Biodiversity Journal, 2020,11 (3): 689–698, https://doi.org/10.31396/Biodiv.Jour.2020.11.3.689.698
http://zoobank.org:pub:8006DBB4-CF4F-4CE4-BAD3-0A7BB4857FC3

Two new *Helobdella* species (Annelida Hirudinida Glossiphoniidae) from the Intermountain region of the United States, formerly considered as *Helobdella stagnalis* Linnaeus, 1758

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ABSTRACT Two *Helobdella stagnalis*-like leech specimens (Annelida Hirudinida Glossiphoniidae) were histologically examined from Nevada in the Great Basin, and from Utah in the Colorado River Basin (USA) to determine whether or not their crops were similar to those in *H. californica* Kutschera 1988. The Nevada form was brown and with pigmentation patterns, whereas the Utah form was plain and white. The dorsoventral histological sectioning of these 3 specimens showed the Utah and Nevada forms had compact salivary glands, hitherto noted only in the South American *Helobdella* and *Haementaria* species. The pharynx of Nevada individuals was S-shaped, and in the Utah form the ejaculatory ducts formed a Gordian knot in the distal-most posterior region, further distinguishing these 2 intermountain *Helobdella*-isolates. Comparing these two taxa to other published *Helobdella* internal morphologies, two new species are proposed: *Helobdella humboldtensis* n. sp. from Nevada (size and pigmentation similar to *H. californica*) and *Helobdella gordiana* n. sp. from Utah, which resembles *H. stagnalis* from Europe. These findings suggest the Intermountain area may be a prime region to study the evolution of members of the *Helobdella* species complex.

KEY WORDS Hirudinida; *Helobdella stagnalis*; Leeches; Evolution; new species.

Received 25.06.2020; accepted 07.08.2020; published online 18.09.2020

INTRODUCTION

In this last century, the two-eyed flat leech *Helobdella stagnalis* Linnaeus, 1758 (Annelida Hirudinida Glossiphoniidae) was considered a Holarctic species with no morphological differences (Klemm, 1985; Sawyer, 1986; Siddall & Borda, 2003). Using molecular phylogeny, Siddall et al. (2005) resurrected the Nearctic (North American) taxon *H. modesta* Verrill 1872, which was distinguishable from the Paleoarctic *H. stagnalis*, based on one specimen from each continent.

Moser et al. (2011) re-examined *H. modesta* (i.e., Verrill's type locality, using specimens from Connecticut) and noted a 15 % genetic divergence between Connecticut (USA) and England (UK), 16% between Connecticut and Ohio, and 18% between Ohio and England. Using 1.8% per million years (see Smith and Dowling 2008 for the intermountain fish *Rhinichthys osculus* mitochondrial DNA), these data indicate evolutionary events between the United Kingdom (Europe) and Ohio (USA) 4.4 Million years ago (Ma), and Connecticut 8.3 Ma, and between Ohio and Connecticut 8.8 Ma. These Miocene-Pliocene population antiquities suggest species stasis since this Miocene evolutionary

burst, and the Beringian trans-continental crossing from North America to Asia at this time.

The *Helobdella* Blanchard, 1896 taxonomic status has changed since the morphological species descriptions by Klemm (1985) and Sawyer (1986), and with the advent of molecular phylogenetic studies (Siddall et al., 2005; Bely & Weisblat, 2006; Moser et al., 2011, 2013; Kutschera et al., 2013). More recent efforts demonstrated even larger changes, with Beresic-Perrins et al. (2017) studying a unique site and adjacent drainages in the Colorado River Basin, and Saglam et al. (2018) describing the anatomy of 5 new species. Recently, Iwama et al. (2019) described the Sweden neo-type specimen of *H. stagnalis* and noted 6 new genetic forms over a large geographical range. As a result of these revisions, the distribution of the *Helobdella* species is unknown in the Intermountain Region of the USA (Wetzel et al., 2020).

We used the historic *Helobdella stagnalis* name in reference to all specimens lacking morphological, distributional, and taxonomic identification. Shortly after 2001, we noted populations of *H. stagnalis* with brown-colored speckles or spots, and with paramedial fine lines. To determine whether or not these specimens were members of the taxon *H. californica* (Kutschera, 1988), the first author submitted 3 specimens, the California Stow Lake Golden Gate Park *H. californica*, the Nevada pigmented *Helobdella*, and the Utah typical light plain *Helobdella*, for dorsal-ventral serial sectioning histology. The results showed the described branched caeca of the "California leech", and its absence in the Nevada and Utah isolates, confirming the pigmented Nevada form was *H. stagnalis*.

With the recent surge of works, we resurrected the drawings made at that time and compared them to the findings of Salas-Montiel et al. (2014), Beresic-Perrins et al. (2017), and Saglam et al. (2018), noticing the Utah and Nevada anatomical features were unlike any in these illustrations. This paper first describes in more detail our reference species *H. californica*. Then, we compare the Intermountain *H. stagnalis* forms, and propose two new North American species.

MATERIAL AND METHODS

Living leeches of the genus *Helobdella* were collected in aquatic ecosystems in Europe (Kassel, Germany) and California (USA). Populations of 5 to 20 individuals were maintained in aquaria and fed with *Tubifex* worms or *Chironomus* larvae. Photographs of these agile predators were taken as described by Kutschera & Weisblat (2015) (Fig. 1). In addition, *H. stagnalis*-like leeches were collected in the Humboldt River drainage, Nevada, and the Colorado River basin (Dolores River, Utah, USA) (Figs. 2, 3).

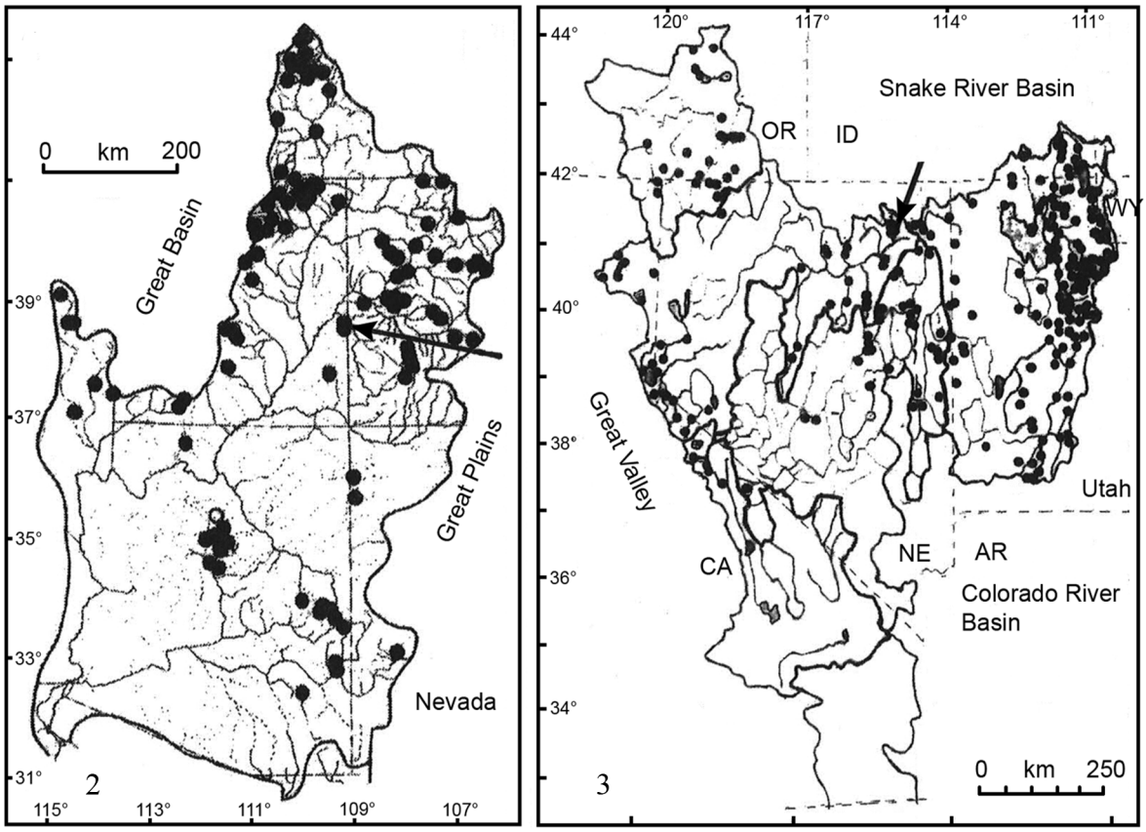
For microscopic observations, some of these leeches were relaxed with 10 % ethanol, fixed with 10 % formaldehyde, and preserved in 70 % ethanol while in the field (see Kutschera et al., 2013). Dorsal-ventral serial sections, (5 microns thick) of whole body mounts were prepared and stained with hematoxylin and eosin (Cathy Mayton, Wasatch Histo Consultants, Winnemucca, Nevada, USA). The drawings of the nervous, the digestive, and female and male reproductive systems (i.e., primary sex organs; gonads: ovaries and testes of these hermaphrodites) were synthesized from dissecting scope observations. Some sections were absent in the anterior region of *Helobdella californica*, due to technical problems with the block re-embedded. All observations and morphological/anatomic studies were repeated six times, using different leeches from the same population.

RESULTS

Comparison of Helobdella stagnalis *and* H. californica

Figure 1 shows a living specimen of a *H. stagnalis*-like leech, collected in a creek in Palo Alto, California, and a representative member of the "Golden Gate Leech", *H. californica* Kutschera 1988. Both species are about the same size and feed on *Chironomus* larvae as well as on *Tubifex* worms. We studied the feeding behavior of these *Helobdella*-species, but were unable to detect any consistent differences. Based on these observations, we conclude that *H. stagnalis* (USA) and *H. californica* (restricted to the Golden Gate Park, see Kutschera, 2011) are closely related species, despite obvious differences in color and pigmentation.

Figure 1. Photograph of living specimens of North American *Helobdella stagnalis*-like leeches. The "Golden Gate Leech" *H. californica* Kutschera 1988 is dark-pigmented; the individual carries one juvenile leech on its belly (arrow) (collected in Golden Gate Park, SF, CA, USA). The taxon on the right is, based on size and morphology, indistinguishable from its European counterpart, *H. stagnalis* L. 1758 (collected in Palo Alto, CA, USA).



Figures 2, 3. Fig. 2: the Great Basin (Nevada). Fig. 3: the Colorado River Basin (Utah). The dots show the sites where *Helobdella stagnalis*-like leeches were collected. The arrow on each map points to the 2 sites selected for *Helobdella* morphological studies.

Anatomical features of **Helobdella californica** *Kutschera 1988*

As mentioned in the Introduction, *H. californica* is considered in this context as an "outlier species" (Fig. 1).

The collected/fixed individuals of this taxon can be characterized as follows:

Site # 56. Collector # 1232. UMNH.ann. 00014 14: 4 specimens. Collected 23 Feb 2001. Pacific Coast: California: San Francisco: Golden Gate Park: Stow Lake. GEO Locate 37.769686, -122.475024. Histology serial sections: Collector # 1232. UMNH.ann.0001764. Technical problems with serial slides resulted in the absence of some anterior sections: C 02535, slides # 1–53, re-embeded # 1–25.

EXTERNAL DESCRIPTION. Color: grey, with both longitudinal and annular patterns (Fig. 1). Length: 4 specimens: 7–14 mm. Histology serial sections: length 8.7 x 2.2 mm.

DIGESTIVE SYSTEM. The pharynx transverses the atrium, descends vertically ventrally, and connects to the horizontal crop. The diffuse pair of salivary glands has bundled ductules with nearly paired origins on the pharyngeal vertical, the right ductules extending from the pharynx to the body wall for 55 microns, and the left ductules extending outward 45 microns. The crop caeca are branched (the species-identifying character), with the crop caecum # 6 not extending posteriorly. The 4 pairs of intestinal caeca angle anteriorly.

REPRODUCTIVE SYSTEM. The male linear right and left sperm duct terminates at testisac # 6 somite VIII. The posterior portions of the sperm ducts unite with the vas deferens from the 6 testisac pairs. The female left ovisac extends linear to testisac # 3 somite XVI, while the right ovisac extends linear to testisac # 5 somite XVII, both with short oviducts.

Description of the Nevada form

Helobdella humboldtensis n. sp.
http://zoobank. org:act:667AEA2C-DBD7-4704-B303-D1643 7775400

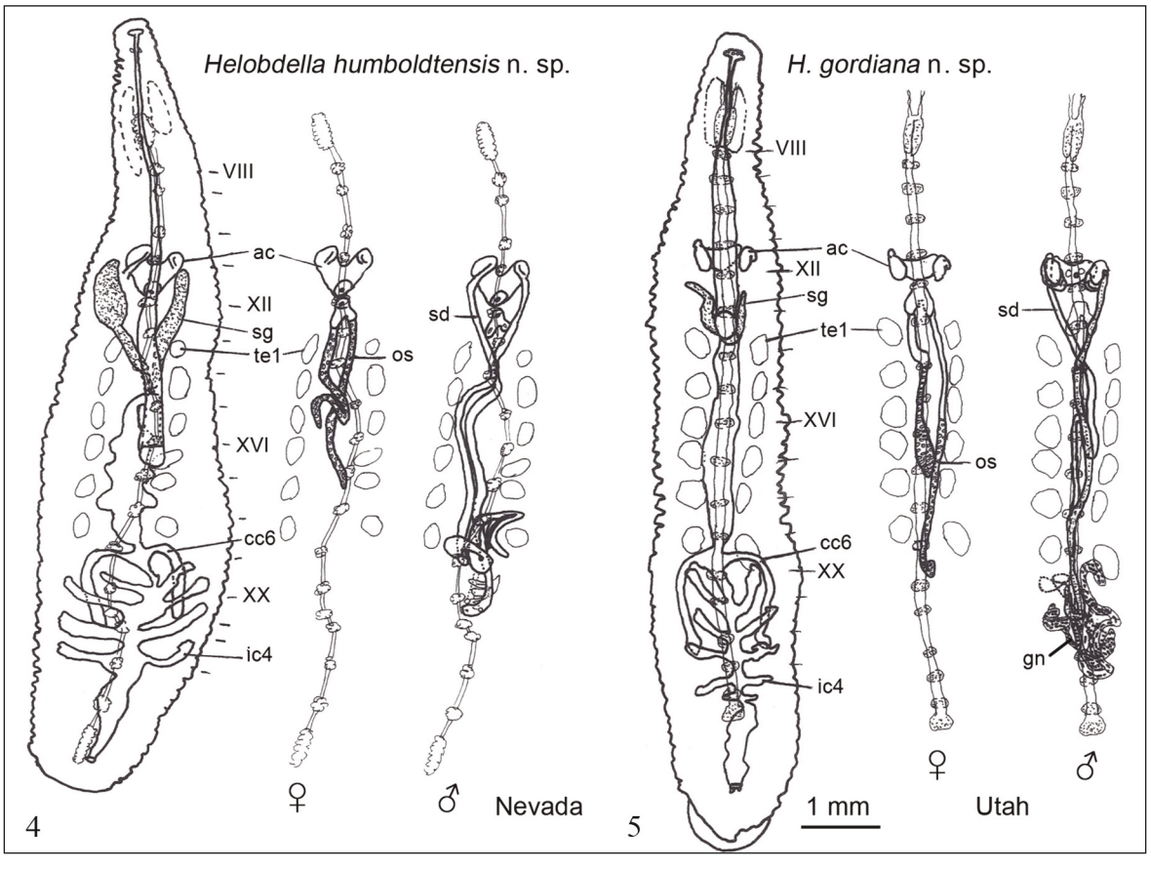
Figure 4 shows the reconstruction of the internal morphology (anatomy) of *Helobdella*-isolates from Nevada. Site # 453. Collection # 914. SIUSNM # 1617002, 15 formalin fixed specimens and UMNH.ann.0001760 (Holotype, histology serial sections). Collected 11 Jun 1989. Great Basin: Lahontan Basin: Humboldt River basin: Marys River: Bathtub Spring; Nevada: Elko County: T42N, R60E, section 15d (Fig. 4): Elevation 1840 meters. GEO Locate 41.530951, -115.183065. Recollected: Site # 453. Collection # A274. UMNH.ann. 0001762, 12 specimens in 95% ethanol and Collection # 3177. UMNH.ann.0001761, 4 formalin fixed specimens. 9 Apr 2020.

DESCRIPTION OF THE HOLOTYPE. Histology: length: 9.8 x 2.7 mm. Eyes (from histology) were separated by 1 eye width, 15 microns depth, and pigment shape as quarter moons. Digestive System (Fig.4, dorsal view): the S-shaped pharynx, after passing posteriorly horizontally over the atrium, descend vertically (the posterior pharyngeal vertical) between testisacs #3 and #4 (Fig. 6, # 7), extends anteriorly to between testisac #2 and # 3), extends vertically (the anterior pharyngeal vertical) (Fig. 6, # 6), and then leftward looping from the anterior side of the pharynx (Fig. 6, # 5), and extends posterior left of the midline as the esophagus and then dorsally vertical to adjoin the crop. This pharyngeal structure has increased total length compared to the L-shaped pharynx. The paired, compact, possessing of large cells salivary gland is directly located within the anterior pharyngeal vertical in somite XIII, beginning and ending with single cells. The right salivary gland adjoined the dorsal anterior pharyngeal vertical at the reach of the horizontal pharynx and extends dorsal anterior for 285 microns (Fig. 6, # 4). The left gland adjoins the base of the anterior pharyngeal vertical as the pharynx loops leftward and posterior, 7 microns ventral to the right salivary gland (Fig. 6, # 6), and extends dorsal anterior for 260 microns. The crop caeca were underdeveloped, perhaps because feeding had long lapsed, as suggested by Meyer and Moore (1954) for the leech *Marvinmeyeria*. The crop caecum # 6 extends posteriorly from somites XIX to XX1 (the 3rd intestinal caecum) (Fig. 4). The 4 pairs of intestinal caeca extend laterally in anterior direction. Rectum appears largely linear.

REPRODUCTIVE SYSTEM (Fig. 4). The male and female gonopores occur in somite XII, with the female ovisacs most ventral and the male sperm duct more dorsal, both ventral to the crop and intestine.

The male left and right sperm ducts extend linear, with the ducts criss-crossing the midline and each other, to a region of contortion at testisac # 5 somite XVIII, where after the left sperm duct terminates at testisac # 6, somite XIX and the right duct terminates at somite XX. The posterior portions of the sperm ducts unite with the vas deferens from the 6 testisac pairs. The female left ovisac extends posterior with double loops at testisac # 3 to somite XV, while the right ovisac extends linear to testisac # 5. Both ovisacs are of the same length, have short oviducts, and are ventral to the male sperm ducts.

Variability in external appearances. The 11 June 1989, USNM 1617002 paratype and 9 April 2020, UMNH 1761, 1762 collections were examined with the UMNH specimens observed live and through the preservation process with relaxation with ethanol, fixing with 10 % formalin, and after fixing with 70 % ethanol. These processes greatly diminished the pigmentation pattern, now visible with 40x examinations. The dorsal median is plain and creme-yellowish in the anterior of compacted bodies and full length in stretched out body. The dorsal is brown with chromatophoric speckles, dense along side of the midline. During the 10 % ethanol-relaxation over a 12 hour period, the speckles disappeared, with pigment found in multiple linear longitudinal muscle lines on the dorsum and ventrum with 1 specimen having horizontal lines.



Figures 4, 5. Reconstructed leech morphology/anatomy from histological serial sections. The Nevada *Helobdella* form (Fig. 4); the Utah *Helobdella* isolate (Fig. 5). Right of the body outline: female and male reproductive organs, respectively. Atrium cornua (ac); salivary glands (sg); testisac, first of 6 pairs (te1); crop caeca, the 6th of 6 pairs (cc6); intestinal caeca, the 4th of 4 pairs (ic4); ovisac (os); ejaculatory (semen) ducts (sd). The somites are noted by right lateral lines and in part by the Roman numbers, determined by the position of the mid body ganglia. Note in figure 4 (the Nevada form) the vertical view of the lengthened pharynx, the posterior pharyngeal vertical turning anterior to the anterior pharyngeal vertical, and then, as the esophagus turning posterior, as dorsal to the crop, and the contortions of the ejaculatory duct. Note in figure 5 (the Utah form) the absence of 5 pairs of crop caeca and the Gordian Knot (gn) (i.e., ejaculatory ducts).

Length collection 15 specimens: USNM 1617002: 6–15 mm; 16 specimens: UMNH 1761, 1762: 10–15 mm. The size and pigmentation is similar to that of *H. californica* (Fig. 1). Eye separation: UMNH 1761, 1762: separated by 1 eye width (5), a half eye width (2), less than half eye width (7), and adjacent (2). The scute was most similar to figure 7 E in Saglam et al. (2018), with a circular anterior position on most of the annulus VIIa1, and a narrower posterior oval portion on the anterior annulus VIIa2.

Description of the Utah form

Helobdella gordiana n. sp.
http://zoobank.org:act:B4CB0E45-1BF6-40C1-B819-FAC42A61 DD26

Figure 5 shows the reconstruction of the internal morphology (anatomy) of *Helobdella* isolates from Utah. Site # 107. Collection # 1195. UMNH.ann. 0000584 (5 formalin-fixed specimens) and UMNH. ann.0001763 (Holotype: histology serial sections). Collected 30 Sept 2000. Colorado River Basin La Sal Mountains: Dolores River: La Sal Creek: West Fork Beaver Creek: western pond; Utah: San Juan County. T28S, R25E, section 6a (Fig. 5): Elevation 2682 meters. GEOLocate 38.404855°, -109.202434°. Re-collect: Site # 107. Collection # A276. UMNH. ann.0001767 (3 specimens, 95 % ethanol). 30 July 2020. The La Sal Mountain is an Oligocene laccolith within the Colorado Plateau Mesozoic geology.

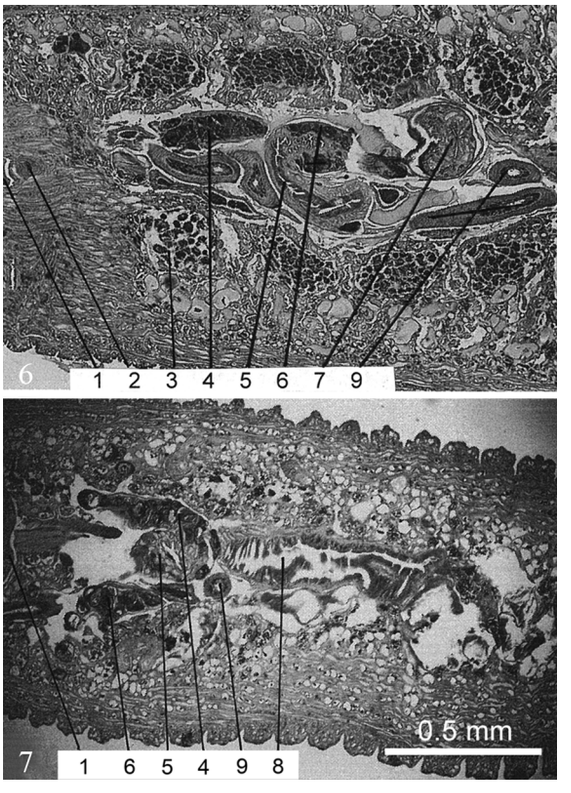
DESCRIPTION OF THE HOLOTYPE. Histology. Length 9.8 x 2.7 mm. Eyes were separated by less than 1/4 eye width with circular partial moon eclipse appearance.

DIGESTIVE SYSTEM (Figure 5, the dorsal view). The L-shaped pharynx, after traversing the atrium, turned vertical (Fig. 7, #5) to the ventrum at testisac #1 and adjoined the horizontal crop. The paired salivary gland with compact large cells budded off the pharyngeal vertical at somite XIII. The right salivary gland (Fig. 7, #4) became emplaced within the pharyngeal vertical and extended anteriorly dorsal 120 microns. The left salivary gland (Fig. 7, # 6) became emplaced within the pharynx 14 microns below the right salivary gland, and extended dorsal and anterior for 190 microns. The salivary gland range begins and ends with single cells. The crop caeca (Fig. 5) are lacking, perhaps because of the extended time since feeding (Meyer and Moore 1954, see above description), except for crop caecum #6 which extends posteriorly from somites XX to XXII. The intestinal caeca # 1, # 2, # 3 extend anteriorly while caecum # 4 extends laterally. There was a (parasitic?) global 60 micron thick cyst midline in the coelomic cavity posterior to intestinal # 4 caecum. Rectum appears largely linear.

REPRODUCTIVE SYSTEM (Fig. 5). The male and female gonopores occur in somite XII, with the female ovisac most ventral and the male sperm duct more dorsal, both ventral to the crop and intestine. The male ducts criss-cross the midline and each other. The left sperm duct loops at testisac #4 somite XVI, reversed directions at testisac #3 somite XV, and then proceeded posterior to the "Gordian Knot" region between somites XXI and XXIII. The right sperm duct extends linear to somite XXI Gordian knot region. The sperm ducts total length increased by 30 % with the Gordian Knot. The posterior portions of the sperm ducts unite with the vas deferens from the 6 testisac pairs. The female left ovisac extends linear to testisac #5 somite XVII, while the right ovisac extends linear beyond testisac #6 somite XX. The oviduct is 1/3 of the total length.

VARIABILITY IN EXTERNAL APPEARANCES. Length: UMNH 0584, 5 paratypes 5–9 mm and UMNH 1767, 3 specimens: 9–10 x 1mm. Color: white to light brown with mid dorsal clear when stretched and speckled when relaxed, changing with 70% ethanol fixation to white with speckled pattern transverse within annulus at 40 x. Eyes separation was ½, 1, and >1 eye width. Scute shape was D-form (see Saglam et al., 2018, Fig. 6). We examined the labial eye and scute shapes in 5 paratypes and 16 additional specimens in 5 lots UMNH 0561, 0562, 0586, 0593, 0596 from the Dolores River La Sal and Rock Creek drainages. The eyes were ovate, lateral ovate, or other, and the distance between the eyes were adjacent, 1 eye width apart, and 2 eye width apart in intrapopulations specimens. The scute shape was also irregular (Saglam et al., 2018, Fig. 6), with scute D noted but not prevalent in the 6 lots. A more extensive collection of the Utah form was collected on 5 June 2020 from Medicine Lake in upper La Sal Creek, 3093 m, GEOLocate 38.415698° -109.247677°: Site # 105. Collection # 3178. UMNH.ann.0001765. Preserved

with formalin (10), field collection # 3178 and with 95 % ethanol (29 adults, 13 juveniles) UMNH. ann.0001766, field collection #A 275. Color: live specimens appearing light and rarely dark, with dorsum heavily speckled, brownish, with irregularly wide mid dorsum light region with speckles. On 2 specimens para-medial dark lines were observed, when the specimen is stretched. The integument is sometimes transparent, with the intestinal caeca visible in 2 specimens and the eggs in the ovisac visible in 1 specimen. The fixed formalin-specimens appear very light, and with 40 x magnification reveal dorsal horizontal and ventral longitudinal rows, whereas the 95 % alcohol specimens were mostly plain and light. Length: adult 8–16 mm, juvenile 5–8 mm. The distance between the eyes varied from 1 eye width (27), half eye width (19), or adjacent width (6), and with 3 specimens having slit eyes. The scute was D-shaped (33) (see Saglam et al., 2018, Fig. 8), while the juvenile's scute was box-shaped with anterior outward curvature. Five specimens carried eggs, although additional specimens may have lost their eggs during preparation. The whitish color and 10 mm length appeared similar to that of the *H. stagnalis*-isolate from Palo Alto, CA (Fig. 1), and specimens from Europe.



Figures 6, 7. Serial histological section of paired salivary glands budding from the pharyngeal vertical. The Nevada Helobdella from (*H. humboldtensis* n. sp.) (Fig. 6); the Utah *Helobdella* type (*H. gordiana* n. sp.) (Fig. 7). The denotation Fig. 6 and Fig. 7 are placed on the anterior, left side of the leech. (1), the male gonopore (Fig. 6) or the atrial horns (Fig. 7); (2), the female gonopore; (3), the anterior #1 testisacs (Fig. 6), (4), the right salivary gland budding from the pharyngeal vertical (Fig. 7); (5), the anterior pharyngeal vertical cross-section (Fig. 7) or the ventral anterior pharyngeal vertical cross-section with the left looping to the esophagus; (6), the left salivary ducts embedded in the ventral pharyngeal vertical (Fig. 6); (7) posterior pharyngeal vertical after the dorsal horizontal pharynx from the atrial cornua turning ventral; (8), crop (Fig. 7); and (9), the ejaculatory duct fragments.

Comparison with other Helobdella stagnalis

The morphological description of the neotype of *Helobdella stagnalis* Linnaeus, 1758 (Iwama et al. 2019) is distinct from the Nevada and Utah forms, the Intermountain types having compact salivary glands with cells embedded within the pharyngeal vertical before budding into the coelom and the right salivary gland occuring above the left. This is the first reported occurrence of compact salivary glands from a North American Helobdellid, in contrast to their presence in *Helobdella* and *Haementaria* in South America (Siddall & Borda, 2003), and in contrast to the diffuse salivary glands in the parenchyma in other North American *Helobdella stagnalis* (Saglam et al., 2018). The J-shaped pharynx of *H. stagnalis* may be distinct from the L-shaped pharynx of the Utah specimen, *H. eriensis* and *H. californica*, and from the contorted S shape seen in the Nevada specimen.

Saglam et al. (2018) were the first to illustrate different anatomies in *Helobdella stagnalis*. They described 4 Holarctic species, based on morphology, including differences in reproductive ducts, eyes, scute, gastric caeca, and rectum shapes. We have compared these species with the Utah and Nevada morphotypes, the Arizona Montezuma Well species (Beresuc-Perrins et al., 2017), and with the species from Mexico (Salas-Montiel et al., 2014), as well as the leech populations from Oregon (Moser et al. 2013). These Helobdellids all have a scute, defining the discussed *Helobdella* lineages.

The eyes and scute (6 specimens) of the Utah form and 2 lots from the Fremont River were com-

pared to the findings of Saglam et al. (2018). The intra-Utah population eyes had variable distance between them, being adjacent (1 specimen), an eye-width apart, and more than an eye width apart, even two eye-widths apart in some cases. The scute shape was also variable, the *eriensis*-shape seen in a few individuals within the 4 lots. These observations are not definitive and may result from preservation techniques in the field, and certainly the lack of studies using electron microscopy.

DIGESTIVE SYSTEM. There were 6 crop caeca, except in *H. temiscoensis* (with 5 caeca). The 6th crop caecum (the 5th crop caecum in *temiscoensis*) extends posteriorly to the 3rd and 4th intestinal caeca, except in *H. serendipitous*, *H. bowermani*, and *H. atli* extending laterally. The anterior intestinal caecum # 1 extends mostly anteriorly. The intestinal caecum # 4 extends posteriorly, except in *H. eriensis*, *H.* Utah and *H.* Nevada, where it is lateral.

MALE REPRODUCTIVE ORGANS. The male organs include the 6 pairs of testisacs (4 pair in *Helobdella octatestisaca*) connected by vas deferens ducts directed posterior between testisac # 1 and # 6, and then adjoining the posterior ejaculatory duct and then anterior to the atrial cornua and the male gonopore. The left and right male ejaculatory ducts have similar twisted lengths, extending to testisac #6 at somite XIX or beyond to somite XXIII. The left sperm duct in the Utah form is double-looped at testisac # 3 somite XV, with both sperm ducts extending posterior to between somites XX and XXIII, respectively, where they intertwine and form the Gordian knot. The Nevada form is twisted with loops at somite XXI, and not intertwined.

FEMALE REPRODUCTIVE ORGANS. The female left ovisac terminates more anterior than the right ovisac, with the Utah form extending posterior to testisac # 5 (left) and to # 6 (right), and the Nevada form to testisac #3 (right, with double loops), and to # 5, with the right and left ovisacs, having same total length. This contrasts with right and left ovisacs being of similar lengths and extending posterior to testisac # 1 (*octatestisaca*, equivalent to testisac # 3), testisac # 3 (*temiscoensis, blinni*), testisac # 4 (*modesta*), testisac # 5 (*stagnalis*), testisac # 6 (*atli*, *echoensis* looped), posterior beyond testisac # 6 twisted (*eriensis*). The ovisacs are linear except for the twisted (*eriensis*), curled (*echoensis*), and double-looped (Nevada). The paired oviducts are short, with *echoensis* having a curled oviduct with a length equal to the ovisac and the Utah form oviduct reaching ~ 1/3 the length of the reference ovisac. The ovisac and oviduct length and secondary shape (curled, looped, twisted) may be useful taxonomic indicators of morphotype species, although Moser et al. (2013) noted that the ovisac length is dependent on the reproductive state of the sexually mature leech.

New Intermountain Helobdella *species*

We propose the Nevada and Utah forms as distinct species, based on their shared compacted salivary glands, as well as the distinct pharynx (the Nevada form) and the presence of the Gordian knot terminus of the ejaculatory duct (the Utah form).

Accordingly, we propose the Nevada form species as *Helobdella humboldtensis* n. sp. (Fig. 4) (Holotype: UMNH.ann.0001760 serial histological slides # 02549: # 1–45); paratype: USNM 1617002; type locality USNM.ann.0001761, 1762. This species is found in the Humboldt River drainage of Lahontan Basin (Great Basin). Name: based upon Alexander von Humboldt (1769–1859), honoring the initiator of many scientific disciplines, including biogeography, and the name of the river that flows past the microtome which sliced the *Helobdella* Gordian knot. The Humboldt River was first noted by European descended British-Canadian fur trader Peter Skene Ogden (1790–1854) in 1828, officially named by Fremont in 1845 with the first description of the endorheic Great Basin, and which meanders for 1600 km across northern Nevada (Houghton, 1976). Humboldt River formed from Miocene interior lakes, drained westward after breaking through the Basin and Range topography some 10 million years ago, for an unknown time entered the Pacific Ocean, and since the Pliocene has terminated within the Great Basin (Hovingh 2017). A. v. Humboldt visited the United States and President Thomas Jefferson (1743–1826) after his explorations of South America (the place of origin of the genus *Helobdella*), and left his name on towns, counties, mountain ranges, rivers, terminal saline sinks, universities, and commercial establishments of the western United States, which he never saw.

We propose the Utah form species as *H. gordiana* n. sp. (Fig. 5), based on the posterior contorted ejaculatory duct (the Gordian Knot)

(Holotype: UMNH.ann.0001763 serial histological slides # 02534: # 1–52); paratypes UMNH.ann. 0000584 and UMNH.ann.0001767 found in the Colorado River drainage basin. Name: the intricate knot tied by King Gordius of Gordiumin Phyrygia, and cut by Alexander the Great (356–323 BC) with the prophecy that only a ruler of Asia could loosen it. In the present era, the skill of the Wasatch Histo Consultants proprietor Cathy Mayton, with her microtome, accomplished a similar feat.

DISCUSSION

Recent *H. stagnalis* phylogenetic studies have provided much confusion with regard to North American forms, largely because the serendipitously sampling of a few specimens (mostly one from one site), first as the Holarctic (European) species, and then within the Nearctic (USA). The repeated assumption of no morphological/anatomical differences within the Holarctic, and then, similarly assuming one Nearctic *H. modesta* species, has proved anomalous (Saglam et al., 2018), wrongly resulting in geographical distribution from Newfoundland (Madill & Hovingh, 2007) to the Pacific Coast (Beresic-Perrins et al., 2017). The utilization of leech morphology herein has pitfalls, namely only 3 specimens were examined and the leech population anatomical variability undetermined. Most leech anatomical illustrations show the crop caeca fully expanded, whereas we found the 3 *Helobdella* specimens with their 5 paired crop caeca essentially deflated, perhaps from the lack of feeding prior to preservation (Meyer & Moore, 1954; Kutschera et al., 2013). The internal organ shapes could be altered by the state of relaxation, the crop inflation-depression, and the sexual maturity of the leech.

The above studies have changed the views of the North American *H. stagnalis* (Fig. 1). The Great Basin surveys noted 362 *Helobdella* sites out of 2442 sites within the arid basin and range endorheic basins with thousands of springs, while the Colorado River drainage basin surveys noted 146 sites out of 937 sites within the arid drainages of the Green, the upper Colorado, the Grand Canyon, and the Lower Colorado rivers (Figs. 2, 3) (Hovingh, 2017). The Intermountain region has been noted for fauna speciation since the fish studies of Hubbs & Miller (1948), and the gastropod *Pyrgulopsis* by Hershler & Liu (2017).

The presence of two distinct species at two different sites suggests that future studies may greatly increase the number of *Helobdella* species in the western United States. The studies of Beresic-Perrins et al. (2017) also suggest such diversity within the Colorado River basin with the identification of *H. blinni*, and noting a phylogenetic species in drainages flowing northward to the Little Colorado River and southward to the Gila River. We conclude that leeches of the genus *Helobdella* may represent model organisms for the study of speciation via geographical separation. More work is required to further explore the true (hidden) biodiversity of this large group of morphologically similar freshwater leeches that evolved, millions of years ago, in South America.

ACKNOWLEDGEMENTS

The work on the biology and morphology of *H. californica* by U.K. was carried out at the Carnegie Institution for Science (Dept. of Plant Biology, Briggs-lab) in Stanford/California 94305 (USA). Support of the Alexander von Humboldt-Stiftung (AvH, Bonn, Germany, Stanford-Fellowships 2010 to 2016) is gratefully acknowledged. This paper is dedicated to the memory of Prof. Winslow R. Briggs (1928–2019), who was not only an eminent lab scientist, but also a wildlife conservationist.

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