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The freshwater leech *Helobdella europaea* (Hirudinea: Glossiphoniidae): an invasive species from South America?

U. Kutschera

With 4 figures

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In freshwater ecosystems throughout Europe, *Helobdella stagnalis* Linnaeus, 1758 is the only member of its genus. In 1982 I discovered a second *Helobdella* species in a fast-running stream in Southern Germany (Schobbach/Moosbach, Vörstätten). Three years later, this second European *Helobdella* species was described as *H. striata* (Kutschera, 1985) and later renamed *H. europaea* (Kutschera 1987). In this report I describe in more detail the discovery, feeding strategies and taxonomic status of this leech. It is shown that *H. europaea* is not only a predator but also a scavenger that feeds on dead bodies of aquatic vertebrates (fish, amphibians). On the basis of mt-DNA sequence data it is documented that *H. europaea* is distinct from *H. stagnalis* and *H. triserialis* (North America). *H. europaea* is identical with (or closely related to) *H. papillornata* discovered in Australia a few years ago. This leech may represent an introduced annelid from South America that reached Germany via the aquarium trade.

1 Introduction

Since Linnaeus' (1758) description of the common freshwater leech *Helobdella stagnalis* there had not been a new species of *Helobdella* found in any European freshwater ecosystem. Herter (1968) introduced the name "two-eyed flat leech" for the type species *H. stagnalis*. However, all species of the genus *Helobdella* described so far have a flattened body and two eyes (Elliott & Mann 1979, Sawyer 1986). Accordingly, this popular name for the taxon *H. stagnalis* is not very appropriate, but used here in accordance with the German literature.

In this contribution I summarize the discovery, identification, description, feeding behaviour, and systematic position of a second *Helobdella* species that was found two decades ago in a fast-running stream in Germany (Fig. 1). Based on molecular phylogenetic evidence it is proposed that this enigmatic glossiphoniid leech may be an introduced annelid from South America.

2 Discovery and identification

Between August 1982 and April 1984 I collected a total of 52 adult leeches (*Helobdella* sp.) in their original habitat, a stream near Freiburg i. Br. (Schobbach/Moosbach, Vörstätten, Germany). Most leeches (length: 15–18 mm) were attached to the underside of flat stones or roots. Breeding animals (with cocoons, larvae, or young) were regularly found (Fig. 2); they were maintained in aquaria that contained flat stones and aquatic plants from the leeches' habitat (*Elodea canadensis, Calitriche palustris, Myriophyllum demersum, Fontinalis antipyretica*). In the same stream, i. e. under identical stones, three other leech species, *Erpobdella octoculata*, *Glossiphonia complanata*, and *Helobdella stagnalis* were found in high abundance.

In captivity, the enigmatic *Helobdella*-individuals fed on oligochaeta (*Tubifex* sp.), water snails (*Physa acuta*, *Radix peregrea*), and crustaceans (*Asselus aquaticus*). All three prey organisms occurred in the habitat of the leeches and therefore may represent their natural diet.

In order to be sure that this leech (Figs. 1, 2) is in fact a new species, I first consulted the zoologist Prof. G. Osche (Institut für Biologie I, Universität Freiburg), who recommended to describe this taxon as a novel species. In summer 1984 I sent some ethanol-preserved specimens to Dr. R. T. Sawyer (University College of Swansea, United Kingdom) and asked this expert for additional advice. On 3. September 1984 Dr. Sawyer responded as follows: "many thanks for your letter and specimens. I must say I find them very interesting. Being originally from the United States I am familiar with various species of *Helobdella*. It does appear to be of the genus *Helobdella* which, as you know, occurs throughout North and South America. Before I am sure it is *Helobdella* I would like to know more about it: 1. Eyes. I see only one pair of well-separated eyes, but in several specimens there are pigmented areas similar to eyes... 2. Cocoons. I see in one specimen that cocoons are attached to the venter... 3. Gonopores. Can you confirm one annulus between gonopores ? 4. Food. Does it show a strong preference ? Very important. 5. Mouthpore. I am surprised to find an anterior mouth. 6. Ecology. What type of habitat ? How fast, cold ? ... I have just completed a key to South and North American *Helobdella* and I will go through this carefully. ... I have a reference to a *Helobdella* accidentally imported to Europe, found in someone's aquarium. Please be assured of my interest in this leech".

Two months later Dr. Sawyer confirmed that my leech belongs to the genus *Helobdella*: "From what you tell me there is little doubt that the species in question is a *Helobdella*... As to the species identification: It looks superficially like *H. triserialis* (= *H. lineata* of USA) but two features you told me about cause some reservation. 1. The fact that it not only eats oligochaetes but actu-

ally prefers them over snails is in my experience very different from *H. triserialis*, which is virtually restricted to snails. 2. Crop caeca five pairs. This is somewhat ambiguous since the first pair can be very small and barely detectable.... It would also be useful to see some living specimens. ... I hope you continue to pursue more information on the leech. Undoubtedly it is of interest whether new or introduced. Please keep me informed".

On 19. March 1985 Dr. Sawyer recommended describing my *Helobdella* as a *nova species*: "Concerning the identification of your leech I am still uncertain. Anatomically, I do not see any criterion with which to separate it from *Helobdella triserialis*. Biologically and geographically, it is identifiable. You say it feeds on *Tubifex* and crustaceans. This would, in my opinion, be sufficient reason to regard it as a new species of *Helobdella*".

My paper on the description of a new leech species, *Helobdella striata* (Kutschera 1985), was published in October 1985, when I had started to work as a Postdoctoral Research Fellow at the Carnegie Institution of Washington in Stanford, California, USA. During the first month of my stay in the United States I discovered in a small creek on the campus of Stanford University a large population of the leech *H. triserialis* (Sawyer, 1986; for a revision of the taxonomy, see Siddall and Borda 2003). Because Dr. Sawyer had informed me

Fig. 1: Adult individual of the freshwater leech *Helobdella europaea* Kutschera, 1987 in relaxed (A) and extended position (B). Bar = 1 cm

in a letter that "the name *striata* may be pre-occupied" and since *H. striata* from Germany was clearly distinguishable from *H. triserialis* (North America) I renamed this taxon *H. europaea* (Kutschera 1987). However, the following observations and molecular data indicate that *H. europaea* is no native species from Europe, but may have been introduced from South America or somewhere else.

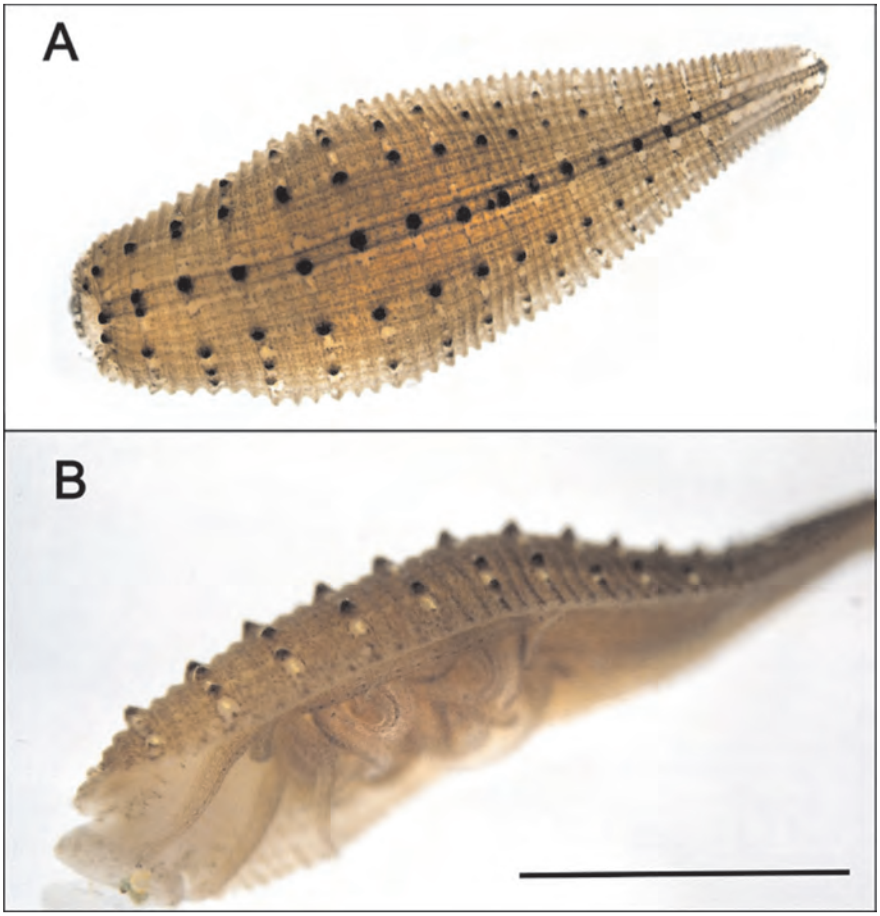

Fig. 2: Adult individuals of *Helobdella europaea*, dorsal (A) and lateral view (B). The caudal sucker is attached to the bottom of the aquarium. Numerous fully developed young are attached to the belly (B). This species is characterized by dark pigmented, cone-shaped papillae, dark longitudinal stripes and white chromatophores on the dorsal side of its body. Bar = 1 cm

3 The occurrence of *H. europaea* in Germany

In April 1987, Mr. H.-A. Pederzani (East Berlin, German Democratic Republic) sent me a letter and reprint of a publication that had been unknown to me. I was informed that the leech I had described as *H. striata* (or a morphologically very similar species) had been found years ago in several freshwater aquaria in Berlin. At this time, my paper (Kutschera 1987) was already accepted for publication. The results described in an article by Pederzani (1980) largely confirmed our observations on the reproductive biology and brooding behaviour of *H. europaea* (Kutschera & Wirtz 1986): this leech feeds its young that it carries on its belly.

In the year 2000 several individuals of *H. europaea* were found in an unnamed pond in Berlin-Tiergarten (Pfeiffer et al. 2004). In addition, I was informed that this leech occurred in an aquarium shop in Halle (most individuals were found in a large container where the leeches were attached to aquatic plants and the shells of water snails).
Hence, *H. europaea* may have been introduced as a "guest" via aquatic plants and occasionally had escaped into the wild. At least in one habitat, a warm freshwater stream in Southern Germany, *H. europaea* had survived two winters and maintained a small but stable population (Kutschera 1985).

According to Nesemann & Neubert (1999) this leech population, investigated in detail during the 1980s, no longer exists. These authors failed to find *H. europaea*-individuals in their German *locus typicus*. Accordingly, Nesemann & Neubert (1999) suggested that the taxon *H. europaea* may be identical with *H. triserialis*: the leeches I had collected may have been imported individuals from North (or South) America that had survived in a German fresh water ecosystem (Fig. 2).

4 Feeding behaviour in captivity

In my original report (Kutschera 1985) and in subsequent articles on the reproductive behaviour of *H. europaea* (Kutschera 1987, Kutschera & Wirtz 1986, 2001, Pfeiffer et al. 2004) the following living prey organisms were identified: Oligochaeta (*Tubifex* sp.), insect larvae (*Chironomus* sp.), water snails (*Physa acuta*, *Radix peregrea*) and Crustacea (*Asellus aquaticus*).

A series of feeding studies, carried out with individuals taken from a large population of *H. europaea* that was maintained in aquaria, led to the following results. Adult and juvenile individuals (average length ~16 and ~3–5 mm, respectively) were starved for seven days and thereafter exposed to a number of aquatic organisms. The hungry leeches sucked the body fluids from the following living or dead prey:

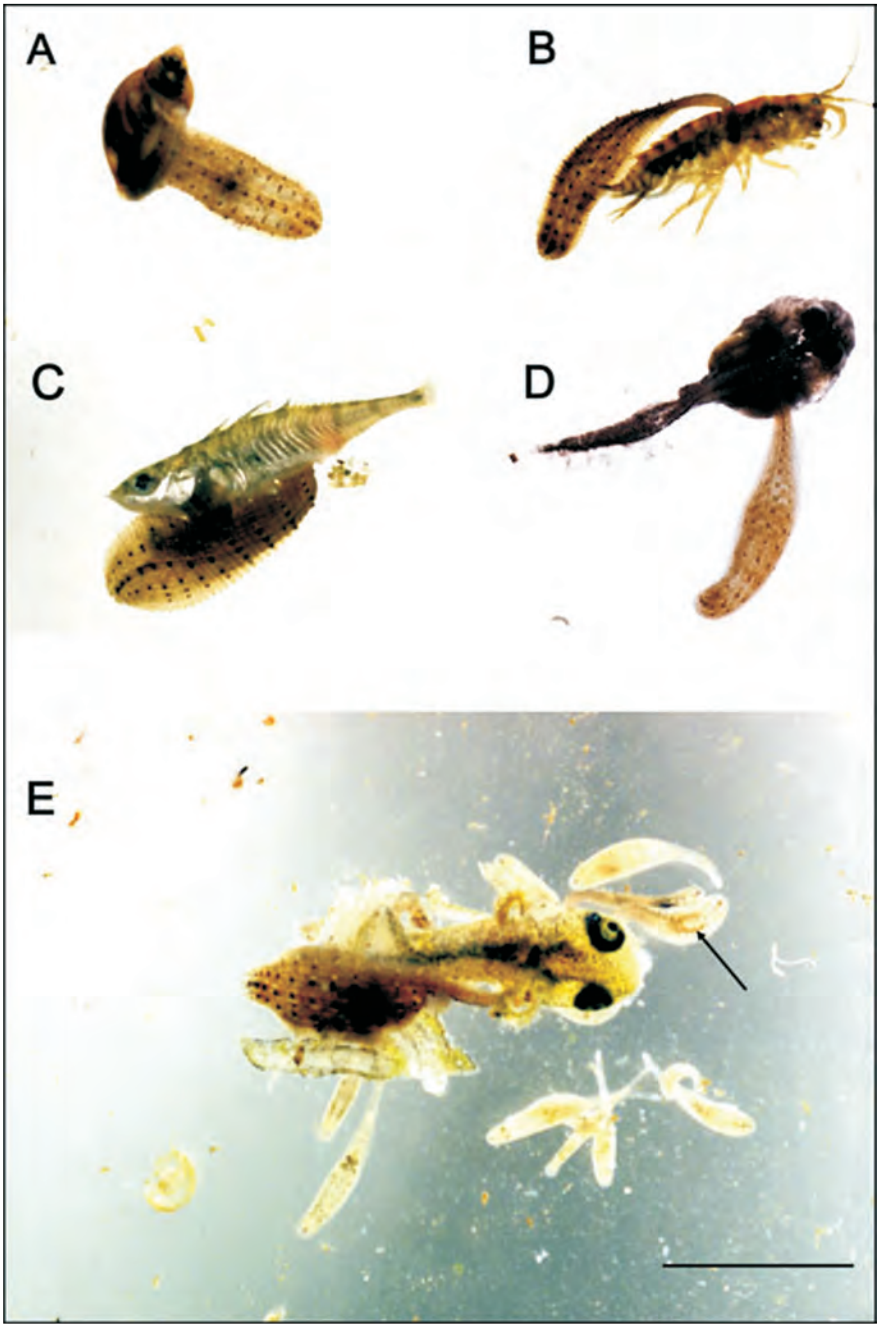
A
B
C
D
E

Fig. 3: Feeding behaviour of adult and juvenile *H. europaea*. The following prey organisms were added to hungry leeches: a living water snail (*Physa fontinalis*) (A), a dead (decaying) body of a *Gammarus pulex* (B), of a young fish (*Gasterosteus aculeatus*) (C), a tadpole (*Rana esculenta*) (D) and a newt larvae (*Triturus vulgaris*) (E). Note that the young leeches that were attached to the belly of the parent (see Fig. 2 B) had left the parent for feeding. *Arrow*: crop caeca of a young leech, filled with body fluids extracted from the dead newt larvae. Bar = 1 cm

1. Invertebrates: water snails (*Physa acuta*, Fig. 3 A; *Bithynia tentaculata*), wounded crustaceans (*Gammarus pulex*) (Fig. 3 B) and small earthworms (*Lumbricus castaneus*), which occasionally fall into the water (not shown).
2. Vertebrates: In numerous trials the following dead animals were attacked: fish (*Gasterosteus aculeatus*) (Fig. 3 C), tadpoles (*Rana esculenta*) (Fig. 3 D), and newt larvae (*Triturus vulgaris*) (Fig. 3 E).

These results show that *H. europaea* is not only a predator, but also a scavenger. Similar results were reported for the common leech *Erpobdella octoculata* (Kutschera 2003). However, I have never observed that hungry *H. europaea*-individuals attacked satiated conspecifics, i. e., no intraspecific predation (cannibalism) occurs in this glossiphoniid species.

5 Phylogenetic position based on molecular data

In order to determine whether the leech *H. europaea* is in fact different from the morphologically very similar taxon *H. triserialis* from North America (see Kutschera 1992), mitochondrial (mt) DNA was isolated from alcohol-preserved specimens of both species. In addition, corresponding DNA extracts were obtained from five other European leech species (*Helobdella stagnalis, Alboglossiphonia heteroclita, Glossiphonia complanata, Hemiclepsis marginata*, and *Theromyzon tessulatum*, family Glossiphoniidae) and an earthworm (*Lumbricus castaneus*). After DNA sequence amplification (710 base pair-fragments of the protein-coding mitochondrial gene cytochrome oxidase subunit I, CO-I, using universal primers), mt-DNA sequencing and sequence alignment (663 bp, excluding primer regions, see Fig. 4) a phylogenetic tree was constructed. The corresponding methods are described in detail by Pfeiffer et al. (2004).

One such DNA-consensus tree, "rooted" using the CO-I sequence of the earthworm *L. castaneus* as outgroup, is shown in Fig. 4. The results demonstrate that all seven members of the Glossiphoniidae represent one monophyletic clade, with a separate branch that includes all three *Helobdella* species. In general, the CO-I-mt-DNA tree agrees well with published data (Apakupakul et al. 1999, Trontelj et al. 1999, Siddall & Borda 2003, Siddall & Burreson 1998). Specifically, the results demonstrate that the taxon *H. europaea* is different from *H. stagnalis* (Germany) and *H. triserialis* (North America). Hence, *H.*

europaea represents a separate species, as suggested in earlier reports (Kutschera 1985, 1987, 1992).

However, when *H. europaea* was compared with *H. papillornata* (Govedich & Davies 1998), a mt-DNA-sequence identity of 98 % was obtained, whereas the identity of alignment positions with *H. triserialis* was only 85 % (Pfeiffer et al. 2004). These results indicate that *H. europaea* from Germany and *H. papillornata* from Australia are identical or very closely related species.

	TTA	TAA	AGT	TTA	ATG	ACC	CTA	GAA	TTG	ATG	AAG	CTC	CAG
H. stagnalis													
H. triserialis	...	...	...	...	...	.T.	...	A..	...	...	.T.	.C.	...
H. europaea	...	...	...	...	...	.T.	...	...	...	...	...	.A.	.T.
A. heteroclita	.G.	...	.A.	...	.A.	.A.	...	A..	...	...	.T.	...	.T.
G. complanata	.A.	...	.A.	...	...	.A.	...	A..	.A.	.A.	...	.A.	.T.
T. tessulatum	.A.	...	.A.	...	...	C..	...	A..	.A.	.A.	.T.	...	.T.
H. marginata	.A.	...	...	...	.A.	.T.	...	AG.	.A.	.G.	.T.	.A.	.T.
L. castaneus	.G.	...	.A.	.A.	T..	C..	.G.	.G.	.A.	.A.	.T.	.C.	.C.

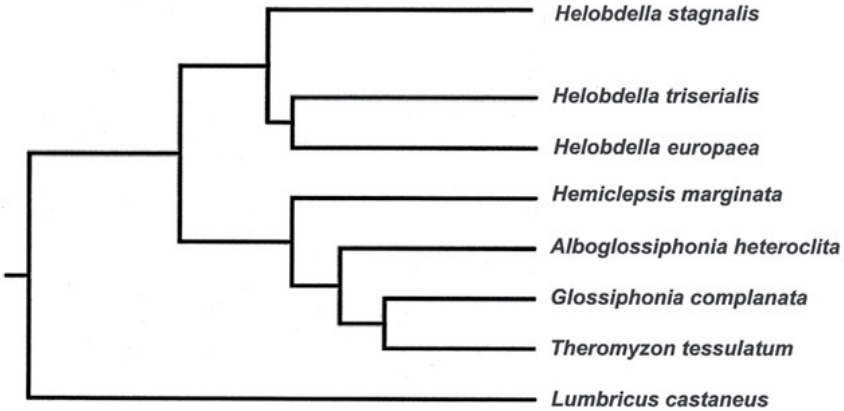

Fig. 4: Aligned DNA sequences (39 base pair segment) from the mitochondrial cytochrome c oxidase subunit I gene (CO-I) of seven leech species and an earthworm (*upper panel*) (data from I. Pfeiffer). A, T, G, C = nucleobases adenine, thymine, guanine and cytosine, respectively. dots = identical nucleobase compared to that of the two-eyed flat leech *H. stagnalis*. Phylogenetic relationships of seven leech species (Glossiphoniidae) with the earthworm *Lumbricus castaneus* as outgroup (*lower panel*) (Adapted from Pfeiffer et al. 2004)

6 Conclusions

The freshwater leech *Helobdella europaea* (Kutschera 1987) was found two decades ago in a fast-running creek in Southern Germany, where a wild population survived at least two subsequent winters. Sawyer (1986, p. 711) noted in his key to the freshwater and terrestrial Euhirudinea of the Palaearctic region (Europe) that "a new species resembling *H. triserialis* was recently found in southern Germany (Kutschera, per. comm.)". The original population of this second *Helobdella* species no longer exists (Nesemann & Neubert 1999). Over the past 20 years, no second free-living *H. europaea* population has been discovered throughout Europe, with the exception of a few individuals collected in a small pond in Berlin-Tiergarten in September 2000 (unpublished observations). Because a number of freshwater aquaria are housed in the buildings of the Zoological Garden of Berlin, these leeches may have escaped into the pond where I found them.

My earlier conclusion that *H. europaea* is a second native German *Helobdella* species (Kutschera 1987) is no longer acceptable. All available evidence indicates that *H. europaea* is an imported annelid that reached Europe via the German aquarium trade. The leeches I collected in the years 1982 to 1985 in the wild were obviously introduced into the stream where I collected them, possibly attached to water plants or prey organisms (shells of aquatic snails).

Since the genus *Helobdella* has its center and evolutionary origin in South America and many freshwater fish (and plants) are imported to Europe from this continent, it is suggested that *H. europaea* (syn. *H. papillornata* found in Australia) may be a species from South America (Siddall & Borda 2003). In contrast to *H. triserialis*, which is restricted to water snails with respect to its feeding strategy, *H. europaea* sucks on a variety of prey organisms, including dead vertebrates. This omnivorous behaviour may explain why this leech had survived in German freshwater habitats and may occur as a "guest" in streams, lakes and ponds on other continents as well (several colleagues have sent me unpublished reports on the occurrence of *H. europaea*-like leeches from Hawaii, South Africa and New Zealand).

In a recent molecular phylogenetic analysis it was shown that *H. europaea* belongs to the South American *Helobdella triserialis* species complex (Siddall & Budinoff, per. comm.). These results are in accordance with our phylogenetic tree (Fig. 4) and support the hypothesis of a widespread introduction of *H. europaea* via the world-wide aquarium trade.

Acknowledgement

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Address of the author: Prof. Dr. U. Kutschera, Institut für Biologie, Universität Kassel, Heinrich-Plett-Str. 40, D-34109 Kassel, Germany. E-mail: kut@uni-kassel.de

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