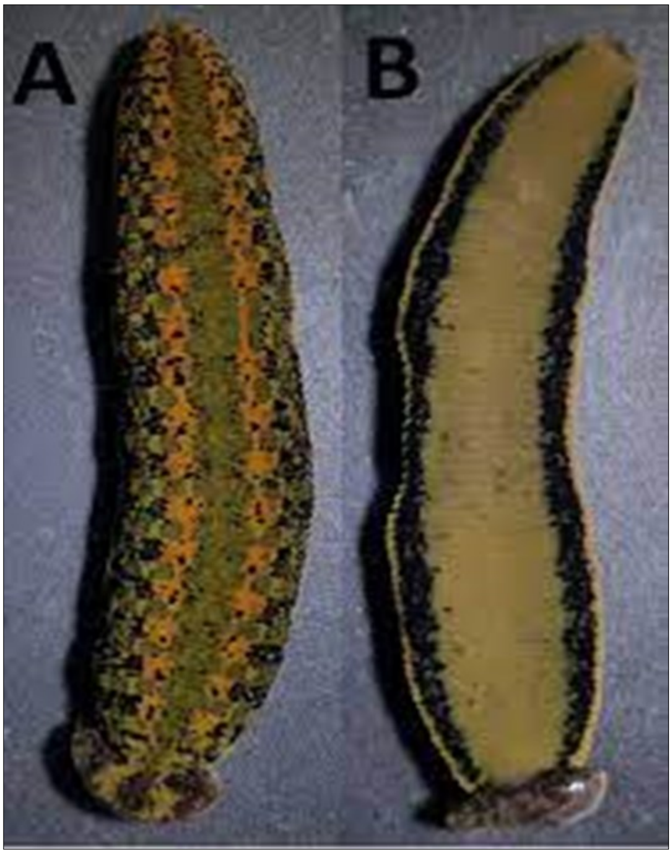

Figure 1. Morphological view of *Hirudo verbana* (Sağlam, 2019).

Their body consists of 34 segments. The anterior and posterior segments differentiate into anterior and posterior cecum (Sig et al., 2017). The anterior caecum has two or three multi-toothed jaws for feeding by sucking blood. These jaws have sharp teeth that make V- and Y-shaped wounds on the host's skin, respectively (Kutchera, 2012; Bahmani et al., 2014; Abdisa, 2018). The posterior caecum helps the leech to attach to the surface and move. The smallest leech species is 1 cm in length, but most species are 2.5 cm in size. Leeches of interest for human and animal health (e.g. H. medicinalis, *H. verbana*, *H. orientalis*) can reach up to 20 cm (Sağlam, 2004). The anatomical structure of Hirudo medicinalis is shown in Figure 2.

Gills in leeches were found only in some species of the family Piscicolidae. In other leeches, the body surface is equipped in such a way that it can receive dissolved or atmospheric oxygen in water and provide gas exchange (Sağlam, 2000). The nervous system of leeches are transmitters specialised to body structures. A large ganglionic nerve surrounds the pharynx and proboscis in the fifth and sixth segments. This ring represents the brain. Leeches have specialised senses such as mechanoreceptors, photoreceptors, baroreceptors, thermoreceptors and sonar equipment (Sawyer 1986) and perform three types of locomotion: crawling, swimming and undulating locomotion (Saglam and Sareyyüpoglu, 1998). Leeches are hermaphrodites and have both male and female reproductive systems, but they reproduce by mutual fertilisation instead of self-fertilisation. During the reproductive periods, the mother leech surrounds the egg with a spongy structure called 'cocoon' with various sex factors secreted for the protection and development of the eggs under difficult conditions (Sawyer, 1986).

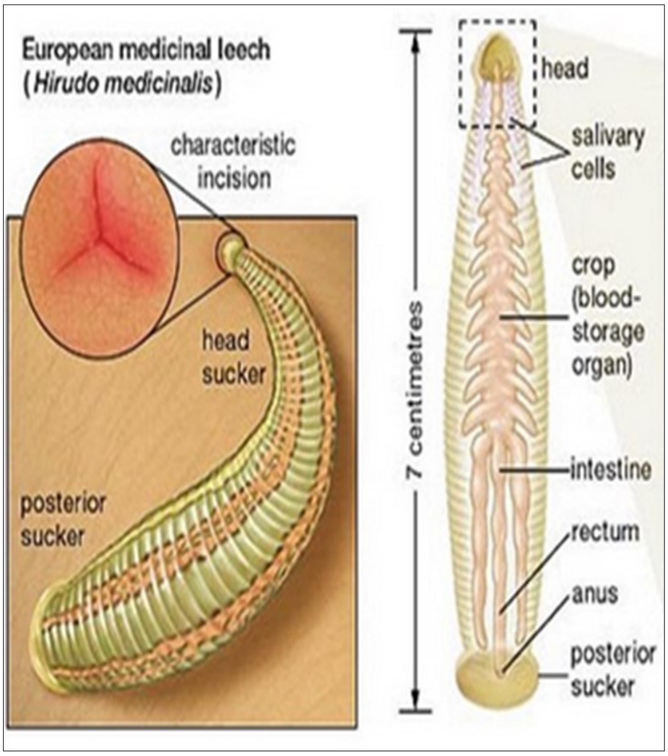

Figure 2. Anatomical structure of *Hirudo medicinalis* (Thakur et al., 2016).

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Digestive system of leeches

The digestive system consists of three main parts: the foregut, the midgut and the hindgut. The midgut consists of two main components: the stomach (crop) and the intestine (intestinum) (Worthen et al., 2006). The rhythmic and peristaltic contractions of the muscular pharynx transfer the blood into the cavity. After the pharynx, there are pouches on the sides of the stomach, which vary according to the leech species. These sacs allow the storage of food. Digestion takes place in the stomach and ciliated intestine. Leeches host symbionts such as Aeromonas hydrophila and Pseudomonas hirudinia in their intestines. These symbionts play a very important role in the digestion of blood (Gileva et al., 2013; Herlin et al., 2017). After blood suction, erythrocytes stored in the stomach are slowly transported to the intestine, where they are broken down and the released nutrients are absorbed. Culture-independent analyses have shown that Aeromonas and Rikenella-like symbionts are present and abundant in the gut as well as the stomach; however, the gut microbiome is also more diverse than that of the stomach. In addition to Aeromonas and Rikenella-like symbionts, *Morganella morganii*, α, γ and δ proteobacteria, Fusobacteria and members of the Firmicutes were also observed. This increased microbial diversity in the intestinal tract, where digestion and absorption of nutrients occur, is similar to that seen in humans and mice (Walter and Ley, 2011). In a study performed by Nelson and Graf in 2012, the bacterial symbionts of *Hirudo verbana* were revealed using the Fluorescent In Situ Hybridisation (FISH) method (Figure3).

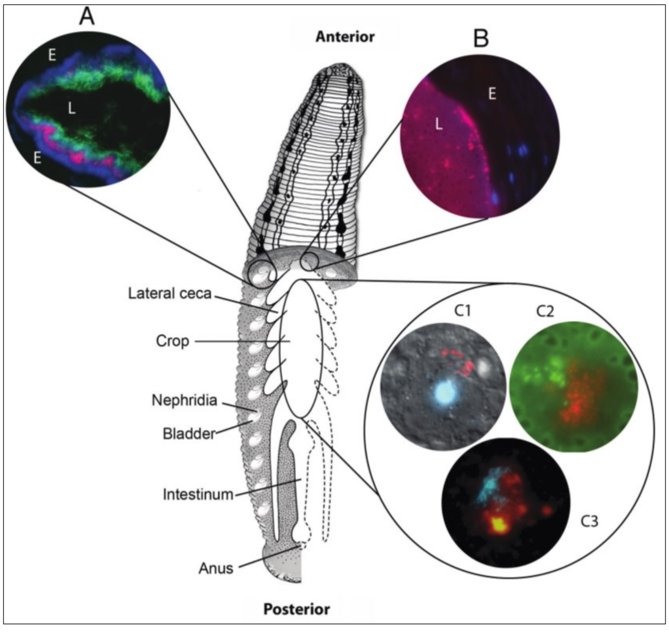

Figure 3. Major alimentary structures and microbial interactions of *Hirudo verbana*.

Drawing demonstrating the anatomy of Hirudo verbana showing the major structures of the alimentary system including the crop, lateral crop ceca, nephridia and bladders, intestinum and anus. Inset images illustrate microbial interactions within the bladder (A), along the crop epithelium (B) and through the crop at large (C) and are as follows: (A) FISH image showing the distribution of bacteria within the bladder lumen (colors correspond to probes targeted as follows: green, β-proteobacteria; red, Bacteroidetes; blue, bacteria); (B) FISH image of the Rikenella-like bacterium (red) along the crop epithelium, epithelial cell nuclei counter-stained with DAPI; (C1) composite image of Aeromonas (red) associated with the cell surface of a leech hemocyte, nucleus counter-stained with DAPI; (C2) FISH im- age of mixed microcolonies showing both Aeromonas (green) and the Rikenella-like bacterium (red), erythrocytes from a blood meal auto-fluoresce green and have darkened interiors; (C3) fluorescent image of lectin staining using succinylated wheat germ agglutinin (WGA-S, red) and DAPI counter- staining of bacterial colonies. For images (A and B), the following indicators are used: (E) indicates the tissue epithelium of the bladder (A) or crop (B) and L indicates the lümen (Nelson and Graf, 2012). (FİSH : Fluorescent İn Situ Hybridization ; DAPI : 4',6-Diamidino-2-Phenylindole)(Nelson and Graf, 2012) .

Leeches, which can suck blood about 10 times their own weight, continue to live with this blood content for 3-6 months with a slow digestion process. Some of them can live up to a year without the need for feeding (Hildebrandt and Lemke, 2011; Sağlam et al., 2020).

Salivary enzymes and bioactive components of leech saliva.

It has a pair of salivary glands on the sides of the pharynx and secretes the secretion it produces through the excretory ducts between its teeth. Salivary secretion, which gives the pheasant its main therapeutic qualities rather than bloodsucking, contains more than 100 active compounds (Das, 2014). To date, more than 20 molecules and their mechanisms of action have been identified, but there are many more molecules waiting to be discovered (Sig et al., 2017). The most famous bioactive compound they secrete is 'hirudin', which has a strong antithrombotic effect, and hyaluronidase, eglin, destabilase, piyavit, collaginase, apyrase, bdellin, decorsin, ornatin, Many biological substances with known vasodilator, anticoagulant, fibrinolytic, antimicrobial, bacteriostatic, analgesic, anti-inflammatory and local anaesthetic effects such as hirustatin and calin have also been isolated (Graf et al., 2006). During blood sucking from the host, leeches secrete this complex mixture of biologically and pharmacologically different active substances to the host (Michaelsan et al., 2007). Information on salivary enzymes and mechanism of action of leeches is given in Table 2

Table 2. Salivary enzymes of leeches and mechanism of action

Chemical Constituent	Mode of Action
Hirudin	Inhibits blood coagulation by binding to thrombin
Calin	Inhibits blood coagulation by blocking the binding of Von Willebrand factor to collagen-mediated platelet aggregation
Destabilase	Glycosidase activity, dissolves fibrins and possesses antimicrobial activity
Hirustatin	Inhibits kallikrein, trypsin, chymotrypsin and neurophilic cathepsin G; accelerates reperfusion and prevents reocclusion in femoral arterial thrombosis in a canine model
Bdellins	Anti-inflammatory; inhibits plasmin, trypsin and acrosin
Hyaluronidase	Reduces viscosity, increases tissue permeability to injected fluids and promotes resorption of excess fluids.
Tryptase Inhibitor	Inhibits proteolytic enzymes of host's mast cells.
Eglins	Anti-inflammatory; inhibits the activity of alpha chymotrypsin, chymase, subtilisin, elastase and cathepsin G.
Factor Xa Inhibitor	Inhibits the activity of coagulation factor Xa by forming equimolar complexes
Carboxypeptidase A	Increases blood inflow at the bite site by inhibiting other factors
Acetylcholine	Acts as a vasodilator
Histamine-like Substance	A vasodilator that increases blood inflow at the bite site

Hirudotherapy and usage areas of leeches

Hirudotherapy is performed with medical leeches. These leeches are members of the family Hirudinidae and the majority belong to the genus Hirudo. Hirudin, a very effective anticoagulant found in leech saliva, was the first bioactive substance isolated by Haycraft in 1884 (Wells et al., 1993). With the discovery of many other medicinally effective bioactive substances in leech saliva, this form of treatment has regained momentum in recent years (Singh, 2010). Among the numerous areas of indication; plastic and reconstructive surgery, cardiovascular diseases, arthrosis, osteoarthritis, periarthritis and rheumatoid arthritis, thrombophlebitis, thrombosis and embolism, haematomas, external ear and chronic ear infections, varicose veins, hypertension, haemorrhoids, gingivitis, dental and gum problems such as paradontitis, chronic skin diseases such as scabies, psoriasis, eczematous dermatitis and chronic ulcers, asthma, endometriosis (Trovato and Agarwal, 2008; Duruhan et al. , 2014).

In recent years, there has been a widespread increase in the use of hirudotherapy especially in reconstructive surgery and skin flaps. Increased capillary flow provides oxygenated blood flow to the region. With their anticoagulant compounds, they eliminate microcirculation disorders, restore damaged vascular permeability of organs and tissues, eliminate hypoxia, lower blood pressure, increase immunity, relieve pain and increase the bioenergetic state of the organism. In transplanted tissues, the difficulty of joining peripheral capillaries is facilitated by these aspects of leeches. After anastomosis, hirudotherapy is used to establish arterial blood flow (Gödekmerdan et al., 2011).

Leeches have various uses other than hirudotherapy. For example It has been widely used in neurophysiological and developmental genetic studies. It is also considered as one of the best laboratory model organisms for toxicological, physiological, biochemical and histological research (Petrauskiene, 2003; Le Marrec-Croq et al., 2013).

Complications of hirudotherapy

The use of medicinal leeches is recognized as a beneficial therapeutic application in various medical and surgical settings; however, it has also been reported that hirudotherapy can lead to various complications, such as infections (Litwinowicz and Blaszkowska, 2013). Gram-negative bacterial species, primarily Aeromonas spp., are known to cause infections, and studies on the intestinal flora of medicinal leeches have revealed that A. hydrophila and A. veronii biovar sobria are predominant species in the leech's digestive tract (Verriere et al., 2016; Ruppé et al., 2018). Medicinal leeches lack proteolytic enzymes, and the digestion of the ingested blood relies on bacterial enzymes provided by symbiotic bacteria, such as *A. hydrophila*, present in the gut flora. However, *A. hydrophila*, a gram-negative bacterium symbiotically residing on the leech's surface, oral flora, and intestine, can cause pneumonia, septicemia, and gastroenteritis in the host from which the blood is extracted. The first reported case of *A. hydrophila* infection associated with leeches occurred in 1983 (Whitlock et al., 1983). In a five-year retrospective study, Sartor et al. found that

4.1% of patients undergoing leech therapy developed infections (Sartor et al., 2002). The prophylactic use of antibiotics is recommended to prevent such infections.

Leeches as vectors

Hematophagous (blood-feeding) leeches, while sustaining their own life, can feed on various hosts and, during the blood-feeding process, may facilitate the transmission and spread of certain pathogens, thus acting as vectors for microorganisms. Various case reports and laboratory studies indicate that leeches could potentially serve as vectors for bacterial, viral, and parasitic infections, including Hepatitis B, HIV, Syphilis (*Treponema pallidum*), and Toxoplasmosis (Abbas et al., 2011; Pietrzak et al., 2012). Leeches have been implicated in the transmission of pathogens such as erysipelas (*Streptococcus* sp.), tetanus (*Clostridium tetani*), hog cholera (hog-cholera virus), equine arteritis virus, equine herpesvirus type 1, bovine parvovirus, feline calicivirus, and *Rickettsia* spp. (Haycox, 1995; Nehili et al., 1994). *Ozobranchus* spp. (the turtle leech) has been reported as a mechanical vector for the turtle herpesvirus associated with fibropapilloma (Greenblatt et al., 2004). In a study conducted by Salimi and Abdi in 2016, viruses responsible for infectious pancreatic necrosis (IPNV) were identified in Hemiclepsis marginata and Hirudo medicinalis leeches collected from free-flowing aquatic environments inhabited by fish and turtles. The findings suggest that these leech species may act as potential vectors for IPNV.

Leeches as vectors of blood parasites

Hemoprotozoan parasites are unicellular protists that exhibit different developmental stages in the tissues and blood of various vertebrate hosts, as well as in the intestines and tissues of blood-feeding invertebrate vectors. Hemoprotozoans are categorized into five main groups: Trypanosomatids from the phylum Euglenozoa, and Haemogregarines, Haemococcidians, Haemosporidians, and Piroplasms from the phylum Apicomplexa.

The host in which the parasite undergoes sexual development is referred to as the "definitive host"; the host in which only asexual development occurs is known as the "intermediate host"; and the host in which the parasite does not undergo any development is termed the "paratenic (transport) host" (O'Donoghue, 2017). Since hemoprotozoan parasites exhibit a continuous cycle between vertebrate and invertebrate hosts throughout their life, identifying the hosts and vectors is crucial for understanding the parasite's life cycle and determining necessary preventive or control measures.

All invertebrate hosts that serve as vectors for blood protozoa (such as ticks, insects, mosquitoes, and leeches) feed by drawing blood from vertebrate hosts. The acquisition of parasites by vectors from vertebrate hosts occurs when the vector uses specialized mouthparts to penetrate the epidermal layers and consume blood, during which the parasites undergo various developments within the vector's organs. The transmission of parasites from vectors to vertebrate hosts occurs through three primary mechanisms: inoculative (parasites are injected by the vector along with saliva containing anticoagulants and other vasoactive substances, known as salivary transmission); contaminative (parasites contaminate the wound when excreted by the vector, known as stercorarian transmission); or consumptive (in this case, the infected vector is ingested by the vertebrate host, resembling a type of predator-prey transmission). Leeches primarily transmit parasites through salivary transmission. In the case of Lankesterella, a haemococcidian agent, leeches are considered paratenic hosts, with the transmission suggested to be consumptive.

According to the information available to date, freshwater and marine leeches play a role in the transmission of hemoflagellates such as Trypanosoma and Cryptobia (Trypanoplasma), as well as intracellular Haemogregarine and Piroplasm to fish, and they are known to impact fish health in various ways (Woo, 2006).

Trypanasoma species in leeches

More than 600 kinetoplastid species transmitted through the blood have been classified into two families based on a range of phenotypic characteristics (host presence, vectors, life cycles, developmental stages, etc.): the family Bodoidae includes the genus Cryptobia, while the family Trypanosomatidae encompasses the genera Sauroleishmania, Leishmania, and Trypanosoma. Leeches serve as vectors for some species of Cryptobia and Trypanosoma (O'Donoghue, 2017). The genus Cryptobia, also known as *Trypanoplasma* spp., is a blood protozoan that resides in the peripheral blood of various freshwater fish species, transmitted by leeches, and can cause severe anemia and mass fish deaths.

Trypanosome species are parasitic hemoflagellates that infect vertebrates, including humans. They are transmitted to these vertebrates by blood-feeding invertebrate vectors, such as arthropods and leeches (Maslov et al., 2019). Trypanosome can be studied in two main phylogenetic groups. The first group comprises terrestrial-origin Trypanosome species that infect mammals, including humans, as well as reptiles, crocodiles, and birds. Their vectors include arthropods such as *Glossina* spp., *Tabanidae* spp., and *Triatoma* spp. (Hamilton et al., 2007; Baldacchino et al., 2013). The second group consists of aquatic trypanosomes that infect fully aquatic fish (Karlsbaak et al., 2005;

Hayes et al., 2014) and semi-aquatic hosts such as turtles, frogs (Siddall and Desser, 1992), and snakes, with leeches serving as their vectors (Hamilton et al., 2007). The groups of Trypanosoma undergo salivarian development in flies and leeches during the anterior station, while they experience stercorarian development in insects during the posterior station. The relationship between leeches and Trypanosoma has been a subject of ongoing research. Leeches of the family Glossiphoniidae and Piscicolidae are ectoparasites of vertebrates such as fish, amphibians, reptiles (turtles, crocodiles) and mammals and have long been considered as the main vector of some Trypanasoma species (Fermino et al., 2015; Smit et al., 2020). For instance, the carp trypanosome *Trypanosoma danilewskyi* is transmitted by the leech species *Hemiclepsis marginata*, a member of the Glossiphoniidae family. *T. danilewskyi* undergoes developmental changes within this leech, migrating from the leech's stomach to the proboscis sheath, and is transferred to another vertebrate host during blood feeding (Qandei, 1962). However, studies regarding the vector role of medicinal leeches (family Hirudinidae), which belong to the suborder Arhynchobdellida and lack a proboscis, as well as the family Haemadipsidae, known for terrestrial leeches, concerning vertebrate trypanosomes are insufficient and remain open to further investigation (Su et al., 2022). In their study, Su et al. (2022) reported the presence of certain flagellates in the gastric contents of *Hirudinaria manillensis* and isolated a new species of Trypanosoma from them. Based on morphological and molecular analyses, this species has been named Trypanosoma *bubalis*. Additionally, a study conducted in Australia using molecular methods confirmed the presence of frog and marsupial trypanosomes in four leech species from the family Haemodipsidae (Hamilton et al., 2005). Additionally, Siddall et al. (2019) reported that they were able to amplify unknown Trypanosoma DNA from 56.7% (25/44) of the terrestrial leeches collected from Australia and New Guinea. Ellis et al. (2021) isolated a new Trypanosoma subspecies, *Trypanosoma cyclops*, from the leech species *Chtonobdella bilineata* in Australia. As evidenced by these studies, various analyses suggest that terrestrial leeches may serve as potential vectors for Trypanosoma (Hamilton et al., 2005). However, the development of these Trypanosoma species within leeches of the family Haemadipsidae is not yet fully understood. In a study conducted by Fermino et al. (2015) in Brazil, epimastigotes and trypomastigotes were found in the intestines of *Haementeria* spp. leeches collected from the mouth of a wild crocodile species, Caiman yacare, captured in the Pantanal wetlands. Both the crocodile and leech trypanosomes were molecularly identified as *Trypanosoma clandestinus*.

In the study conducted by Siddall and Desser (1992), starved leeches Desserobdella picta and *Placobdella ornata* were allowed to feed on a juvenile bullfrog (Rana catesbeiana) infected with a heavy load of *Trypanosoma pipientis* and an adult turtle (Chelydra serpentina) infected with *Trypanosoma chrysemydis*. Subsequently, the transmission of *Trypanosoma pipientis* to laboratory-reared frog larvae via Desserobdella picta and the transfer of *T. chrysemydis* from Placobdella ornata to laboratory-reared turtles demonstrated the vector potential of these leeches and the persistence of the infection.

Hemmingsen et al. in 2005 performed a study on red king crabs in the Barents Sea. As a result of this study, it was reported that the sea leech *Johanssonia arctica*, which uses red king crabs to release their cocoons, acts as a vector for *Trypanosoma murmanensis*. Soltys and Woo reported that *Placobdella rugosa* leeches can be infected with *Trypanosoma brucei* by feeding with infected mice. When infected leeches were examined periodically, various forms of Trypanasoma were observed (Soltys and Woo, 1968).

Apicomplexan parasites in leeches

Haemogregarines, which are apicomplexan parasites, have obligate heteroxene life cycles that require development in invertebrate vectors before they can pass to vertebrate hosts. These coccidia undergo cyclic merogony and gametogony in vertebrates, but fertilisation and sporogony occur in invertebrate vectors (Lee et al., 2000). It is also seen in fish, amphibians, birds and mammals, especially in reptiles. The classification into four families has become increasingly accepted. Thus: Haemogregarinidae family includes the genera Haemogregarina, Cyrilia and Desseria; Hepatozoidae family includes the genus Hepatozoon; Karyolysidae family includes the genera Karyolysus and Hemolivia; and Dactylosomatidae family includes the genera Dactylosoma and Babesiosoma. The genera of the Haemogregarinidae family and their hosts and vectors are given in detail in (Table 3).

Cyrilia Lainson, 1981 from freshwater fish; Desseria Siddall, 1995 and Haemogregarina Danilewsky, 1885 from freshwater and marine fish and Haemogregarina species in turtles have been reported to undergo various developmental stages in leeches (Siddall, 1995). The development in leeches of the genus Babesiosoma (fish and frog), which is thought to be closely related to

Table 3. Genera of Haemogregarine Parasites (Lee et al., 2000).

Assemblage	Genus	No spp.	Site of development in vertebrate		Vertebrate hosts	Intervertebrate hosts
			Meronts	Gamonts		
F:Haemogregarinidae	Cyrilia	4	Erythrocytes	Erythrocytes	Fish	Leeches
F:Haemogregarinidae	Desseria	40	Erythrocytes	Erythrocytes	Fish	Leeches
F:Haemogregarinidae	Haemogregarina	46	Erythrocytes	Erythrocytes	Turtles	Leeches
F: Hepatozoidae	Hepatozoon	300	Viscera	Erythrocytes, leucocytes	Mammals, birds, reptiles, amphibia, fish	Leeches, arthropods
F: Karyolysidae	Hemolivia	4	RE cells	Erythrocytes	lizards, tortoises, toads	Ticks
F: Karyolysidae	Karyolysus	5	Viscera+-rbc	Erythrocytes	lizards	Mites
F:Dactylosomatidae	Dactylosoma	10	Erythrocytes	Erythrocytes	chameleons, frogs, teleosts	Leeches
F:Dactylosomatidae	Babesiosoma	7	Erythrocytes	Erythrocytes	frogs, fish	Leeches

the haemogregarines, has also been confirmed (Barta and Desser, 1989). Siddall and Burreson (1994) reported the developmental stages of fish haemogregarines in the sinus system of sea leeches. Turtle haemogregarins were detected in the blood sinuses of leech vectors (Siddall and Desser, 1991). Cyrilia spp. are characterised by sporogony in intestinal epithelial cells. These sporozoites are presumed to migrate to the salivary glands of the leech and become infective for a new host (Lainson, 1981). Desseria spp. undergo multiple sporogonitic divisions after syngamy in the leech, producing 16-32 sporozoites. The turtle haemogregarine Haemogregarina balli, Placobdella ornata, has been studied in leeches and reported to be characterised by sporogony producing eight sporozoites in the intestinal epithelium (Siddal and Desser, 1990). In addition, merozoites were observed to be produced in the extra-intestinal tissues of leeches after sporogony (Siddall, 1995). Interestingly, the development of Babesiosoma stableri in the leech Batracobdella picta also involves an oocyst containing eight sporozoites and post-sporogonic production of merozoites in the salivary glands (Barta and Desser, 1989).

Haemococcidia, another apicomplexan parasite, are quite different from other haemoprotozoa in that they utilise only invertebrates as paratenic host hosts without parasite development. Transmission to vertebrates is not the typical blood-borne transmission (inoculative/salivary or contaminative/stercorarian); instead, it occurs by vertebrates ingesting invertebrates (e.g. mosquitoes and leeches eaten by reptiles and amphibians). Lankesterella species of haemococcidia in frogs and lizards, comprising two families in total, form sporozoites in host blood cells and utilise leeches as paratenic hosts (O'Donoghue, 2017). In addition, leeches are also vectors of fish plasmodium and the Haemohormidiidae family, which is the piroplasma of fish, frogs and turtles (O'Donoghue, 2017).

Rickettsial agents in leeches

In addition to all these, leeches are also known as vectors for some rickettsial agents. It was reported that DNA of rickettsial agents such as *Bartonella grahamii* was found in Haemadipsa rjukjuana terrestrial leeches (Kang et al., 2016). In Laos, leeches of the genus Haemadipsida have been reported to be another potential vector for Rickettsia infections (Slesak et al., 2015). In a study performed by Kikuchi and Fukatsu in Japan in 2001, Torix tagoi and Hemiclepsis marginata, members of Glossiphoniidae family, were found to be positive for Rickettsia spp. The fact that the juveniles of *T. tagoi* cocoons were also positive for *Rickettsia* spp. suggested that vertical transmission may be possible (Kikuchi and Fukatsu, 2001). In another study conducted in 2005, Rickettsia DNA was detected in *Torix tukubana*, *Torix tagoi* and *Hemiclepsis marginata* leech samples collected from the field. All cocoons of infected females of *T. tagoi* and *H. marginata* were also tested positive for *Rickettsia* spp. (Kikuchi and Fukatsu, 2005).

The genus Ehrlichia consists of several obligate gram -negative intracellular bacterial species that are transmitted to vertebrates by ticks sucking blood (Dumler et al., 2007). In a study performed by Zhou et

Table 4. Some blood parasite species and hosts that leeches act as vectors

Agent	Vector	Host
Cryptobia salmositica (Trypanoplasma spp.)	*Piscicola salmositica*	*Oncorhychus spp.*
Cryptobia borreli	*Piscicola geometra*	*Cyrinus carpis*
Trypanasoma danilewski	*Piscicola geometra, Hemiclepsis marginata*	Carps
Trypanasoma bubalisi	*Hirudinaria manillensis*	Asian Buffalo *(Bubalis bubalisi)*
Trypanasoma cyclops	*Chtonobdella bilineata* (Terrestrial leech)	Kangaroo (Wallabia)
Trypanasoma clandestinus	*Haementeria spp.*	Crocodiles *(Caimon yacare)*
Trypanasoma murmanensis	*Johanssania artica*	Marine fish
Trypanasoma cabitis	*Hemiclepsis marginata*	-
Trypanasoma cabitis	*H. marginata*	Freshwater fish
Trypanasoma phaleri	*Desserobdella phalera*	Freshwater fish, Bowfin (Mudfish)
Trypanasoma rotatorium	*D. picta*	Frog
Trypanasoma pipientis	*Desserobdella picta*	Amphibians and reptiles
Trypanasoam chrysemydis	*Placobdella ornata*	Amphibians and reptiles
Trypanasoma brucei	*Placobdella phalera*	Mice (experimental)
Cyrilia spp	Freshwater leeches	Freshwater fish
Desseria spp	Freshwater or marine leeches	Freshwater or marine fish
Haemogregarina balli	*Placobdella ornata*	Freshwater fish
Haemogregarina stepanowi	*Placobdella costata*	Freshwater turtles
Babesiosoma stableri	*Batracobdella picta*	-
Lankesterella spp.	Paratenic host – Leech	Frog, lizard
Bartonella grahami	*Haemodipsa rjukjuana*	-
Rickettsia spp.	*Torix tagoi ve Hemiclepsis marginata* (Terrestrial leech)	-
Ehrlichia spp.	*Hirudinaria spp.*	-

al. in Hubei province of China in 2019, it was reported that *Ehrlichia* spp. DNA was detected in 39 of 620 Hirudinaria genus leech samples (Shu-Han Zou et al., 2019). However, whether leeches permanently carry Ehrlichia spp. and vector-host transmission is a subject that needs further research. Some blood parasite species and hosts that leeches act as vectors are shown in Table 4.

Conclusion

In conclusion, it is seen that the vectors of leeches, which are blood-sucking ectoparasites, are open to research. In the studies that have been performed so far, it has been observed that leeches can transmit many important disease agents both in human medicine and veterinary medicine during their feeding by sucking blood. Blood sucking arthropods (ticks, fleas, lice, mosquitoes, etc.) are known to be the vectors of blood parasites as well as leeches. However, the studies were interpreted as insufficient in parasitological aspects. In contrast to other vectors, leeches show diversity with their marine, freshwater and terrestrial species, and therefore they can interact with many more organisms than other vectors. They have a wide range of hosts as they can feed on aquatic organisms as well as semi-aquatic and terrestrial organisms. The increasing interest and need for leech treatment (hirudotherapy) method, which is both an ectoparasite in nature and extends from the past to the present, makes it necessary for us to investigate these yet unknown aspects of leeches.

Medical leeches have been one of the living natural resources used for centuries to treat certain diseases and protect people's health. However, in 2004, the US Food and Drug Administration (USA - FDA) approved the use of leeches in the field of plastic and reconstructive surgery. In 2014, with the entry into force of the ''Traditional and Complementary Medicine Practices Legislation'', which also covers leech application in Turkey, it has started to be applied as a treatment method in the light of modern medicine and science. However, leech hunting is still being carried out in the world using traditional methods and uncontrolled practices. This situation increases the risk of blood parasites that leeches can transmit.

In addition, with the development of molecular analysis techniques and the discovery of bioactive substances found in the salivary enzymes of leeches, leeches have become the focus of interest of the pharmaceutical industry. This demand for medicinal leeches all over the world has led to the collection of living individuals from their natural habitats, domestic and foreign trade, and thus to the development of a leech economy.

The research of leeches, which fill such an important place from the past to the present, not only as an ectoparasite, but also from their healing properties, will ensure a more conscious use based on scientific principles and will make it inevitable to give the necessary value to these organisms. For this reason, we think that it is important to increase the number of qualified scientific studies and to evaluate the data obtained from the researches as a whole by collecting them frequently with reviews. Raising awareness and consciousness on this issue is also important for leeches to maintain their existence and their roles in the ecosystem.

It is seen that there is a need for further studies on the subject due to the potential of leeches as vectors and thus the potential to transmit zoonotic pathogens. In our country, the presence of blood parasite agents in leeches has not been sufficiently investigated so far and there is no information on the transmission of these agents. Therefore, it is thought that this review will provide information for future studies on the subject.

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