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# The taxonomic status of the San Francisco Bay area Leech *Helobdella triserialis* (SF) (Annelida Hirudinida Glossiphoniidae) with notes on its ecology

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**ABSTRACT** Over the past decades, freshwater leeches of the genus *Helobdella* Blanchard, 1896 (Annelida: Hirudinida: Glossiphoniidae) have been used as model organisms for developmental studies. Notably, the species "*Helobdella triserialis* US", discovered during the 1970s in ponds in San Francisco (California , USA) was kept in lab-populations and served as representative of the genus, as documented in numerous publications. Here, I show that this enigmatic "San Francisco Bay area leech" has been misclassified and confused with the South American species *H. triserialis* (E. Blanchard, 1849), and later with *H. papillata* (Moore, 1952). Using specimens collected in Matadero Creek, Palo Alto (California , USA), novel morphological, anatomical, behavioral and molecular data (novel mitochondrial 618 bp-COI gene sequence) were generated. Based on these results, it is shown that this taxon represents a new North American species, described here as *Helobdella farmeri* n. sp. In addition, the occurrence of extant and extinct populations of *Helobdella* sp. in natural habitats of Northern California is documented over the past three decades.

**KEY WORDS** Annelida; Hirudinida; *Helobdella*; new species, biodiversity.

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## INTRODUCTION

In October 1985, the author discovered a dense population of leeches of the genus *Helobdella* Blanchard, 1896 (Annelida Hirudinida Glossiphoniidae) in a small creek on campus of Stanford University (California, USA). These small, hermaphroditic freshwater invertebrates (length of adult animals ca. 12 to 15 mm) co-occurred with populations of water snails *Physella gyrina* (Say, 1821), which served as host organisms of these aquatic parasites. Close examination revealed that these *Helobdella* individuals resembled the taxon *H. europaea* (Kutschera, 1987), a species described at a time when my systematic study of the Stanford-leech-population began.

Based on the literature available during the 1980s, the leeches were assigned to the South American taxon *Helobdella triserialis* (E. Blanchard, 1849), and a few years later, a report on sexual reproduction, parental care, and feeding behavior of these "Stanford-annelids" was published (Kutschera, 1992).

Over decades, this leech species, which also occurred during the 1970s and 1980s in ponds and streams in San Francisco (California, USA) served as model organism for developmental studies

(Weisblat et al., 1978; Sawyer, 1986; Bely & Weisblat, 2006; Kutschera & Weisblat, 2015). Due to the fact that the taxonomic status of this model species was a matter of debate, Kutschera & Weisblat (2015) referred to these enigmatic annelids as the "San Francisco Bay area leech *Helobdella triserialis* (SF)". Here, I document that this taxon, denoted as "*Helobdella triserialis*-US", represents a new, undescribed species, with information on its occurrence and ecology in different habitats in Northern California over the past 32 years.

The type specimen of the newly described taxon *Helobdella farmeri* n. sp., and paratypes were collected between 2008 and 2019 in Matadero Creek, Palo Alto, CA, USA.

## MATERIAL AND METHODS

Between October 1985 and March 2019, freshwater leeches of the genus *Helobdella* were collected in ponds and streams in the San Francisco Bay area of Northern California, USA. Most individuals were attached to the underside of stones, leaves or pieces of bark. The leeches were gently removed from their substrate using soft forceps, and kept in water taken from their natural habitat. Groups of leeches (3 to 4 individuals) were cultivated in petri dishes equipped with pond water and a flat "hiding stone" at room temperature. In addition, breeding populations of ca. 20 to 50 adult individuals were kept in the laboratory (glass jars) as described by Kutschera et al. (2013). The leeches were fed on water snails and frozen/thawed "bloodworms" (*Chironomus* larvae). Selected individuals were photographed for documentation of morphological features, behavior and feeding activity.

The samples were fixed in 10% formaline and stored in 70% ethanol at room temperature.

Histological studies on fixed-stained paratypes of the new species described (see below) were carried out as described by Hovingh and Kutschera (2020) and documented via series of light-micrographs. For DNA extraction, paratypes, collected in Matadero Creek, were fixed in 70% ethanol. Small pieces of tissue were cut from the posterior sucker and used as source of mitochondrial DNA. For genetic characterization, three adult individuals, collected in Matadero Creek, were analyzed, using the methods for determination of part of the mitochondrial gene of cytochrome c oxidase subunit I (COI) as described by Schmerer et al. (2013) (for details, see Pfeiffer et al., 2004). The newly acquired sequence was published on June 8, 2023 at GenBank. Sequence alignments were carried out according to standard protocols and given as percentage distance, with reference to the newly acquired COI-sequence of *Helobdella farmeri* n. sp. (= 100%).

## RESULTS

### *Natural habitats and biodiversity of leeches*

In 1975, Roy T. Sawyer (Weisblat et al., 1978) investigated the aquatic ecosystems in Golden Gate Park (San Francisco, California, USA). In Stow Lake, he discovered populations of leeches he assigned to the taxon *Helobdella triserialis*. Based on individuals of these invertebrates, which were previously found exclusively in South America, lab-populations were established during the late 1970s/early 1980s at the University of California (Berkeley) and other Institutions.

In October 1985, I investigated the then clear water of Stow Lake and found, attached to stones in the flat-water zones, numerous individuals of Sawyer's "*H. triserialis*-US" (Fig. 1). Moreover, in the same aquatic habitat, often under the same small, flat stones, I collected "*Helobdella stagnalis*"-like leeches that turned out to be a new species, later described as *H. californica* (Kutschera, 1988, 2011).

In 2007, the water of Stow Lake was no longer clear, but had developed a green color due to the growth of large populations of aquatic micro-algae (eutrophication). As a result, the leech populations of the 1980s had disappeared.

At that time (October 1985) I began to study the small, unnamed freshwater habitats on campus of Stanford University, and discovered, in one of these creeks that runs perpendicular to "Palm Drive", a population of "*H. triserialis* US". In this slowly running water system, no other leech species occurred, but numerous water snails *Physella gyrina* co-existed with these leeches, and served as prey organism (Fig. 2). However, in 2007, this leech population that I had studied between October 1985 and September 1987, no longer existed. Only fallen,

rotten leaves were collected in this small creek, with no aquatic animals attached.

In March 2008, when I investigated the fauna of Matadero Creek, Palo Alto, California, I discovered a population of "*H. triserialis* US" that co-occurred with small water snails (*Physella gyrina*) and other aquatic invertebrates, such as insect larvae (Fig. 3). These specimens has collected between 2008 and 2019 in Matadero Creek (coordinates: 37°25'27" N - 122°08'01''W; collection site: intersection Matadero Avenue/Josina Av., Palo Alto, CA 94306, USA). The Matadero Creek (Fig. 3) begins in Los Altos Hills, traverses the area of Stanford University (Fig. 2) and Palo Alto, until it enters the Palo Alto Flood Basin, joining Adobe Creek, before culminating in southwest San Francisco Bay.

As mentioned by Kutschera & Weisblat (2015), in the Matadero Creek also some individuals of "*Helobdella stagnalis*-like leeches" occur that are characterized by a uniform, creamy body colour and a nuchal plate in the neck of the head region (see also Hovingh & Kutschera, 2020). The taxonomic status of these aquatic invertebrates is currently under investigation.

## *Confusing taxonomy of* "Helobdella triserialis US"

During the 1980s, when I studied the now extinct "Stow Lake" and "Stanford creek"-leech populations (Figs. 1, 2), the systematic position of these hirudineans appeared to be settled. As described in detail by Sawyer (1986), they were thought to represent sub-populations of the South American species *H. triserialis*, and my report on the biology of these aquatic annelids followed this taxonomy (Kutschera, 1992).

However, based on the study of individuals collected in Bolivia-South America, Siddall and Borda (2003) concluded that "*H. triserialis* US" from Michigan is identical with the taxon *H. papillata* (Moore, 1952). Accordingly, the Californian "*H.*


Figures 1, 2. Natural habitats of populations of the "San Francisco Bay area leech *Helobdella triserialis* (SF)" during the 1980s: Stow Lake in Golden Gate Park, San Francisco, CA (Fig. 1) and an unnamed, small creek running perpendicular to Palm Drive on Campus of Stanford University (Fig. 2). The photographs were taken in April 1986.


Figures 3, 4. Natural habitat and *locus typicus* of the leech *Helobdella farmeri* n. sp.: Matadero Creek in Palo Alto, CA 94306, USA (Fig. 3). Photograph of a group of leeches collected from the underside of stones in March 2009, and an individual of an "*H. stagnalis*-like" leech from the same habitat (Fig. 4).

*triserialis* US", were assigned to this taxon. Three years later, Bely & Weisblat (2006) documented via gene sequence-analyses that the "San Francisco-Stow Lake *H. triserialis*-US"-individuals are not identical with *H. papillata* (Moore, 1952), as proposed by Siddall & Borda (2003). Nevertheless, in accordance with the pertinent literature on the taxonomy of North American freshwater leeches (see Wetzel et al. 2020), Kutschera, Langguth, Kuo, Weisblat & Shankland (2013), in their description of *H. austinensis* n. sp., referred to the *Helobdella*-leeches from Matadero Creek, Palo Alto, California, as "*H. papillata*".

This assignment was questioned by Schmerer et al. (2013), who pointed out that the taxonomic status of these "Matadero Creek-*Helobdella*-leeches" is unclear. Due to these confusing, and contradictory interpretations, Kutschera & Weisblat (2015) coined the name "San Francisco Bay area leech *Helobdella triserialis* (SF)", but it should be noted that the original Stow Lake-SF-population, to which this name refers, no longer exists. More recently, Iyer et al. (2019) referred to this taxon as "H. triserialis*...originally collected in San Francisco CA in the 1970s*" and remarked that "*it* (i.e. this species) *was lost from the laboratory and disappeared from its original location*". Finally, Morhun et al. (2021) used the name "H. triserialis *California USA*", in apparent accordance with Kutschera & Weisblat's (2015) terminology.

In 1986, I collected numerous individuals of "*H. triserialis* US" in the creeks of the Botanical Garden of Golden Gate Park, SF-USA. Between 2007 and 2019, I was looking for these aquatic invertebrates, but could not find a single "San Francisco Bay area leech" in the aquatic ecosystems of this area, where populations of *Helobdella californica* are abundant (Kutschera 1988, 2011). Obviously, these wild breeding colonies of "*H. triserialis* US" likewise no longer exist from 2007 onwards. Only individuals from Matadero Creek (Figs. 3, 4) could be collected (between 2008 and 2019), on which the following description of "*H. triserialis* US" as a *nova species* rests.

## *Systematics*

***Helobdella farmeri*** n. sp.
https://www.zoobank.org/FC0D7DF5-E0DB-48AA-B8E8-4847A8F55311

LOCUS TYPICUS. Matadero Creek, Palo Alto, California CA 94306, USA (Figs. 3, 4).

TYPE MATERIAL. One individual of average size and morphology was selected from six leeches collected in 2015 in the wild, fixed in 10% formalin,

and stored in 70% ethanol. This holotype (Fig. 5), and five paratypes from the same locality, were deposited in the Department of Invertebrate Zoology and Geology, California Academy of Sciences, 55 Music Concourse Drive, Golden Gate Park, San Francisco, CA 94118 (USA). Holotype: Ethanol-fixed leech, CASZ No. 228696; Paratypes: 5 ethanol-fixed individuals, CASZ No. 228697.

DESCRIPTION OF THE HOLOTYPE. Morphology: Size of the ethanol-fixed holotype: body length at rest: 12.5 mm; max. width: 4.5 mm.

Shape and annulation (Figs. 4, 5): lanceolate body form, broadest in the anterior part of the animal. Head region is comprised of segments I through IV; no mid-dorsal scute present, as in *H. stagnalis* and related taxa (see Fig. 4). One pair of triangular eyes at segments III and IV. The yellow-brown dorsal side of the body convex, with an orthogonal array of white pigment spots; in most specimens, six white spots arranged perpendicular to the body axis. Twelve longitudinal, dark stripes, inclusive of two more pronounced dark-pigmented lines in the middle of the body, reaching from the head region (eyes) to the posterior sucker; no conical papillae present. Ventral side flat-concave, uniformly light-brown, with no pigment spots.

Anterior sucker oval, mouth pore in sub-terminal position. Posterior (caudal) sucker concave-circular, diameter about 1/3 of the maximum body width (Figs. 5, 6).

Annulation along the body axis: Segments I, II / III, IV (head region): uni- / bi-annulate, respectively; midbody region (V through XXIV) tri-annulate, subdivided (a1, a2, a3); segments XXV and XXVI bi-annulate, and XXVII uni-annulate.

VARIABILITY. Paratypes: length: 8.0 to 15.0 mm; max. width: 4.0 to 6.0 mm. Living animals in resting position are about the same size. As documented in Figure 4, living specimens are relatively uniform in color pattern and pigment spot-distribution; among 100 individuals investigated, a minority of 5 was darker-pigmented than the average (brown instead of yellow-brown), and 2 leeches of the population consisted of yellow-colored individuals that nevertheless displayed the "8 to 14 longitudinal lines/6 white-pigment-spots"pattern observed in 100 specimens.

Anatomy. The internal organization of the organs was reconstructed based on series of sections through para-types collected in the wild (Fig. 6).

Alimentary tract: Pharynx eversible (i.e., proboscis, enclosed in a sheath), diffuse salivary glands, plus gastric and intestinal caeca. Six branched crop caeca, the nos. 2 to 6 posteriorly curved (depicted in Kutschera, 1987, and visible during/after feeding on water snails or frozen/thawed *Chironomus* larvae, Fig. 8). Intestine with four conspicuous lobes, reaching from segments XXIX through XXIV; anus at segments XXVI/XXVII, located on the dorsal side of the body.

Reproductive system. The primary sexual organs consist of the atrium, with the male gonopore on segment XIIa1/a2, and the female gonopore, separated by one annulus, on XIIa2/a3. Six pairs of testisacs that can be observed in sexually mature individuals on the ventral side of the body. Two tube-shaped ovisacs with branches, visible in adult individuals before egg-deposition, extending through body segment XVII.

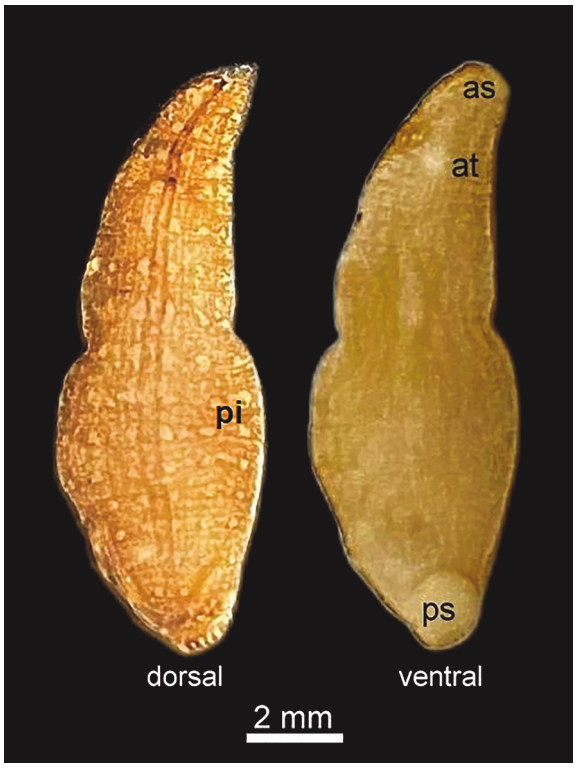


Figure 5. Dorsal and ventral views of the formaline-fixed/ethanol-stored holotype of *Helobdella farmeri* n. sp Note the six white pigment spots arranged perpendicular to the axis of the body , and the longitudinal stripes. as = anterior sucker; at = atrium; pi = pigment spot; ps = posterior sucker.

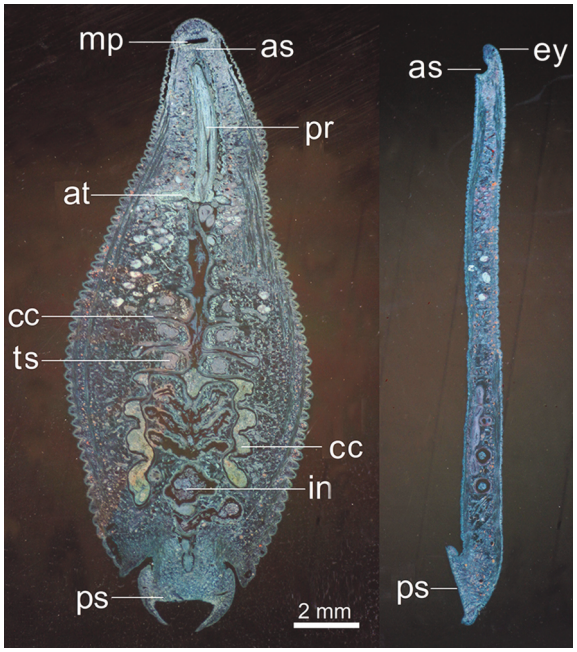


Figure 6. Stained longitudinal slices of a paratype of *Helobdella farmeri* n. sp., showing parts of the digestive and reproductive system of an adult, sexually mature animal. as = anterior sucker; at = atrium; cc = crop caeca; ey = eye; in = intestine; mp = mouth pore; sg = salivary glands; ts = testisac.

ETYMOLOGY. The taxon-specific name, *farmeri,* is in honor of Dr. Steve Farmer, PhD (Stanford University) head of the Systems Biology Group in Palo Alto, CA 94306, USA, who invited the author repeatedly to visit his organization. On the evenings and weekends of these visits to Palo Alto, I collected the Matadero Creek-leeches described here as *a nova* species, and performed behavioral studies on *Helobdella*-specimens kept in glass jars and fed them on frozen/thawed insect larvae. In addition, the name refers to "The Farm", a nickname of Stanford University, where a dense population of *H. farmeri* n. sp. was discovered by the author in October 1985 (Fig. 2), as documented in two scientific reports based on studies of these leeches (Kutschera 1987; 1992).

### *Phylogenetic analysis of samples collected in Matadero Creek*

In 2010, I sent three adult leeches that I had collected in Matadero Creek via D.A. Weisblat (UC Berkeley, CA, USA) to M. Shankland, University of Texas at Austin, Texas, USA. Based on these individuals, a breeding colony of "*H. triserialis* US" was established at this institution. To evaluate the taxonomic status of these leeches, Schmerer et al. (2013) analysed tissue samples from descendants of these leeches kept under artificial lab-conditions: "*The* H. triserialis *colony was established in 2011 with first generation progeny of wild leeches collected in Palo Alto, CA, by Ulrich Kutschera… species identity was verified by sequencing a fragment of (the mitochondrial gene) cytochrome c oxidase subunit I (COI) amplified with primers…*" (Schmerer et al., 2013).

The authors obtained a sequence (GenBank Acc.-No. KC771417) that was at 628 of 630 nucleotide positions identical to the "*H. triserialis* US (San Francisco)"-sequence deposited at GenBank years ago under DQ995303. This high level of COI-sequence identity (99.7%) documents that "*H. triserialis* US (Palo Alto)" should be assigned to the (now extinct) San Francisco-Stow Lake-population studied during the 1970s and 1980s. The distance between Stow Lake-SF and Matadero Creek-Palo Alto, California, is ca. 50 km. Freshwater leeches are frequently moved from one place (creek, stream, pond) to the next by mobile water birds, who migrate from one pond to nearby aquatic ecosystems.

In order to verify the finding of Shankland et al. (2013), I repeated their sequencing study, using the same methods as have been employed by these authors. However, in my experiments, "*H. triserialis* US"-leeches from the wild (Matadero Creek, Palo Alto, CA) were used as source for DNA extraction.

Three independent experiments yielded the same result: a new 618 bp-DNA-COI-sequence, deposited at GenBank on June 8, 2023, under No. OR075101 (*Helobdella* sp. f TH-2023 voucher Hel2 cytochrome c oxidase subunit I gene, partial cds; mitochondrial). This COI-sequence was found to be 100% identical to Schmerer et al.'s (2013) sequence No. KC771417, obtained from next-generation leeches cultivated in the laboratory at the U. of Austin, Texas.

Sequence alignment-studies yielded the following results, with the new 618 bp-fragment OR075101 labelled as 100%. The next-closely related *Helobdella*-taxa at the COI-level were found to be *H. socimulcensis* Caballero 1937 (see Salas-

Montiel et al. 2014) from South America (97.3%), and *H. europaea* Kutschera, 1987, discovered by the author in a stream in Germany, and in the meantime found worldwide in a variety of aquatic ecosystems (95.5%). However, the sequence similarity with *H. papillata* from Michigan (Moore, 1952; Siddall & Borda, 2003; Moser et al., 2011, 2013) was found to be much lower (83.6%). Accordingly, this novel analysis shows that the assignment of "*H. triserialis* US" (i. e. *H. farmeri* n. sp.) to the taxon *H. papillata* is not justified (see Kutschera et al., 2013; Wetzel et al., 2020).

In summary, these sequence alignments and quantitative data show that the newly described taxon *Helobdella farmeri* n. sp. from Matadero Creek (Figs. 3, 4) is genetically identifiable at the level of its unique COI-sequence (OR075101, identical with KC771417). These facts are illustrated in Fig. 7, which includes another photograph of a typical individual of *Helobdella farmeri* n. sp., documenting the low intraspecific variability of this leech species. Figure 8 corroborates this finding and shows that adult *H. farmeri* n. sp. not only suck off the body fluids of water snails ( Kutschera, 1992),

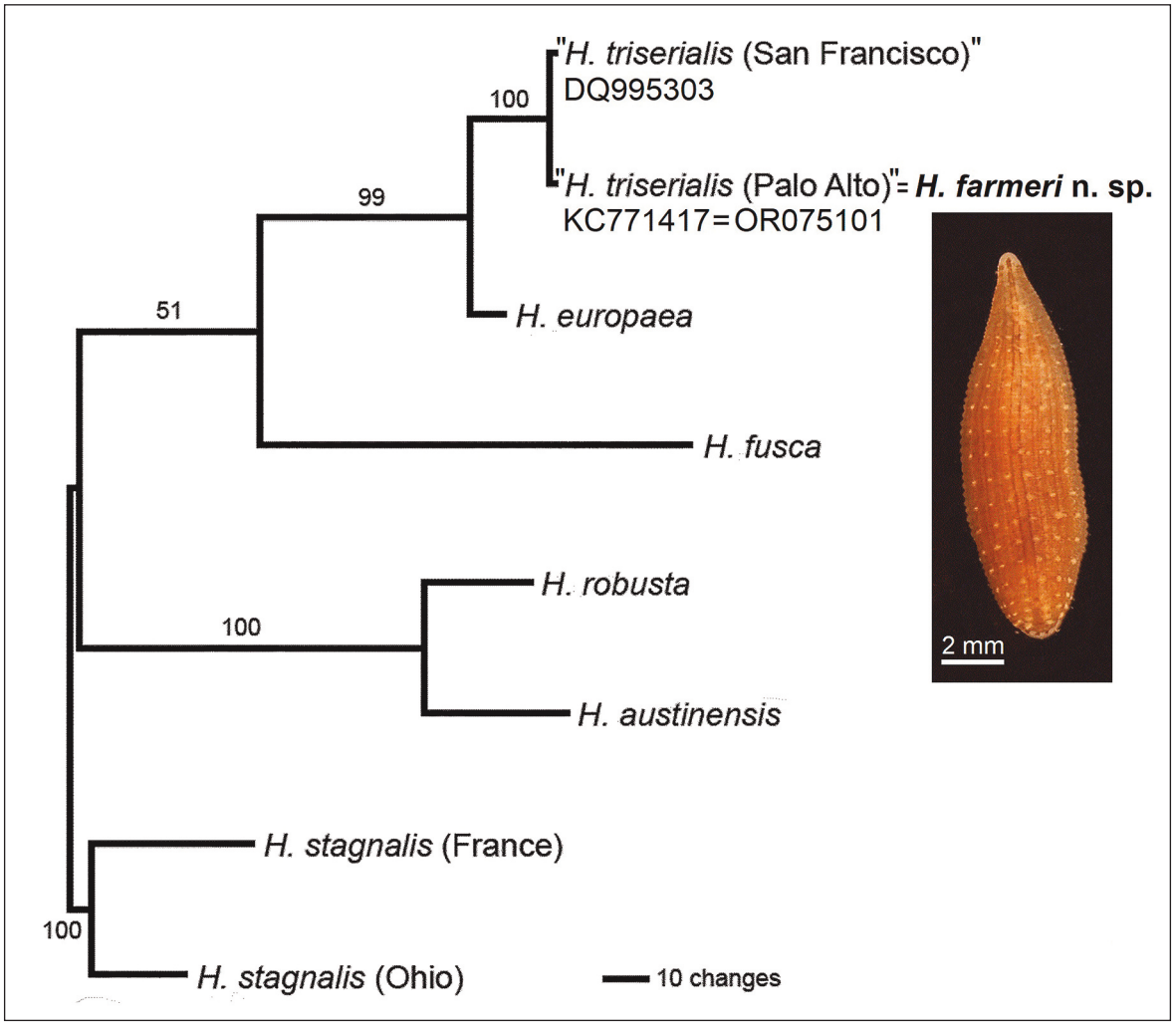


Figure 7. Mitochondrial gene tree of partial cytochrome c oxidase I (COI) sequences from a selection of *Helobdella* species. The GenBank acc. numbers are given for "*H. triserialis* San Francisco", "*H. triserialis* Palo Alto", and *H. farmeri* n. sp., which is 100 % identical with the former isolate at the COI-level. The GenBank acc. ns. for the other species are DQ995298 (*H. europaea*), AF329038 *(H. fusca*), DQ995301 (*H. robusta*), DQ995306 *(H. austinensis*), AF116018 for *H. stagnalis*-France, and AF329040 for *H. stagnalis*–Ohio. A photograph of a paratype was added to the phylogram (adapted and modified from Schmerer et al., 2013).

but also feed on frozen/thawed insect larvae. During these feeding episodes, sometimes two or more leeches attach via their posterior sucker to the prey organism and insert their eversible proboscis to draw body fluids into their digestive tract.

## DISCUSSION

In 1849, the French zoologist Emile Blanchard (1819–1900) described the freshwater leech *Helobdella (Glossiphonia) triserialis* based on specimens collected in Chile (South America) (E. Blanchard, 1849). In contrast to the European type species of this genus, *Helobdella (Hirudo) stagnalis* Linnaeus, 1758, Blanchard's *H. triserialis* lacks a scute in the neck region of the body, and is characterized by dorsal stripes and yellow pigment spots, as well as a much lower agility than that observed in *H. stagnalis* (Kutschera & Weisblat, 2015).

Decades later, Raphael Blanchard (1857–1919) provided a concise definition of leeches of the newly established genus *Helobdella,* which include the morphological features of two eyes and one annulus between the male-female gonopores in these hermaphrodites. R. Blanchand (1896) finally established the name "*Helobdella triserialis*", which is still valid today. Hence, the papers of E. Blanchard (1849) and R. Blanchard (1896) are key publications in this area of biodiversity research. Today, more than 50 *Helobdella*-species have been described from freshwater ecosystems around the world (Beresic-Perrins et al., 2017; Saglam et al., 2018; Iwama et al., 2019; Bassett et al., 2022; Bolotov et al., 2022).

In a report of the freshwater leeches on campus of Stanford University, the diagnostic features distinguishing *Helobdella europaea* from "*H. triserialis*-US" were summarized (Kutschera, 1987). The key figure of this article, showing the branched crop caeca in the latter species, were recently reproduced by Morhun et al. (2021), who provided phylogenetic data documenting that "*H. triserialis*-US" may be regarded as a mislabeled, undescribed species, as suggested by Schmerer et al. (2013) and by Kutschera & Weisblat (2015).

Thirty one years ago, the feeding behavior and sexual reproduction/parental care pattern of the "San Francisco Bay area leech *Helobdella triserialis* (SF)" was described in detail (Kutschera 1992; see also Kutschera & Wirtz, 2001). However, it should be noted that the leeches I collected during the 1980s on campus of Stanford University (Fig. 2), and, at lower abundance, also in Stow Lake, San Francisco (Fig. 1), and in ponds of the Botanical Garden/Golden Gate Park, were on average larger and more sturdy than the Matadero Creek-Palo Alto-individuals, on which the present report is based. This striking phenotypic plasticity may be due to the fact that the Stanford-Palm Drive-leech population of the 1980s co-existed with aquatic snails, which often crept out of the water to escape their predators (usually, an adult leech completely empties the shell of its prey organism, as documented by Kutschera et al. (2013). In contrast to this "Palm Drive" habitat, in Matadero Creek (Fig. 3), freshwater smails occurred at low abundance, and insect larvae (*Chironomus*, see Fig. 8) were found, often attached to the underside of stones, where the *Helobdella*-leeches occurred. I suggest that the Stanford-*Helobdella*-populations of the 1980s were exclusively maintained on water snails as prey organisms, whereas the less frequently observed Matadero Creek-leeches fed on snails and insect larvae. This difference in feeding options may explain the observation that the snail-fed *Helobdella*-individiuals in the Creek at Palm Drive, Stanford, were, on average, ca. 10 to 20% larger and sturdier than their conspecifics I found in Matadero Creek from 2008 to 2019.

In summary, the results of this study show that Kutschera and Weisblat's "San Francisco Bay area leech *Helobdella triserialis* (SF)" represents a new taxon that is described here as *Helobdella farmeri* n. sp. (Figs. 4–8). This new North American species is characterized by unique morphological-anatomical features as well as by a taxon-specific COI-gene-sequence. With this establishment of a *nova species*, the decade-old discussions as to the status of this enigmatic leech, used as model organism during the 1980/90s (Weisblat et al., 1978; Kutschera & Weisblat, 2015) may be finished. However, progress in biodiversity research will continue, so that other "*Helobdella*-problems" in leech systematics, ecology and evolution will remain a challenge for future research.

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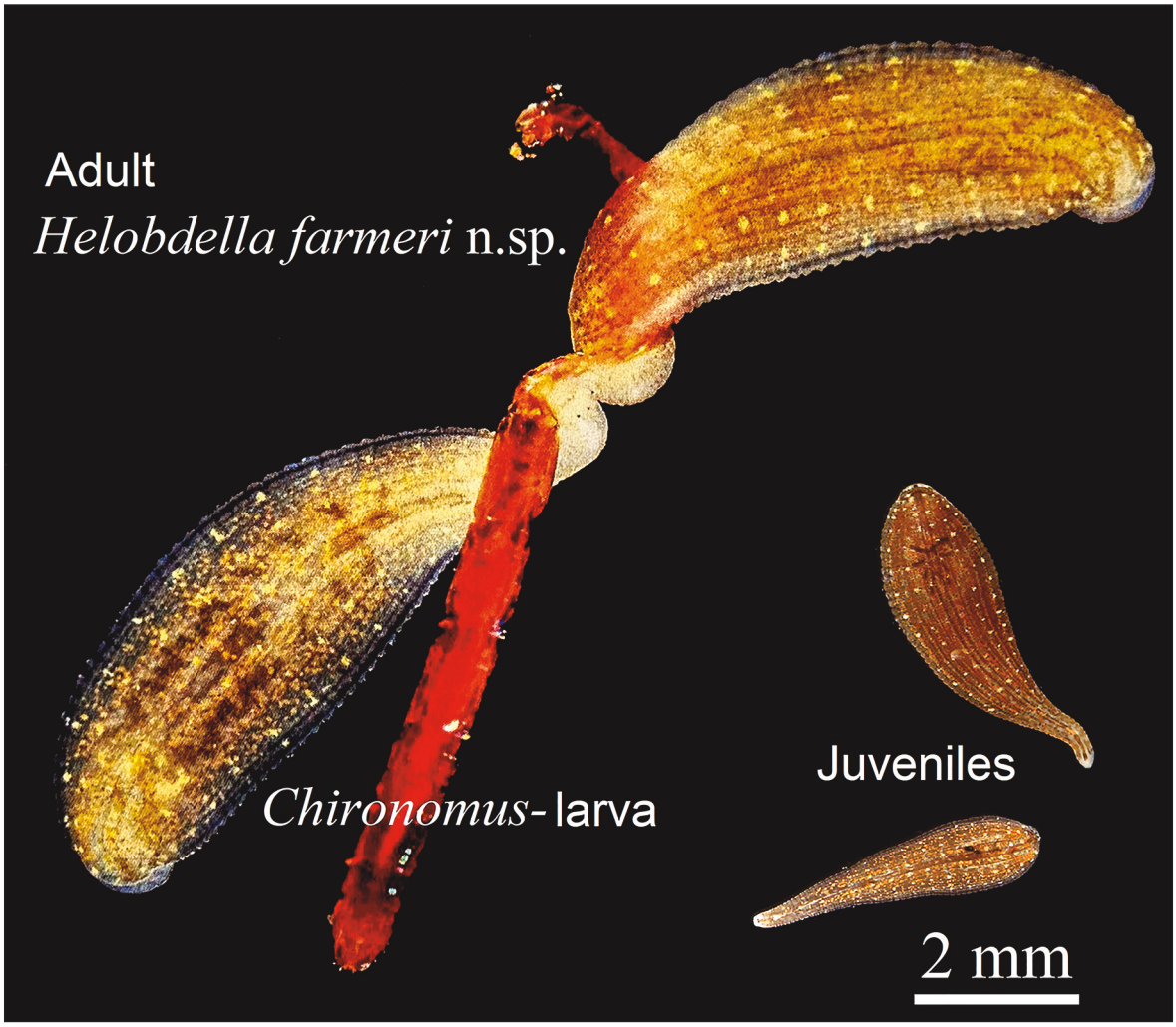


Figure 8. Photograph of two juvenile and 2 adult *Helobdella farmeri* n. sp. in dorsal view. The leeches were kept in a petri dish and fed on a frozen-thawed *Chironomus* larva, from which the adult individuals sucked body fluids (haemolymph).

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