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Biology and Biodiversity of the Marine Leeches of California

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Biodiversity and Biology of the Marine Leeches of California

A thesis submitted in partial satisfaction
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by

Bernard Youngsoo Kim

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ABSTRACT OF THE THESIS

Biodiversity and Biology of the Marine Leeches of California

by

Bernard Youngsoo Kim

Master of Science in Biology
University of California, Los Angeles, 2013
Professor Donald Buth, Chair

The marine leeches of California are found on many host species, but the biology, distribution, and ecology of these leeches is not well understood. In this thesis I describe two previously unknown species: the leeches *Mysidobdella californiensis*, found on mysid shrimp hosts in Bodega Bay, CA, and *Heptacyclus cabrilloi*, found on giant kelpfish in San Pedro, CA. I also describe a method that addresses the problem of measuring the size of leeches, which is considered to be difficult because of the inconsistencies in the body proportions of soft-bodied invertebrate organisms. I utilize digital photography to measure leech size and use maximum-likelihood estimation to fit a model of size/age cohort distributions to a sample of leeches.

The thesis of Bernard Youngsoo Kim is approved.

Malcolm Gordon
Kirk Lohmueller
Donald Buth, Committee Chair

University of California, Los Angeles
2013

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ACKNOWLEDGEMENTS

The material presented herein in Chapter 1 is previously published in the *Journal of Parasitology* and is reproduced here in accordance with the author rights provided by Elsevier.

Chapter 1-1 is a version of the following publication:

Burreson, E., Kim, B., and J. Kalman Passarelli. 2012. A new species of *Mysidobdella* (Hirudinida: Piscicolidae) from mysids along the California Coast. *Journal of Parasitology* 98(2):341-343

Chapter 1-2 is a version of the following publication:

Burreson, E., J. Kalman Passarelli, and B. Kim. 2012. A new species of marine leech (Hirudinida: Piscicolidae) from Giant Kelpfish, *Heterostichus rostratus* Girard, in Southern California. *Journal of Parasitology* 98(4):788-790

Both manuscripts in Chapter 1 were co-authored by Dr. Eugene Burreson, Dr. Julianne Kalman Passarelli, and Bernard Y. Kim. The authors are listed by order of contribution at the beginning of each section. Dr. Eugene Burreson described the leeches and provided most figures; Dr. Julianne Kalman Passarelli provided additional specimens and supported collection efforts at Cabrillo Marine Aquarium, San Pedro, CA; and I collected specimens, field data, and provided digital photographs.

Chapter 2 was co-authored by Dr. Donald Buth and Bernard Y. Kim. The authors are listed by order of contribution at the beginning of the section. I developed the methods and wrote part of the paper, and Dr. Donald Buth provided essential advisement and authored a significant portion of the paper. This paper is being prepared for submission to the *Journal of Parasitology*.

J. Parasitol., 98(2), 2012
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A NEW SPECIES OF MYSIDOBDELLA (HIRUDINIDA: PISCICOLIDAE) FROM MYSIDS ALONG THE CALIFORNIA COAST

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ABSTRACT: *Mysidobdella californiensis* n. sp. is described from the mysid *Holmesimysis sculpta* from Bodega Bay on the central California coast and from *Holmesimysis costata* var. from San Pedro on the southern California coast. The internal anatomy of *M. californiensis* is similar to that of the only other species in the genus, *Mysidobdella borealis* from the north Atlantic Ocean, except that *M. californiensis* lacks the medial, unpaired seminal receptacle present in M. borealis. Externally, *M. californiensis* is slightly larger and more robust than *M. borealis*, with a much larger caudal sucker. The most striking difference between the species is the unusually large, trumpet-shaped, fluted oral sucker in *M. californiensis*. At Bodega Bay, the prevalence of *M. californiensis* on its host was 17% with an average intensity of 1.46 (range 1–3) leeches per host.

The only marine leech previously known to associate with mysids is *Mysidobdella borealis* (Johansson, 1899). This leech has been reported from mysids at Spitzbergen (Johansson, 1899), in the White Sea (Selensky, 1927) and Kara Sea (Vasileyev, 1939) of northern Russia, Greenland (Epshtein, 1961), and is widely distributed on mysids in the north Atlantic Ocean as far south as New Jersey on the western side (Burreson and Allen, 1978; Allen and Allen, 1981) and France on the eastern side (E. M. Burreson, unpubl. obs.). The leech has also been reported from the Bering Sea near the tip of the Aleutian Island chain (Vasileyev, 1939).

In the summer and fall of 2010, leeches were found on the mysid *Holmesimysis sculpta* (Tattersall, 1933), which, during this time, were present in large numbers along the central California coast. Leeches also have been collected periodically beginning in 2004 from the mysid *Holmesimysis costata* var. (Holmes, 1900) collected in kelp beds at San Pedro, California, by aquarists at the Cabrillo Marine Aquarium. The mysid leeches from the central and southern coasts of California are all the same new species and are described here.

MATERIALS AND METHODS

An unprecedented increase (based on 20 yr of experience; J. Newman, Bodega Marine Laboratory, pers. comm.) of the mysid *H. sculpta* occurred along the central California coast during summer and fall of 2010, apparently a reproductive swarm. Mysid shrimp were swept into the water clarification system at Bodega Marine Laboratory, Bodega Bay, California, during the swarm. The mysids accumulated inside the filtration chambers and were collected by laboratory staff as fish food. When leeches were noticed on the mysids, additional efforts were made to collect directly from the water clarifier. A daily sweep was performed using a fine mesh dip net within all the filter chambers. Despite daily efforts, mysids and leeches appeared in the water clarifier only once during a 2-wk period. All mysids were stored in a small flow-through tank. Mysids with leeches attached were removed from the tank with a dip net and transferred to small dishes. Leeches were removed with small forceps using a dissecting microscope.

The description is based on the examination of leeches collected at the Bodega Marine Laboratory and from kelp beds in San Pedro, California. As well as observations of live leeches, 8 leeches from Bodega and 7 from San Pedro were relaxed in weak ethanol and preserved in AFA. One of the leeches from Bodega was embedded in paraffin, sectioned longitudinally at 5 mm, mounted on glass slides, and stained with hematoxylin and eosin using the methods of Burreson and Kalman (2006). Seventeen leeches from Bodega Bay were preserved directly in 95% ethanol. Measurements were taken using an ocular micrometer.

Mysids were identified using the characters listed in Kathman et al. (1986).

REDESCRIPTION

Mysidobdella Selensky 1927

Revised generic diagnosis: Size small, not known to exceed 15 mm total length, body cylindrical in life, not sharply divided into trachelosome and urosome. Integument smooth, lacking papillae, tubercles, gills, and pulsatile vesicles. Caudal sucker slightly subterminal, not wider than maximum body width; oral sucker very deeply cupped and attached to trachelosome by long and tapered nuchal constriction. Oral sucker with inner annular membrane and small sucker-like structures around mouthpore. Midbody segments 3 (13) annulate. Five pairs of testisacs, medial unpaired seminal receptacle present or absent. Coelomic system reduced; posterior crop ceca fused, with fenestrae; esophageal diverticula present. Marine; symbiotic on mysids.

DESCRIPTION

Mysidobdella californiensis n. sp.
(Figs. 1–4)

Diagnosis: With the characters of genus. Total length including suckers not known to exceed 9 mm; oral sucker unusually large and very deeply cupped, trumpet-shaped. One pair of punctiform eyes on nuchal constriction; paired punctiform ocelli dorsally and ventrally on 13 urosome segments and last segment of trachelosome. No ocelli on oral or caudal sucker. Pigmentation in life brown, interrupted laterally in each segment. Male reproductive system simple with moderately large bursa; 5 pairs of testisacs that do not atrophy in mature individuals. Accessory gland cells on atrial cornu lacking. Female reproductive system lacking medial, unpaired seminal receptacle in segment XII.

External characters: Body round in life, but subcylindrical to flat after preservation, indistinctly divided into trachelosome and urosome. Average total length of mature individuals 7 mm (range 6–9 mm); average body width 0.75 mm (range 0.6–0.8 mm). Mouthpore centrally located in very deeply cupped, trumpet-shaped oral sucker, attached to trachelosome by tapered nuchal constriction (Fig. 1). Oral sucker 1.2 mm in length, 0.8 mm in diameter. Annular membrane located on inner surface of oral sucker; internal surface of oral sucker around mouthpore with small sucker-like structures (Fig. 2A). Body surface smooth, lacking papillae, tubercles, gills, and pulsatile vesicles. Caudal sucker 0.7 mm in diameter, not wider than body width. One pair of punctiform eyes on nuchal constriction (Fig. 1), but no ocelli on oral or caudal sucker. One pair of punctiform ocelli dorsally and ventrally on last segment of trachelosome and first 13 segments of urosome. Pigmentation in life somewhat variable, uniformly brown to reddish brown on body and suckers, interrupted laterally on trachelosome and urosome to form metameric unpigmented areas.

Internal characters: Coelomic and digestive system similar to M. borealis. Terminal portions of male reproductive system lack accessory gland cells on atrial cornu. Five pairs of testisacs that do not appear to atrophy in large individuals. Female reproductive system with typical paired ovisacs, but lacking medial, unpaired seminal receptacle (Fig. 2B).

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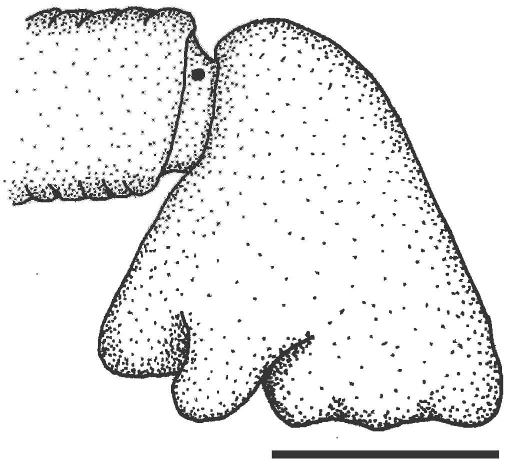

FIGURE 1. Oral sucker of *Mysidobdella californiensis* n. sp., lateral view. Scale bar 5 0.5 mm.

Taxonomic summary

Type host: Mysid *H. sculpta* (Tattersall, 1933). Voucher specimens deposited in the Natural History Museum of Los Angeles County Marine Biodiversity Center as MBPC 10803.

Other known hosts: Mysid *H. costata* var. (Holmes, 1900). Voucher specimens deposited in the Natural History Museum of Los Angeles County Biodiversity Center as MBPC 11493–97.

Site of infection: External, dorsally, or laterally on cephalothorax or abdomen.

Type locality: University of California, Davis Bodega Marine Laboratory, Bodega Bay, California (38.32uN, 123.07uW).

Other locations: San Pedro, California kelp beds (33.05uN, 118.17uW).

Prevalence and intensity: Prevalence at Bodega Marine Laboratory 13/75 infected (17%); average intensity 1.46, range 1–3 leeches per mysid.

Etymology: Named after the general location of California.

Specimens deposited: Polychaete Collection, Natural History Museum of Los Angeles County (LACM-AHF POLY). Holotype LACM-AHF POLY 2982, Paratype LACM-AHF POLY 2983 (Bodega, 17 specimens), Paratype LACM-AHF POLY 2984 (Bodega, 4 specimens) Paratype LACM-AHF POLY 2985 (San Pedro, 4 specimens) Paratype histological sections LACM-AHF POLY 2986-2989 (slides 1–4).

Remarks

Mysidobdella californiensis clearly belongs in *Mysidobdella* because of its association with mysids, its large oral sucker attached by a nuchal constriction, the annular membrane and small sucker-like structures in the oral sucker, and details of the male reproductive system. *Mysidobdella californiensis* differs from the only other member of the genus, *M. borealis*, by its much larger and more deeply cupped oral sucker (Fig. 4), more than 3 times the size of that of *M. borealis*; its larger caudal sucker, about twice the diameter of that of *M. borealis*; and the absence of the medial, unpaired seminal receptacle in segment XII. The generic diagnosis is revised to reflect that the latter structure may or may not be present.

Little is known of the biology of *M. californiensis*, but based on knowledge of its congener, *M. borealis*, it is likely a specific parasite of mysids. In the laboratory leeches rapidly attached to mysids with the oral sucker upon contact and then shifted to a position dorsally or laterally on the cephalothorax or abdomen (Fig. 3). Prevalence and intensity estimates were made by random samples from a holding tank at Bodega Marine Laboratory and may not represent that found in nature.

DISCUSSION

Many species of marine leeches are known to associate with arthropods (Meyer and Barden, 1955). Usually this association occurs in areas with sandy or muddy bottom where the leeches use

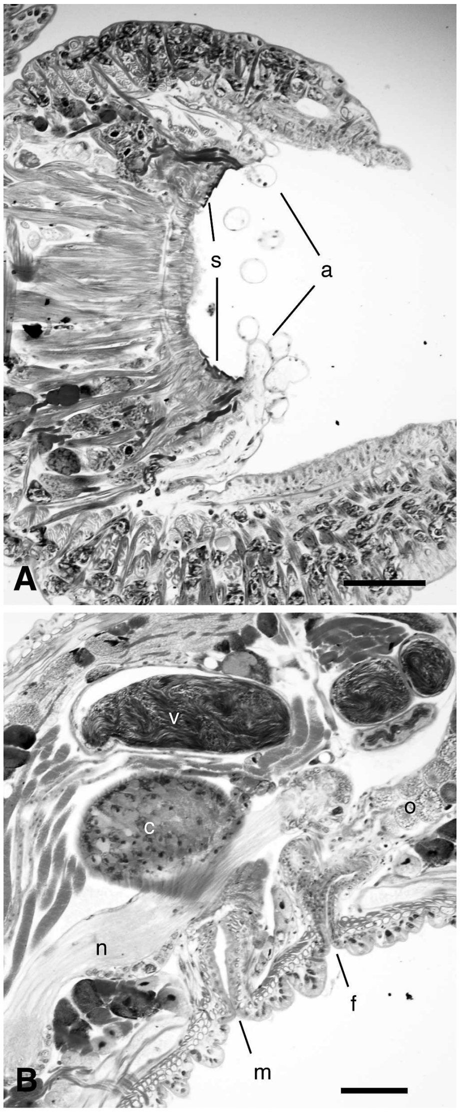

FIGURE 2. Histological sections of *Mysidobdella californiensis* n. sp. (A) Sagittal section through oral sucker showing annular membrane (a) and small sucker-like structures (s). Hematoxylin and eosin stain. Scale bar 5 100 mm. (B) Sagittal section through clitellum showing atrial cornua (c), female gonopore (f), male gonopore (m), ventral nerve cord (n), ovisac (o), and vas deferens filled with sperm (v). Hematoxylin and eosin stain. Scale bar 5 100 mm. Note lack of seminal receptacle pore posterior to female gonopore; compare figure 9, Burreson and Allen (1978).

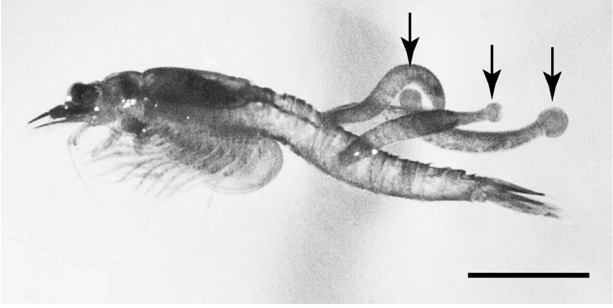

FIGURE 3. Three individuals of *Mysidobdella californiensis* n. sp. (arrows) on *Holmesimysis sculpta*. Scale bar 5 mm.

the hard carapace of the arthropod for attachment and perhaps cocoon deposition. All these leeches, with the exception of those associating with mysids, have known fish hosts and in no case does the leech actually feed on hemolymph of the arthropod host.

The exact nature of the relationship between species of *Mysidobdella* and mysids is still uncertain. All observational evidence (Burreson and Allen, 1978) of *M. borealis* and its host *Neomysis americana*, the lack of fish red blood cells in the gut of all sectioned leeches, and the failure of the leech to attach to a wide variety of local fishes in laboratory trials, suggest that the leech actually feeds on the hemolymph of the mysid host. However, the presence of mysid hemolymph in leech gut contents has not been confirmed with DNA evidence. The rapid attachment of *M. californiensis* to mysids in the laboratory also suggests a specific relationship. Assuming that both *M. borealis* and *M. californiensis* feed exclusively on mysid hemolymph, these are the only marine leeches known that feed on the hemolymph of invertebrates and not on the blood of fishes or sea turtles.

It was not possible to identify the mysid host from San Pedro with absolute certainty; it shows a mix of characters used to distinguish the 5 known species of *Holmesimysis* (*H. costata*, *H. nuda*, *H. nudensis*, *H. sculpta*, and *H. sculptoides*). However, the specimens were most similar to *H. costata*, which has previously been reported to occur in southern California and are, therefore, designated *H. costata* var. Because of the confusion of mysid identification in the literature, the distribution of either mysid host for *M. californiensis* and, thus, the potential distribution of *M. californiensis* is unclear.

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Thanks to Dr. Donald G. Buth, UCLA, for his assistance with many aspects of the study, Don Cadien, Los Angeles County Sanitation District for identification of the mysids, and Leslie Harris, Polychaete Collection Manager, Natural History Museum of Los Angeles County, for accession of the specimens. This publication is a contribution in part of the Bodega Marine Laboratory, University of California at Davis.

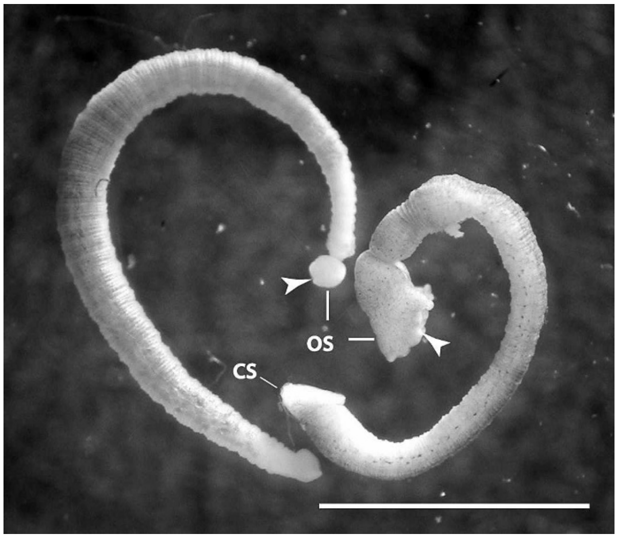

FIGURE 4. Comparison of suckers of *Mysidobdella californiensis* n. sp. (right) and paratype of *Mysidobdella borealis* (USNM 55687) (left). cs, caudal sucker; os, oral sucker. Arrowheads point to opening of oral sucker. Scale bar 5 mm.

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J. Parasitol., 98(4), 2012

A NEW SPECIES OF MARINE LEECH (HIRUDINIDA: PISCICOLIDAE) FROM GIANT KELPFISH, *HETEROSTICHUS ROSTRATUS* GIRARD, IN SOUTHERN CALIFORNIA

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ABSTRACT: Collections of giant kelpfish at inner Cabrillo Beach, San Pedro, California revealed the presence of an undescribed species of *Heptacyclus*, described here as *Heptacyclus cabrilloi* n. sp. The leech is small, only up to 14 mm total length including suckers; it has 2 pair of slightly crescentiform eyes on the oral sucker, 1 pair of punctiform ocelli on the second annulus of the trachelosome, 13 pairs of punctiform ocelli dorsally and ventrally on the urosome, and 14 marginal punctiform ocelli on the caudal sucker. Pigmentation is yellowish-tan with an unpigmented mid-dorsal stripe on both trachelosome and urosome, and with unpigmented halos around eyespots and urosome ocelli. Male reproductive system with 5 pairs of large, spherical testisacs. Mycetomes present, accessory gland cells on atrial cornu absent. Prevalence was 24.1% in June, 2011 with a range of 1–2 leeches per fish and 25.8% in October, 2011 with a range of 1–6 leeches per fish.

Heptacyclus Vasileyev, 1939 is one of the most speciose genera of marine leeches in the Northern Hemisphere, with 10 described species. The genus was synonymized with *Malmiana* Strand, 1942 by Williams and Burreson (2006) based on molecular and morphological evidence; *Heptacyclus* has priority. The type species, *Heptacyclus virgatus* Vasileyev, 1939 is known from the Bering Sea and northern Pacific Ocean; *Heptacyclus scorpius* (Malm, 1863) and *Heptacyclus brunneus* (Johansson, 1896) are widely distributed in the north Atlantic Ocean; *Heptacyclus bubalis* (Srivastava, 1966), *Heptacyclus yorki* (Srivastava, 1966), and *Heptacyclus myoxocephali* Srivastava, 1966 are restricted to British waters; *Heptacyclus philothermus* (Sawyer, Lawler, and Overstreet, 1975) is known from the Gulf of Mexico; and *Heptacyclus viridus* (Burreson, 1977), *Heptacyclus diminutus* (Burreson, 1977) and *Heptacyclus buthi* (Burreson and Kalman, 2006) are known from the northeastern Pacific Ocean along the west coast of the United States. With the exception of H. philothermus, all species of *Heptacyclus* parasitize cottid fishes.

Collections of giant kelpfish, *Heterostichus rostratus* Girard, 1854, near San Pedro, California revealed the presence of an undescribed species of *Heptacyclus*, described here.

MATERIALS AND METHODS

Giant kelpfish were collected by beach seine in February, June, and October 2011 during the Inner Cabrillo Beach Survey conducted quarterly by the Cabrillo Marine Aquarium in San Pedro, California. Leeches were removed with small artist's brushes and either immediately relaxed in weak ethanol, prior to fixation in AFA, or returned alive to Cabrillo Marine Aquarium for photographing prior to relaxation and fixation. Three individuals were placed directly into 95% ethanol.

Descriptions are based on observations and photographs of live leeches and examination of 12 individuals fixed in AFA, 2 individuals fixed in 95% ethanol, and serial transverse sections of 2 leeches embedded in paraffin, sectioned at 5 mm, and stained with hematoxylin and eosin by the methods of Burreson and Kalman (2006). Measurements were made with an ocular micrometer. Drawings were made with the aide of a camera lucida. Segments and associated ganglia are designated by roman numerals. All leeches have 34 segments and associated nerve ganglia. The ganglia for the first 6 segments compose the brain, and then there are 21 individual ganglia, one in the center of each subsequent segment, until the posterior ganglionic mass composed of 7 fused ganglia. In the Piscicolidae, ganglion XII always occurs between the gonopores.

DESCRIPTION

Heptacyclus cabrilloi n. sp.

(Figs. 1–3)

Diagnosis: Small leeches, up to 14 mm total length, including suckers. Suckers well developed, caudal up to 1.2 mm in diameter, oral up to 0.5 mm in diameter. Oral sucker with 2 pair slightly crescentiform eyes; 1 pair punctiform ocelli on second annulus of trachelosome. Caudal sucker eccentrically attached to urosome, with 14 punctiform ocelli around margin. Paired punctiform ocelli dorsally and ventrally on 13 urosome segments. Pigmentation of trachelosome and urosome uniformly yellowish-tan with unpigmented mid-dorsal stripe and unpigmented halos around dorsal paired punctiform ocelli on urosome. Caudal sucker uniformly yellowish-tan with unpigmented halos around marginal ocelli. Oral sucker uniformly yellowish-tan with unpigmented halos around eyes and irregular unpigmented area anterior to first pair of eyes. Mycetomes present; crop expands only slightly between 5 pair of testisacs. Postceca fused with fenestrae at ganglia. Vector tissue and accessory gland cells on atrial cornu absent. Ventral, dorsal, and lateral coelomic sinuses present.

External morphology: Body smooth, lacking papillae or tubercles, subcylindrical, indistinctly divided into trachelosome and urosome (Fig. 1). Six annuli per segment, subdivided to 12. Maximum total length including suckers 14 mm; maximum width 0.8 mm. Oral sucker up to 0.5 mm in diameter; caudal sucker up to 1.2 mm in diameter. Pigmentation on trachelosome and urosome uniformly yellowish-tan in mature leeches with unpigmented mid-dorsal stripe (Fig. 1); in immature leeches, pigmentation interrupted laterally segmentally, giving appearance of transverse bands. Paired punctiform ocelli dorsally and ventrally on 13 urosome segments and dorsally on second annulus of trachelosome (Fig. 1). Unpigmented halos surround dorsal ocelli on urosome, but not on trachelosome (Fig. 1). Pigmentation on oral sucker uniformly yellowish-tan, with unpigmented halos around 2 pair of slightly crescentiform eyes, and irregular unpigmented areas anterior to first pair of eyes (Fig. 2A). Pigmentation on caudal sucker uniformly yellowish-tan with unpigmented halos surrounding 14 punctiform ocelli and extending to margin of sucker (Fig. 2B).

Digestive system: Mouthpore located at center of oral sucker. Proboscis extends to ganglion IX. Large mycetomes connected to esophagus at X/XI, extending anteriorly to IX/X. Crop expands slightly between testisacs in segments XIV through XVIII. Intestine with 1 pair of large ceca in XIX and smaller ceca segmentally until opening into rectum in XXV. Postceca fused with fenestrae at each ganglion.

Reproductive system: Five pair of large spherical testisacs occur intersegmentally from XIV/XV through XVIII/XIX. Vasa deferentia run anteriorly to XII and expand into thin-walled, convoluted epididimides filled with sperm (Fig. 3). Vasa deferentia become thick-walled and continue anteriorly to anterior portion of XI, where they turn ventrally and enter large atrial cornua on anteroventral surface. No accessory gland cells on atrial cornua. Common atrium fuses with very small bursa that opens through male gonopore in anterior portion of XII (Fig. 3). Paired ovisacs occur in posterior portion of XII and in XIII. Muscular common

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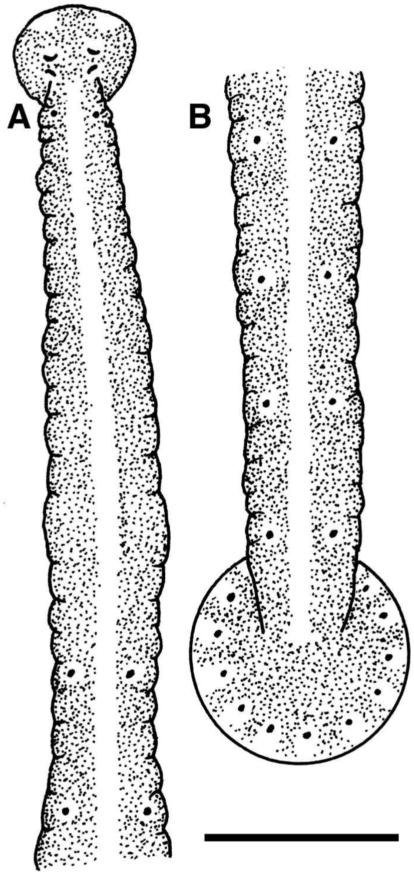

FIGURE 1. Line drawing of *Heptacyclus cabrilloi* n. sp. (A) Anterior one-third, dorsal view. (B) Posterior one third, dorsal view. Scale bar 5 1.0 mm.

oviduct opens through female gonopore in posterior portion of XII. Vector tissue and conducting tissue absent.

Coelomic system: Pulsatile vesicles absent. Ventral, dorsal, and lateral sinuses present in urosome segments. The presence of testicular sinuses, and segmental or intersegmental connecting sinuses, could not be determined from the material available.

Taxonomic summary

Type host: Giant kelpfish, *Heterostichus rostratus* Girard, 1854. Host voucher specimens deposited in the Cabrillo Marine Aquarium (CMA 2011.07.0018 and CMA 2011.07/0019), San Pedro, California. No other known hosts.

Site of infection: External on body and fins.

Type locality and collection dates: Inner Cabrillo Beach, San Pedro, California 33u429380N, 118u169580W. February, June, and October 2011.

Prevalence and intensity of infection: June, 2011, 9/42, 21.4%; 1.1, range 1–2 leeches per host. October 2011, 54/209, 25.8%; 1.5, range 1–6 leeches per host.

Specimens deposited: Polychaete Collection, Natural History Museum of Los Angeles County (LACM-AHF POLY), Los Angeles, California. Holotype: LACM-AHF POLY XXXXXX, paratype specimens: LACM-AHF POLY 3131, 3132, paratype serial sections: LACM-AHF POLY 3133A-3133M. Additional specimens are deposited in the Cabrillo Marine Aquarium under numbers CMA 2012.07.0003-0008.

Etymology: Named for the early Spanish explorer Juan Rodriguez Cabrillo, the first European to visit the San Pedro, California area. Many local landmarks, including the leech type locality of Cabrillo Beach, bear his name.

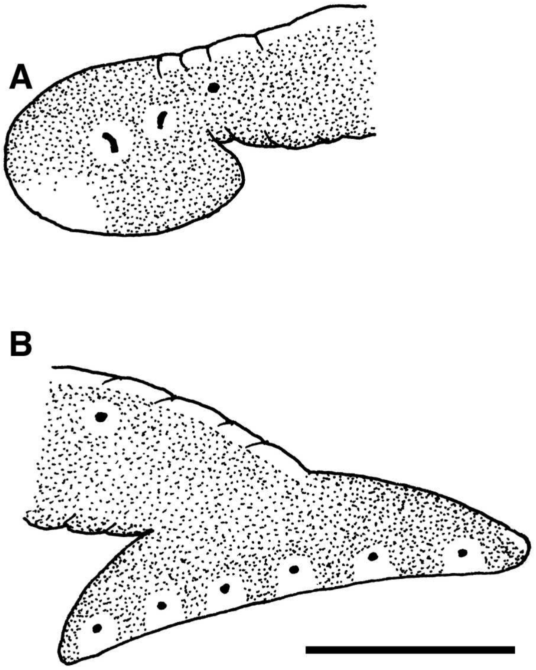

FIGURE 2. Line drawing of Heptacyclus *cabrilloi* n. sp. suckers. (A) Oral sucker, showing eyes. (B) Caudal sucker, showing ocelli. Scale bar 5 0.5 mm.

Remarks

Heptacyclus cabrilloi is assignable to the genus *Heptacyclus* because of the following combination of characters: smooth body lacking papillae, tubercles, or pulsatile vesicles; 2 pair of eyes on oral sucker; 1 pair of eyes on second annulus of trachelosome; ocelli on relatively large, eccentrically-attached caudal sucker; 5 pair of testisacs; atrial cornua lacking accessory gland cells; and small bursa. *Heptacyclus cabrilloi* differs from known species of *Heptacyclus* in the following ways: *H. virgatus*, *H. scorpius*, *H. brunneus*, and *H. viridus* are much larger (24–35 mm) and have dark brown or green pigmentation in the form of longitudinal stripes (Vasileyev, 1939; Khan and Meyer, 1976; Burreson, 1977; Appy and Dadswell, 1981;); *H. philothermus* has only 1 pair of eyes on the oral sucker and lacks ocelli on

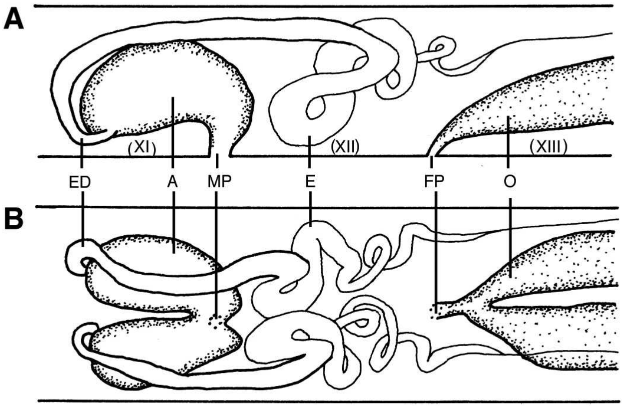

FIGURE 3. *Heptacyclus cabrilloi* n. sp., terminal portions of male and female reproductive systems. (A) Lateral view. (B) Dorsal view. A, atrial cornu; E, epididymus; ED, ejaculatory duct; FP, female gonopore, MP, male gonopore; O, ovisac. Roman numerals indicate position of respective ganglia.

the caudal sucker (Sawyer et al., 1975); *H. buthi* is smaller (~9 mm), has brown pigmentation, and parasitizes intertidal fishes (Burreson and Kalman, 2006); *H. bubalis* and *H. yorki* are known only from single specimens and are not well characterized, but *H. bubalis* lacks ocelli on the caudal sucker and has only 4 pair of testisacs, while *H. yorki* lacks metameric ocelli on the urosome and also lacks the pair of eyes on the second annulus of the trachelsome (Srivastava, 1966); *H. myoxocephali* apparently lacks eyes and ocelli, but it was described from preserved specimens and eyes and ocelli may have faded (Srivastava, 1966). *Heptacyclus cabrilloi* is most similar to *Heptacyclus diminutus* from Oregon; however, *H. diminutus* has reddish-brown pigmentation, punctiform eyes on the oral sucker, and very small testisacs that lie ventral to the crop (Burreson, 1977). *Heptacyclus cabrilloi* also differs from all known species by having unpigmented halos around the segmental ocelli on the urosome.

DISCUSSION

Vasileyev (1939) established *Heptacyclus* for large marine leeches collected in the Bering Sea. The name refers to 7 annuli per segment of the type species, *H. virgatus*. Historically, annulation was one of the most important morphological characters used in descriptions of new species, especially for terrestrial and freshwater forms. However, for leeches in the Piscicolidae, annulation is often difficult to discern even after careful relaxation and fixation, and other external characters and internal anatomy are more useful for determining relationships (Williams and Burreson, 2006). For that reason, many species legitimately placed in *Heptacyclus*, based on external and internal anatomy, do not have 7 annuli per segment.

With the exception of *H. philothermus*, all previously known species of *Heptacyclus* parasitize cottid fishes. *Heptacyclus philothermus* parasitizes a variety of non-cottid fishes in the Gulf of Mexico (Sawyer et al., 1975) and lacks many of the characters of typical members of this genus; thus, it likely belongs in another genus. *Heptacyclus diminutus* parasitizes the cottid fish cabezon (*Scorpaenichthys marmoratus*) but also infests black rockfish, *Sebastes melanops* (Scorpaenidae) (Burreson, 1977). The leech *H. cabrilloi* is known only from giant kelpfish, *He. rostratus* (Clinidae), which it matches closely in golden-yellow pigmentation. If *He. rostratus* is the only host, *H. cabrilloi* would be the only valid species in *Heptacyclus* that does not parasitize a cottid host. A cottid host, however, cannot be ruled out until more information is obtained on leeches present on local fishes.

ACKNOWLEDGMENTS

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INTRASPECIFIC VARIATION IN GROWTH AND REPRODUCTION OF THE MARINE LEECH, *HEPTACYCLUS BUTHI*

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Abstract: *Heptacyclus buthi* was harvested from fish hosts in rocky intertidal zones of Sonoma and Marin Counties, California in 2008 (N = 162) and 2010 (N = 51). The size of the leeches was quantified using a method that approximated the sagittal cross-section of each specimen. Size-frequency curves were modeled to estimate the number of size-class cohorts in each year. If *H. buthi* is an annual species like many of its relatives, the single cohort modeled for in 2010 and the comparable "older" cohort in 2008, both with a broad range of sizes, may represent one component of its reproductive life history. A second, younger, more-numerous, less-variable cohort modeled from the 2008 sample may represent a second reproductive bout during that year that was prevented in the subsequent La Niña period of 2010-11.

INTRODUCTION

The marine leech *Heptacyclus buthi* was originally described as *Malmiana buthi*, a piscicolid leech ectoparasitic on fishes from rocky intertidal regions in Sonoma County, California by Burreson and Kalman (2006). Little is known about the biology of *H. buthi* beyond its original description and its phylogenetic relationships to other marine leeches (Williams and Burreson, 2006). We had the opportunity to collect large numbers of this species in 2008 and 2010. These samples included many individuals that varied considerably in size. This variation had the potential to address the question: Can age cohorts be estimated for *H. buthi*?

The age and growth of many organisms can be ascertained from recaptured marked individuals or from marks that accrue periodically in hard tissues such as vertebrate bone. These approaches are not feasible for small invertebrate parasites due to the inconsistencies in the way in which many specimens are preserved. A potentially viable alternative is the Peterson method, which involves the comparison of length-frequency distributions in population samples (Cailliet et al., 1986). The Peterson method assumes that the lengths, or other measure of size, of specimens in a particular age-class will be distributed normally around a mean (Figure 1). When plotted, a series of normal curves for each of the age-classes should be revealed. This method is often useful to distinguish younger age-classes as slower growth and variation among older individuals would tend to yield overlap among the temporal cohorts, e.g. as is the case for fishes (Westrheim and Ricker, 1978). Typical size-frequency curves are shown in Everhart et al. (1975 fig. 5-1) and in Cailliet et al. (1986 fig.12.1). Herein we apply the Peterson method to address the questions of reproductive periodicity and age of *H. buthi*.

MATERIALS AND METHODS

Cottid and clinid fish hosts (*Oligocottus snyderi*, *Oligocottus maculosus*, *Clinocottus globiceps*, *Clinocottus analis*, and *Gibbonsia metzi*) were captured with dip nets from the type locality in the rocky intertidal zone of Horseshoe Cove on the Bodega Marine Reserve (38°19’01”N, 123°04’16”W) and from five other intertidal coastal areas in Sonoma and Marin Counties, California. Fishes were transported in buckets of seawater back to the Bodega Marine Laboratory where they were examined and all leeches were removed with small paintbrushes. The leeches were placed individually in wells of spot plates. Each well was filled with seawater that was allowed to warm to ambient temperature. Leeches were anesthetized by adding 70% ethanol dropwise to the seawater, then fixed in alcohol-formalin-acetic acid solution (AFA), and transferred to 70% ethanol. A few leeches retained for molecular studies skipped the AFA fixation stage and were transferred to 95% ethanol. Leeches were allowed to die in a “natural,” often curved, position rather than stretched or straightened post-mortem. In October 2008, a total of 88 fish hosts yielded 162 leeches and in October 2010, 22 fish hosts yielded 51 leeches. All specimens were deposited in the Marine Biodiversity Collection in the Natural History Museum of Los Angeles County: 2008 (#11302 through 11389) and 2010 (#11390 through 11411).

As a proxy for length as used in many Peterson method applications, leech size was measured using digital photography. Each fixed leech was placed on its side on a well-lit white paper background then photographed with a digital camera. Calipers set at 20.0 mm were placed in each frame as a reference for measurement. Lens distortion correction was applied to each digital photograph, and then the 2-dimensional area of each leech was measured with ImageJ (1.4.6, http://rsb.info.nih.gov/ij/). This method yielded an area measurement equivalent to the largest sagittal cross-section of each relaxed leech.

Statistical analyses were performed with the R packages mclust and mixdist (R Development Core Team, 2008). The mclust package was used to select a model of normal distributions based on Bayesian Information Criteria (BIC) and mixdist was used to fit the distributions to the data with a maximum likelihood method.

RESULTS

Ninety-two percent of the leeches examined in this study came from *Oligocotttus snyderi*, the preferred host of *H. buthi* (Burreson and Kalman, 2006). The size distributions of all leeches are shown in Figure 2 for both 2008 and 2010.

The statistical results of model selection are shown in Table 1. The best fit model that describes the 2008 data predicts two distinct distributions, whereas the best fit model that describes the 2010 data predicts one distinct distribution (Figure 3).

DISCUSSION

The size variation in our samples of *H. buthi* was adequate for the estimation of age cohorts in this species. However, the best fitting models for our size-class data yield different interpretations for the 2008 and 2010 temporal samples. The difference could be due to a number of variables or might be revealing a biologically real heterogeneity in the reproductive patterns of *H. buthi*. The larger 2008 sample can be modeled as having two reproductive cohorts, a younger more-numerous group and an older, more-variable group. For organisms that have an annual reproductive mode, this pattern would be interpreted as that of two year classes, i.e. that *H. buthi* lives for two years, and that the growth rate in the second year is highly variable. However, the assumption of a single annual reproductive period may be incorrect. Piscicolids *Heptacyclus* (*Malmiana*) *brunnea*, *H.* (*M.*) *scorpii*, and *Oceanodbella sexoculata* all have the potential for a second reproductive effort with a year as shown in laboratory studies (Khan and Meyer, 1976, 1978). However, these three piscicolids are believed to be annuals in terms of life cycle duration (Khan and Meyer, 1976, 1978). Therefore if *H. buthi* is also an annual, this species had two reproductive bouts in 2008.

The smaller 2010 sample could be modeled as having just a single, highly variable cohort that is similar to the "older" cohort in the 2008 sample (Figure 3). This pattern is consistent with that of an annual organism that has a single extended reproductive period and highly variable individual growth rates. The difference between the 2008 and 2010 models could be due to (1) sample size, (2) host composition, (3) location of capture, and/or (4) a climatic difference between the collecting years. The number of leeches in the 2008 sample was over three times that of the 2010 sample. However, the same range of sizes was obtained in both years and the same broad cohort was modeled in both. Hosts other than *Oligocottus snyderi* were a minor factor in both temporal samples; eight leeches from three other hosts in 2008 and seven from two other hosts in 2010. The 2008 sample was obtained from a 75 km stretch of Pacific coastline from north of, south of, and at the type locality, whereas the 2010 sample was from a 75 m stretch of rocky intertidal zone at the type locality. These samples are not that dissimilar as 99/162 leeches in 2008 were also taken from the type locality. The fourth variable may have had the greatest impact on the difference in the number of reproductive bouts in 2008 vs. 2010. In terms of oceanic temperatures in the sampled region, 2008-9 was considered to be a "near normal" season that preceded a 2009-10 El Niño period, whereas 2010-11 was a strong La Niña period (http://www.cpc.ncep.noaa.gov/products/analysis_monitoring/ensostuff/ensoyears.shtml; http://ggweather.com/enso/oni.htm). 2008 began with cooler water temperatures that rose to "normal" in the latter part of the year. 2010 began at the height of a warm period that cooled toward a La Niña during the latter two-thirds of that year. Temperature is known to affect the seasonal occurrence, abundance, mating, development, mobility, and cocoon deposition in leeches (Llewellyn, 1965; Sawyer and Hammond, 1973; Daniels and Sawyer, 1975; Khan and Meyer, 1978). These annual different temperature patterns may be the case of the different reproductive patterns between 2008 and 2010.

Heptacyclus buthi may be similar to its congeners with an annual life cycle, with a single extended reproductive period (Khan and Meyer, 1976, 1978). This would account for the single cohort in 2010 and the comparable "older" cohort in 2008, and the broad range of sizes observed in both years for these cohorts. The cooler La Niña conditions for the latter part of 2010 may have been sufficient to prevent *H. buthi* from having a second reproductive period that year. We were fortunate to have sampled *H. buthi* during two recent periods of temperature extremes. Future sampling during El Niño and La Niña episodes is needed to verify the relationship between temperature and the number of annual reproductive bouts in *H. buthi*.

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Table 2-1. Statistics for models.

Year	Best-fit Model	Log likelihood	df	BIC	Mean	σ
2008	Univariate, unequal variance with two components	-245.38	5	-516.197	0.8236, 2.3903	0.2138, 1.2712
2010	Univariate, one component	-88.468	2	-184.801	2.372	1.405

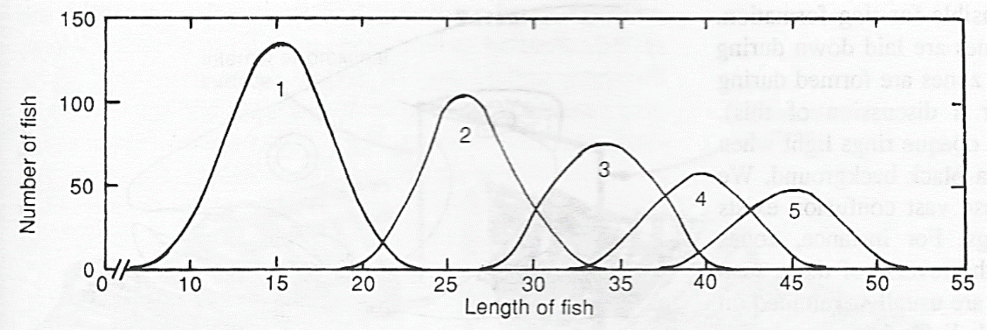

Figure 2-1. Size frequency curve used for the Peterson method (from Cailliet et al. 1986).

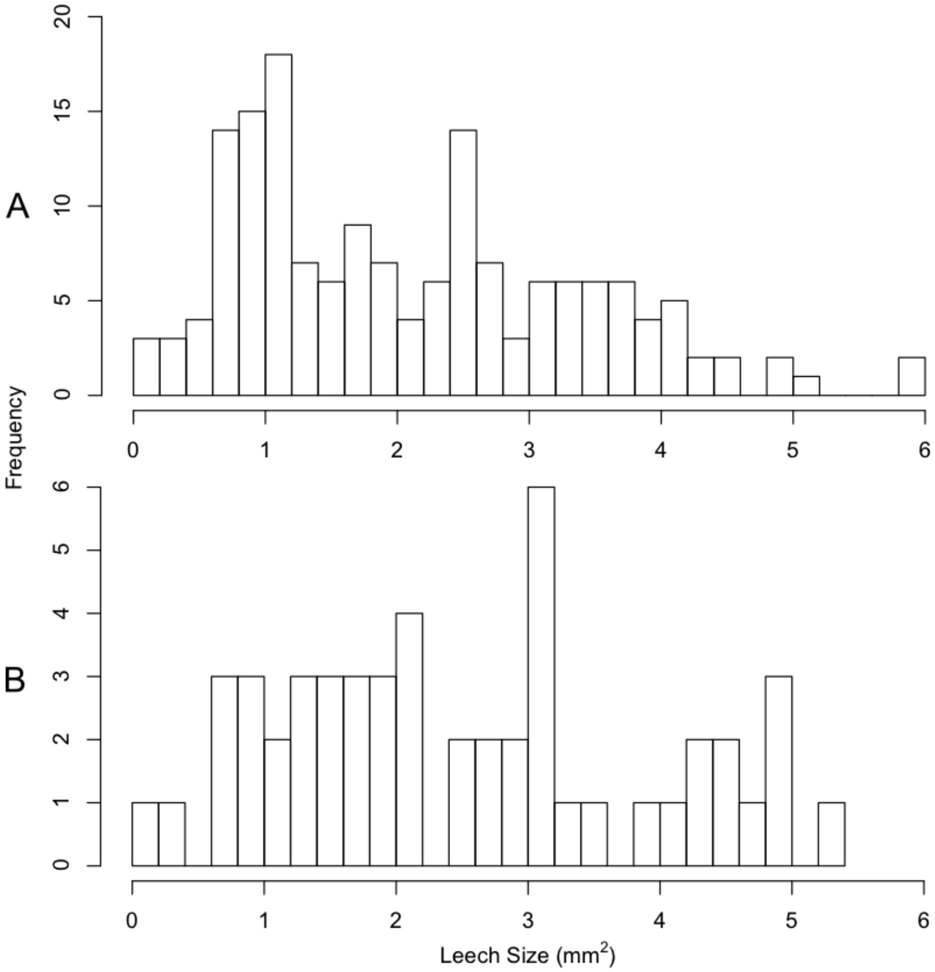

Figure 2-2. Size distribution histograms of *H. buthi* from A: 2008 and B: 2010, by count.

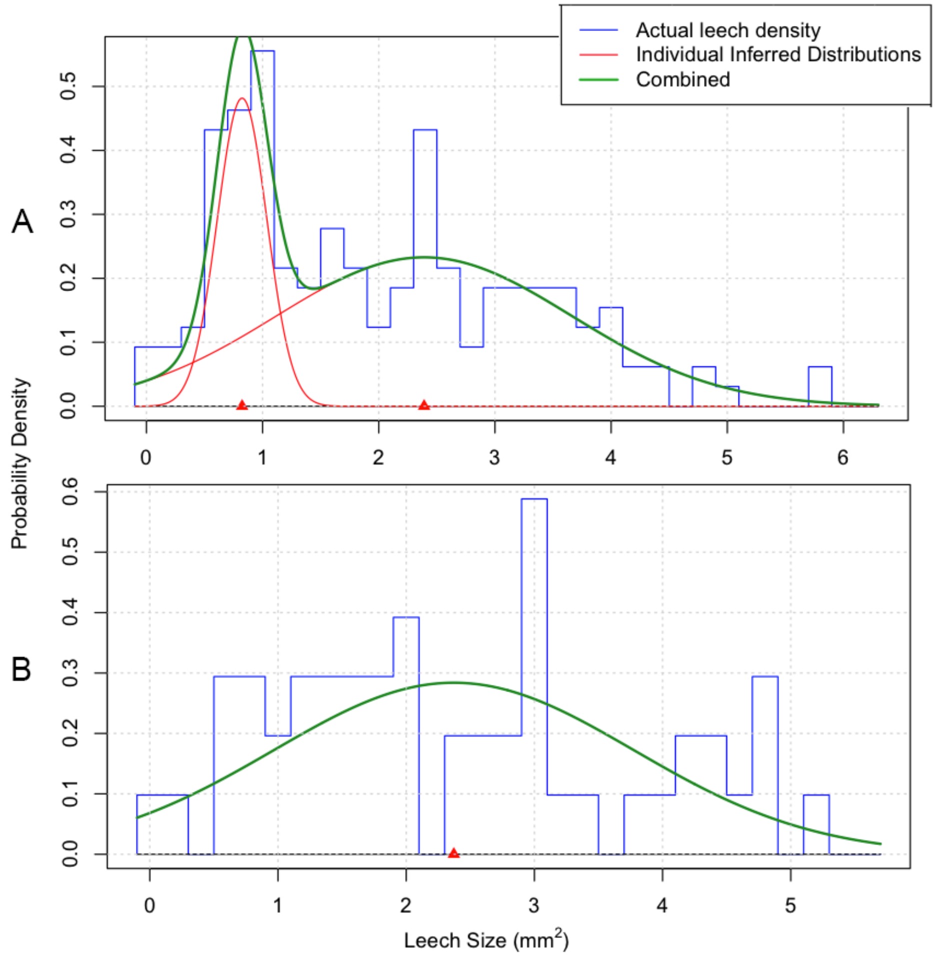

Figure 2-3. Best fit distributions of *H. buthi* from A: 2008 and B: 2010. Mean values for the curves are indicated with triangles. The size distribution histogram is shown in the background (blue).

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