A taxonomic revision of *Octomacrum* Mueller, 1934 (Monogenea: Polyopisthocotylea: Octomacridae), including an 18S DNA based phylogeny

By
Jonathon J.H. Forest

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Approved:	Dr. David Cone Supervisor
Approved:	Dr. Roland Cusack Committee member
Approved:	Dr. Timothy Frasier Committee member
Approved:	Dr. Florian Reyda External examiner
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A taxonomic revision of *Octomacrum* Mueller, 1934 (Monogenea: Polyopisthocotylea: Octomacridae), including an 18S DNA based phylogeny

by Jonathon J. H. Forest

August, 2011

Abstract

The genus of fish parasite, *Octomacrum*, consists of six species that parasitize certain catostomids and cyprinids in North America and Europe. Little is known about these parasites, and the original descriptions lack detail and standardization of descriptive terminology. The present study undertakes a taxonomic revision of the genus, using new material when available and extensive museum collections of all six species in the genus: *O. lanceatum*, *O. microconfibula*, *O. semotili*, *O. spinum*, *O. mexicanum* and *O. europaeum*. A dichotomous key to all six species using features of the attachment clamps with new figures is provided. A molecular phylogeny based on 18S DNA provides enough variation to infer relationships between 2 members of the genus, while grouping the remaining four into an unresolved clade. This study concludes that in spite of poor descriptions for many of the species, the taxonomy of this group has remained stable with few misidentifications.

August, 2011

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Introduction

The Class Monogenea Carus, 1863, is a diverse and widespread group of aquatic parasites consisting of 958 nominal species as of 1956 (Bychowsky, 1956) and an estimated 3000 to 4000 by 1998 (Whittington, 1998), with thousands likely yet to be discovered (Whittington, 1998). They parasitize aquatic vertebrates, including most fishes (Poulin and Morand, 2000), amphibians, and occasional cephalopods and aquatic reptiles (Bychowsky, 1956). The Monogenea are not known to infect birds, but one, *Oculotrema hippopotami* Stunkard, 1924, has a specified microhabitat on the eye of the hippopotamus (Bychowsky, 1956). Monogeneans have developed elaborate haptoral attachment organs to fix themselves to the host surface. Most are ectoparasites and attach to body surfaces, gills, and fins. Some monogeneans are endoparasites and invade such organ systems as the gut, respiratory and circulatory systems, and the kidney and associated ducts (Kearn, 1994). The haptoral attachment organs are diverse and vary depending on the microhabitat of a specific species of parasite (Bychowsky, 1956).

The Class has traditionally been divided into two Orders, the Monopisthocotylea Odhner, 1912 and Polyopisthocotylea Odhner, 1912. Monopisthocotyleans are histozoic grazers and have a haptor comprised of mostly hooks and supporting structures (Malmberg, 1970; Hoffman, 1999). Polyopisthocotyleans are more or less stationary blood feeders and have complex haptors on the posterior end that consist mainly of numerous scleritized clamping structures and muscular suckers (Bychowsky, 1956; Yamaguti, 1963; Khotenovsky, 1985; Hoffman, 1999).

This traditional view is still commonly used in the field; however, other taxonomic classifications have been proposed, most notably, Boeger and Kritsky (1993)

who separate Monogenoidea Bychowsky, 1937 (Monogenea) into Subclasses Polyonchoinea Bychowsky, 1937 (Monopisthocotylea) and Oligonchoinea Bychowsky, 1937 (Polyopisthocotylea). In the present study, the author continues to use the more commonly referred to Monopisthocotylea and Polyopisthocotylea Subclasses seen in general reviews (Yamaguti, 1963; Hoffman, 1999).

The Monogenea observed in the present study, *Octomacrum* Mueller, 1934, (Fig. 1) is a polyopisthocotylean that includes 6 nominal species worldwide (Fig. 2; Table 1) from cyprinid (minnow) and catostomid (sucker) fishes. Mueller (1934) established the genus with the description of the type species *O. lanceatum* from *Catostomus commersoni* and *Erimyzon sucetta oblongus* hosts (both catostomids) in New York State, U.S.A. There have been 5 subsequent descriptions within the genus: *O. microconfibula* Hargis, 1952 in Virginia; *O. europaeum* Roman and Bychowsky, 1956 in Romania; *O. semotili* Dechtiar, 1966 in Ontario; *O. spinum* Dansby and Shoemaker, 1973 in West Virginia; and *O. mexicanum* Lamothe-Argumedo, 1980 in Michoacán, Mexico, all of which are based on specimens from cyprinids.

Few studies have attempted to determine the origin and evolutionary history of these parasites. Lambert and Le Brun (1988) speculate a recent common ancestor of *Octomacrum* and *Diplozoon* occurring in the Pacific resulting in the present distributions of Diplozoidae in Eurasia and Africa and Octomacridae mainly in North America. This study, however, ignores the presence of *Octomacrum*, *O. europaeum*, in Europe.

Species of *Octomacrum* infect gills of their fish host, feeding on blood while clasping onto the lamellae with the posterior haptor (Mueller, 1934; Bychowsky, 1956; Beverley-Burton, 1984; Hoffman, 1999). They have evolved 4 pairs of attachment

clamps within the haptor. These clamps consist of hard-parts that can include posterior and anterior mid-sclerites and a varying number of lateral sclerites being embedded within a muscular sucker (Fig. 3) (Sproston, 1945; Bychowsky, 1956; Llewellyn, 1956, 1960; Shaw, 1979). These clamps are also the main diagnostic feature used to distinguish the 6 species in the genus. Compared to other polyopisthocotyleans, the clamps of *Octomacrum* are considered to be relatively complex (Bychowsky, 1956) in that the muscular sucker is diminished and the parasite appears to rely mainly on a clamping action to secure itself to the gills. Other features of *Octomacrum*, such as form of the testis and presence of the genital pore, help distinguish *Octomacrum* from the other 2 members of the Suborder Discocotylinea Bychowsky, 1957, namely Discocotylidae Price, 1936 and Diplozoidae Tripathi, 1959. The Discocotylidae has a Y-shaped vagina, numerous testes, a genital pore lacking musculature and intestinal crura that extend into the haptor, whereas *Octomacrum* lacks a vagina, has a single, multi-lobed testis, a genital pore surrounded by obvious musculature and crura that end blindly in the peduncle before entering the haptor (Mueller, 1934, Hoffman, 1999). The Diplozoidae is most easily distinguished by having a single intestinal crus, no genital pore and fusion of two individuals in mature worms, whereas *Octomacrum* has bilateral intestinal crura, a genital pore with surrounding muscle and mature worms remain as separate individuals (Hoffman, 1999).

A number of poor original descriptions due to errors in morphometric analysis, the lack of any type of standardization in anatomy, and no previous critical examination of clamp morphology, may result in improper membership of species within the genus. Therefore, it seems prudent that the genus *Octomacrum* receive a taxonomic revision.

This taxonomic revision of *Octomacrum* follows that of Maxted (1992) and compiles all available literature, examines museum type and voucher material when possible, and collects new material for supplementary morphometric data and molecular sequences (18S DNA gene). Observations on development of the attachment clamps of one of the species is reported. Finally, a molecular phylogeny based on the 18S DNA gene is presented with which to discuss the host specificity and radiation of these parasites within the cyprinid and catostomid fishes.

Materials and Methods

Study Localities and Sampling

Cyprinid and catostomid fish were collected from localities in Nova Scotia and Ontario during summer sampling seasons (May to September) of 2008 to 2010. Samples were also collected and shipped to Saint Mary's University by colleagues worldwide (Fig. 4; Table 2).

The method of sampling fish varied. Seine nets and baited traps were used in Ontario, while baited traps, seine nets, gill nets, Illinois fyke nets and electrofishing were used in Nova Scotia. Live fish were transported to the Harkness Laboratory of Fisheries Research in Ontario, or the Saint Mary's University Taxonomy Laboratory in Nova Scotia for necropsy. Fish were anesthetised in an overdose of MS222, then aged (Tesch, 1970), sexed (using internal reproductive organs) and total length measured. The gills were removed and examined microscopically. If parasites were present, they were fixed immediately in 10 % phosphate buffered formalin for future morphological work, or stored in either 95 or 100 % molecular grade ethanol (EtOH) for subsequent DNA analysis, or examined live in wet mounts for any structure that was more easily visible with live worms.

Taxonomy

Species of *Octomacrum* were identified using the original species descriptions of Mueller (1934), Hargis (1952), Roman and Bychowsky (1956), Dechtiar (1966), Dansby and Shoemaker (1973) and Lamothe-Argumedo (1980) as well as examination of all available type specimens. Type and voucher museum material came from Canadian Museum of Nature (CMNPA or NMCP) Ottawa, Ontario, Canada; Harold W. Manter

Laboratory of Parasitology (HWML) Lincoln, Nebraska, U.S.A.; United States National Parasite Collection (USNPC) Beltsville, Maryland, U.S.A.; Colección Nacional de Helmintos (CNHE) Mexico City, Distrito Federal, Mexico; and Biologické Centrum Akademie Věd Česke Republicky Institute of Parasitology (CSAV) Branisovska, Česke Budejovice, Czech Republic. For identifications, parasites were either mounted live when available, and studied in wet mounts or as 10 % formalin fixed specimens that were prepared as wet or whole mounts (see details below). Identifications were based on the shapes of the diagnostic clamps as per the original species descriptions, as well as supplementary information provided by Beverley-Burton (1984) and Matejusová and Koubková (2002) (Fig. 5). Clamp terminology is taken and modified mainly from Bychowsky (1956), Sproston (1945), Llewellyn (1956, 1960) and Shaw (1979). Parasites were studied, measured, and photographed using a Zeiss compound microscope with AxioVision Rel. 4.5 image taking software. Mature worms were distinguished from juveniles by the presence of fully developed reproductive organs and vitellaria. The generic morphological description is modified from Yamaguti (1963). All measurements taken are between extreme points of the respective body part, are in μm and are illustrated in Figure 6.

Clamp hard-part morphology was studied in greater detail using a modified digestion method of Harris et al. (1999). Ethanol stored specimens were removed from 100 % EtOH, placed into 70 % EtOH for 10 minutes, then into distilled water to fully rehydrate. The haptor was removed and the upper body returned to 100 % EtOH. The haptor was placed on a clean, round glass coverslip in 25 μl of distilled water (dH_2O). As much as possible of a second wash of dH_2O was removed with a pipette. Then, while

observing the haptor with aid of a stereoscope, another 25 µl of dH_2O was added. If the sample still had debris, a third wash was done. Into a last wash of dH_2O, 2.5 µl of premade 10 X digestion buffer (75 mM Tris-HCL pH 8.0, 10 mM EDTA, 5 % SDS and proteinase K (Qiagen) to a final concentration of 100 µg/ml) was added and the preparation incubated at 50 °C for 10 minutes. If the tissue was not fully digested a further 2.5 µl of digestion buffer was added and incubated for another 10 minutes at 55 °C. Once the tissue was digested, the buffer was removed with a pipette and the specimen was examined as a wetmount by light microscopy (LM).

Staining

Specimens of *Octomacrum* were stained using techniques reported by Pritchard and Kruse (1982). Formalin fixed material was washed twice in dH_2O for 30 minutes to 1 hour each, followed by a 20 minute wash in glacial acetic acid (100 %). The samples were then washed once more in dH_2O overnight. They were stained with Mayer's hematoxylin or Mayer's carmalum (recipe from Pritchard and Kruse, 1982) for 30 minutes. After staining, parasites were washed in dH_2O for 10 minutes and transferred through a dehydration sequence starting from 35 % EtOH to 50 % and 70 % each for 10 minutes. A destain was administered using 1 % acid alcohol, the specimen carefully observed until the outer layers appeared to clear. Specimens then were fully dehydrated by being transferred from 70 % EtOH to 85 %, 90 % and two steps of 100 %, each for 10 minutes. Once specimens were fully dehydrated they were cleared using xylene, first with 50/50 xylene/100 % EtOH, then two steps of full xylene for 5 minutes each step. Once cleared, specimens were prepared as permanent mounts in Canada balsam.

Histology

Histological techniques were employed to prepare stained tissue sections of *Octomacrum*. The embedding procedure was modified from Humason (1967) which involved a dehydration stage of 70 % EtOH for 30 minutes, 95 % ethanolic eosin (100 mg Eosin spirit soluble, 90.0 ml absolute ethanol, 0.5 ml glacial acetic acid and 9.5 ml dH_2O) for 30 minutes, 95 % EtOH for 15 minutes, and 2 steps of 100 % EtOH for 30 minutes each. An intermediate step consisted of 30 minutes in a 50/50 solution of EtOH/xylene. This was followed by clearing in two changes of xylene for 20 minutes each, and an overnight bath in xylene saturated paraffin wax in an oven set just above the wax melting point (~ 58 °C). The following day the worm was transferred to melted wax for 1 hour, then transferred into fresh wax for another hour. The parasite was finally placed in fresh wax, the wax being allowed to solidify. The wax block was fixed to a chuck and placed in the microtome. Sections were cut between 7 and 10 μm and ribbons were placed in water, heated and flattened onto glass microscope slides and allowed to dry.

Deparaffinization of the wax sections occurred as follows. Four steps of 2 minutes in xylene, replacing with fresh xylene every step. One minute in 100 % EtOH followed by 1 minute in new 100 % EtOH, 30 seconds in 95 % EtOH, 45 seconds in 70 % EtOH, 1 minute wash in dH_2O. These sections were placed in coplin jars with diluted Giemsa (Sigma-Aldrich) stain to $1/5^{th}$ its stock strength and incubated at 37 °C for 3 hours, rinsed in dH_2O and differentiated in 2 quick dips in 0.5 % aqueous acetic acid. This was followed by a 5 second dehydration through 50, 70, 95 and 2 100 % dips of EtOH and a final clearing in xylene. Slides were permanently mounted in Canada balsam.

Line Drawings

Anatomical drawings were completed by selecting a representative specimen for each species of *Octomacrum*, which would later be augmented with further observations of other specimens of the same species, and using a drawing tube mounted to a light microscope to trace the worm. These hand drawings were scanned into the program CorelDraw9 and were re-traced using the freehand drawing tool option. These lines were reshaped to match the original hand drawing and any imperfections from the hand drawing were eliminated. This process was repeated for all six species for whole-body drawings as well as clamp, hook, and egg drawings when available.

Statistical Analysis

Statistical tests were performed on two groups of *O. microconfibula*, 1 group from common shiner, *Luxilus cornutus*, in Ontario; and 1 group from golden shiner, *Notemigonus crysoleucas*, in Nova Scotia. This analysis tests significance in any differences between morphometrics of these two groups. Morphometrics considered normal, based on an Anderson-Darling test of normality using statistical program Minitab15 (Minitab Inc.) were compared using a t-test (whole body length, greatest body width, right buccal sucker length, right buccal sucker width, left buccal sucker width, pharynx length, genital pore length, genital pore width, haptor length, haptor width, anterior right clamp length, anterior right clamp width, anterior left clamp length, anterior left clamp width, second right clamp width, second left clamp length, second left clamp width, third right clamp length, third right clamp width, third left clamp length, third left clamp width, posterior right clamp length, posterior right clamp width, posterior left clamp length, posterior left clamp width, right anchor blade, right anchor handle, left

anchor blade, left anchor handle) and data considered not normal by the same test were compared using the nonparametric Mann-Whitney test (left buccal sucker length, pharynx width, second right clamp length). Significance is maintained at $\alpha < 0.05$. This test was confirmed using the Bonferroni correction method which showed a correlation of 0.9.

Molecular Techniques

DNA Extraction

DNA was extracted from EtOH stored specimens through either phenol:chloroform or Qiagen DNEasy Blood and Tissue kits (Qiagen). For phenol:chloroform extractions, specimens were taken from 100 or 95 % EtOH and put into 150 µl of lysis buffer (10 mM Tris at pH 8.0; 10 mM EDTA at pH 8.0; 2 % SDS; 0.1 M NaCl; 40 mM DTT) (Budowle et al. 2000). The tube contents were mixed thoroughly and proteinase K (Qiagen) was added to each tube to a final concentration of 0.1 mg/µl. Samples were mixed well again and left to lyse overnight at room temperature. The following day proteinase K (0.1 mg/µl) was again added to each sample and incubated in a 65 °C water bath for 1 hour. Specimens were then incubated at 37 °C for 1 hour and left at room temperature overnight. The next day, 150 µl of phenol:chloroform (Sigma) was added to each sample and mixed for 5 minutes before being centrifuged at 12,000 x g for 1 minute. Tubes were carefully removed and the top aqueous layer, containing the DNA, was pipetted off and put into a new 1.5 ml tube. A second shot of 150 µl phenol:chloroform was added and the specimens, mixed again for 5 minutes and centrifuged at 12,000 x g for 1 minute. The aqueous layer was again pipetted off and put in a new 1.5 µl tube and 150 µl of chloroform added (Fluka), mixed for five minutes, and

spun at 12,000 x g for 1 minute. The top layer was pipetted into a new tube and spun at 12,000 x g for a further 5 minutes. The DNA was removed and put into a new 1.5 ml tube. Ammonium acetate (0.9 M) and 300 µl of ice-cold 95 % EtOH were added to each sample and mixed for 5 minutes. These samples were then placed in a -20 °C freezer overnight. The following day, samples were spun at 12,000 x g for 10 minutes to form a DNA pellet at the bottom of the tube. The ethanol was poured off and 100 µl of 70 % EtOH was added and spun at 12,000 x g for 10 minutes. Ethanol was then carefully decanted again and a kimwipe used to remove any traces of EtOH while being careful not to disturb the pellet. Tubes were then left uncapped for 20 minutes so the EtOH could evaporate. The pellet was then redissolved in 75 µl of $TE_{0.1}$ (10 mM Tris-HCl; 0.1 mM EDTA), mixed well and incubated at 65 °C for 2 minutes in the water bath.

Extractions through Qiagen kits followed manufacturer's instructions with the exception of eluting DNA in 50 µl of Buffer AE instead of the suggested 200 µl.

Quantification

Samples were quantified using a Nanodrop 2000 spectrophotometer (Thermo Scientific). Premade calf thymus DNA of known concentrations (1 ng/µl, 5 ng/µl, 10 ng/µl and 50 ng/µl) were used to standardize the session. Two µl of sample and the blank ($TE_{0.1}$) were used in each trial of the Nanodrop and DNA concentrations were found for each specimen of *Octomacrum*. If samples were shown to have possible chloroform contamination, based on a higher than 1.8 of the 260/280 absorbance ratio taken from the spectrophotometer, DNA was placed in 0.6 ml tubes and put into an open polymerase chain reaction (PCR) machine at 65 °C for 5 minutes to evaporate the chloroform and

leave only DNA. Samples were then standardized down to 1 ng/µl working solutions. Once quantification was complete, DNA was stored at -30 °C.

Primer Selection

Two sets of primers, targeting different regions, were designed for this study. Primers designed to amplify 18S DNA were designed from Campos et al. (1998) using the program Primer3 (Rozen and Skaletsky, 2000). *Octomacrum* sequence data from Campos et al. (1998) found in GenBank was copied into the program, forward and reverse primers selected, and the top primer combination was selected with a sequence of 20 bp for the forward primer (REOctoM1F 5' - CAG ACA GCC TGA AGC GAA AG – 3'), 21 bp for the reverse (REOctoM1R 5' - TTC ACG GGG GAT AAT TAC AAA – 3'). Primers to amplify the internal transcribed spacer (ITS) region, consisting of ITS1, 5.8S, and ITS2, were designed using reported primers for the genes surrounding the ITS region, 18S and 28S. The reverse compliment, obtained by flipping the primer and replacing the base with it's counterpart (G becomes C, T becomes A, and vice versa), was taken from the reverse running 18S primer, JLR25 (Campos et al., 1998) and from the forward running 28S primer, C1 (Mollaret et al., 2000). These new primers should now run in the proper direction to amplify the ITS region, however, they were not used due to a lack of primer binding.

Amplification

DNA was amplified with a Titanium Taq kit (Clontech) and modified protocol. For each 20 µl reaction, the following PCR reagents were used in a MyCycler thermal cycler (BioRad): 1 X Titanium Taq buffer, 0.3 µM forward primer, 0.3 µM reverse primer, 1 X dNTP mix, 1 X Taq polymerase, 3 ng of template DNA, and dH_2O to bring

the reaction to the final volume. The reaction times and temperature for these 20 µl reactions were as follows: 10 minutes at 94 °C, 30 cycles of 30 seconds at 94 °C, 1 minute at 52 °C and 1 minute at 72 °C, followed by 45 minutes at 65 °C and a final hold of 4 °C.

Five µl of PCR product were size-separated and visualized on 1.5 % agarose gels with a 100 bp ladder (Promega) and dyed with a 6 X loading dye. Gels ran for 55 minutes at 90 V and 400 mA and were stained for 45 minutes in ethidium bromide. A Bioimaging system (Syngene, ChemiGenius^2) imager was used to view stained gels (Fig. 7).

Sequencing and Analysis

Once PCR amplification of the 18S DNA gene was deemed successful, with clear bands from electrophoresis, PCR products were sequenced externally by GENEWIZ Inc., New Jersey, U.S.A.

Molecular Phylogenetics

A BLAST (Basic Local Alignment Search Tool) search was performed based on returned sequence data to identify similar sequences (Altschul et al., 1997). Sequences were edited using 4Peaks vers. 1.7 (Mekentosj) by confirming each base was correct and removing poorly sequenced ends and primer sequences. Consensus sequences were made by taking the reverse compliment of the reverse sequence and aligning with the forward sequence. Consensus sequences from different individuals in the same species that were identical were deemed the same haplotype and only one sequence was used. Sequences were aligned using Clustal X vers. 2 (Larkin et al., 2007) three different times; once with gap opening and extension penalties at their default values (15 and 6.66

respectively), once with penalties at twice their values, and once at half their values. Once alignments were complete and variable sites observed, these sites were double-checked in the original sequence data to ensure they represented true variation and not sequencing errors.

The transition and transversion ratio, as well as the rate of heterogeneity, were then found using Tree-Puzzle vers. 5.2 (Schmidt et al., 2002) and recorded. The outgroup was then selected from the BLAST results, choosing a previously known closely related species, *Discocotyle sagittata*, and an alignment that included all species of *Octomacrum* as well as the outgroup was done with gap opening and extension penalties set to default.

Phylogenetic trees were constructed using the programs Tree-Puzzle vers. 5.2 and PHYLIP vers. 3.69 (Felsenstein, 1989) and viewed using the program FigTree vers. 1.3 (Morariu, 2008). Maximum likelihood and Fitch trees were constructed using 1000 bootstrap repeats. Bootstraps were created using the seqboot package in PHYLIP. The previously calculated transition/transversion ratio as well as the rate of heterogeneity were used to create distance matrices using the dnadist package in PHYLIP. A Fitch tree was created using the Fitch package and the consense package to compile all 1000 trees. A maximum likelihood tree was created using Tree-Puzzle and the quartet puzzling software, after which the consense package was used again to compile all 1000 bootstrap trees. The HKY85/F84 model of evolution was used based on it's ability to allow unequal base frequencies and distinguish between transition and transversion rates. These trees were viewed using FigTree, rooting the tree on *D. sagittata*, changing font sizes, and labelling the nodes with the bootstrap percentages.

Results

1. Taxonomy

Class Monogenea Carus, 1863

Order Polyopisthocotylea Odhner, 1912

Suborder Discocotylinea Bychowsky, 1957

Family Octomacridae Yamaguti, 1963

Genus *Octomacrum* Mueller, 1934

Emended diagnosis (Fig. 1):

Body opaque, lanceolate, compressed dorsoventrally, with moderately developed peduncle, and symmetrical haptor. Body tegument smooth. Mouth subterminal with 2 lateral buccal suckers. Small buccal cavity extending into pharynx, immediately posterior to buccal suckers. Pharynx becoming intestinal crus; bifurcation anterior to unarmed genital pore, surrounded by obvious musculature. Crura with short diverticula; crura ending blindly, before haptor. Ovarium elongate, often coiled clockwise, less often counter-clockwise; extensive, more developed eggs located anteriorly. Obvious, pigmented, bifurcated yolk reservoir at mid-body. Genito-intestinal canal present dextrally. Vagina absent. Testis post ovarium, single, lobed mass. Vitellaria from genital pore to haptor. Haptor with 4 pairs of lateral clamps, fourth (posterior) pair often noticeably smaller. Single pair of posterior anchors between fourth clamp pair; anchor with curved blade and straight handle. Single egg filament anterior and posterior, often coiled anteriorly. Parasites of Catostomidae and Cyprinidae. Type species *Octomacrum lanceatum* Mueller, 1934.

Remarks:

This generic diagnosis is modified from Yamaguti (1963) with additional mention of the musculature surrounding the genital pore, a smooth tegument, specifics of buccal cavity and pharynx, presence of an obvious bifurcate yolk reservoir and correction of infections occurring on both Catostomidae and Cyprinidae.

Octomacrum lanceatum Mueller, 1934 (Figs. 5a, 8a – d, 19-22; Tables 1, 3, 9)

Synonym: *Octobothrium sagittatum* Wright, 1879.

Type hosts: *Catostomus commersoni* Lacépède, 1803 and *Erimyzon sucetta* Lacépède, 1803 (Catostomidae).

Type locality: Frederick Creek, Constantia, New York, U.S.A. (43°14'52"N, 76°00'04"W).

Other hosts and localities: *Catostomus macrocheilus* Girard, 1856 (Catostomidae) British Columbia (Arai and Mudry, 1983), *Catostomus discobolus* Baird and Girard, 1853 (Catostomidae) Utah (Brienholt and Heckmann, 1980), *Carpiodes carpio* Rafinesque, 1820 (Catostomidae) Illinois (Robinson and Jahn, 1980), *Catostomus catostomus* Forster, 1773 (Catostomidae) British Columbia (Bangham and Adams, 1954), *Mylocheilus caurinus* Richardson, 1836 (Cyprinidae) British Columbia (Bangham and Adams, 1954), *Luxilus cornutus* Mitchill, 1817 (Cyprinidae) Wisconsin (Fischthal, 1947), *Notropis heterolepis* Eigenmann and Eigenmann, 1893 (Cyprinidae) Ontario (Bangham, 1941), *Pimephales promelas* Rafinesque, 1820 (Cyprinidae) Ontario (Beverley-Burton, 1984), and allegedly *Oncorhynchus tshawytscha* Walbaum, 1792 (Salmonidae) British Columbia (Arai and Mudry, 1983).

Site: Gills.

Specimens examined:

Measurements based on museum slides listed as *O. lanceatum* or those recorded as *Octomacrum* sp. that were later identified as *O. lanceatum* (n = 30). Syntype specimens (USNPC 32570.1, USNPC 32570.2, USNPC 32570.3, USNPC 32570.4). Other specimens (CMNPA 2009-0002, CMNPA 2009-0006.1, CMNPA 2009-0006.2, CMNPA 2009-0006.3, CMNPA 2009-0006.4, CMNPA 2009-0006.5, CMNPA 2009-0006.6, CMNPA 2009-0004.1, CMNPA 2009-0004.2, CMNPA 2009-0004.3, CMNPA 2009-0004.4, CMNPA 2009-0004.5, CMNPA 2009-0004.6, CMNPA 2009-0005.1, CMNPA 2009-0005.2, CMNPA 2009-0005.3, CMNPA 2009-0005.4, NMCP 1987-2357, CMNPA 1989-0115, CMNPA 1989-0114, CMNPA 1989-0113, CMNPA 1989-0112, CMNPA 1989-0111, CMNPA 1989-0110, NMCPC 1984-6045, CMNPA 2009-0001.1, CMNPA 2009-0001.2, CMNPA 1987-0109, CMNPA 2009-0003.1, CMNPA 2009-0003.2, CMNPA 2009-0003.3, CMNPA 1989-0108, NMCP 1987-2259, NMCP 1987-2400.1, NMCP 1987-2400.2, NMCP 1987-2400.3, HWML 49148, HWML 20499 – 1, HWML 20499 – 2, HWML 20498, HWML 37110 – 1, HWML 37110 – 2, USNPC 74014.1, USNPC 74014.2, USNPC 74014.3, USNPC 32570 - 2, USNPC 77644, USNPC 80234, USNPC 80235). Measurements are presented as type specimen average size ± the standard deviation (range of measurements; specimens measured) followed by similar measurements of all other specimens represented within square brackets.

Redescription:

Summary morphometrics are provided in Figs 19-22 and Table 3. Whole body (Fig. 8a) 5125 ± 254.5 (4945 – 5305; n = 2) [5757 ± 1329 (3356 – 9677; n = 47)] long, greatest body width 1239 ± 77.9 (1184 – 1294; n = 2) [1294 ± 269.8 (743 – 1831; n =

50)] at midbody. Mouth subterminal, slit like, with lateral buccal suckers; right buccal sucker 100 ± 3.0 (98 – 102; n = 2) [125 ± 22.6 (70 – 167; n = 47)] long, 87 ± 3.2 (85 – 90; n = 2) [117 ± 18.5 (66 – 151; n = 47)] wide; left buccal sucker 92 ± 6.4 (87 – 96; n = 2) [125 ± 23.0 (72 – 168; n = 48)] long, 80 ± 4.0 (78 – 83; n = 2) [118 ± 20.2 (55 – 147; n = 48)] wide. Pharynx immediately posterior to buccal suckers; 100 ± 5.1 (96 – 103; n = 2) [133 ± 24.2 (90 – 184; n = 36)] long, 64 ± 2.0 (63 – 65; n = 2) [82 ± 17.2 (51 – 119; n = 36)] wide. Almost no esophagus. Crura with diverticula along length, crura ending blindly within peduncle. Round, unarmed genital pore surrounded by muscular sucker immediately posterior to intestinal bifurcation, 119 ± 0 (119 – 119; n = 1) [118 ± 18.7 (83 – 178; n = 40)] long, 131 ± 0 (131 – 131; n = 1) [130 ± 20.7 (82 – 185; n = 40)] wide. Ovary midbody, coiled clockwise; posterior ova smaller. Testis immediately posterior to ovary, as large, multi-lobed mass. Tanned eggs (Fig. 8d), when present, most often in close proximity to genital pore; 2 egg filaments, short posterior filament, long coiled anterior filament. Vitellaria located throughout main body, from genital pore to peduncle, never entering haptor. Genito-intestinal canal not seen in types or additional material. Haptor more often long than wide, 696 ± 67 (634 – 791; n = 4) [936 ± 214.4 (592 – 1468; n = 49)] long, 614 ± 90 (542 – 734; n = 4) [816 ± 207.5 (285 – 1266; n = 49)] wide. Four pairs of attachment clamps (Fig. 8b), first pair largest and each subsequent pair smaller. Clamps with 4 main hard-parts embedded in surrounding muscle tissue; mid-sclerite having flared posterior section and shorter, non-flared anterior section; three lateral sclerites on each side of mid-sclerite; dorsal and ventral anterior, lateral sclerites similar in length, slightly longer ventral, posterior lateral sclerite; posterior mid-sclerite and anterior lateral sclerites pitted; two accessory sclerites ventral

to the anterior mid-sclerite. Specific clamp morphometrics are available in Tables 3 and 10. Posterior anchors (Fig. 8c) between fourth pair of clamps; right anchor blade, [19 ± 4.0 (14 – 29; n = 13)]; handle, [37 ± 4.5 (30 – 48; n = 12)]; left anchor blade, [19 ± 1.7 (16 – 23; n = 15)]; handle, [37 ± 3.4 (30 – 42; n = 14)].

Remarks:

Mueller's (1934) original description provided exact details of the internal anatomy of *O. lanceatum*, based primarily on stained histological sections. The genus was considered new on the basis of the absence of a vagina, absence of 2 posterior anchors and the clamps are not stalked. In fact, the type material submitted by Mueller and examined in this study does not show the presence of any posterior anchors, however, their presence has since been observed in subsequent material of *O. lanceatum*, first reported by Hargis (1952), and all other species in the genus.

The original description of *O. lanceatum* includes only a limited number of dimensions which appear to have been reported accurately, and generally fall into the ranges I report herein with some discrepancies (Table 3). These discrepancies illustrate how much variation in morphometrics can occur within a species in this genus. For instance, Mueller reports a total body size of 5000 – 6000 whereas material examined in this study shows that body size of *O. lanceatum* can range from anywhere between approximately 3300 and 9700. Understanding that this large variation can occur in mature worms is essential when trying to identify species of *Octomacrum*.

Octomacrum lanceatum can be distinguished from other species of *Octomacrum* by its comparatively large body size (typically the only species to reach a length of over 5000, and the only species reported to exceed 6700) (Fig. 14), having a haptor that is

consistently longer than wide (on average approximately 936 x 816) compared to all other species that either have haptors wider than long or of similar size, by a genital pore that in relation to overall body size is small (18 % of the body width at the genital pore compared to 45 % of the body width at the genital pore in *O. microconfibula*), and by the form and arrangements of the haptoral clamps. It is these clamps that are the main identifying factor, especially when considering the large variation in mature worm size that can happen within a species. *Octomacrum lanceatum*, besides possessing the largest clamps (Fig. 5) also has the most complex clamps (along with *O. microconfibula*) with the most hard-parts. *Octomacrum lanceatum* is one of three species to have both a posterior and anterior mid-sclerite (along with *O. microconfibula* and *O. spinum*), one of three species to possess accessory sclerites (along with *O. microconfibula* and *O. europaeum*) and one of three species to have anterior ventral lateral sclerites that are pitted (along with *O. microconfibula* and *O. mexicanum*). This is the only species to have an anterior mid-sclerite that is half the size or less than its posterior mid-sclerite and have neither the anterior nor posterior mid-sclerite with an open flared tip (like *O. microconfibula* and *O. europaeum*).

The distribution of *O. lanceatum* is widespread in Canada being reported from Nova Scotia (present study) to British Columbia (Bangham and Adams, 1954) and as far south as Virginia and west as Illinois in the United States (Table 1).

This is the only species in the genus that infects catostomid fish as well as cyprinids. *Octomacrum lanceatum* has been reported from 6 different catostomid hosts and 5 cyprinid hosts whereas the other 5 species have only been known to infect a maximum of 5 cyprinid species and no catostomids (Table 1).

Octomacrum lanceatum has been reported from a single salmonid host (Arai and Mudry, 1983). This report is likely a misidentification due to the close resemblance of *Octomacrum* to a similar species of polyopisthocotylid parasite, *Discocotyle sagittata*, known from salmonid hosts (Hoffman, 1999). Species of *Octomacrum* can be distinguished from *Discocotyle* by the structure of the testes (a singular mass in *Octomacrum* and numerous in *Discocotyle*), the vagina (absent in *Octomacrum* and Y-shaped in *Discocotyle*), the genital pore (surrounded by musculature in *Octomacrum* and lacking musculature in *Discocotyle*), the expanse of the intestinal crura (ending blindly in or just before the peduncle of *Octomacrum* and continuing through the peduncle and into the haptor of *Discocotyle*), and the form of the eggs (a single mature egg in *Octomacrum* with a short, straight filament posteriorly and a long, many times coiled filament anteriorly and multiple mature eggs in *Discocotyle* lacking any filament) (Mueller, 1934; Beverley-Burton, 1984).

The original genus description done by Mueller, 1934, shows very detailed reports of the genito-intestinal tract of *O. lanceatum*. These fine details can only be observed by using histological techniques to see the canals and ducts that Mueller observed. Although histology and live observations of worms were done and observations taken, the fine details of this part of *Octomacrum* anatomy was not evident in the material I prepared. The presence of a gastero-intestinal canal is not uncommon within the Polyopisthocotylea and can be seen in a number of parasites such as species of *Discocotyle*, *Lintaxine* Sproston, 1946, *Kuhnia* Sproston, 1945 and *Mazocraeoides* Price, 1936 (Beverley-Burton, 1984; Hoffman, 1999).

Sequence data for *O. lanceatum* from the 18S DNA gene (616 bp) can be seen in Figure 15 showing the alignment with the other species of *Octomacrum*, and in Appendix B. A BLAST search returned 99 % similar sequence data of a number of other monogenean parasites including *D. sagittata* and *O. mexicanum*. These sequence data are taken from 2 individual *O. lanceatum* parasites, both from Lochabor Lake, Antigonish, Nova Scotia with both forward and reverse sequences. There was no variation in sequence data between these individuals.

Although there are two hosts listed as type hosts from the original description of *O. lanceatum*, only the host *Erimyzon sucetta* is represented by the available type material which consists of 2 syntypes consisting of a total 4 specimens (USNPC 32570).

This species is well represented by the material available from museums. Slides, including the syntypes, were easily available and in large numbers from 3 museums in Canada and the United States, and the material was generally in good condition.

This species is one of the most studied beyond the original description. Boeger and Kritsky (1993) used morphological characteristics of this species and *O. semotili* to place Octomacridae within a phylogenetic study of the Monogenea. A single sequence of this species of the 28S DNA gene has been used in a number of Monogenea phylogenetic studies (Mollaret et al., 2000; Jovelin and Justine, 2001; Olson and Littlewood, 2002) and this same sequence has been used to infer the relationship between Octomacridae and Diplozoidae (Sicard et al., 2002). Finally, Hathaway et al. (1995) studied spermatogenesis of this species and reveal ultrastructure and morphological details about the sperm.

Octomacrum microconfibula Hargis, 1952 (Figs. 5b, 9a-d, 19-22; Tables 1, 4, 5, 10)

Type host: *Notemigonus crysoleucas* Mitchill, 1841 (Cyprinidae).

Type locality: Westhampton Lake, Virginia, U.S.A. (37°34'33"N, 77°32'20"W).

Other hosts and localities: *Mylocheilus caurinus* Richardson, 1836 (Cyprinidae) British Columbia (Arai and Mudry, 1983), *Phoxinus eos* Cope, 1861 (Cyprinidae) New Brunswick (Cone, 1980), *Margariscus margarita* Cope, 1867 (Cyprinidae) New Brunswick (Cone, 1980), *Richardonius balteatus* Richardson, 1836 (Cyprinidae) British Columbia (Shepard and Mace, 1980).

Site: Gills. Parasite located between adjacent rows of lamellae, attached to the inner surface of one filament, typically half way between the gill arch and tip of the filament.

Specimens examined:

Measurements based on museum slides identified as *O. microconfibula* and slides labeled as *Octomacrum* sp. later identified as *O. microconfibula* (n = 7). Holotype specimen (USNPC 37382). Other specimens (NMCP 1987-2655, NMCPC 1984-7069, NMCP 1987-2356.1, NMCP 1987-2356.2, HWML 40048). Measurements presented as type specimen average size ± standard deviation (range of measurements; specimens measured) followed by similar measurements of all other specimens represented within square brackets.

Redescription:

Summary morphometrics are available in Figs. 19-22 and Table 4. Whole body (Fig. 9a) 5565 ± 287.2 (5362 – 5768; n = 2) [4706 ± 0133 (3559 – 5768; n = 4)] long, greatest body width 1208 ± 111 (1129 – 1286; n = 2) [1052 ± 234 (731 – 1286; n = 4)] at midbody. Mouth subterminal, slit like, with lateral buccal suckers; right buccal sucker 67

± 8.1 (61 – 73; n = 2) [73 ± 8.4 (61 – 80; n = 4)] long, 58 ± 9.0 (51 – 64; n = 2) [67 ± 14.7 (51 – 187; n = 4)] wide; left buccal sucker 70 ± 7.0 (65 – 75; n = 2) [75 ± 7.4 (65 – 83; n = 4)] long, 58 ± 7.5 (53 – 64; n= 2) [72 ± 17.9 (53 – 94; n = 4)] wide. Pharynx immediately posterior to buccal suckers 92 ± 9.8 (85 – 99; n = 2) [98 ± 9.1 (85 – 106; n = 4)] long, 70 ± 15.4 (59 – 81; n = 2) [74 ± 12.5 (59 – 88; n = 4)] wide. Indistinct esophagus. Crura with diverticula along length; crura ending blindly within peduncle. Unarmed, square with round corners genital pore surrounded by muscular sucker directly posterior to intestinal bifurcation, 158 ± 20.7 (144 – 173; n = 2) [178 ± 35 (144 – 227; n = 4)] long, 150 ± 15.3 (139 – 161; n = 2) [181 ± 47.8 (139 – 249; n = 4)] wide. Ovary midbody, coiled clockwise; posterior ova smaller. Testis immediately posterior to ovary, as large, multi-lobed mass. Tanned eggs (Fig. 9d), when present, most often in close proximity to genital pore; 2 egg filaments, short posterior filament, long coiled anterior filament. Vitellaria located throughout main body, from genital pore to peduncle, never entering haptor. Genito-intestinal canal not seen in types or additional material. Haptor more often wide than long, [370 ± 98.6 (301 – 440; n = 2)] long, [401 ± 102.3 (329 – 473; n = 2)] wide. Four pairs of attachment clamps (Fig. 9b), first pair largest and each subsequent pair smaller. Clamps with 4 main hard-parts embedded in haptoral tissue, lacking noticeable muscle tissue; mid-sclerite having flared, heart-shaped anterior section and straight, thin, similar length posterior mid-sclerite; three lateral sclerites on each side of mid-sclerite; dorsal and ventral anterior, dorsal shorter than ventral and claw-shaped; posterior ventral lateral sclerite, similar in length to anterior ventral lateral sclerite; anterior and posterior mid-sclerite and anterior dorsal lateral sclerite pitted; four accessory sclerites, two ventral to each the anterior and posterior mid-sclerite. Clamp

morphometrics are available in Table 4 with other body morphometrics and in Table 10 as a genus wide comparison. Posterior anchors (Fig. 9c) between fourth pair of clamps; right anchor blade, 19 ± 0.6 (19 – 19; n = 2) [19 ± 0.4 (19 – 19; n = 3)]; handle, 39 ± 1.7 (38 – 41; n = 2) [39 ± 2.0 (37 – 41; n = 3)]; left anchor blade, 21 ± 0.4 (21 – 21; n = 2) [18 ± 3.9 (13 – 21; n = 4)]; handle, 41 ± 2.5 (39 – 43; n = 2) [36 ± 6.9 (27 – 43; n = 4)].

Three juvenile specimens of *O. microconfibula* were measured (NMCP 1987-2356.1, NMCP 1987-2356.2, HWML 40048) as were specimens collected from Nova Scotia on golden shiner, and from Ontario on common shiner. The summaries of these morphometrics can be seen in Table 4 and as a comparison of fresh material collected from the 2 geographic regions with statistical analysis in Table 5.

Remarks:

Hargis (1952) described *O. microconfibula* using detailed morphometrics that typically fall in range with the observations in this study, with some variation. Though reported by Hargis to have a total body length of 4350, the present study has seen mature worms as small as approximately 2500 and as large as approximately 6200 (both from new material collected by the author). Likewise, even in the diagnostically important haptoral clamps, originally reported as approximately 90 long by 100 wide have been seen as small as 65 long by 65 wide (fresh material) and as large as 107 long (fresh material) by 156 wide (museum material). This illustrates further the need of structure, form and arrangements of the clamp hard-parts in identifying and not morphometrics alone.

Octomacrum microconfibula, the second species described in the genus and so named because of the "small clamps" that it possess compared with those of the only

other species known at the time, *O. lanceatum*, contains one of the most detailed descriptions in terms of morphometrics with few errors (Table 4). The first pair left clamp originally reported as 833 long and second pair right clamp reported as 9 long. These measurements are likely meant to be 83 and 90 respectively.

Octomacrum microconfibula is distinguished from other members in the genus by what is the largest genital pore (45 % of body width at genital pore compared to the smallest of 18 % of body width in *O. lanceatum*), a haptor more often wide than long (also seen in *O. semotili*), and the features of the haptoral clamps. Clamps of *O. microconfibula* have a similar size anterior and posterior mid-sclerite (along with *O. spinum*), have a distinctive heart-shape flare at the tip of the anterior mid-sclerite (along with *O. europaeum*), have claw-shaped anterior dorsal lateral sclerites (along with *O. europaeum*) and are one of the most complex forms of clamps (along with *O. lanceatum*). Clamps of *O. microconfibula* are the only ones in the genus to have claw-shaped and pitted anterior dorsal lateral sclerites, and have both anterior and posterior mid-sclerite accessory sclerites. This species also shows the most diminished musculature surrounding the clamps.

This species is well represented in the available museum material. Though not as easily available as *O. lanceatum*, there are still a number of available slides, both holotype and voucher, from three different museums in Canada and the United States, and generally the material is in good condition. In some cases not all the anatomy can be viewed, such as the posterior anchors, or in some cases the body is distorted but the haptor is of good quality. In addition, there are also representative juvenile voucher specimens available from both the Canadian Museum of Nature and the Harold Manter

Laboratory of Parasitology. This provides some of the only information on juvenile specimens, in addition to a single juvenile paratype of *O. spinum* (which lacks any definitive clamp information).

These few museum specimens of *O. microconfibula* juveniles, coupled with observations in the present study of *O. microconfibula* juveniles, provide information for the first reported account of juvenile *Octomacrum* development. The clamp and anchor development may show how the parasite initially attaches to the gills of the host and what mechanisms take over in attachment. The anchors of *O. microconfibula* appear to be fully developed, even as a juvenile (Fig. 16). This is likely the primary attachment method of *Octomacrum* as a juvenile. Clamp development likely begins shortly after infection, with the furthest posterior pair of clamps growing first and progressing anteriorly. Once clamp development begins and the body of the parasite increases in size, the clamps appear to take over as the primary method of attachment on the gills for the hooks have become relatively small.

Octomacrum microconfibula has a wide Canadian distribution from Nova Scotia (Forest, 2011) to British Columbia (Shepard and Mace, 1980) and as far south as Virginia (Hargis, 1952). It can be found on 5 previously reported cyprinid hosts (Table 1) as well as the newly observed in this study, *Luxilus cornutus*, a new host record for the species.

Octomacrum microconfibula 18S DNA sequence data from both golden shiner, *Notemigonus crysoleucas*, in Nova Scotia (608 bp) and common shiner, *Luxilus cornutus*, in Ontario (589 bp) can be seen in Figure 15 in the genus alignment, as well as in Appendix B. Performing a BLAST search reveals a 99 % similarity to other monogenean parasites. Three and five individuals of both forward and reverse sequences make up the

sequence data for *O. microconfibula* from golden shiner and common shiner respectively. Specimens from golden shiner show no variation within themselves, nor do those from common shiner. These two sequences are classified as the same species based on the morphologically identical clamps, even though nearly all body morphometrics were statistically different (except the left anchor blade and the left and right anchor handles) (Table 5). This again demonstrates the variation that can be observed within a species. The molecular data show a single base pair variation between these two haplotypes (Fig. 17) at 421 bp. This small variation may represent the beginnings of an evolutionary change based on either geographical distance or host infection.

Octomacrum europaeum Roman and Bychowsky, 1956

(Figs. 5c, 10a – c, 19-22; Tables 1, 6, 10)

Type host: *Alburnoides bipunctatus* Bloch, 1782 (Cyprinidae).

Type locality: Romania (45°56'05"N, 21°30'06"E).

Site: Gills.

Specimens examined:

Measurements based on museum slides identified as *O. europaeum* (n = 2) (CSAV 2822-9 - 1, CSAV 2822-9 - 2). Measurements are presented as voucher material average size ± the standard deviation (range of measurements; specimens measured).

Supplemental diagnosis:

Summary morphometrics are seen in Figs. 19-22 and Table 6. Whole body (Fig. 10a) 1493 (n = 1) long, greatest body width 379 (n = 1) at midbody. Mouth subterminal, slit like, with lateral buccal suckers; right buccal sucker 43 ± 10.0 (36 – 50; n = 2) long,

42 ± 16.2 (30 – 53; n = 2) wide; left buccal sucker 35 ± 17.5 (23 – 48; n = 2) long, 42 ± 17.1 (30 – 54; n = 2) wide. Pharynx immediately posterior to buccal suckers 67 ± 17.1 (55 – 79; n = 2) long, 37 ± 17.9 (25 – 50; n = 2) wide. Almost no esophagus. Crura with diverticula along length, crura ending blindly within peduncle. Round, unarmed genital pore surrounded by muscular sucker immediately posterior to intestinal bifurcation, 91 ± 37.4 (65 – 118; n = 2) long, 104 ± 43.3 (73 – 134; n = 2) wide. Ovary midbody, coiled counter-clockwise; posterior ova smaller. Testis immediately posterior to ovary, as large, multi-lobed mass. Eggs not visible in this material. Vitellaria located throughout main body, from genital pore to peduncle, never entering haptor. Genito-intestinal canal not seen in specimens. Haptor approximately square, 235 (n = 1) long, 247 (n = 1) wide. Four pairs of attachment clamps (Fig. 10b), second pair largest, first and third pair slightly smaller and fourth pair noticeably smaller. Clamps with 4 main hard-parts embedded in surrounding muscle tissue; mid-sclerite having flared, heart-shaped anterior section and lacking posterior section; three lateral sclerites on each side of mid-sclerite; anterior dorsal, ventral and posterior ventral similar in length; anterior dorsal sclerite thicker than other lateral sclerites and claw-shaped at the terminal end; mid-sclerite pitted; two accessory sclerites ventral to the anterior mid-sclerite. Summary clamp morphometrics are available in Table 6 with all morphometrics of *O. europaeum* and in Table 10 comparing clamp morphometrics between species.

Remarks:

This species is the only one in the genus that has had subsequent morphological work performed (Matejusová and Koubková, 2002) (Table 6). The material observed in

this study generally falls within the morphometrics described previously and is consistent in making this the smallest member of the genus.

This is the single species in the genus found in Europe and is known from fairly small geographic regions in Romania and the Czech Republic. In addition, the only host it has been reported from, riffle minnow *Alburnoides bipunctatus*, is currently red-listed and therefore difficult to obtain material from. Only one specimen of *O. europaeum* was acquired and was put towards molecular use (see below). The type material of this species was also unavailable and only 2 voucher slides were tracked down, and of those 2, one is damaged (CSAV 2822-9 – 2) and only useful in a few morphometrics. This makes *O. europaeum* the most under represented species in the genus.

Octomacrum europaeum is unique in being the smallest reported genus at 1250 in length (Roman and Bychowsky, 1956) as well as the only species to be found in European waters. This species also has the shortest, or most underdeveloped, peduncle giving less a lanceolate shape and more of a segmented upper body and haptor. Clamps of *O. europaeum* are most similar to that of *O. microconfibula* in that they share many of the same sclerites, including the claw and heart-shaped anterior dorsal lateral sclerites and anterior mid-sclerites respectively. *Octomacrum europaeum* is one of only two species to not possess a posterior mid-sclerite (along with *O. semotili*) and therefore also does not have the associated posterior mid-sclerite accessory sclerites that *O. microconfibula* has. Similarly, only the mid-sclerite, no lateral sclerites, are pitted in this species. This species also has more obvious musculature surrounding the sclerites than does *O. microconfibula.*

18S DNA sequence data (611 bp) was obtained from a single specimen of *O. europaeum*. Sequence data can be seen in the genus alignment (Fig. 15) and in Appendix B. A BLAST search shows 99 % similarity to a number of other monogenean parasites, including the outgroup *Discocotyle sagittata*, also found in Europe. As seen in Fig. 15, *O. europaeum* shows some of the highest variation to other members of the genus and has a few notable sites in common with the outgroup (for example at 43 bp which is the only variation to occur outside the 342 to 449 bp region).

This is the only species to not receive a redescription in this study due to the lack of type material, but rather receives a supplemental diagnosis to the original description.

Octomacrum semotili Dechtiar, 1966 (Figs. 5d, 11a – d, 19-22; Tables 1, 7, 10)

Type host: *Semotilus atromaculatus* Mitchill, 1818 (Cyprinidae).

Type locality: Algonquin Park, Ontario, Canada (45°32'60"N, 78°35'60"W).

Other hosts and localities: *Phoxinus eos* Cope, 1861 (Cyprinidae) Ontario (Dechtiar, 1972), *Phoxinus neogaeus* Cope, 1867 (Cyprinidae) Ontario (Dechtiar, 1972), *Margariscus margarita* Cope, 1867 (Cyprinidae) Ontario (Dechtiar, 1972).

Site: Gills. Parasite located between adjacent rows of lamellae, attached to the inner surface of one filament, typically half way between the gill arch and tip of the filament.

Specimens examined:

Measurements based on museum slides identified as *O. semotili* (n = 9). Holotype (USNPC 61682) and paratype specimen (USNPC 61683). Other specimens (NMCP 1987-2672, NMCP 1987-2671, NMCP 1987-2670, NMCP 1987-2669, NMCP 1987-2654, NMCP 1987-1897.1, NMCP 1987-1897.2, NMCP 1987-1897.3, NMCP

1987-1893.1, NMCP 1987-1893.2). Measurements presented as type specimen average size ± the standard deviation (range of measurements; specimens measured) followed by similar measurements of all other specimens represented within square brackets.

Redescription:

Summary morphometrics are available in Figs. 19-22 and Table 7. Whole body (Fig. 11a) 2771 ± 799.7 (2206 – 3337; n = 2) [3185 ± 970 (1710 – 4397; n = 12)] long, greatest body width 774 ± 160.1 (661 – 887; n = 2) [864 ± 193.9 (544 – 1137; n = 12)] at midbody. Mouth subterminal, slit like, with lateral buccal suckers; right buccal sucker 65 ± 3.4 (62 – 67; n = 2) [70 ± 15.4 (53 – 100; n = 12)] long, 73 ± 2.4 (71 – 74; n = 2) [72 ± 15.2 (55 – 104; n = 12)] wide; left buccal sucker 68 ± 6.2 (64 – 73; n = 2) [70 ± 13.4 (48 – 97; n = 12)] long, 70 ± 0 (70 – 70; n = 2) [70 ± 11.6 (58 – 96; n = 12)] wide. Pharynx immediately posterior to buccal suckers; 100 ± 19.4 (86 – 114; n = 2) [90 ± 18.0 (62 – 114; n = 12)] long, 59 ± 1.9 (58 – 61; n = 2) [60 ± 14.1 (44 – 92; n = 12)] wide. Almost no esophagus. Crura with diverticula along length, crura ending blindly within peduncle. Unarmed, square with round corners genital pore surrounded by muscular sucker immediately posterior to intestinal bifurcation, 169 ± 0.8 (168 – 170; n = 2) [158 ± 43.4 (99 – 251; n = 12)] long, 169 ± 5.3 (165 – 173; n = 2) [159 ± 44.5 (100 – 249; n = 12)] wide. Ovary midbody, coiled counter- clockwise; posterior ova smaller. Testis immediately posterior to ovary, as large, multi-lobed mass. Tanned eggs (Fig. 11d) when present, most often in close proximity to genital pore; 2 egg filaments, short posterior filament, long coiled anterior filament. Vitellaria located throughout main body, from genital pore to peduncle, never entering haptor. Genito-intestinal canal not seen in types or additional material. Haptor more often wide than long, typically in a trapezoidal

shape, 358 ± 96.3 (290 – 426; n = 2) [360 ± 87.4 (227 – 503; n = 12)] long, 452 ± 145.1 (350 – 555; n = 2) [424 ± 111 (258 – 628; n = 12)] wide. Four pairs of attachment clamps (Fig. 11b), first three pairs of similar size, posterior pair noticeably smaller. Clamps with 3 main hard-parts embedded in surrounding muscle tissue; anterior mid-sclerite having equally flared ends, lacking posterior mid-sclerite; two lateral sclerites on each side of mid-sclerite; anterior and posterior ventral sclerites similar in length; anterior mid-sclerite pitted; accessory sclerites absent. Morphometrics of the clamps are in Table 7 along with all morphometrics of *O. semotili* and in Table 10 showing variation of clamps within the genus. Posterior anchors (Fig. 11c) between fourth pair of clamps; right anchor blade, 15 ± 3.7 (12 – 18; n = 2) [17 ± 2.6 (12 – 21; n = 10)]; handle, 31 ± 5.2 (27 – 35; n = 2) [32 ± 5.0 (26 – 41; n = 10)]; left anchor blade, 12 (n = 1) [17 ± 2.5 (12 – 20; n = 10)]; handle, 25 (n = 1) [32 ± 7.0 (25 – 42; n = 9)].

Remarks:

A number of descriptive morphometrics were presented in the original description of *O. semotili*, which generally are in accordance with the material observed in this study. A single error was reported in the original description, stating the right buccal sucker has a length of 750, which in all likelihood was meant to be reported as 75 (Table 7).

Of the species that infect waters within Canada, *O. semotili* is the second best represented (following *O. lanceatum*) with two museums in Canada and the United States providing holotype, paratype and voucher material with slides in good condition.

Of the Canadian species of *Octomacrum*, *O. semotili* shares the closest resemblance to *O. microconfibula* based on their similar size (compared to that of the third Canadian species, *O. lanceatum*) and the general shape of the haptor (more often

wide than long), however the similarities end in regards to the clamps where the only feature not common to all species that is shared by *O. semotili* and *O. microconfibula* is a pitted anterior mid-sclerite. The clamps of *O. semotili* can be distinguished from *O. microconfibula* by having noticeably more musculature surrounding sclerites. The clamps of this species are also the most basic of all species in the genus, being the only ones to not have 3 pairs of lateral sclerites (*O. semotili* has no anterior nor posterior dorsal lateral sclerite).

The distribution of *O. semotili* is limited to Canadian waters, with reports of infection on 4 cyprinid species. This species has only been reported from Ontario (Dechtiar, 1966; 1972; present study) and Manitoba (Beverley-Burton, 1984) (Table 1).

Sequence data for *O. semotili* from the 18S DNA gene (562 bp) can be seen in a genus alignment (Fig. 15) and in Appendix B. This sequence is the result of forward and reverse sequences from 10 replicates from 2 hosts (*Phoxinus eos*, redbelly dace and *Semotilus atromaculatus*, creek chub) in Ontario with no variation between any of the sequences. When a BLAST search is performed, the sequence of *O. semotili* shows 99 % similarity between a number of monogenean parasites.

Octomacrum spinum Dansby and Shoemaker, 1973

(Figs. 5e, 12a – c, 19-22; Tables 1, 8, 10)

Type host: *Campostoma anomalum* Rafinesque, 1820 (Cyprinidae).

Type locality: Twelvepole Creek, West Virginia, U.S.A. (37°53'02"N, 82°07'15"W).

Site: Gills.

Specimens examined:

Measurements based on museum slides identified as *O. spinum* (n = 2). Holotype (USNPC 71060) and paratype specimens (USNPC 71061). No voucher material was observed. Measurements are presented as type specimen average size (number of specimens measured).

Redescription:

Summary morphometrics are available in Figs. 19-22 and Table 8. Whole body (Fig. 12a) 1885 (n = 1) long, greatest body width 278 (n = 1) at midbody. Mouth subterminal, slit like, with lateral buccal suckers; right buccal sucker 36 (n = 1) long, 35 (n = 1) wide; left buccal sucker 34 (n = 1) long, 32 (n = 1) wide. Pharynx immediately posterior to buccal suckers, not measurable in type material. Almost no esophagus. Crura with diverticula along length, crura ending blindly within peduncle. Round, unarmed genital pore surrounded by muscular sucker immediately posterior to intestinal bifurcation, 78 (n = 1) long, 75 (n = 1) wide. Ovary midbody, coiled clockwise; posterior ova smaller. Testis immediately posterior to ovary, as large, multi-lobed mass. Eggs were not present in type material. Vitellaria located throughout main body, from genital pore to peduncle, never entering haptor. Genito-intestinal canal not seen in type or other material. Haptor often similar size in length and width, 265 (n = 1) long, 278 (n = 1) wide. Four pairs of attachment clamps (Fig. 12b), second pair largest, first and third pair similar size, fourth pair smallest. Clamps with 4 main hard-parts embedded in muscle tissue only at base of sclerites; anterior mid-sclerite having flared posterior section and thinner, non-flared posterior section; three lateral sclerites on each side of mid-sclerite; anterior and posterior ventral lateral sclerites similar in length, posterior dorsal lateral sclerite shorter; anterior and posterior mid-sclerites pitted; no accessory sclerites present.

Clamp morphometrics are available in Tables 8 and 10. Posterior anchors (Fig. 12c) between fourth pair of clamps; right anchor blade, 18 (n = 1), handle, 31 (n = 1); left anchor blade, 17 (n = 1), handle, 33 (n = 1).

A juvenile paratype specimen (USNPC 71061) along with 2 recently collected *O. spinum* from *Campostoma anomalum* in Tennessee were also measured. These measurements are provided in Table 8.

Remarks:

Octomacrum spinum is the second species in the genus (along with *O. europaeum*) to have very little material available through museums. The only two species examined in this study are the holotype and paratype from the original species description, with the paratype as a juvenile with very few morphometrics available due to a side-view mount. The single available mature worm, the holotype, does however show clearly, body features of *O. spinum* including important clamp details.

This species was also collected from *C. anomalum*, the only recorded host for this parasite, from two rivers in Tennessee for both molecular (see below) and morphological analysis. The morphometrics in this study are generally larger than those originally reported (original total body size 1117 compared to museum material at 1885 and fresh material as large as 3119) including the clamp measurements (originally 48 long by 49 wide, museum material 59 long by 68 wide and fresh material 78 long by 93 wide) (Table 8). This variation, even within the hard parts of the clamps at almost double the original description, again illustrates the variation that can occur within species in this genus.

The distribution of this species is limited to mid-eastern United States with the only recorded reports coming from West Virginia and the newly reported Tennessee in this study, infecting only one reported cyprinid host (Table 1).

Octomacrum spinum is the smallest species in North America with a largest reported body size of 3119 (present study) and smallest of 1117 (Dansby and Shoemaker, 1973). This coupled with its high host specificity and small geographic distribution are all useful in identifying this species. The clamps of *O. spinum* are one of only two species to have anterior and posterior mid-sclerites of approximately the same size and both with pits (along with *O. microconfibula*). The species also is similar to *O. microconfibula* in that is has diminished musculature surrounding the clamps. This species is easily distinguished from *O. microconfibula*, however, due to the lack of a heart-shaped end of the anterior mid-sclerite (seen in *O. microconfibula*) and the lack of an anterior dorsal lateral bar (seen as claw-shaped in *O. microconfibula*). It is also the only species in the genus to possess posterior dorsal lateral sclerites.

Sequence data for the 18S DNA gene of *O. spinum* (599 bp) can be seen in a genus alignment (Fig. 15) and in Appendix B. This sequence data is a consensus of the forward and reverse sequences of 5 replicate specimens from Tennessee with no variation between individuals. A BLAST search reveals sequences of *O. spinum* to be 99 % similar to other species of monogenean parasites.

A single study has been conducted that includes *O. spinum* since the original description in 1973. The prevalence of this species has been reported to increase as temperature increases in the warmer months (Joy et al., 1978).

Octomacrum mexicanum Lamothe-Argumedo, 1980

(Figs. 5f, 13a – d, 19-22; Tables 1, 9, 10)

Type host: *Algansea lacustris* Steindachner, 1895 (Cyprinidae).

Type locality: Patzcuaro Lake, Michoacán, Mexico (19°38'06"N 101°38'29"W).

Site: Gills.

Specimens examined:

Measurements based on museum slides identified as *O. mexicanum* (n = 7). Paratype specimens (CNHE 232-2 – 1, CNHE 232-2 – 2, CNHE 232-2 – 3). Other specimens (HWML 39539 - 1.1, HWML 39539 - 1.2, HWML 39539 - 1.3, HWML 39539 – 2, CNHE 252-20 - 1.1, CNHE 252-20 - 1.2, CNHE 252-20 - 2). Measurements are presented as type specimen average size ± the standard deviation (range of measurements; specimens measured) followed by similar measurements of all other specimens represented within square brackets.

Redescription:

Summary morphometrics are provided in Figs. 19-22 and Table 9. Whole body (Fig. 13a) 4030 ± 296.6 (3791 – 4362; n = 3) [5164 ± 1035 (3791 – 6673; n = 10)] long, greatest body width 805 ± 108.2 (701 – 917; n = 3) [977 ± 245.1 (701 – 1437; n = 10)] at midbody. Mouth subterminal, slit like, with lateral buccal suckers; right buccal sucker 55 ± 4.1 (52 – 58; n = 2) [58 ± 7.1 (47 – 69; n = 9)] long, 64 ± 4.3 (60 – 69; n = 3) [61 ± 5.7 (53 – 69; n = 10)] wide; left buccal sucker 56 ± 2.5 (54 – 59; n = 3) [59 ± 6.6 (51 – 72; n = 10)] long, 62 ± 5.2 (57 – 68; n = 3) [62 ± 6.6 (52 – 70; n = 10)] wide. Pharynx immediately posterior to buccal suckers; 79 ± 17.1 (63 – 97; n = 3) [94 ± 16.1 (63 – 120; n = 10)] long, 69 ± 15.3 (57 – 86; n = 3) [61 ± 11.0 (47 – 86; n = 10)] wide. Almost no

esophagus. Crura with diverticula along length, crura ending blindly within peduncle. Unarmed, square with round corners genital pore surrounded by muscular sucker immediately posterior to intestinal bifurcation, 144 ± 21.2 (124 – 166; n = 3) [188 ± 38.0 (124 – 239; n = 10)] long, 153 ± 14.3 (137 – 165; n = 3) [188 ± 31.6 (137 – 234; n = 10)] wide. Ovary midbody, coiled clockwise; posterior ova smaller. Testis immediately posterior to ovary, as large, multi-lobed mass. Tanned eggs (Fig. 13d) when present, most often in close proximity to genital pore; 2 egg filaments, short straight posterior filament, long coiled anterior filament. Vitellaria located throughout main body, from genital pore to peduncle, never entering haptor. Genito-intestinal canal not seen in types or additional material. Haptor square, 527 ± 177.2 (403 – 730; n = 3) [509 ± 127.5 (390 – 730; n = 10)] long, 447 ± 98.7 (339 – 533; n = 3) [548 ± 131.7 (339 – 764; n = 10)] wide. Four pairs of attachment clamps (Fig. 13b), first three pairs similar in size, all noticeably larger than fourth pair. Clamps with 4 main hard-parts embedded in surrounding muscle tissue; anterior mid-sclerite having flared posterior section, lacking anterior mid-sclerite; three lateral sclerites on each side of mid-sclerite; anterior and posterior ventral sclerites similar in length, shorter anterior dorsal sclerite; posterior mid-sclerite and anterior ventral sclerites pitted; no accessory sclerites present. Clamp morphometrics can be seen in Tables 9 and 10. Posterior anchors (Fig. 13c) between fourth pair of clamps; right anchor blade, 18 ± 1.3 (17 – 19; n = 2) [17 ± 2.0 (15 – 19; n = 3)]; handle, 32 ± 1.3 (31 – 33; n = 2) [28 ± 7.3 (20 – 33; n = 3)]; left anchor blade, 15 (n = 1) [17 ± 1.5 (15 – 18; n = 2)]; handle, 32 (n = 1) [28 ± 5.9 (24 – 32; n = 2)].

Remarks:

This species is fairly well represented by the museum material available from museums in both Mexico and the United States with specimens in good condition. This material generally falls into the ranges of those reported in the original description, with slight variations in morphometrics such as original total body size reported as approximately 4000 but has been seen in museum material to be as small as 3791 and as large as 6673 (Table 9).

The distribution of this species is unique and highly specified. The only reports of this parasite are from a single cyprinid host, *Algansea lacustris*, from one lake in Mexico, Lake Patzcuaro (Table 1). The very high host specificity of this species is a useful identifying factor. Also, this parasite has shown a notable decline within the past 30 years. Previous prevalence of infection had been close to 30 % and has dropped to recently seen less than 1 % (Pérez Ponce de León, 2011 pers. comm.).

Octomacrum mexicanum is the second largest reported species (following *O. lanceatum*) (Fig. 14) reaching upwards of 6673 (present study) and can often be identified at a glance by its uniquely formed peduncle. The peduncle is longer and thinner than any other species in the genus, clearly separating the main body from the haptor. The clamps of *O. mexicanum* are one of only three species to possess either an anterior or posterior mid-sclerite (along with *O. europaeum* and *O. semotili*), never both, however, it is the only species that possess a posterior mid-sclerite with no anterior mid-sclerite. There are no accessory sclerites in the clamps of *O. mexicanum* (similar to *O. semotili* and *O. spinum*) and the anterior ventral lateral sclerites are pitted (similar to *O. lanceatum*). This species also has the most defined clamp musculature in the genus.

Forward and reverse sequences of two individuals of *O. mexicanum* from *A. lacustris* in Patzcuaro Lake, Mexico were used in creating sequence data for the 18S DNA gene (598 bp) (Fig. 15, Appendix B). No variation was observed in these sequences. A BLAST search reveals 99 % similarity to other monogenean parasites.

Besides *O. lanceatum*, which has had sequence data reported from the 28S DNA gene, this is the only species to have any previous molecular analysis reported. Sequence data of *O. mexicanum* from the 18S DNA gene was used to place this species within a broader phylogeny of Monogenea study.

2. Key to the species of *Octomacrum* based on clamp morphology:

1a: Anterior and posterior mid-sclerites present.....2

1b: Anterior or posterior, never both, mid-sclerite present.....4

2a: Posterior and anterior mid-sclerites of similar length.....3

2b: Posterior mid-sclerite at least twice as long as anterior.....*O. lanceatum*

3a: Anterior dorsal lateral sclerite pitted and claw-shaped. Anterior mid-sclerite with heart-shaped flare. Accessory sclerites present.....*O. microconfibula*

3b: Anterior dorsal lateral sclerite not pitted nor claw-shaped. Anterior mid-sclerite lacking heart-shaped flare. Accessory sclerites not present .....*O. spinum*

4a: Mid-sclerite flared with heart-shape end. Accessory sclerites present. Claw-shaped anterior dorsal lateral sclerites.....*O. europaeum*

4b: Mid-sclerite not flared in heart-shape. No accessory sclerites present. No claw-shaped lateral sclerites.....5

5a: Anterior ventral lateral sclerites pitted. Anterior dorsal lateral sclerites present.....*O. mexicanum*

5b: Anterior ventral lateral sclerites not pitted. Anterior dorsal lateral sclerites not present.....*O. semotili*

3. Molecular Troubleshooting

Extractions with Qiagen kits often did not provide quantifiable DNA. Rather than Qiagen extractions, phenol:chloroform extractions were performed on all specimens of *Octomacrum*. These extractions all provided quantifiable DNA ranging between 1 ng/µl and 90 ng/µl concentrations.

Though the 18S DNA was eventually settled upon for phylogenetic analysis, a number of other regions were initially chosen (with respective primers) that each failed to amplify. Primers used:

18S:

Campos et al. (1998)

JLR24 5' – CGG AAT TCG CTA GAG GTG AAA TTC TTG G – 3'

JLR25 5' – CCG AAT TCC GCA GGT TCA CCT ACG G – 3'

Gilmore (2010, pers. comm.)

PBS18SF 5' - CGC GCA ACT TAC CCA CTC TC – 3'

PBS18SR 5'- ATT CCA TGC AAG ACT TTT CAG GC – 3'

CO1:

Folmer et al. (1994)

LCO1490 5' – GGT CAA CAA ATC ATA AAG ATA TTG G – 3'

HCO2198 5' – TAA ACT TCA GGG TGA CCA AAA AAT CA – 3'

Moszczynska et al. (2009)

Plat-diploCOX1F 5' – CGT TTR AAT TAT ACG GAT CC – 3'

Plat-diploCOX1R 5' – AGC ATA GTA ATM GCA GCA GC – 3'

ITS1, 5.8S, ITS2:

Campos et al. (1998)

RCJLR25 5' – CCG TAG GTG AAC CTG CGG AAT TCG G – 3'

Mollaret et al. (2000)

RCC1 5' – ATG CTT AAA TTC AGC GGG T – 3'

Cytochrome-b:

Verma and Singh (2003)

mcb398 5' – TAC CAT GAG GAC AAA TAT CAT TCT G – 3'

mcb869 5' – CCT CCT AGT TTG TTA GGG ATT GAT CG – 3'

12S:

Kocher et al. (1989)

L1091 5' – AAA AAG CTT CAA ACT GGG ATT AGA TAC CCC ACT AT – 3'

H1478 5' – TGA CTG CAG AGG GTG ACG GGC GGT GTG T – 3'

Generally, these primers would either yield no PCR product, or would bind at multiple points in the genome, creating numerous bands and PCR product that would not

sequence properly. Only cytochrome-b showed promise, as the streaking could be cut down by changing the PCR times slightly as well as adding bovine serum albumin (BSA), a protein designed to bind with other proteins in the reaction, effectively "cleaning" the PCR. Even with a cleaner cytochrome-b product, the gel still showed multiple sites of primer binding and when the product was sequenced, it was inconsistent as only 1 of the 8 samples sequenced nicely.

Newly designed ITS1, 5.8S, ITS2 primers (taken from reverse compliment primers of previous studies *O. lanceatum* 28S and *O. mexicanum* 18S) also failed to yield PCR product of *Octomacrum* in this study.

4. Phylogenetic Analysis

Sequence results for each species and the selected outgroup, *Discocotyle sagittata*, can be seen in Fig. 15 and Appendix B. Fitch and maximum likelihood phylogenetic trees were created from edited sequences (no variation when changing gap opening and extension penalties). The highest resolution, and most consistent with previous phylogenies, tree is the maximum likelihood and is shown in Fig. 18. Tree results show that 1000 of 1000 bootstrap repeats place *O. europaeum* in a clade separate from all other species and 990 of 1000 bootstrap repeats separate *O. lanceatum* from these sequences. Within the unresolved clade, 750 of 1000 bootstrap repeats separate *O. microconfibula* and *O. semotili*, the remaining Canadian species, from *O. spinum* and *O. mexicanum*, located in more southern regions. *Octomacrum microconfibula* from common shiner in Ontario is also placed with the southern species, likely reflecting this host species widespread geographical distribution (Scott and Crossman, 1973).

Discussion:

This study addresses issues with a lack of consistency in morphology and terminology of *Octomacrum*, as well as corrects the errors of previous reports and provides new insight into individual species and the genus as a whole. Five of the six nominal species of *Octomacrum* have been redescribed in this study, and the sixth, *O. europaeum*, has been given supplemental diagnoses. This study shows that the taxonomy of this group has been relatively stable and that based on morphology, all six members of the genus clearly fit into the Discocotylinea.

Octomacrum is a parasite of catostomid and cyprinid fish. There has been a single report of *Octomacrum* on a salmonid host (Arai and Mudry, 1983), however this is likely a misidentification, confusing *Discocotyle* for *Octomacrum*. Discocotylidae is another Family in the Suborder Discocotylinea along with Octomacridae and Diplozoidae. *Discocotyle* and *Octomacrum* are similar in many aspects and can easily be confused. A single species of *Discocotyle*, *Discocotyle sagittata* Diesing 1850, is found in Canada and infects only salmonid fish (Beverley-Burton, 1984).

The evolutionary history of *Octomacrum* is relatively unknown. A single study to speculate on the distributions of these parasites (Lambert and Le Brun, 1988) suggests a recent common ancestor of Octomacridae and Diplozoidae occurring in the Pacific resulting in distributions of Diplozoidae expanding throughout Eurasia and parts of Africa, while Octomacridae is localized to North America. This study, however, choose to ignore the "rare exception" of *O. europaeum*, found in Europe among the Diplozoidae. Based on the majority of the species of *Octomacrum* occurring in North America and this single exception of *O. europaeum* occurring in Europe, one could argue the possibility

that *O. europaeum* has been misidentified and is not actually a member of the Octomacridae.

Ideally, the sequence data provided in the present study could be analyzed with other 18S DNA sequence from as many species as possible within the Discocotylinea (Discocotylidae, Diplozoidae, Octomacridae) to validate the status of *O. europaeum* as a member of *Octomacrum*, however, only a single sequence of the 18S DNA gene is available for any species within the Discocotylinea that is not a species of *Octomacrum* and it has been used as the outgroup for the present study.

Although the use of molecular data as mentioned above would help solidify the position of *O. europaeum*, the biology of these polyopisthocotylid worms (the Discocotylinea) clearly places *O. europaeum* within *Octomacrum*. The presence of this species strictly on freshwater fish exclude the possibility of its membership within Discocotylidae, and the morphological differences of *O. europaeum* (single mature worms, bifurcate intestinal crura) exclude its membership in the Diplozoidae (fusion of mature worms, single intestinal crus). For these reasons, it seems possible that rather than have a common ancestor in the Pacific, as speculated previously, the present distribution of *Octomacrum* may coincide with continent separation resulting in most species localized in North America and one species in Europe. This is further corroborated by the molecular component of this study, as seen below.

A second study was conducted to determine if Octomacridae and Diplozoidae are sister species (Sicard et al., 2002), based on the fact that they are the only two polyopisthocotylean parasite clades that infect primarily freshwater fish. Sicard et al. found that they are not as closely related as previously thought, having no evidence of a

recent divergence from a common ancestor, likely meaning these two families colonized freshwater fish independently. Sicard et al. (2002) relied solely on molecular data of two previously sequenced species of *Octomacrum*, *O. lanceatum* 28S DNA and *O. mexicanum* 18S DNA, to attempt to clarify the origins of the Diplozoidae. Further work involving more complete taxon sampling and use of additional markers is needed to determine the relationships of these two Families and the third Family, Discocotylidae, in the Suborder, Discocotylinea.

Octomacrum body size appears to correlate to host body size (Fig. 14). This is intuitive as large parasites will attach to large hosts, similarly with small worms and small hosts, as host size will act as a restricting factor on parasite infection given a lack of space for large parasites on small gills. This has also been previously observed in other specialist parasites similar to *Octomacrum* such as *Dactylogyrus* spp. (Sasal et al., 1999).

The terminology of the clamps is an issue within the genus *Octomacrum* and standardization of the clamp component terminology is necessary to establish an effective system of identifying species. Firstly, as a general rule, any reference to "sucker" is eliminated given that *Octomacrum* are known to lack suckers, and instead have well developed hard-parts that are necessary for the clamping action required to secure *Octomacrum* to the gills. It is also necessary to easily distinguish sclerites in the anterior and posterior halves of the clamps as well as the distinction of dorsal and ventral sclerites. For this reason a system of naming is proposed that first separates sclerites by either the anterior and posterior clamp half, then specifies dorsal or ventral orientation when applicable. Due to the arrangement of the mid-sclerites, the only distinction required is separation by anterior or posterior half.

It is this clamp morphology that is the focus for the key due to the variation that occurs within and between species in regards to morphometrics. Some other features may prove useful in identifications, such as overall body size (large in *O. lanceatum*, small in *O. europaeum*), size of genital pore (small in *O. lanceatum* compared to *O. microconfibula*), body shape (more lanceolate in *O. semotili* than *O. europaeum*), peduncle development (with clear separation between body and haptor in *O. mexicanum* and less so in *O. europaeum*), and shape of haptor (more often long than wide in *O. lanceatum* and vice versa in *O. semotili*). These features, however, can show a high degree of variability between individuals within a species. This study provides an example of this in the form of *O. microconfibula* and the differences in morphometrics that can be seen between those found in Nova Scotia and those in Ontario (Table 5). These two groups were identified as the same species based on the form of the attachment clamps, however, based only on morphometric analysis, 29 of the 32 measurements taken show a significant difference between the two groups of *O. microconfibula*. The three measurements that show no significant variation are those of the posterior anchors, shown in the present study to have limited variation between juvenile and adult specimens (Fig. 16). This study provides an example of the variation that can occur within a species, which is likely to be seen amongst other polyopisthocotylid parasites, and therefore gives further evidence to the use of clamp form as the main diagnostic factor when identifying species of *Octomacrum*. It is the first study to report the morphometrics and varying morphology of each species of *Octomacrum*, but to focus an identifying key on the features of the clamps.

Seven previously unidentified species of *Octomacrum* have been reported from 6 different hosts: *Couesius plumbeus*, British Columbia and *Richardsonius balteatus*, British Columbia (Bangham and Adams, 1954), *Semotilus atromaculatus*, Ontario and *Margariscus margarita*, Ontario (Bangham and Venard, 1946), *Hybognathus hankinsoni*, Ontario (Bangham, 1941) and *Campostoma anomalum*, Arkansas (Cloutman, 1974). An unidentified species of *Octomacrum* has also been found on *Notropis telescopus* in Alabama (Adrian, 2010 pers. comm.), which is a new host record for *Octomacrum*. The morphological information provided in this study, including the key based on features of the clamps, may prove a useful addition to the knowledge of *Octomacrum* and could help identify these previously unidentified species.

The present study begins to infer the relationships of some of the species within the genus *Octomacrum*. This study provides 18S DNA sequences for all 6 species of *Octomacrum* and infers relationships within some species in the genus such as this similarities between North American species and the divergence of the European species. The 18S gene has been used numerous times in past phylogenetic studies to infer relationships within the Monogenea (Olson and Tkach, 2005). According to the 18S genetic data in the present study (Fig. 18), the European species, *O. europaeum*, occurs as a separate sister branch, representing the North American species of *Octomacrum*. This result corresponds to the host identities, since *O. europaeum* is the only species that is found on an "Old World" cyprinid host (Briolay et al., 1998). Presumably the European and North American species shared a common ancestor prior to separation of North America and Eurasia, which is a case of presumed vicariant speciation given the close relationship of cyprinids and catostomids, suckers only recently diverging from cyprinids.

This suggests that the ancestral population in North America diverged from this cyprinid ancestor onto catostomids and cyprinids. The position of *O. lanceatum* as the most evolved branch, separate from the other North American species, corroborates the evidence of a recent divergence in catostomids because it is the only species that has been found on catostomid fish in North America. Based on this gene it looks like *O. microconfibula* and *O. semotili* have also diverged from the remaining species that occur in more southern habitats due to the 75 % bootstrap value grouping these remaining species.

Discocotyle sagittata was selected as the outgroup for the phylogenetic analysis for a number of reasons. Morphologically, this genus is the closest in resemblance to *Octomacrum*, with only a few differences. Another close relative to *Octomacrum*, *Diplozoon* spp., are also similar morphologically, however *Diplozoon* has an interesting lifecycle strategy that involves fusion of two individuals to form a single mature parasite. This quite clearly separates the genus from *Octomacrum*, coupled with the evidence provided by Sicard et al. (2002) that Octomacridae and Diplozoidae share no recent common ancestry, and therefore suggests that species of *Diplozoon* may not be the closest relative of *Octomacrum*, even though they represent the only polyopisthocotylids that strictly infect freshwater fish. Since *Discocotyle* infects fish that can be both fresh and saltwater based, anadromous salmonids, and the genus can be found transglobally, whereas *Diplozoon* is limited to Eurasia and Africa, *Discocotyle* seems a logical choice for an outgroup.

A further molecular study on *Octomacrum* will be necessary to properly show reliable relationships within the genus. This will require a different gene, or additional

genes, to be sequenced and phylogenetically analyzed because the 18S DNA gene, though it provides some useful information, does not show enough variation to shed light on most species relationships. In the present study, the 18S DNA gene only has a small region of variation (less than 100 bp in size; Fig. 15) in the approximately 490 bp that were analyzed. This small region of variation is likely a main contributor to the resulting BLAST searches, which return a number of monogenean parasites with 99 % similarity. A future study will need to use a different region of the genome, one that has a higher degree of variation between species, in order to get a more reasonable and resolved idea of the relationships of these species. This may prove difficult, however, as *Octomacrum* is a difficult template to work with, as seen in the present study in which 8 primer sets (including 2 newly designed primer sets) targeting 5 regions were attempted before successfully finding primers that would properly bind to a gene.

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Figure 1: Generic diagram of the genus, *Octomacrum*, showing key morphological features (ventral view). A, buccal suckers; B, pharynx; C, intestinal crus; D, genital pore surrounded by muscular sucker; E, intestinal diverticula; F, yolk reserve; G, vittelaria; H, ovum; I, testis; J, peduncle; K, clamp; L, posterior anchor; M, haptor.

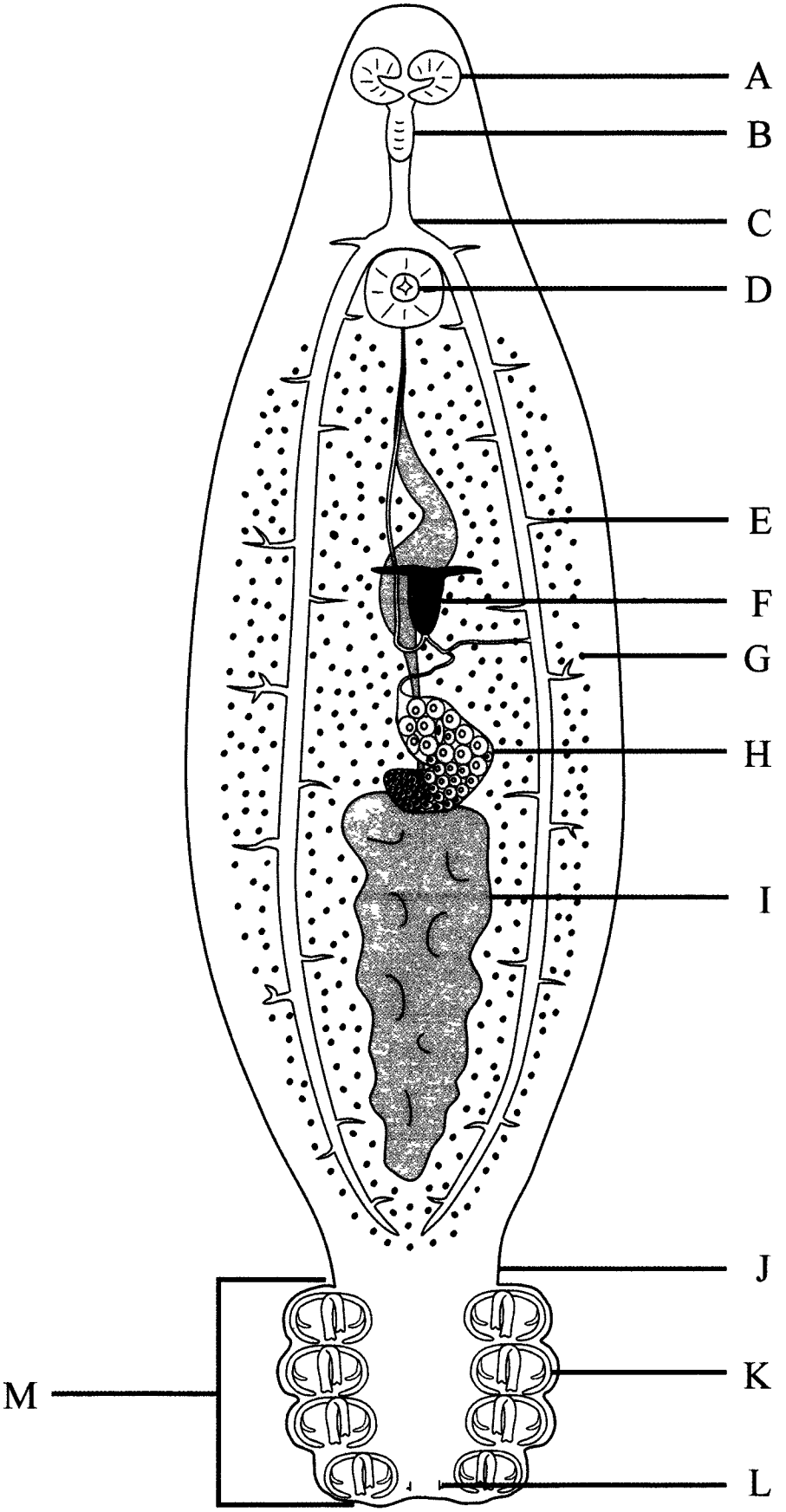
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Figure 2: General distribution of species of *Octomacrum*. *Octomacrum europaeum* localized to Romania and surrounding countries. *Octomacrum mexicanum* located in a single lake in Mexico. *Octomacrum spinum* found generally in mid-eastern United States. *Octomacrum lanceatum*, *O. microconfibula* and *O. semotili* with broad distribution across Canada and northern United States.

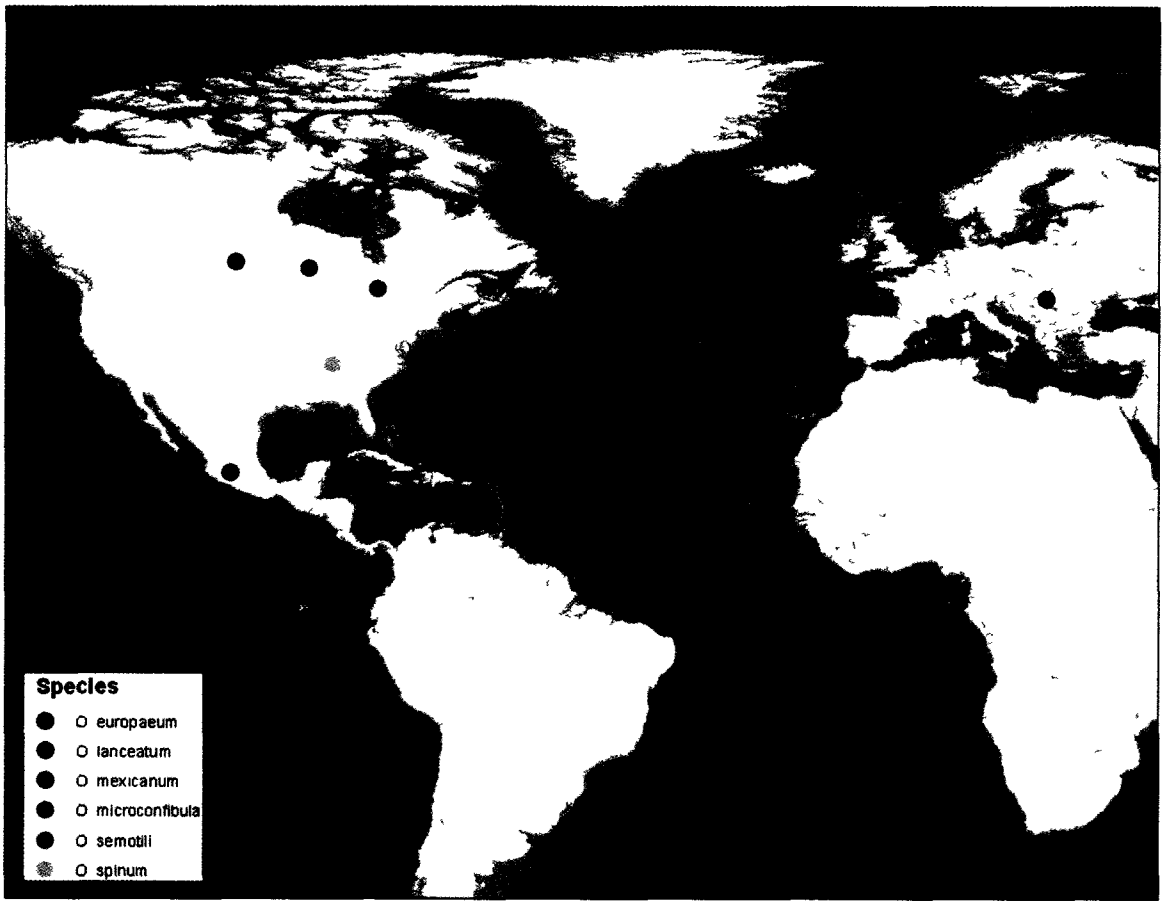
Species
O europaeum
O lanceatum
O mexicanum
O microconfibula
O semotili
O spinum

Figure 3: Generic clamp representation with labeled hard-parts. A, anterior mid-sclerite; B, posterior mid-sclerite; C, anterior ventral lateral sclerite; D, posterior dorsal lateral sclerite; E, accessory sclerites; F, posterior ventral lateral sclerite.

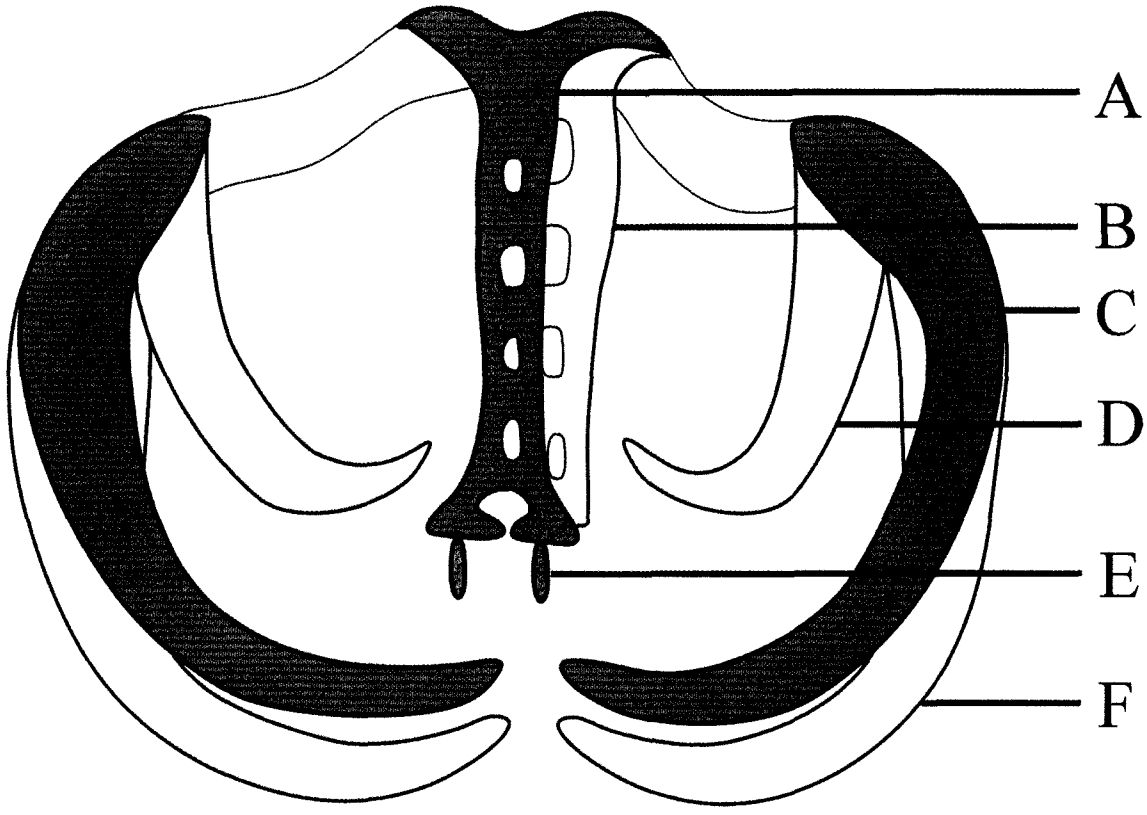
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Figure 4: Sample sites for species of *Octomacrum*. Yellow sites are those sampled by the author (Nova Scotia: Vinegar Lake, Cranberry Lake, Lawerencetown Lake, Long Lake, Dorey Lake [*O. microconfibula*]; Ontario: Brewer Lake, Lake Opeongo, Costello Creek, Costello Lake [*O. microconfibula*, *O. semotili*). Red sites were sampled by colleagues (Nova Scotia: Lower Sackville River, Feely Lake Brook, West River, River Denys, Lake Ainslie, Lochabor Lake [*O. lanceatum*]; Romania: Bega River [*O. europaeum*]; Tennessee: Bradley Creek, Stones River [*O. spinum*]; New York: Schoharie Creek; Mexico: Lake Patzcuaro [*O. mexicanum*]) and if parasites found were shipped to Saint Mary's University Taxonomy Laboratory.

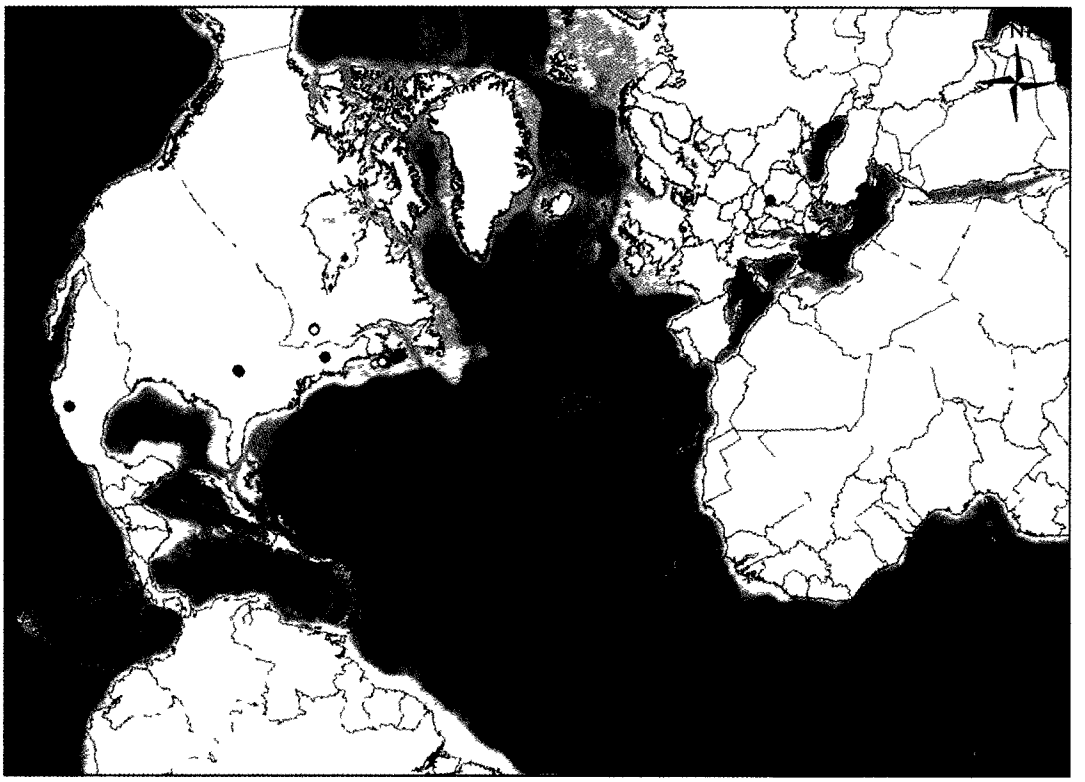

Figure 5: Line drawings of attachment clamps (ventral view) for all six species of *Octomacrum* at the same scale, 60 μm. a) *O. lanceatum*; b) *O. microconfibula*; c) *O. europaeum*; d) *O. semotili*; e) *O. spinum*; f) *O. mexicanum*.

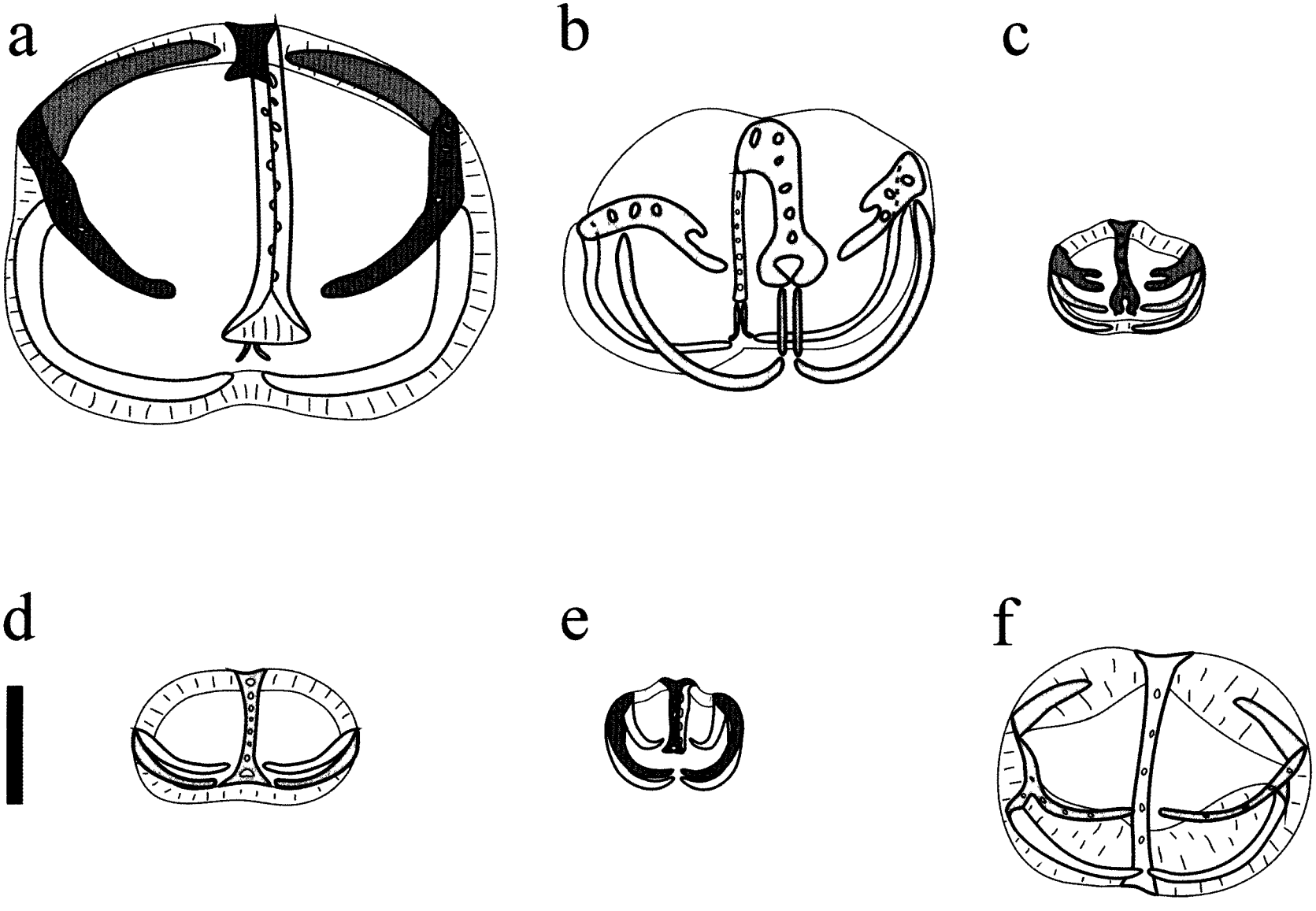
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Figure 6: Diagram of measurements used in morphometric analysis for all species of *Octomacrum*. a) whole parasite body; b) clamp; c) posterior anchor. A, whole body; B, greatest body width; C, buccal sucker length; D, buccal sucker width; E, pharynx length; F, pharynx width; G, genital pore length; H, genital pore width; I, haptor length; J, haptor width; K, clamp length; L, clamp width; M, anchor handle; N, anchor blade.

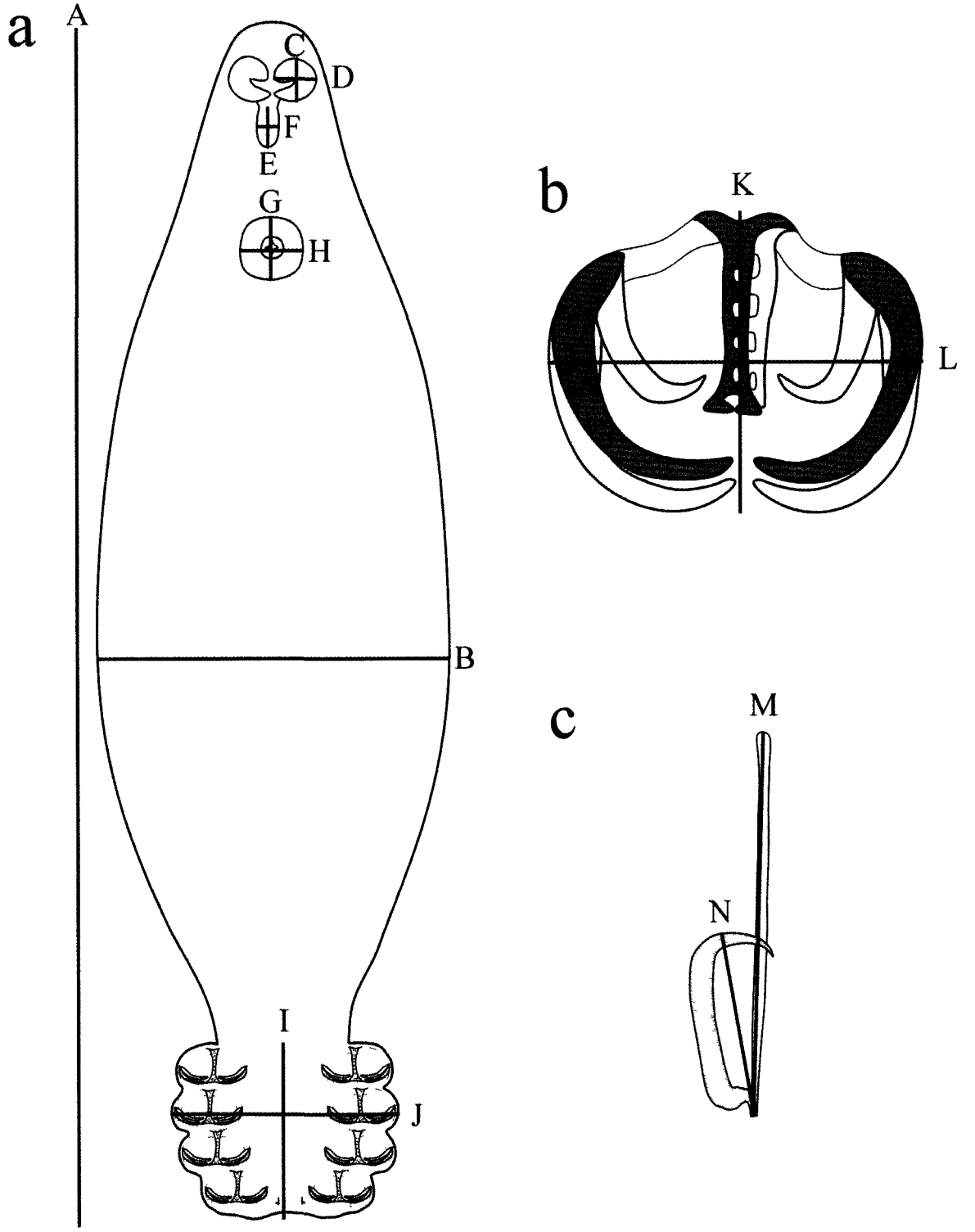
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Figure 7: Agarose gels showing PCR product amplification of 18S DNA at ~720 bp. A-C) *O. microconfibula* from golden shiner, *Notemigonus crysoleucas*, in Nova Scotia; D-E) *O. mexicanum* from Patzcuaro chub, *Algansea lacustris*, in Mexico; F) *O. europaeum* from riffle minnow, *Alburnoides bipunctatus*, in Romania; G-H) *O. lanceatum* from white sucker, *Catostomus commersoni*, in Nova Scotia; I-M) *O. microconfibula* from common shiner, *Luxilus cornutus*, in Ontario; N-R) *O. semotili* from redbelly dace, *Phoxinus eos*, in Ontario; S-W) *O. semotili* from creek chub, *Semotilus atromaculatus*, in Ontario; X-2) *O. spinum* from stoneroller minnow, *Campostoma anomalum*, in Tennessee; 3) 100 bp ladder; 4) 1500 bp; 5) 800 bp; 6) 500 bp.

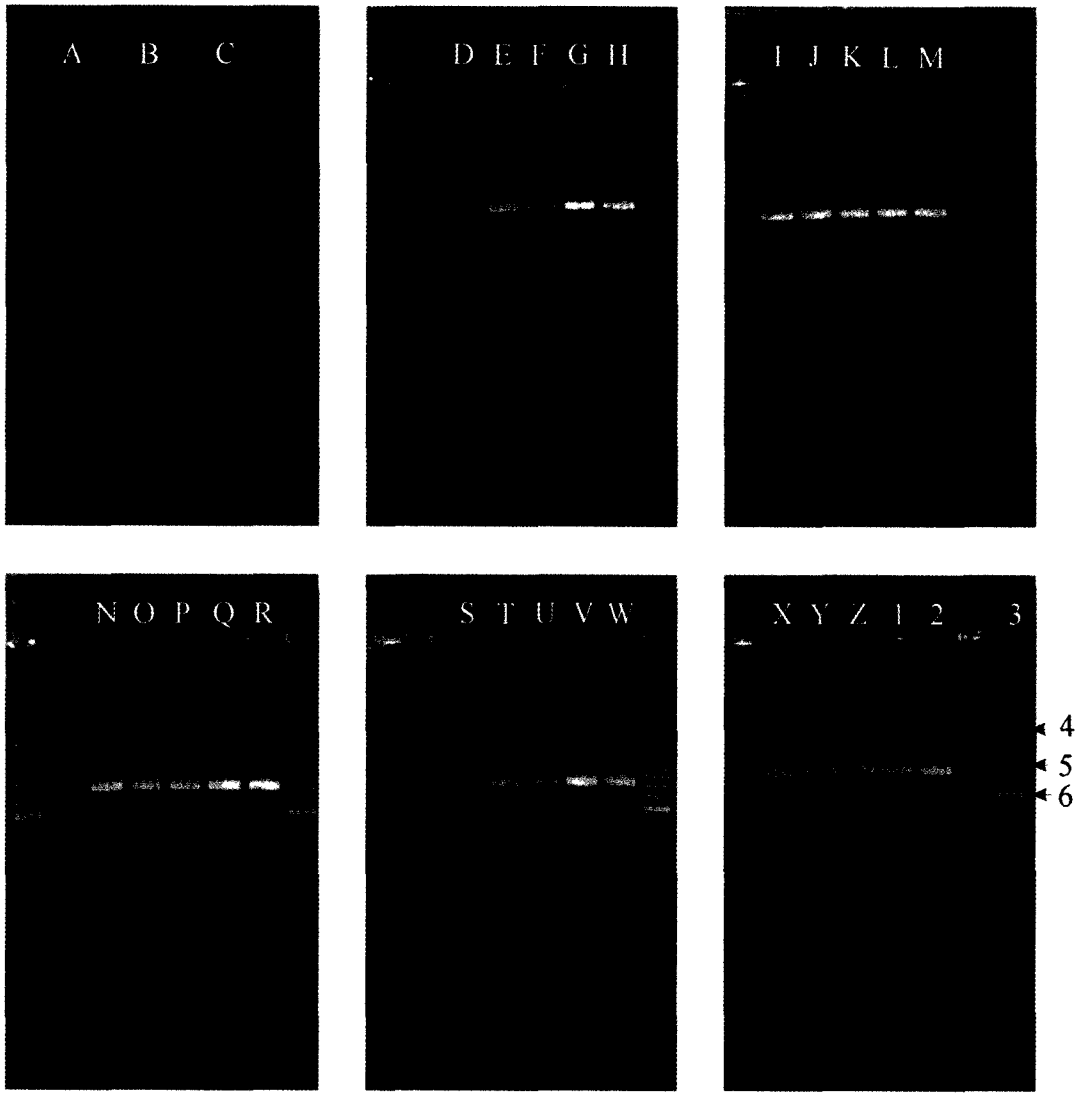
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Figure 8: *Octomacrum lanceatum* (ventral view). a) whole body, scale bar 400 μm; b) clamp, scale bar 60 μm; c) posterior anchor, scale bar 10 μm; d) egg, scale bar 150 μm.

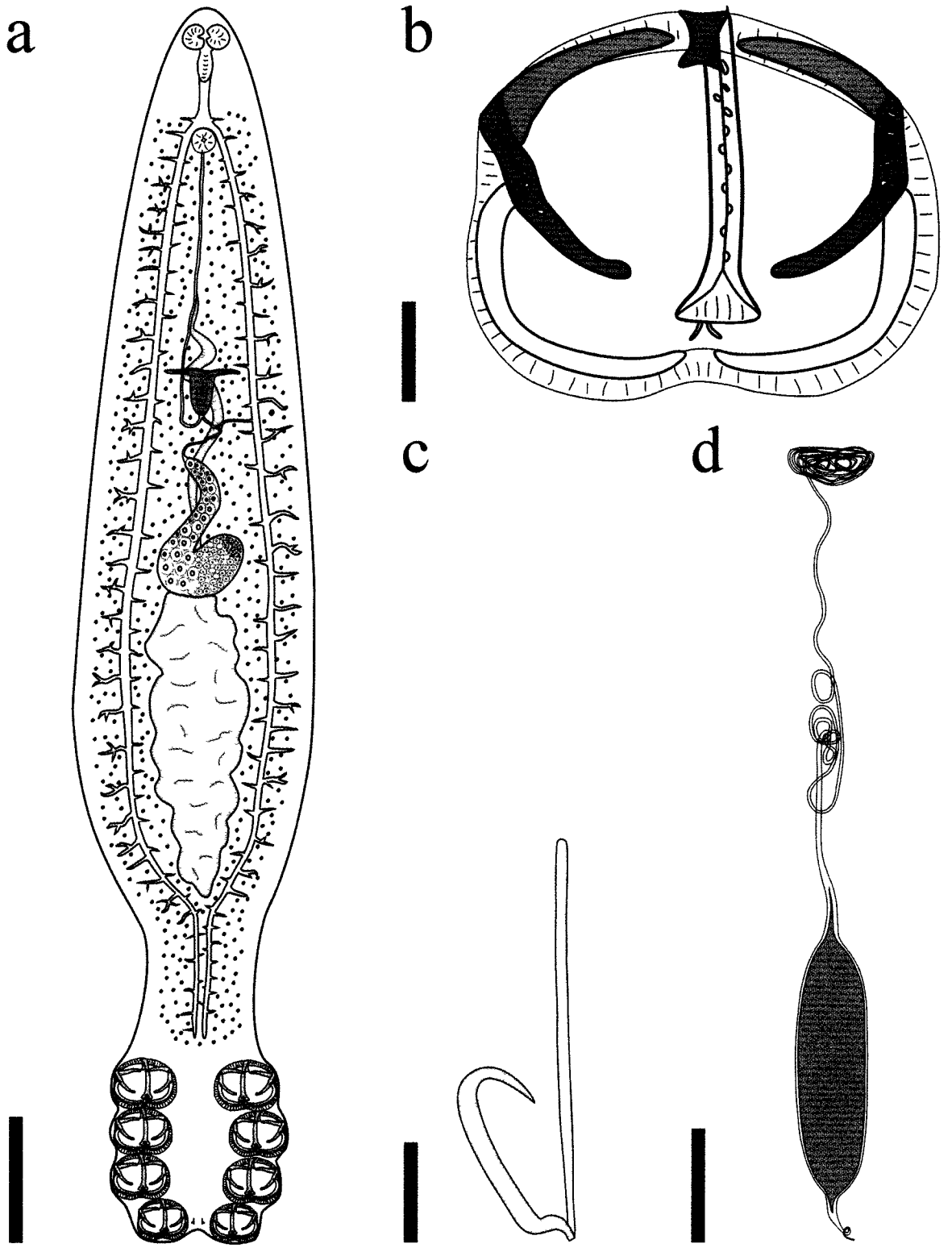
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Figure 9: *Octomacrum microconfibula* (ventral view). a) whole body, scale bar 300 μm; b) clamp, scale bar 25 μm; c) posterior anchor, scale bar 10 μm; d) egg, scale bar 100 μm.

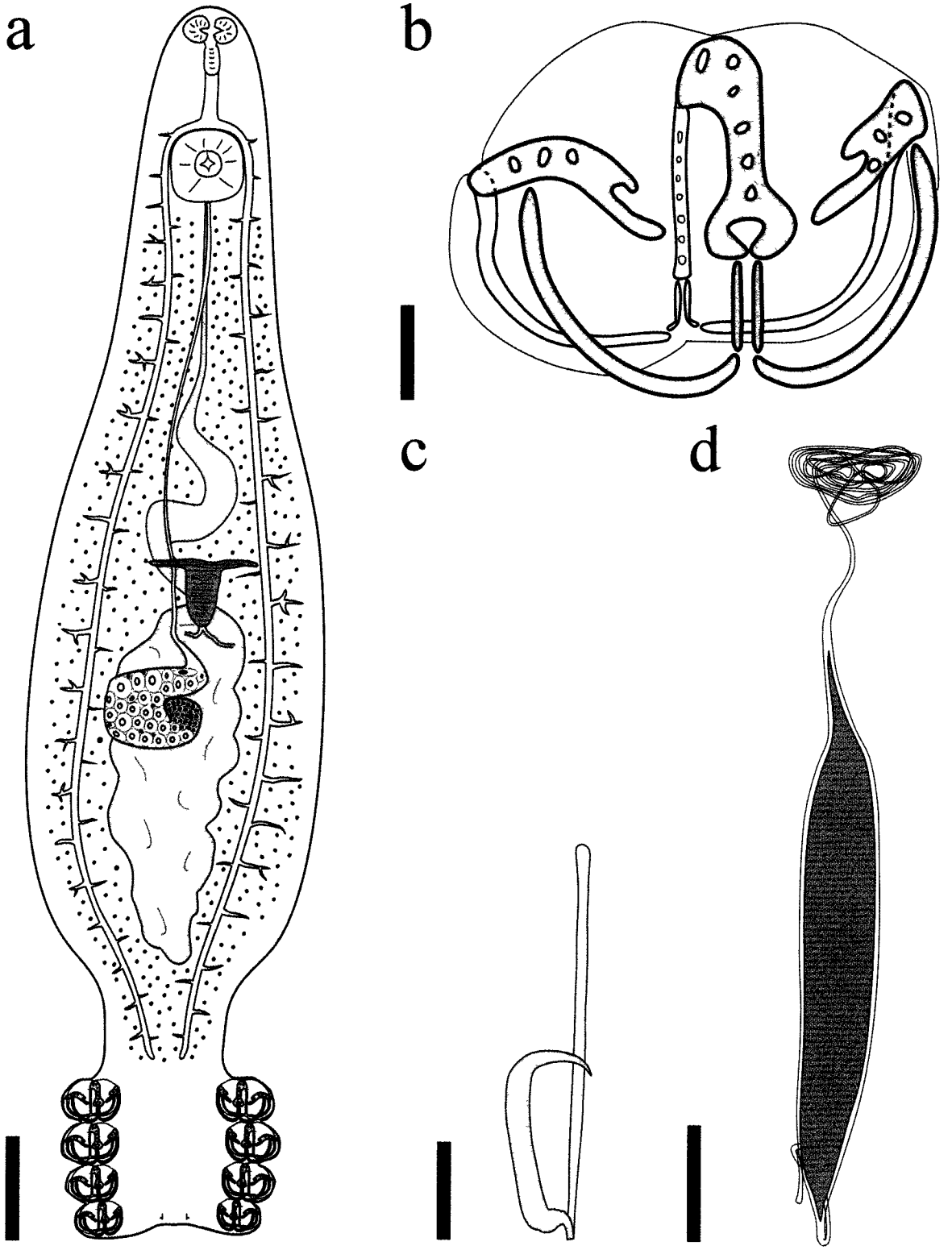
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Figure 10: *Octomacrum europaeum* (ventral view). a) whole body, scale bar 100 μm; b) clamp, scale bar 25 μm; c) posterior anchor, scale bar 10 μm.

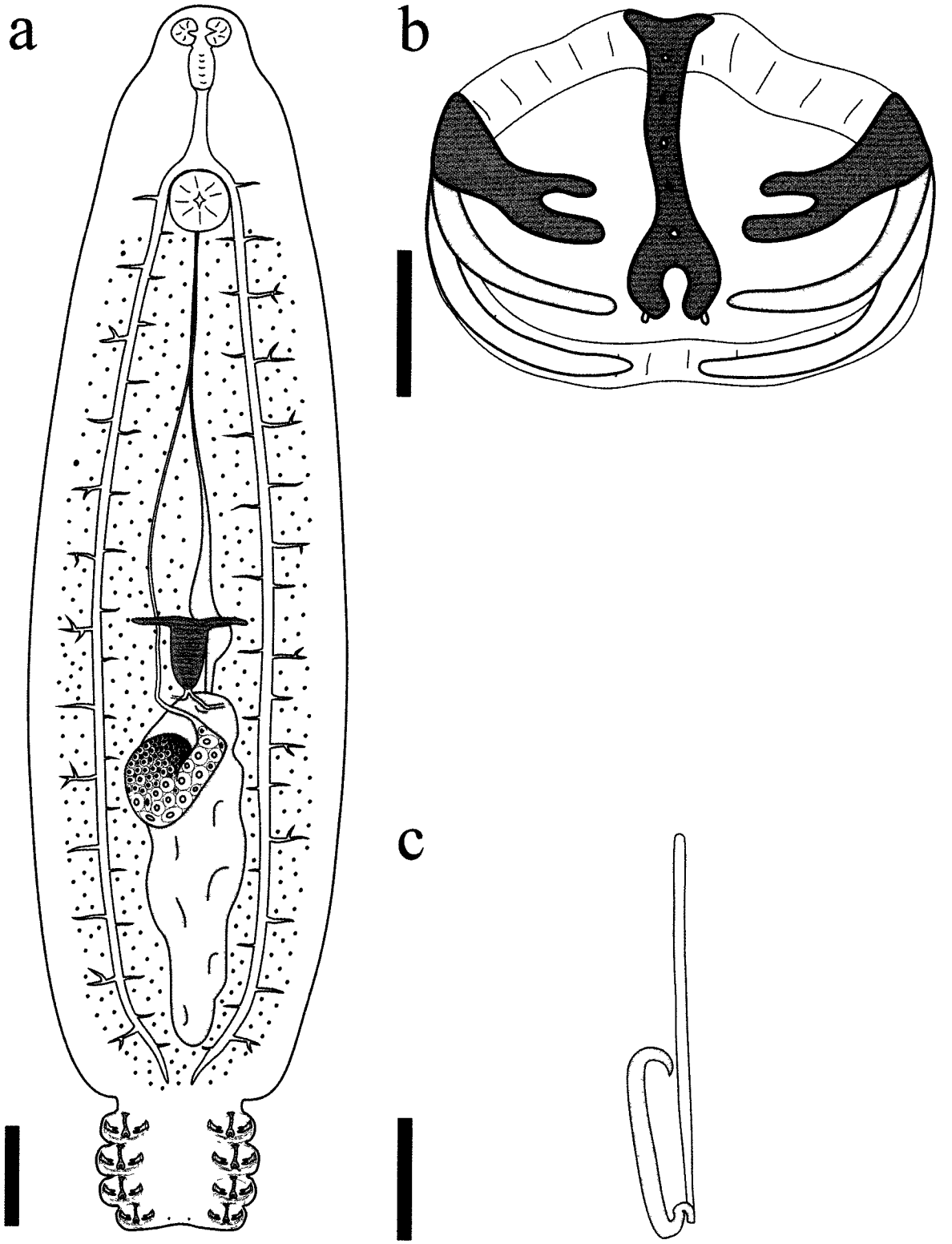
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Figure 11: *Octomacrum semotili* (ventral view). a) whole body, scale bar 300 μm; b) clamp, scale bar 15 μm; c) posterior anchor, scale bar 10 μm; d) egg, scale bar 100 μm.

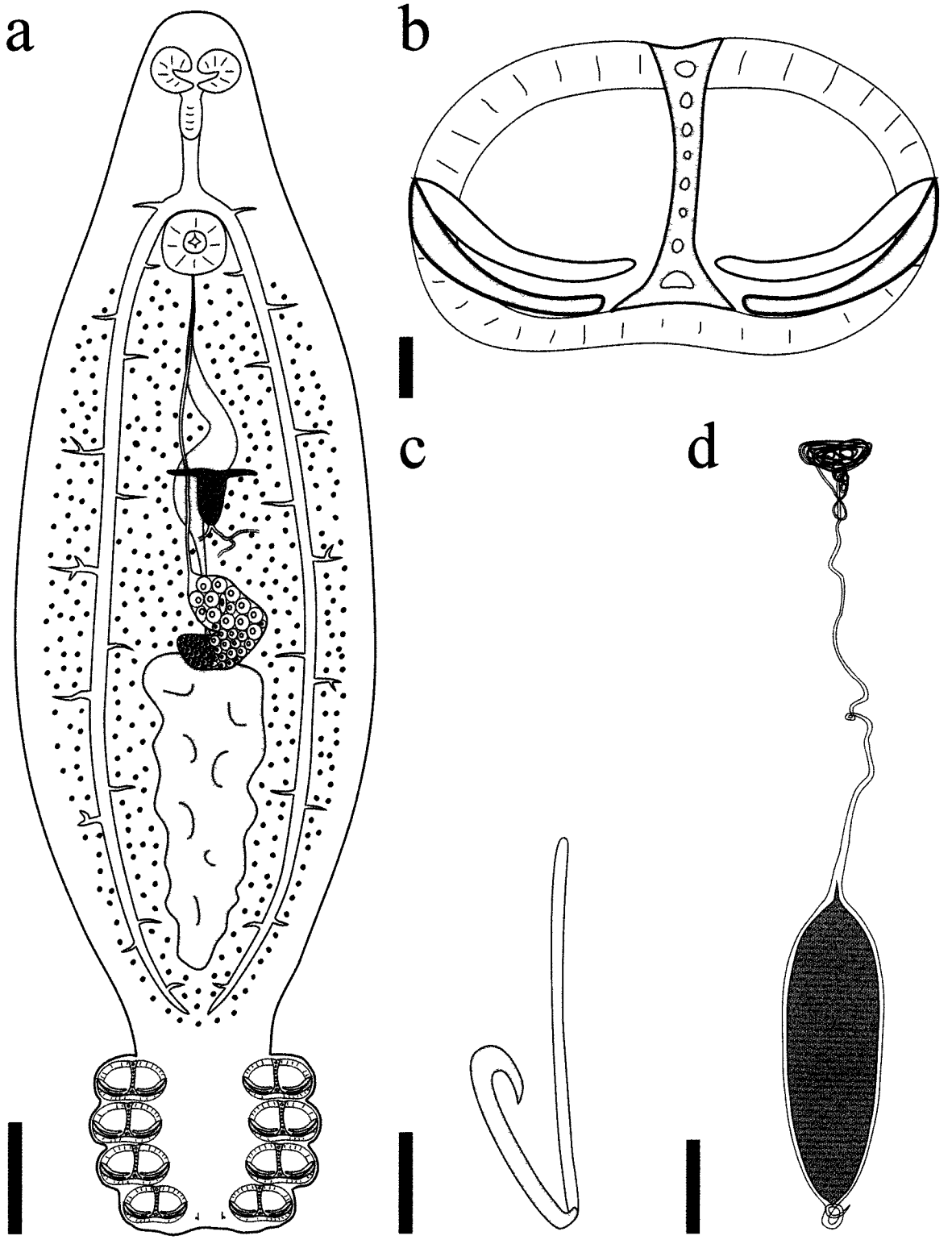
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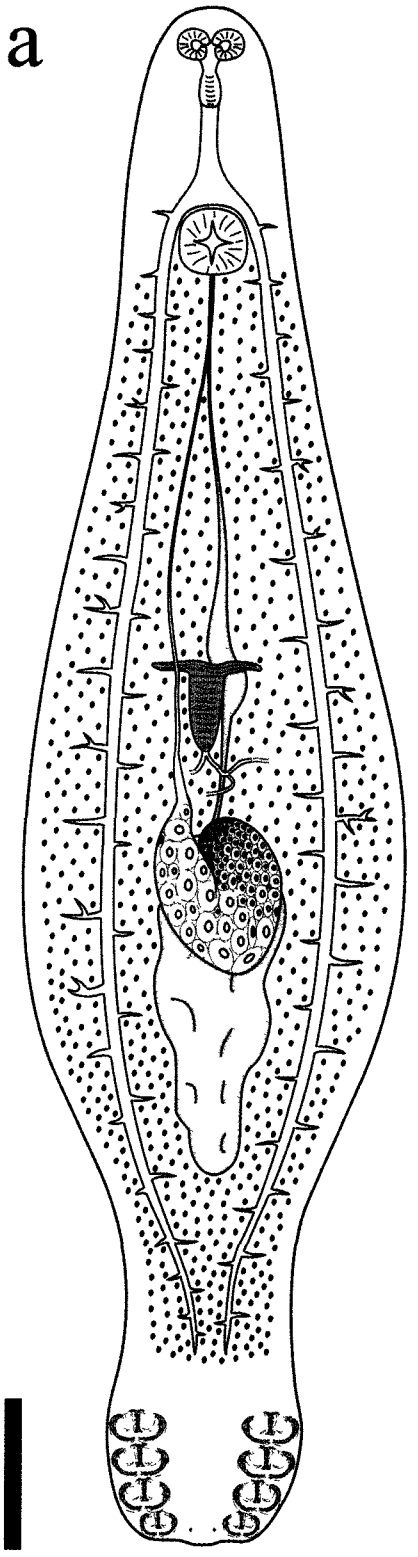
Figure 12: *Octomacrum spinum* (ventral view). a) whole body, scale bar 200 μm; b) clamp, scale bar 10 μm; c) posterior anchor, 5 μm.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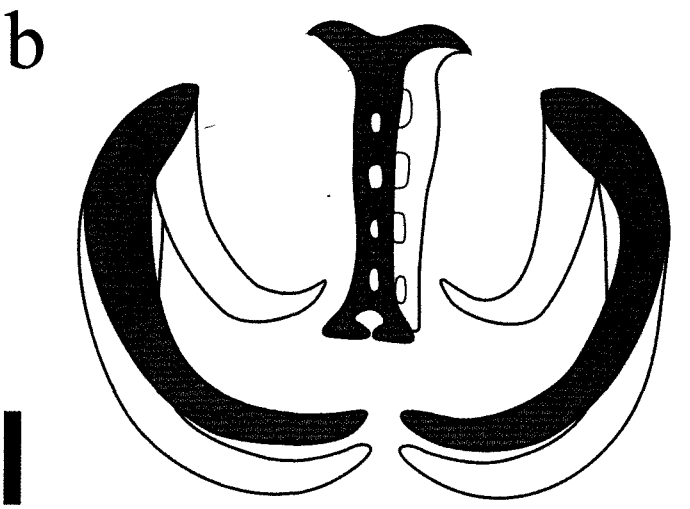
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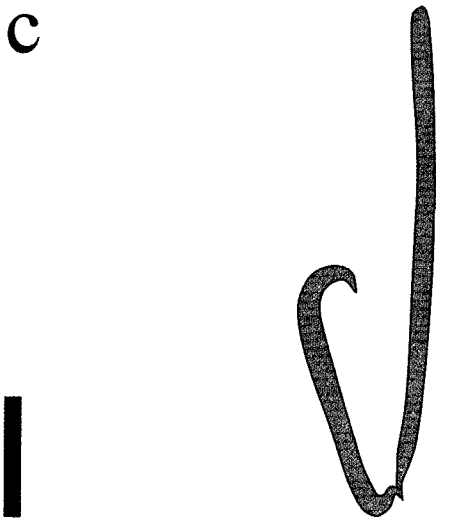
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Figure 13: *Octomacrum mexicanum* (ventral view). a) whole body, scale bar 350 μm; b) clamp, scale bar 50 μm; c) posterior anchor, scale bar 5 μm; d) egg, scale bar 100 μm.

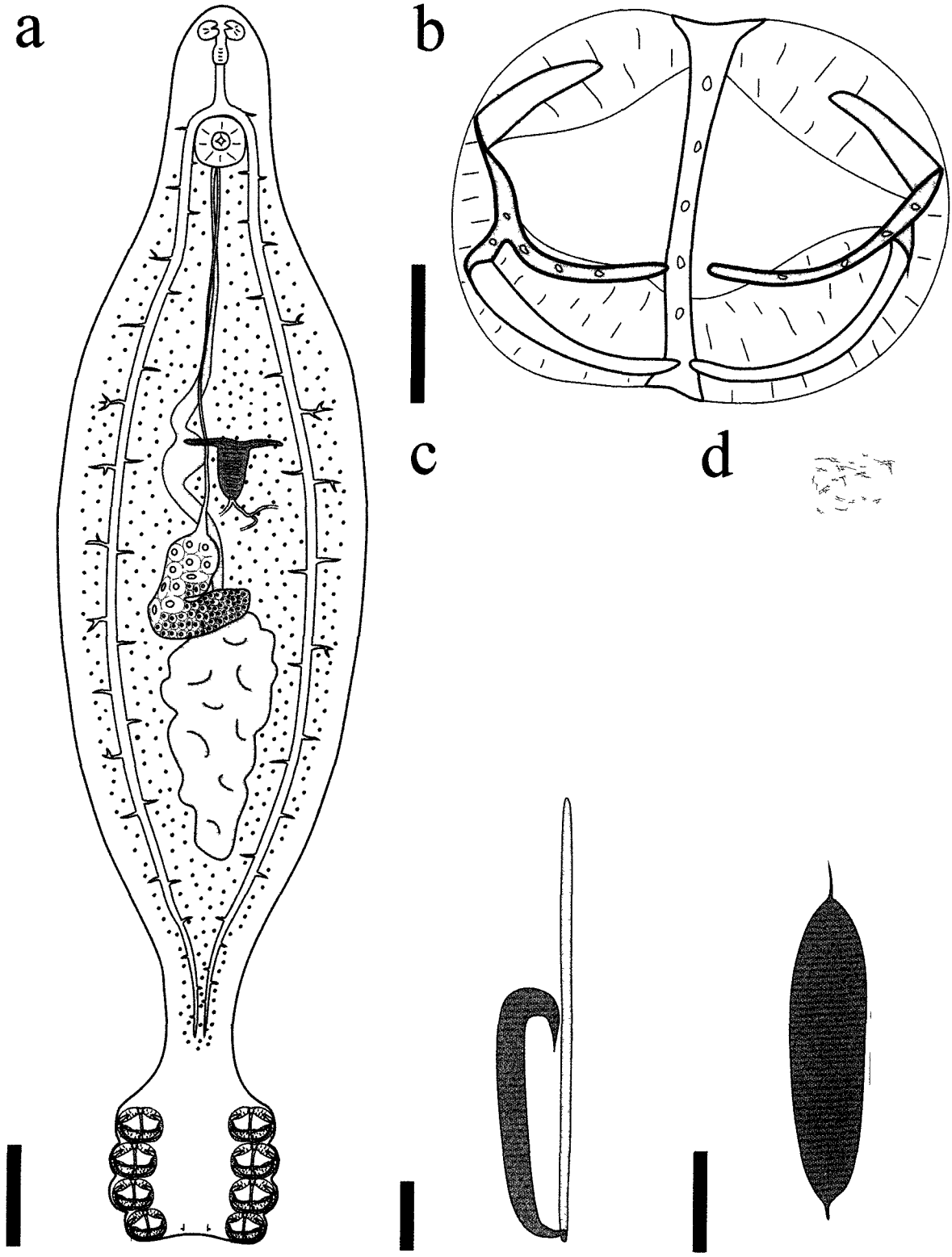
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Figure 14: General trend of *Octomacrum* size compared with average host size showing the larger species of *Octomacrum* are most often found on the larger hosts, and smaller species on smaller hosts. From the left, *O. lanceatum*, *O. mexicanum*, *O. microconfibula*, *O. semotili*, *O. spinum*, *O. europaeum*. Parasite scale bar, 750 μm; Host scale bar, 5 cm.

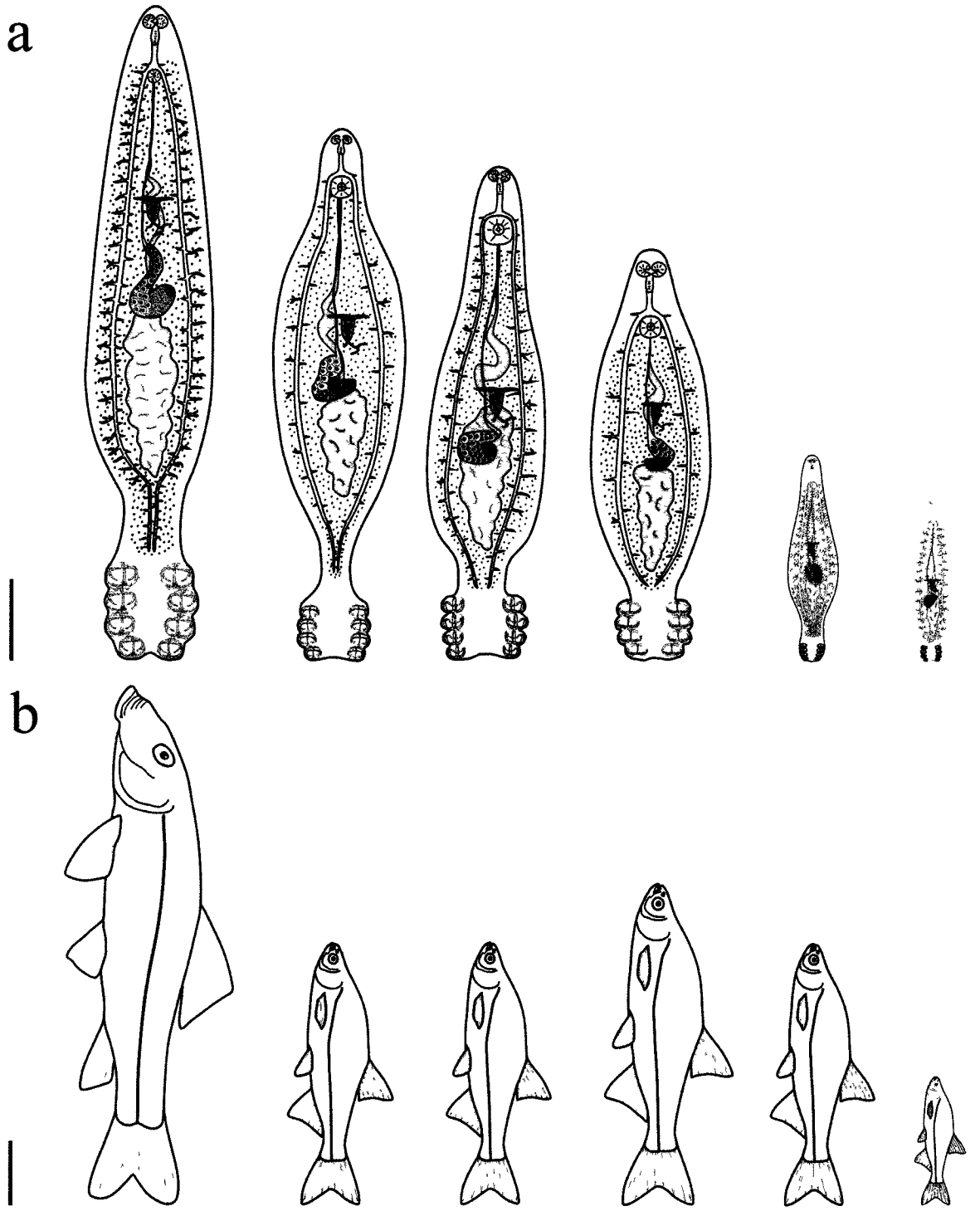

a
b

Figure 15: Sequence base pair comparison (447 bp) between all six species of *Octomacrum*, as well as a second sequence of *O. microconfibula* from a second host and the selected outgroup, *Discocotyle sagittata*, showing variable sites along the sequenced region of the 18S DNA gene.

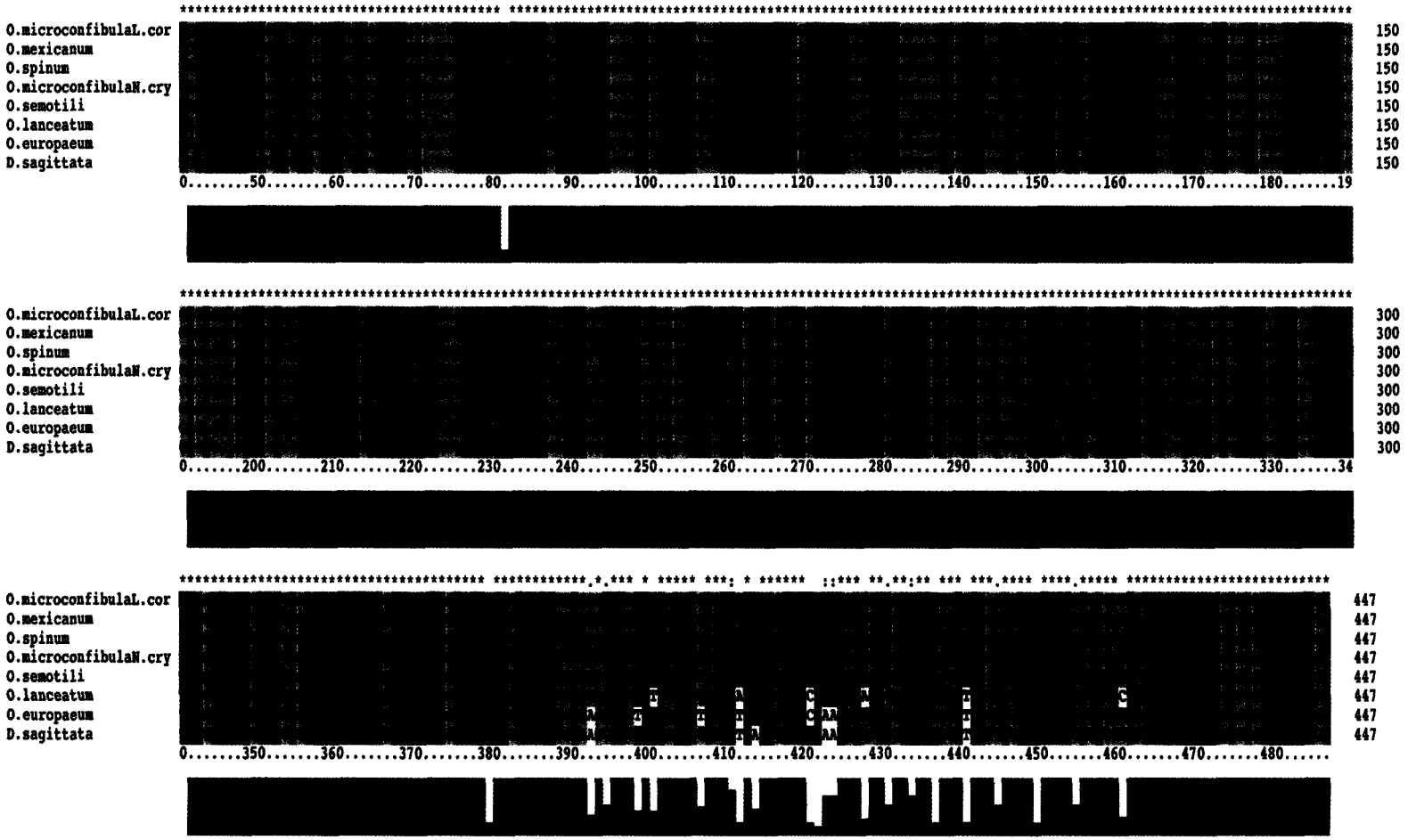
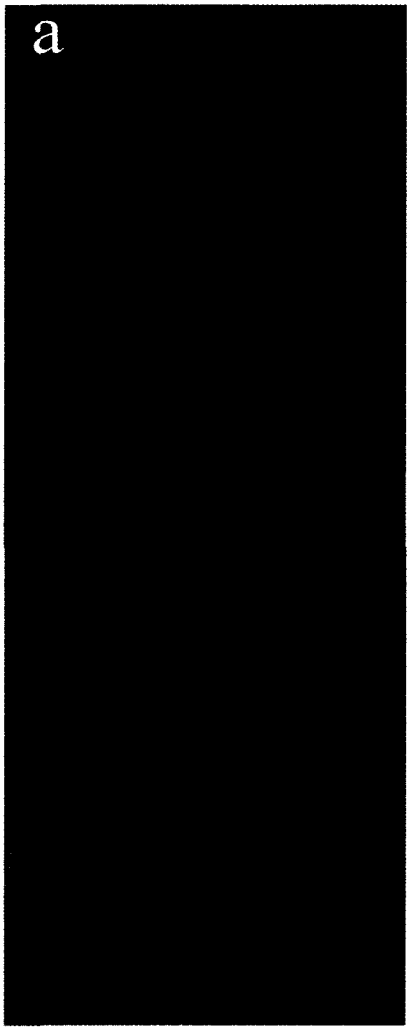
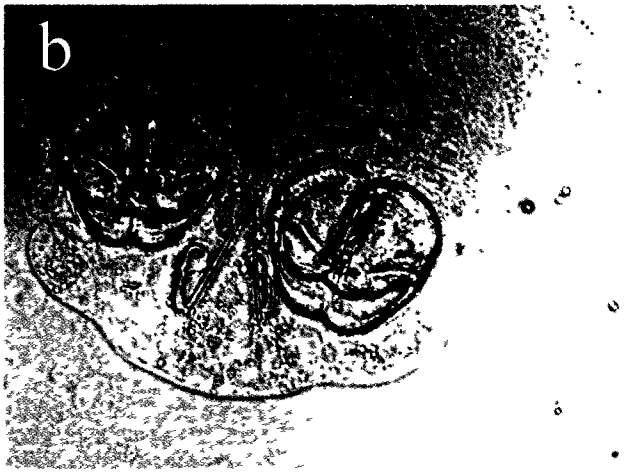

O.microconfibulaL.cor
O.mexicanum
O.spinum
O.microconfibulaN.cry
O.semotili
O.lanceatum
O.europaeum
D.sagittata
150
0.....50.....60.....70.....80.....90.....100.....110.....120.....130.....140.....150.....160.....170.....180.....19
300
0.....200.....210.....220.....230.....240.....250.....260.....270.....280.....290.....300.....310.....320.....330.....34
447
0.....350.....360.....370.....380.....390.....400.....410.....420.....430.....440.....450.....460.....470.....480.....

Figure 16: Photomicrographs of juvenile *O. microconfibula*. a) whole body image of juvenile *O. microconfibula* showing 2 fully formed posterior clamps with developing third pair, scale bar 100 μm; b) magnified image of the fully formed, but not full sized posterior clamps with the two, already full sized, posterior anchors between them, scale bar 20 μm; c) a second juvenile *O. microconfibula* showing the posterior three pairs of clamps fully developed, however, lacking the anterior pair, scale bar 20 μm.


a


b

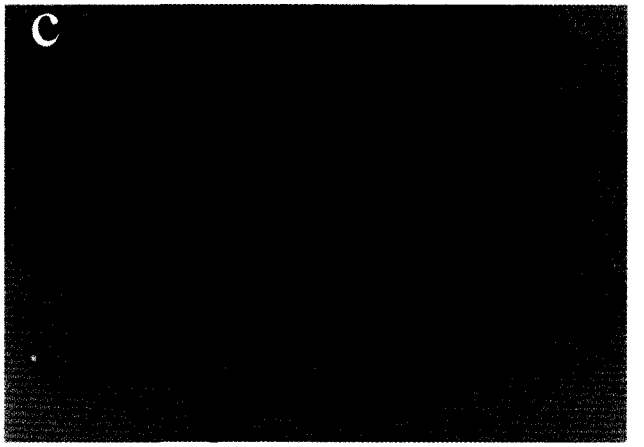

c

Figure 17: Sequence base pair comparison (589 bp) between two haplotypes of *O. microconfibula* showing the single variable site along the sequenced region of the 18S DNA gene located at 421bp. O.microN.cry is *O. microconfibula* from golden shiner in Nova Scotia and O.microL.cor is *O. microconfibula* common shiner in Ontario.

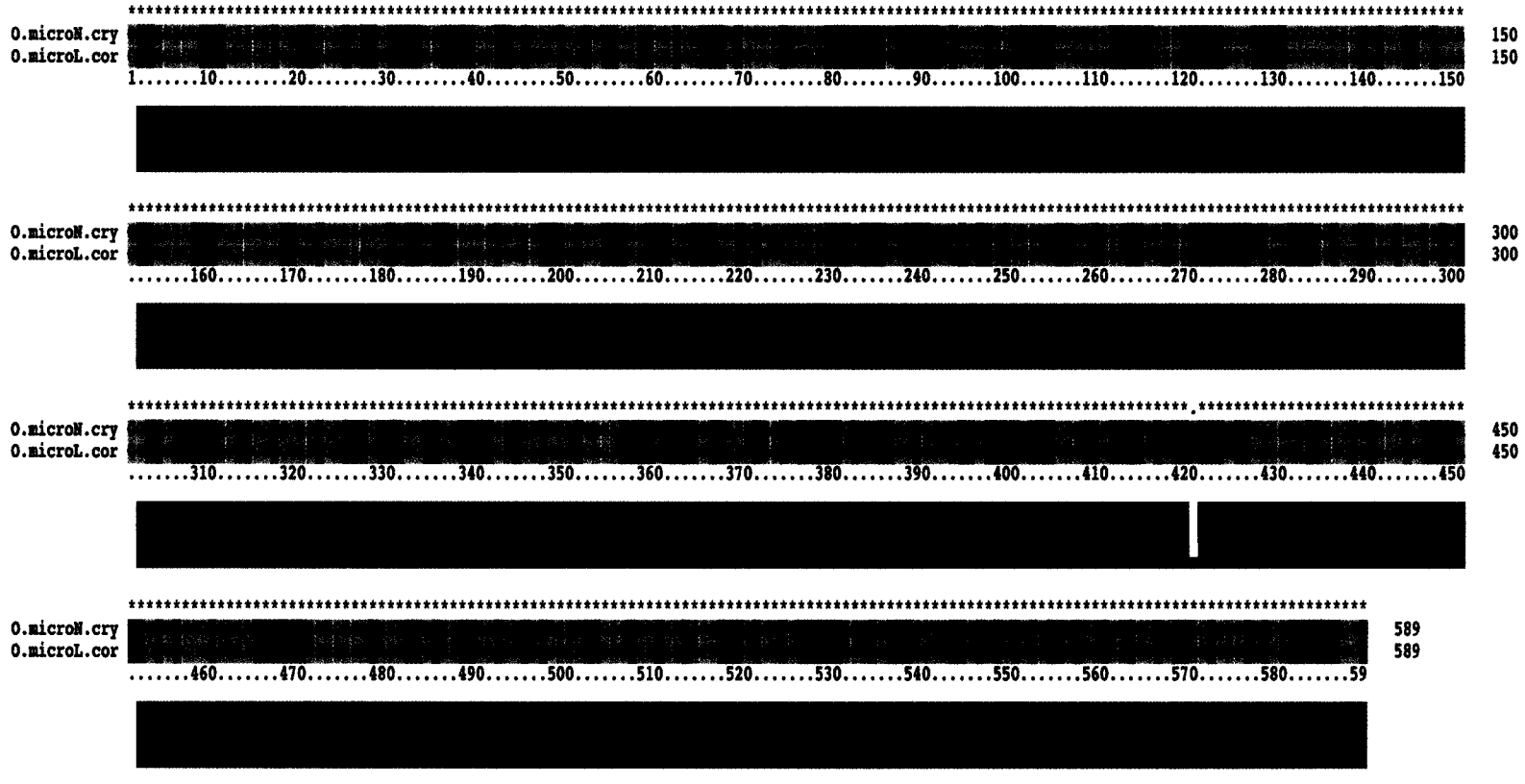

0.microN.cry
0.microL.cor
150
150
1.....10.....20.....30.....40.....50.....60.....70.....80.....90.....100.....110.....120.....130.....140.....150
0.microN.cry
0.microL.cor
300
300
.....160.....170.....180.....190.....200.....210.....220.....230.....240.....250.....260.....270.....280.....290.....300
0.microN.cry
0.microL.cor
450
450
.....310.....320.....330.....340.....350.....360.....370.....380.....390.....400.....410.....420.....430.....440.....450
0.microN.cry
0.microL.cor
589
589
.....460.....470.....480.....490.....500.....510.....520.....530.....540.....550.....560.....570.....580.....59

Figure 18: Maximum likelihood phylogenetic tree based on HKY85/F84 model of evolution. Tree rooted on *Discocotyle sagittata*. Clamp images are superimposed beside each sequence branch and sequence grouped by broad geographic distribution. Both *O. europaeum* and *O. lanceatum* are resolved in their position at 100 and 99% of 1000 bootstrap repeats respectively. Sequences *O. microconfibula* from Nova Scotia and *O. semotili* are separate from the southern North American species 75% of 1000 bootstrap repeats.

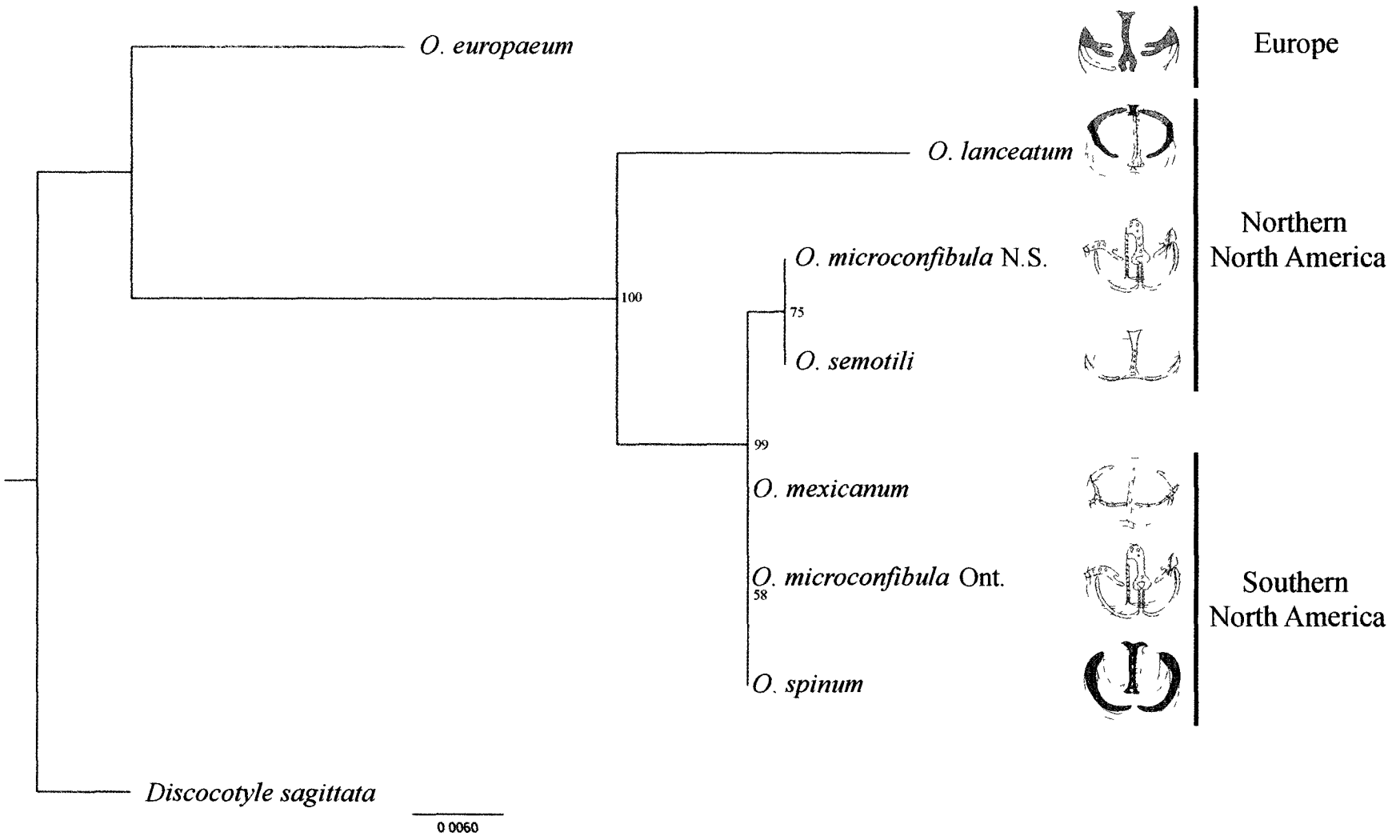
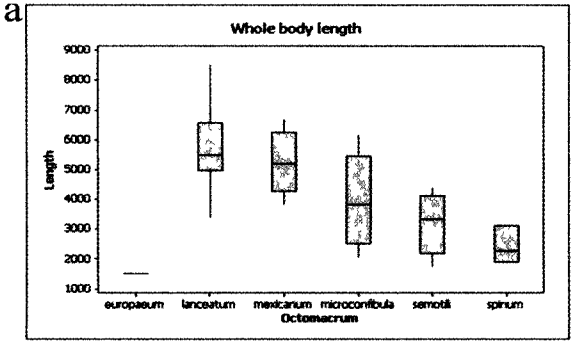
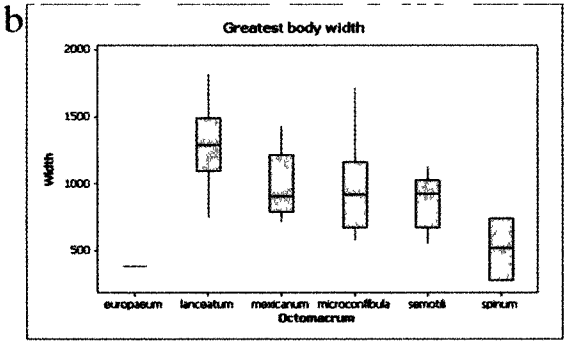

O. europaeum
O. lanceatum
O. microconfibula N.S.
O. semotili
O. mexicanum
O. microconfibula Ont.
O. spinum
Discocotyle sagittata
100
75
99
58
0 0060
Europe
Northern
North America
Southern
North America

Fig. 19: Morphometrics of all six species of *Octomacrum* represented by boxplots. Interquartile range is boxed in gray with the mean line. Minimum and maximum values represented by extremes of vertical lines. All measurements are in μm. a) whole body length; b) greatest body width; c) right buccal sucker length; d) right buccal sucker width; e) left buccal sucker length; f) left buccal sucker width; g) pharynx length; h) pharynx width; i) genital pore length.

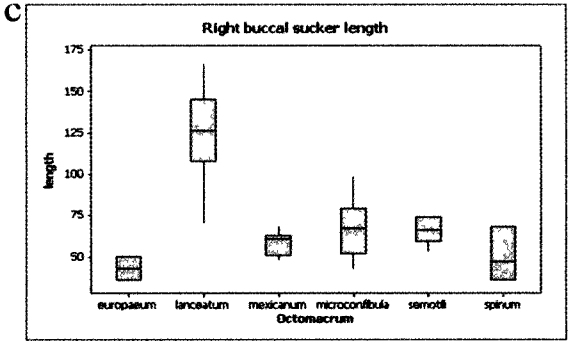
a


Whole body length
Length
9000
8000
7000
6000
5000
4000
3000
2000
1000
europaeum
lanceatum
mexicanum
microconifibula
semotili
spinum
Octomacrum

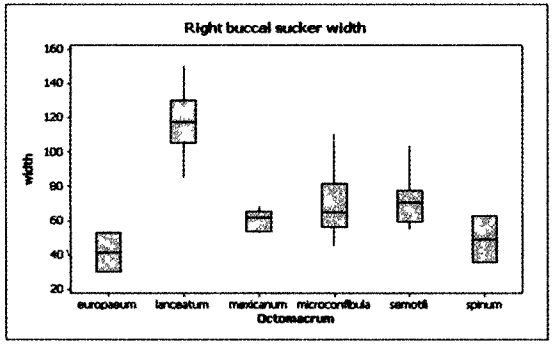
b


Greatest body width
width
2000
1500
1000
500
europaeum
lanceatum
mexicanum
microconifibula
semotili
spinum
Octomacrum

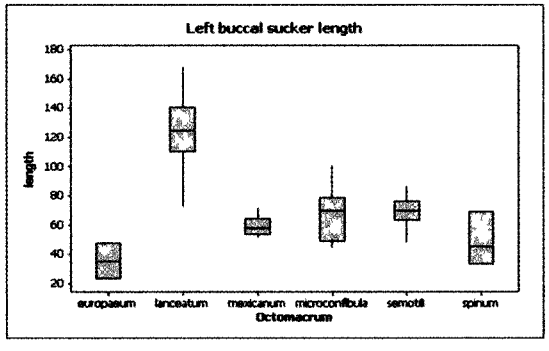
c


Right buccal sucker length
length
175
150
125
100
75
50
europaeum
lanceatum
mexicanum
microconifibula
semotili
spinum
Octomacrum

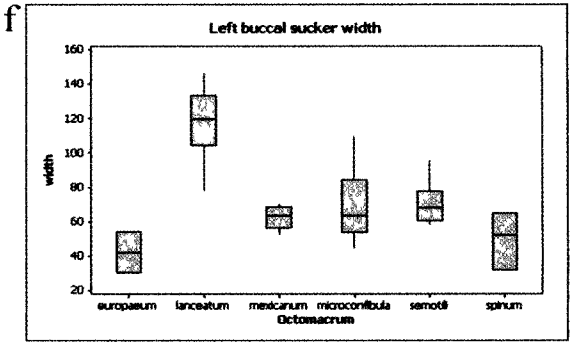
d


Right buccal sucker width
width
160
140
120
100
80
60
40
20
europaeum
lanceatum
mexicanum
microconifibula
semotili
spinum
Octomacrum

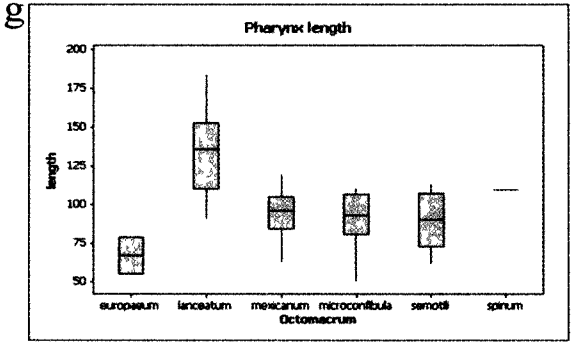
e


Left buccal sucker length
length
180
160
140
120
100
80
60
40
20
europaeum
lanceatum
mexicanum
microconifibula
semotili
spinum
Octomacrum

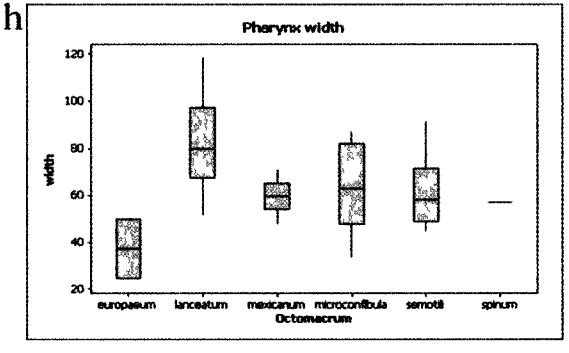
f


Left buccal sucker width
width
160
140
120
100
80
60
40
20
europaeum
lanceatum
mexicanum
microconifibula
semotili
spinum
Octomacrum

g


Pharynx length
length
200
175
150
125
100
75
50
europaeum
lanceatum
mexicanum
microconifibula
semotili
spinum
Octomacrum

h


Pharynx width
width
120
100
80
60
40
20
europaeum
lanceatum
mexicanum
microconifibula
semotili
spinum
Octomacrum

i

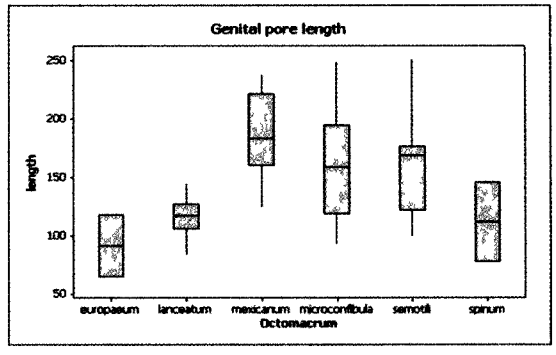

Genital pore length
length
250
200
150
100
50
europaeum
lanceatum
mexicanum
microconifibula
semotili
spinum
Octomacrum

Fig. 20: Morphometrics of all six species of *Octomacrum* represented by boxplots. Interquartile range is boxed in gray with the mean line. Minimum and maximum values represented by extremes of vertical lines. All measurements are in μm. a) genital pore width; b) haptor length; c) haptor width; d) anterior right clamp length; e) anterior right clamp width; f) anterior left clamp length; g) anterior left clamp width; h) second right clamp length; i) second right clamp width.

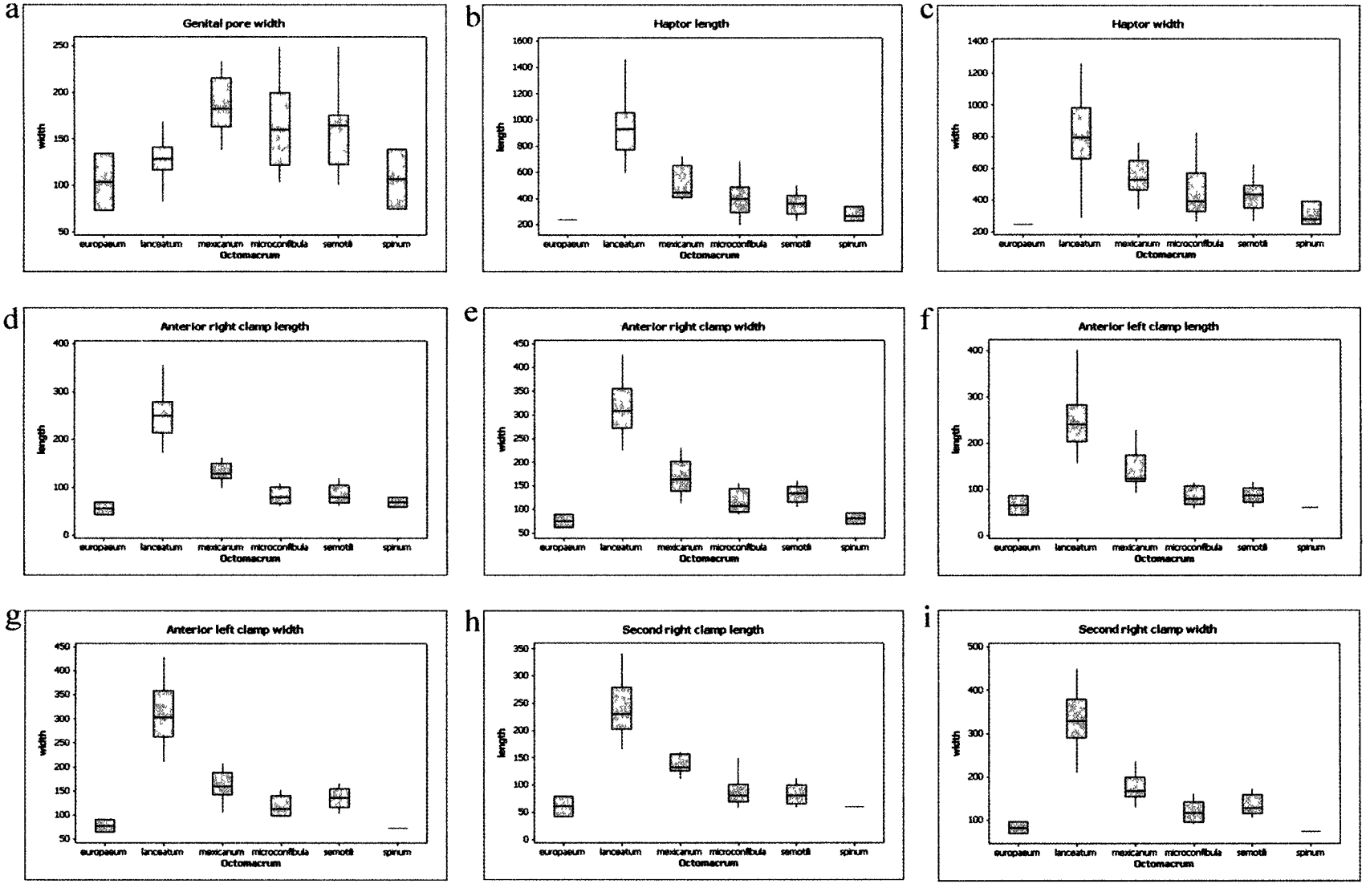
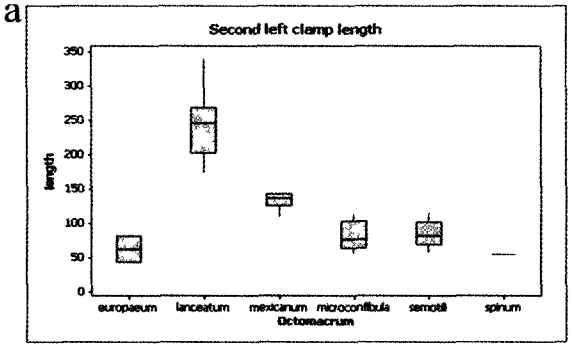
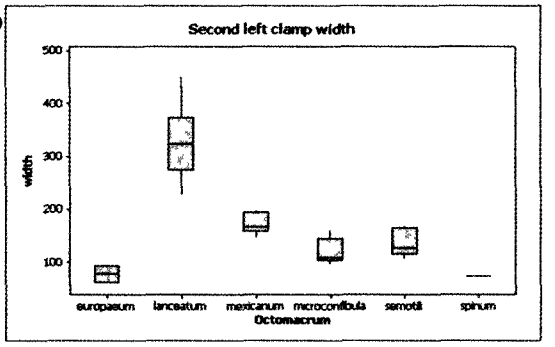

a
Genital pore width
width
europaeum lanceatum mexicanum microconifibula semotili spinum
Octomacrum
b
Haptor length
length
europaeum lanceatum mexicanum microconifibula semotili spinum
Octomacrum
c
Haptor width
width
europaeum lanceatum mexicanum microconifibula semotili spinum
Octomacrum
d
Anterior right clamp length
length
europaeum lanceatum mexicanum microconifibula semotili spinum
Octomacrum
e
Anterior right clamp width
width
europaeum lanceatum mexicanum microconifibula semotili spinum
Octomacrum
f
Anterior left clamp length
length
europaeum lanceatum mexicanum microconifibula semotili spinum
Octomacrum
g
Anterior left clamp width
width
europaeum lanceatum mexicanum microconifibula semotili spinum
Octomacrum
h
Second right clamp length
length
europaeum lanceatum mexicanum microconifibula semotili spinum
Octomacrum
i
Second right clamp width
width
europaeum lanceatum mexicanum microconifibula semotili spinum
Octomacrum

Fig. 21: Morphometrics of all six species of *Octomacrum* represented by boxplots. Interquartile range is boxed in gray with the mean line. Minimum and maximum values represented by extremes of vertical lines. All measurements are in μm. a) second left clamp length; b) second left clamp width; c) third right clamp length; d) third right clamp width; e) third left clamp length; f) third left clamp width; g) posterior right clamp length; h) posterior right clamp width; i) posterior left clamp length.

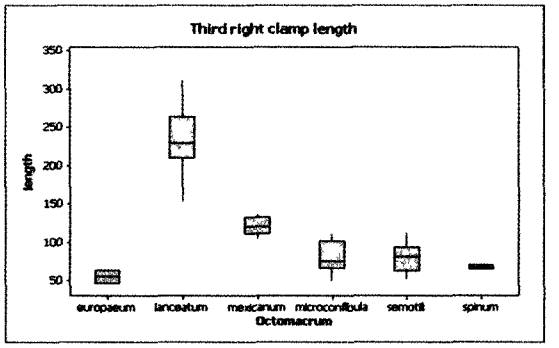
a


Second left clamp length
length
europaeum
lanceatum
mexicanum
microconifibula
semotili
spinum
Octomacrum

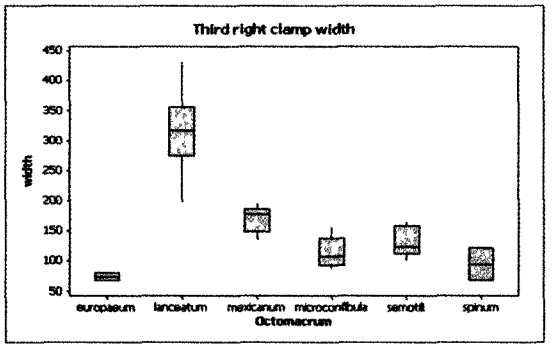
b


Second left clamp width
width
europaeum
lanceatum
mexicanum
microconifibula
semotili
spinum
Octomacrum

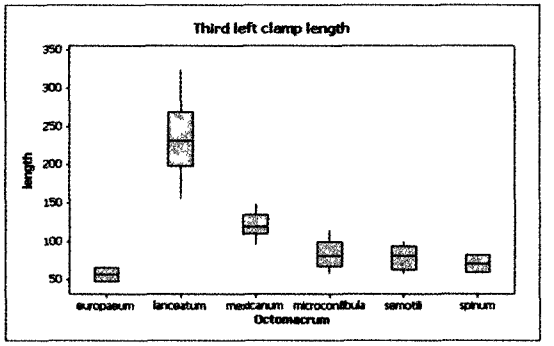
c


Third right clamp length
length
europaeum
lanceatum
mexicanum
microconifibula
semotili
spinum
Octomacrum

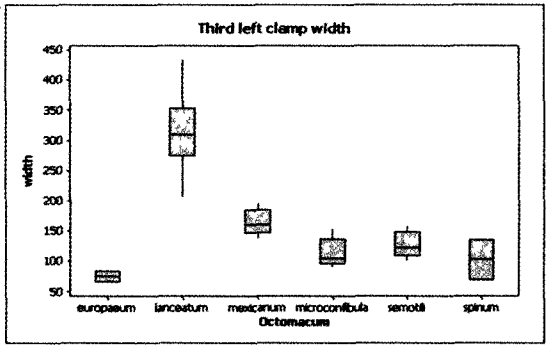
d


Third right clamp width
width
europaeum
lanceatum
mexicanum
microconifibula
semotili
spinum
Octomacrum

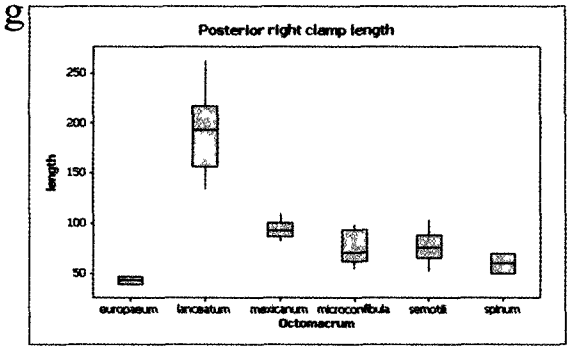
e


Third left clamp length
length
europaeum
lanceatum
mexicanum
microconifibula
semotili
spinum
Octomacrum

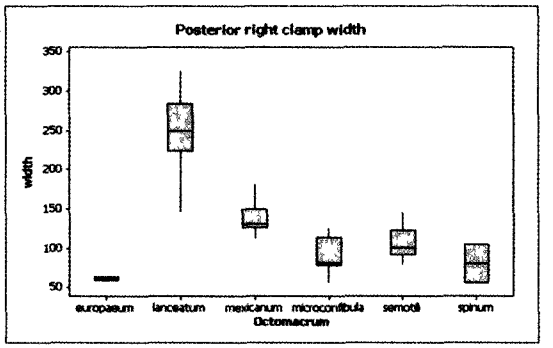
f


Third left clamp width
width
europaeum
lanceatum
mexicanum
microconifibula
semotili
spinum
Octomacrum

g


Posterior right clamp length
length
europaeum
lanceatum
mexicanum
microconifibula
semotili
spinum
Octomacrum

h


Posterior right clamp width
width
europaeum
lanceatum
mexicanum
microconifibula
semotili
spinum
Octomacrum

i

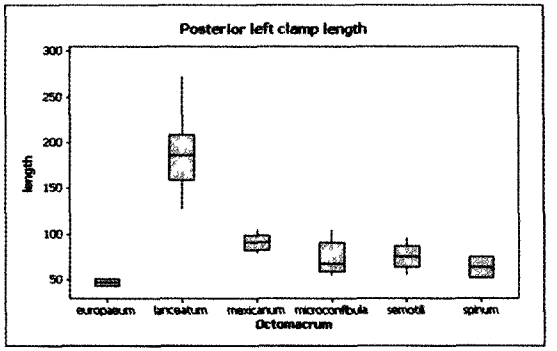
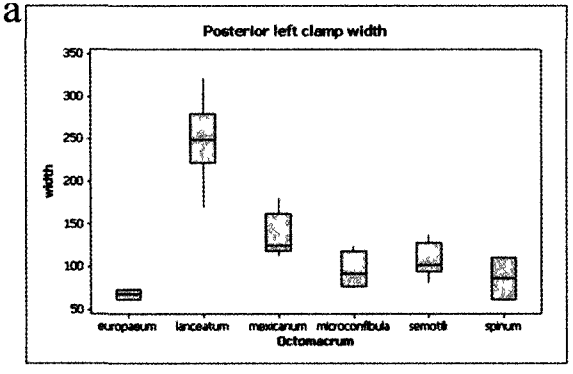
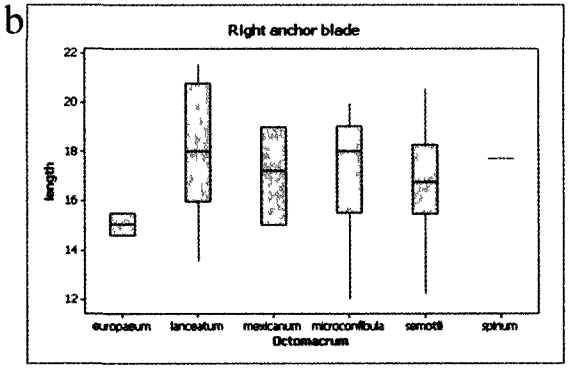

Posterior left clamp length
length
europaeum
lanceatum
mexicanum
microconifibula
semotili
spinum
Octomacrum

Fig. 22: Morphometrics of all six species of *Octomacrum* represented by boxplots. Interquartile range is boxed in gray with the mean line. Minimum and maximum values represented by extremes of vertical lines. All measurements are in μm. a) posterior left clamp width; b) right anchor blade; c) right anchor handle; d) left anchor blade; e) left anchor handle.

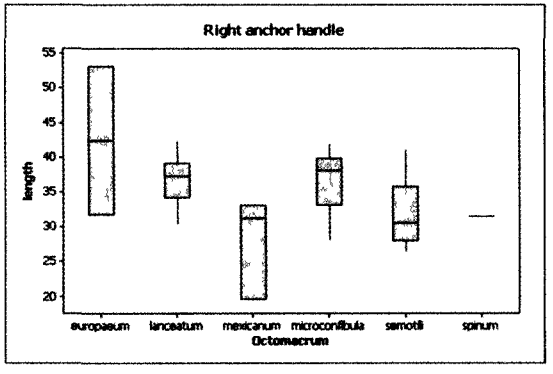
a


Posterior left clamp width
width
350
300
250
200
150
100
50
europaeum
lanceatum
mexicanum
microconfibula
semotili
spinum
Octomacrum

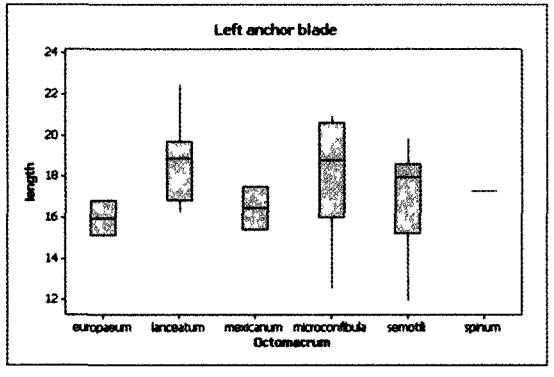
b


Right anchor blade
length
22
20
18
16
14
12
europaeum
lanceatum
mexicanum
microconfibula
semotili
spinum
Octomacrum

c


Right anchor handle
length
55
50
45
40
35
30
25
20
europaeum
lanceatum
mexicanum
microconfibula
semotili
spinum
Octomacrum

d


Left anchor blade
length
24
22
20
18
16
14
12
europaeum
lanceatum
mexicanum
microconfibula
semotili
spinum
Octomacrum

e

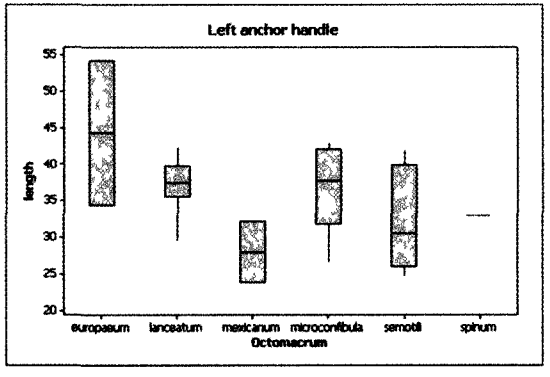

Left anchor handle
length
55
50
45
40
35
30
25
20
europaeum
lanceatum
mexicanum
microconfibula
semotili
spinum
Octomacrum

Table 1: Species of *Octomacrum* with their associated reported hosts and localities. Hosts are separated into Catostomidae (cat), Cyprinidae (cyp) and Salmonidae (sal). NY, New York; WI, Wisconsin; BC, British Columbia; ON, Ontario; PA, Pensylvania; MB, Manitoba; ME, Maine; MA, Masachusettes; ID, Idaho; UT, Utah; WV, West Virginia; VA, Virginia; IL, Illinois; NB, New Brunswick; NS, Nova Scotia; AR, Arkansas; AL, Alabama.

Species		Host	Authority	Type locality (Other locality)
O. lanceatum Mueller 1934	Type host:	*Catostomus commersoni* (cat)	Mueller 1934	NY (WI, BC, ON, PA, MB, ME)
		Erimyzon sucetta (cat)	Mueller 1934	NY (MA)
	Other host:	*Catostomus macrocheilus* (cat)	Arai and Mudry 1983	BC (ID)
		Mylocheilus caurinus (cyp)	Bangham and Adams 1954	BC
		Catostomus discobolis (cat)	Brienholt and Heckmann 1980	UT
		Luxilus cornutus (cyp)	Fischthal 1947	WI (WV, ON)
		Notropis spp. (cyp)	Hargis 1952	VA
		Notropis heterolepis (cyp)	Bangham 1941	ON
		Carpiodes carpio (cat)	Robinson and Jahn 1980	IL
		Catostomus catostomus (cat)	Bangham and Adams 1954	BC
		Pimephales promelas (cyp)	Beverley-Burton 1984	ON
		Oncorhynchus tshawytscha (sal)	Arai and Mudry 1983	BC
O. micronconfibula Hargis 1952	Type host:	*Notemigonus crysoleucas* (cyp)	Hargis 1952	VA (NB, ON, MB, NS)
	Other host:	*Mylocheilus caurinus* (cyp)	Arai and Mudry 1983	BC
		Phoxinus eos (cyp)	Cone 1980	NB
		Margariscus margarita (cyp)	Cone 1980	NB
		Richardsonius balteatus (cyp)	Shepard and Mace 1980	BC
O. europaeum Roman & Bychowsky 1956	Type host:	*Alburnoides bipunctatus* (cyp)	Roman & Bychowsky 1956	Romania
O. semotili Dechtiar 1966	Type host:	*Semotilus atromaculatus* (cyp)	Dechtiar 1966	ON
	Other host:	*Phoxinus eos* (cyp)	Dechtiar 1972	ON
		Phoxinus neogaeus (cyp)	Dechtiar 1972	ON
		Margariscus margarita (cyp)	Dechtiar 1972	ON (MB)
O. spinum Dansby & Shoemaker 1973	Type host:	*Campostoma anomalum* (cyp)	Dansby & Shoemaker 1973	WV
O. mexicanum Lamothe-Argumedo 1980	Type host:	*Algansea lacustris* (cyp)	Lamothe-Argumedo 1980	Mexico
Octomacrum sp.	Host:	*Couesius plumbeus* (cyp)	Bangham and Adams 1954	BC (ON)
		Richardsonius balteatus (cyp)	Bangham and Adams 1954	BC
		Hybognathus hankinsoni (cyp)	Bangham 1941	ON
		Semotilus atromaculatus (cyp)	Bangham and Venard 1946	ON
		Margariscus margarita (cyp)	Bangham and Venard 1946	ON
		Campostoma anomalum (cyp)	Cloutman 1974	AR
		Notropis telescopus (cyp)	Adrian 2010 (pers. comm.)	AL

Table 2: Worldwide collections of *Octomacrum*. Geographical coordinates presented as either localities sampled by the author (limited to within Canada) or those sampled by colleagues (Canada, U.S.A., Mexico and Romania).

Collection by	Locality	Lake	Latitude	Longitude
Author	Ontario, Canada	Brewer Lake	45°35'33.52"N	78°18'25.36"W
		Lake Opeongo	45°38'20.05"N	78°22'47.91"W
		Costello Creek	45°36'25.79"N	78°20'18.56"W
		Costello Lake	45°35'56.91"N	78°19'49.55"W
	Nova Scotia, Canada	Vinegar Lake	44°40'30.87"N	64°03'00.27"W
		Cranberry Lake	44°40'05.58"N	63°46'14.51"W
		Lower Sackville River	44°45'58.62"N	63°39'24.94"W
		Lake Ainslie	46°04'41.00"N	61°08'41.97"W
		Lawerencetown Lake	44°40'23.08"N	63°21'26.20"W
		Long Lake	44°37'31.71"N	63°38'24.73"W
		Feely Lake Brook	44°48'06.20"N	63°41'47.49"W
		West River	44°50'59.85"N	63°47'19.20"W
		Dorey Lake	44°38'54.04"N	64°03'27.52"W
		River Denys	45°50'17.46"N	61°09'55.84"W
Colleagues	Bistra, Romania	Bega River	45°56'04.99"N	21°30'06.50"E
	Tennessee, U.S.A.	Bradley Creek	35°55'11.05"N	86°18'22.92"W
		Stones River	35°59'56.55"N	86°27'30.08"W
	New York, U.S.A.	Schoharie Creek	42°45'34.66"N	74°20'30.07"W
	Michoacán, Mexico	Lake Patzcuaro	19°37'05.75"N	101°38'29.08"W
	Nova Scotia, Canada	Lochabor Lake	45°25'03.44"N	62°01'45.21"W

Table 3: Summary morphometrics of *O. lanceatum* including originally described morphometrics (Mueller, 1934) and museum material from the present study (both type and voucher). All measurements are in μm and are presented as the mean ± standard deviation (range of measurements; number of specimens examined).

Morphometric	Original description	Museum	
		type	voucher
		mean ± st. dev (range; n)	mean ± st. dev (range; n)
whole body	5000-6000	5125 ± 254.5 (4945 – 5305; n = 2)	5757 ± 1329 (3356 – 9677; n = 47)
greatest body width	1500-2000	1239 ± 77.9 (1184 – 1294; n = 2)	1294 ± 269.8 (743 – 1831; n = 50)
right buccal sucker length	-	100 ± 3.0 (98 – 102; n = 2)	125 ± 22.6 (70 – 167; n = 47)
right buccal sucker width	100	87 ± 3.2 (85 – 90; n = 2)	117 ± 18.5 (66 – 151; n = 47)
left buccal sucker length	-	92 ± 6.4 (87 – 96; n = 2)	125 ± 23.0 (72 – 168; n = 48)
left buccal sucker width	100	80 ± 4.0 (78 – 83; n = 2)	118 ± 20.2 (55 – 147; n = 48)
pharynx length	-	100 ± 5.1 (96 – 103; n = 2)	133 ± 24.2 (90 – 184; n = 36)
pharynx width	-	64 ± 2.0 (63 – 65; n = 2)	82 ± 17.2 (51 – 119; n = 36)
genital pore length	-	119 ± 0 (119 – 119; n = 1)	118 ± 18.7 (83 – 178; n = 40
genital pore width	-	131 ± 0 (131 – 131; n = 1)	130 ± 20.7 (82 – 185; n = 40)
haptor length	800	696 ± 67 (634 – 791; n = 4)	936 ± 214.4 (592 – 1468; n = 49)
haptor width	685	614 ± 90 (542 – 734; n = 4)	816 ± 207.5 (285 – 1266; n = 49)
anterior right clamp length	270	209 ± 17.7 (189 – 232; n = 4)	251 ± 49.2 (170 – 381; n = 40)
anterior right clamp width	340	274 ± 10.5 (261 – 284; n = 4)	316 ± 53.8 (224 – 427; n = 40)
anterior left clamp length	270	189 ± 23.2 (169 – 219; n = 4)	247 ± 54.6 (154 – 403; n = 42)
anterior left clamp width	340	267 ± 12.2 (256 – 285; n = 4)	309 ± 57.1 (209 – 431; n = 42)
second right clamp length	-	183 ± 15.5 (164 – 199; n = 4)	240 ± 46.1 (164 – 343; n = 44)
second right clamp width	-	277 ± 23.6 (247 – 301; n = 4)	332 ± 62.1 (207 – 452; n = 45)
second left clamp length	-	197 ± 16.6 (175 – 215; n = 4)	243 ± 46.4 (172 – 341; n = 45)
second left clamp width	-	277 ± 23.5 (263 – 312; n = 4)	330 ± 60.1 (227 – 453; n = 45)
third right clamp length	-	181 ± 21.7 (158 – 210; n = 4)	236 ± 43.3 (152 – 312; n = 48)
third right clamp width	-	251 ± 20.9 (231 – 274; n = 4)	316 ± 56.3 (197 – 433; n = 48)
third left clamp length	-	184 ± 16.6 (167 – 200; n = 3)	234 ± 42.8 (154 – 324; n = 45)
third left clamp width	-	263 ± 20.8 (245 – 286; n = 3)	314 ± 53.8 (205 – 436; n = 45)
posterior right clamp length	-	142 ± 5.9 (136 – 148; n = 3)	190 ± 34.2 (133 – 263; n = 46)
posterior right clamp width	-	194 ± 15.8 (181 – 211; n = 3)	250 ± 40.7 (146 – 327; n = 46)
posterior left clamp length	-	148 ± 15.0 (127 – 158; n = 4)	187 ± 32.6 (127 – 274; n = 46)
posterior left clamp width	-	195 ± 17.9 (169 – 208; n = 4)	250 ± 44.0 (169 – 396; n = 46)
right anchor blade	-	-	19 ± 4.0 (14 – 29; n = 13)
right anchor handle	-	-	37 ± 4.5 (30 – 48; n = 12)
left anchor blade	-	-	19 ± 1.7 (16 – 23; n = 15)
left anchor handle	-	-	37 ± 3.4 (30 – 42; n = 14)

Table 4: Summary morphometrics of *O. microconfibula* including originally described morphometrics (Hargis, 1952), museum material from the present study (type, voucher and juvenile voucher), and material from both Nova Scotia and Ontario collected recently. All measurements are in µm and are presented as the mean ± standard deviation (range of measurements; number of specimens examined). * indicates suspected error in measurements.

Morphometric	Original description	Museum			Present study	
		type	voucher	juvenile	Nova Scotia	Ontario
		mean ± st dev (range, n)	mean ± st dev (range, n)	mean ± st dev (range, n)	mean ± st dev (range, n)	mean ± st dev (range, n)
whole body	4350	5565 ± 287 2 (5362 – 5768, n = 2)	4706 ± 0133 (3559 – 5768, n = 4)	2048 ± 237 0 (1886 – 2321, n = 3)	4092 ± 1300 (2513-6177, n = 10)	2415 ± 335 (2032-3087, n = 9)
greatest body width	906	1208 ± 111 (1129 – 1286, n = 2)	1052 ± 234 (731 – 1286, n = 4)	363 ± 32 7 (326 – 383, n = 3)	1032 ± 418 (590-1726, n = 10)	699 ± 72 5 (574-811, n = 10)
right buccal sucker length	58	67 ± 8 1 (61 – 73, n = 2)	73 ± 8 4 (61 – 80, n = 4)	61 ± 27 6 (39 – 92, n = 3)	76 ± 19 5 (52-99, n = 10)	50 ± 4 9 (42-59, n = 10)
right buccal sucker width	49	58 ± 9 0 (51 – 64, n = 2)	67 ± 14 7 (51 – 187, n = 4)	65 ± 18 (53 – 86, n = 3)	80 ± 18 8 (58-111, n = 10)	55 ± 6 0 (45-66, n = 10)
left buccal sucker length	63	70 ± 7 0 (65 – 75, n = 2)	75 ± 7 4 (65 – 83, n = 4)	61 ± 21 9 (44 – 85, n = 3)	77 ± 21 0 (49-101, n = 10)	49 ± 4 3 (44-56, n = 10)
left buccal sucker width	52	58 ± 7 5 (53 – 64, n= 2)	72 ± 17 9 (53 – 94, n = 4)	66 ± 19 9 (50 – 88, n = 3)	81 ± 19 1 (54-110, n = 10)	54 ± 5 3 (44-63, n =10)
pharynx length	86	92 ± 9 8 (85 – 99, n = 2)	98 ± 9 1 (85 – 106, n = 4)	74 (n = 1)	111 ± 23 8 (85-156, n = 10)	68 ± 12 4 (50-88, n = 10)
pharynx width	70	70 ± 15 4 (59 – 81, n = 2)	74 ± 12 5 (59 – 88, n = 4)	45 (n = 1)	64 ± 13 7 (44-86, n = 10)	49 ± 8 8 (33-62, n = 10)
genital pore length	139	158 ± 20 7 (144 – 173, n = 2)	178 ± 35 (144 – 227, n = 4)	125 ± 1 1 (124 – 126, n = 2)	184 ± 47 8 (116-249, n = 10)	119 ± 14 8 (93-148, n = 10)
genital pore width	135	150 ± 15 3 (139 – 161, n = 2)	181 ± 47 8 (139 – 249, n = 4)	131 ± 6 5 (126 – 136, n = 2)	184 ± 46 0 (120-247, n = 10)	122 ± 16 3 (103-159, n = 10)
haptor length	371	-	370 ± 98 6 (301 – 440, n = 2)	387 (n = 1)	501 ± 111 2 (359-690, n = 10)	287 ± 69 1 (196-431, n = 9)
haptor width	604	-	401 ± 102 3 (329 – 473, n = 2)	447 (n = 1)	604 ± 142 3 (386-829, n = 10)	322 ± 50 1 (260-398, n = 9)
anterior right clamp length	93	81 (n = 1)	94 ± 18 9 (81 – 107, n = 2)	145 (n = 1)	83 ± 15 0 (65-109, n = 10)	68 ± 7 0 (60-78, n = 9)
anterior right clamp width	108	99 (n = 1)	128 ± 40 9 (99 – 156, n = 2)	167 (n = 1)	124 ± 24 8 (88-151, n = 10)	100 ± 7 8 (92-114, n = 9)
anterior left clamp length	*833	-	97 ± 24 6 (79 – 114, n = 2)	97 (n = 1)	90 ± 16 3 (65-113, n = 10)	70 ± 6 7 (56-77, n = 9)
anterior left clamp width	104	-	123 ± 31 1 (101 – 145, n = 2)	170 (n = 1)	126 ± 22 5 (94-154, n = 10)	104 ± 8 3 (96-120, n = 9)
second right clamp length	*9	81 (n = 1)	90 ± 15 1 (81 – 107, n = 3)	79 ± 21 9 (65 – 104, n = 3)	96 ± 23 2 (73-150, n = 10)	64 ± 5 9 (57-78, n = 9)
second right clamp width	108	117 (n = 1)	122 ± 34 5 (90 – 159, n = 3)	127 ± 48 9 (93 – 183, n = 3)	127 ± 23 2 (100-161, n = 10)	103 ± 8 4 (89-115, n =9)
second left clamp length	89	84 (n = 1)	91 ± 19.2 (76 – 112, n = 3)	90 ± 28 7 (67 – 122, n = 3)	93 ± 17 26 (72-115, n = 10)	60 ± 4 8 (54-68, n = 9)
second left clamp width	113	108 (n = 1)	124 ± 32 6 (103 – 162, n = 3)	126 ± 56 1 (94 – 191, n =3)	132 ± 22 4 (103-158 n = 10)	105 ± 7 2 (96-120, n = 9)
third right clamp length	87	70 (n = 1)	86 ± 23 4 (70 – 113, n = 3)	85 ± 37.2 (63 – 128, n = 3)	92 ± 16 1 (66-111, n = 10)	65 ± 9 8 (49-75, n = 9)
third right clamp width	107	107 (n = 1)	116 ± 32 5 (90 – 153, n = 3)	122 ± 51 5 (84 – 181, n = 3)	124 ± 22 4 (94-157, n = 10)	102 ± 9 4 (87-119, n = 9)
third left clamp length	89	81 (n = 1)	90 ± 13 7 (81 – 105, n = 3)	84 ± 37 6 (60 – 128, n = 3)	93 ± 17 5 (70-115, n = 10)	64 ± 6 7 (57-78, n = 9)
third left clamp width	103	105 (n = 1)	117 ± 31 6 (94 – 153, n = 3)	122 ± 54 0 (91 – 184, n = 3)	124 ± 20 5 (97-149, n = 10)	103 ± 10 3 (89-114, n = 9)
posterior right clamp length	69	60 (n = 1)	76 ± 19 3 (60 – 97, n = 3)	79 ± 32 6 (57 – 117, n = 3)	81 ± 13 5 (64-100, n = 10)	67 ± 11 2 (54-90, n = 9)
posterior right clamp width	79	78 (n = 1)	95 ± 27 0 (78 – 126, n = 3)	107 ± 43 (82– 157, n = 3)	104 ± 16 0 (78-124, n = 10)	81 ± 12 8 (55-99, n = 9)
posterior left clamp length	67	58 (n = 1)	75 ± 25 2 (58 – 93, n = 2)	80 ± 33 0 (59 – 118, n = 3)	85 ± 14 8 (63-105, n = 10)	64 ± 5 6 (54-71, n = 9)
posterior left clamp width	80	79 (n = 1)	102 ± 32 7 (79 – 125, n = 2)	105 ± 40 9 (78 – 152, n = 3)	102 ± 15 6 (76-124, n = 10)	84 ± 8 8 (74-100, n = 9)
right anchor blade	20	19 ± 0 6 (19 – 19, n = 2)	19 ± 0 4 (19 – 19, n = 3)	18 ± 1 6 (17 – 19, n = 2)	18 ± 1 3 (16-20, n = 10)	15 ± 2 0 (12-17, n = 7)
right anchor handle	34	39 ± 1 7 (38 – 41, n = 2)	39 ± 2 0 (37 – 41, n = 3)	32 ± 0 8 (31 – 32, n = 2)	38 ± 2 6 (32-42, n = 10)	34 ± 4 1 (28-39, n = 7)
left anchor blade	20	21 ± 0 4 (21 – 21, n = 2)	18 ± 3 9 (13 – 21, n = 4)	14 ± 1.2 (13 – 15, n = 2)	19 ± 1 7 (16-21, n = 10)	18 ± 1 4 (16-20, n = 7)
left anchor handle	34	41 ± 2 5 (39 – 43, n = 2)	36 ± 6 9 (27 – 43, n = 4)	34 ± 1 8 (33 – 35, n = 2)	39 ± 3 4 (31-43, n = 10)	36 ± 3 9 (32-42, n = 7)

Table 5: Morphometrics and statistical values of samples of *O. microconfibula* from Nova Scotia on golden shiner and Ontario on common shiner. P-values represent significance obtained from t-tests (*indicates those obtained by Mann-Whitney tests). All measurements are in μm and are presented as the mean ± standard deviation (range of measurements; number of specimens examined).

Morphometric	***O. microconfibula*** golden shiner mean ± st. dev (range) (n)	***O. microconfibula*** common shiner mean ± st. dev (range) (n)	**Significance** p =
whole body	4092 ± 1300 (2513-6177) (10)	2415 ± 335 (2032-3087) (9)	0.003
greatest body width	1032 ± 418 (590-1726) (10)	699 ± 72.5 (574-811) (10)	0.035
right buccal sucker length	76 ± 19.5 (52-99) (10)	50 ± 4.9 (42-59) (10)	0.002
right buccal sucker width	80 ± 18.8 (58-111) (10)	55 ± 6.0 (45-66) (10)	0.003
left buccal sucker length	77 ± 21.0 (49-101) (10)	49 ± 4.3 (44-56) (10)	*0.001
left buccal sucker width	81 ± 19.1 (54-110) (10)	54 ± 5.3 (44-63) (10)	0.001
pharynx length	111 ± 23.8 (85-156) (10)	68 ± 12.4 (50-88) (10)	0.000
pharynx width	64 ± 13.7 (44-86) (10)	49 ± 8.8 (33-62) (10)	*0.011
genital pore length	184 ± 47.8 (116-249) (10)	119 ± 14.8 (93-148) (10)	0.002
genital pore width	184 ± 46.0 (120-247) (10)	122 ± 16.3 (103-159) (10)	0.002
haptor length	501 ± 111.2 (359-690) (10)	287 ± 69.1 (196-431) (9)	0.000
haptor width	604 ± 142.3 (386-829) (10)	322 ± 50.1 (260-398) (9)	0.000
anterior right clamp length	83 ± 15.0 (65-109) (10)	68 ± 7.0 (60-78) (9)	0.014
anterior right clamp width	124 ± 24.8 (88-151) (10)	100 ± 7.8 (92-114) (9)	0.016
anterior left clamp length	90 ± 16.3 (65-113) (10)	70 ± 6.7 (56-77) (9)	0.005
anterior left clamp width	126 ± 22.5 (94-154) (10)	104 ± 8.3 (96-120) (9)	0.017
second right clamp length	96 ± 23.2 (73-150) (10)	64 ± 5.9 (57-78) (9)	*0.001
second right clamp width	127 ± 23.2 (100-161) (10)	103 ± 8.4 (89-115) (9)	0.010
second left clamp length	93 ± 17.26 (72-115) (10)	60 ± 4.8 (54-68) (9)	0.000
second left clamp width	132 ± 22.4 (103-158) (10)	105 ± 7.2 (96-120) (9)	0.005
third right clamp length	92 ± 16.1 (66-111) (10)	65 ± 9.8 (49-75) (9)	0.000
third right clamp width	124 ± 22.4 (94-157) (10)	102 ± 9.4 (87-119) (9)	0.016
third left clamp length	93 ± 17.5 (70-115) (10)	64 ± 6.7 (57-78) (9)	0.001
third left clamp width	124 ± 20.5 (97-149) (10)	103 ± 10.3 (89-114) (9)	0.013
posterior right clamp length	81 ± 13.5 (64-100) (10)	67 ± 11.2 (54-90) (9)	0.033
posterior right clamp width	104 ± 16.0 (78-124) (10)	81 ± 12.8 (55-99) (9)	0.003
posterior left clamp length	85 ± 14.8 (63-105) (10)	64 ± 5.6 (54-71) (9)	0.001
posterior left clamp width	102 ± 15.6 (76-124) (10)	84 ± 8.8 (74-100) (9)	0.006
right anchor blade	18 ± 1.3 (16-20) (10)	15 ± 2.0 (12-17) (7)	0.002
right anchor handle	38 ± 2.6 (32-42) (10)	34 ± 4.1 (28-39) (7)	0.073
left anchor blade	19 ± 1.7 (16-21) (10)	18 ± 1.4 (16-20) (7)	0.247
left anchor handle	39 ± 3.4 (31-43) (10)	36 ± 3.9 (32-42) (7)	0.136

Table 6: Summary morphometrics of *O. europaeum* including originally described morphometrics (Roman and Bychowsky, 1956), subsequent morphometrics (Matejusová and Koubková, 2002) and museum material from the present study (voucher). All measurements are in μm and are presented as the mean ± standard deviation (range of measurements; number of specimens examined).

Morphometric	Original description	Matejusová and Koubková (2002)	Museum voucher mean ± st. dev (range; n)
whole body	1250	1750	1493 (n = 1)
greatest body width	650	350	379 (n = 1)
right buccal sucker length	-	55	43 ± 10.0 (36 – 50; n = 2)
right buccal sucker width	-	30	42 ± 16.2 (30 – 53; n = 2)
left buccal sucker length	-	-	35 ± 17.5 (23 – 48; n = 2)
left buccal sucker width	-	-	42 ± 17.1 (30 – 54; n = 2)
pharynx length	-	80	67 ± 17.1 (55 – 79; n = 2)
pharynx width	-	30	37 ± 17.9 (25 – 50; n = 2)
genital pore length	-	125	91 ± 37.4 (65 – 118; n = 2)
genital pore width	140	125	104 ± 43.3 (73 – 134; n = 2)
haptor length	-	-	235 (n = 1)
haptor width	-	-	247 (n = 1)
anterior right clamp length	60	89	55 ± 18.4 (42 – 68; n = 2)
anterior right clamp width	90	101	75 ± 19.8 (61 – 89; n = 2)
anterior left clamp length	-	-	64 ± 30.5 (42 – 86; n = 2)
anterior left clamp width	-	-	77 ± 18.4 (64 – 90; n = 2)
second right clamp length	-	95	61 ± 26.7 (42 – 80; n = 2)
second right clamp width	-	114	82 ± 19.2 (68 – 96; n = 2)
second left clamp length	-	-	62 ± 27.0 (43 – 81; n = 2)
second left clamp width	-	-	78 ± 22.6 (62 – 94; n = 2)
third right clamp length	-	92	55 ± 12.6 (46 – 64; n = 2)
third right clamp width	-	109	74 ± 8.9 (68 – 80; n = 2)
third left clamp length	-	-	57 ± 12.9 (48 – 66; n = 2)
third left clamp width	-	-	75 ± 12.7 (66 – 84; n = 2)
posterior right clamp length	-	84	43 ± 5.4 (39 – 47; n = 2)
posterior right clamp width	-	93	61 ± 3.0 (59 – 63; n = 2)
posterior left clamp length	-	-	47 ± 5.7 (43 – 51; n = 2)
posterior left clamp width	-	-	68 ± 8.8 (61 – 74; n = 2)
right anchor blade	-	15	15 ± 0.6 (15 – 16; n = 2)
right anchor handle	-	54	42 ± 15 (32 – 53; n = 2)
left anchor blade	-	-	16 ± 1.2 (15 – 17; n = 2)
left anchor handle	-	-	44 ± 14.1 (34 – 54; n = 2)

Table 7: Summary morphometrics of *O. semotili* including originally described morphometrics (Dechtiar, 1966) and museum material from the present study (type and voucher). All measurements are in μm and are presented as the mean ± standard deviation (range of measurements; number of specimens examined). * indicates suspected error in measurement.

Morphometric	Original description	Museum	
		type	voucher
		mean ± st. dev (range; n)	mean ± st. dev (range; n)
whole body	3190	2771 ± 799.7 (2206 – 3337; n = 2)	3185 ± 970 (1710 – 4397; n = 12)
greatest body width	940	774 ± 160.1 (661 – 887; n = 2)	864 ± 193.9 (544 – 1137; n = 12)
right buccal sucker length	*750	65 ± 3.4 (62 – 67; n = 2)	70 ± 15.4 (53 – 100; n = 12)
right buccal sucker width	63	73 ± 2.4 (71 – 74; n = 2)	72 ± 15.2 (55 – 104; n = 12)
left buccal sucker length	-	68 ± 6.2 (64 – 73; n = 2)	70 ± 13.4 (48 – 97; n = 12)
left buccal sucker width	-	70 ± 0 (70 – 70; n = 2)	70 ± 11.6 (58 – 96; n = 12)
pharynx length	101	100 ± 19.4 (86 – 114; n = 2)	90 ± 18.0 (62 – 114; n = 12)
pharynx width	65	59 ± 1.9 (58 – 61; n = 2)	60 ± 14.1 (44 – 92; n = 12)
genital pore length	193	169 ± 0.8 (168 – 170; n = 2)	158 ± 43.4 (99 – 251; n = 12)
genital pore width	180	169 ± 5.3 (165 – 173; n = 2)	159 ± 44.5 (100 – 249; n = 12)
haptor length	325	358 ± 96.3 (290 – 426; n = 2)	360 ± 87.4 (227 – 503; n = 12)
haptor width	371	452 ± 145.1 (350 – 555; n = 2)	424 ± 111 (258 – 628; n = 12)
anterior right clamp length	89	86 ± 27.4 (67 – 105; n = 2)	85 ± 19.4 (60 – 120; n = 11)
anterior right clamp width	123	139 ± 27.7 (119 – 158; n = 2)	132 ± 20.7 (104 – 162; n = 11)
anterior left clamp length	-	75 ± 22.2 (59 – 91; n = 2)	87 ± 19.9 (59 – 115; n = 10)
anterior left clamp width	-	135 ± 28.1 (115 – 155; n = 2)	135 ± 22.3 (101 – 167; n = 10)
second right clamp length	92	80 ± 30.3 (58 – 101; n = 2)	84 ± 18.9 (58 – 113; n = 12)
second right clamp width	122	138 ± 32.0 (115 – 160; n = 2)	133 ± 24.2 (105 – 174; n = 12)
second left clamp length	-	74 ± 23.0 (58 – 90; n = 2)	84 ± 19.0 (58 – 117; n = 12)
second left clamp width	-	142 ± 39.6 (114 – 170; n = 2)	135 ± 26.1 (105 – 170; n = 12)
third right clamp length	87	74 ± 21.4 (59 – 89; n = 2)	80 ± 18.4 (51 – 113; n = 12)
third right clamp width	115	138 ± 33.0 (115 – 162; n = 2)	131 ± 24.3 (99 – 166; n = 12)
third left clamp length	-	74 ± 19.2 (60 – 87; n = 2)	79 ± 15.6 (57 – 100; n = 12)
third left clamp width	-	134 ± 35.3 (109 – 159; n = 2)	127 ± 22.8 (100 – 159; n = 12)
posterior right clamp length	74	68 ± 21.2 (53 – 83; n = 2)	76 ± 15.7 (51 – 104; n = 12)
posterior right clamp width	100	118 ± 38.9 (91 – 146; n = 2)	107 ± 20.9 (78 – 146; n = 12)
posterior left clamp length	-	66 ± 15.6 (55 – 77; n = 2)	76 ± 13.2 (55 – 97; n = 12)
posterior left clamp width	-	117 ± 30.6 (95 – 138; n = 2)	109 ± 19.4 (81 – 138; n = 12)
right anchor blade	25	15 ± 3.7 (12 – 18; n = 2)	17 ± 2.6 (12 – 21; n = 10)
right anchor handle	45	31 ± 5.2 (27 – 35; n = 2)	32 ± 5.0 (26 – 41; n = 10)
left anchor blade	-	12 (n = 1)	17 ± 2.5 (12 – 20; n = 10)
left anchor handle	-	25 (n = 1)	32 ± 7.0 (25 – 42; n = 9)

Table 8: Summary morphometrics of *O. spinum* including originally described morphometrics (Dansby and Shoemaker, 1973), museum material from the present study (a mature holotype and juvenile voucher), and material from Tennessee recently collected from stoneroller minnow. All measurements are in μm and are presented as the mean ± standard deviation (range of measurements; number of specimens examined).

Morphometric	Original description	Museum		Present study
		mature holotype (n = 1)	juvenile paratype (n = 1)	mean ± st. dev (range; n)
whole body	1117	1885	741	2690 ± 606.7 (2261 – 3119; n = 2)
greatest body width	190	278	193	630 ± 155.7 (520 – 740; n = 2)
right buccal sucker length	30	36	-	58 ± 15.1 (47 – 68; n = 2)
right buccal sucker width	30	35	-	56 ± 9.8 (49 – 63; n = 2)
left buccal sucker length	30	34	-	57 ± 16.8 (45 – 69; n = 2)
left buccal sucker width	30	32	-	59 ± 9.7 (52 – 65; n = 2)
pharynx length	-	-	-	110 (n = 1)
pharynx width	-	-	-	57 (n = 1)
genital pore length	71	78	-	146 (n = 1)
genital pore width	71	75	-	139 (n = 1)
haptor length	202	265	188	282 ± 79.7 (226 – 338; n = 2)
haptor width	190	278	125	316 ± 102.9 (244 – 389; n = 2)
anterior right clamp length	48	59	-	78 (n = 1)
anterior right clamp width	49	68	-	93 (n = 1)
anterior left clamp length	-	60	-	-
anterior left clamp width	-	72	-	-
second right clamp length	-	60	-	-
second right clamp width	-	74	-	-
second left clamp length	-	50	-	-
second left clamp width	-	74	-	-
third right clamp length	-	64	-	70 (n = 1)
third right clamp width	-	67	-	122 (n = 1)
third left clamp length	-	59	-	82 (n = 1)
third left clamp width	-	69	-	135 (n = 1)
posterior right clamp length	-	49	-	70 (n = 1)
posterior right clamp width	-	55	-	105 (n = 1)
posterior left clamp length	-	52	-	75 (n = 1)
posterior left clamp width	-	62	-	111 (n = 1)
right anchor blade	-	18	17	-
right anchor handle	27	31	26	-
left anchor blade	-	17	-	-
left anchor handle	-	33	-	-

Table 9: Summary morphometrics of *O. mexicanum* including originally described morphometrics (Lamothe-Argumedo, 1980) and museum material from the present study (type and voucher). All measurements are in μm and are presented as the mean ± standard deviation (range of measurements; number of specimens examined).

Morphometric	Original description	Museum	
		type	voucher
		mean ± st. dev (range; n)	mean ± st. dev (range; n)
whole body	3967	4030 ± 296.6 (3791 – 4362; n = 3)	5164 ± 1035 (3791 – 6673; n = 10)
greatest body width	812	805 ± 108.2 (701 – 917; n = 3)	977 ± 245.1 (701 – 1437; n = 10)
right buccal sucker length	57	55 ± 4.1 (52 – 58; n = 2)	58 ± 7.1 (47 – 69; n = 9)
right buccal sucker width	64	64 ± 4.3 (60 – 69; n = 3)	61 ± 5.7 (53 – 69; n = 10)
left buccal sucker length	-	56 ± 2.5 (54 – 59; n = 3)	59 ± 6.6 (51 – 72; n = 10)
left buccal sucker width	-	62 ± 5.2 (57 – 68; n = 3)	62 ± 6.6 (52 – 70; n = 10)
pharynx length	96	79 ± 17.1 (63 – 97; n = 3)	94 ± 16.1 (63 – 120; n = 10)
pharynx width	62	69 ± 15.3 (57 – 86; n = 3)	61 ± 11.0 (47 – 86; n = 10)
genital pore length	147	144 ± 21.2 (124 – 166; n = 3)	188 ± 38.0 (124 – 239; n = 10)
genital pore width	168	153 ± 14.3 (137 – 165; n = 3)	188 ± 31.6 (137 – 234; n = 10)
haptor length	489	527 ± 177.2 (403 – 730; n = 3)	509 ± 127.5 (390 – 730; n = 10)
haptor width	446	447 ± 98.7 (339 – 533; n = 3)	548 ± 131.7 (339 – 764; n = 10)
anterior right clamp length	123	159 ± 52.3 (128 – 220; n = 3)	140 ± 35.5 (97 – 220; n = 9)
anterior right clamp width	155	159 ± 53.6 (111 – 217; n = 3)	169 ± 38.8 (111 – 231; n = 9)
anterior left clamp length	137	163 ± 57.5 (119 – 228; n = 3)	140 ± 45.5 (89 – 228; n = 9)
anterior left clamp width	147	216 ± 148.7 (104 – 384; n = 3)	180 ± 81.6 (104 – 384; n = 9)
second right clamp length	111	157 ± 56.1 (122 – 222; n = 3)	143 ± 31.4 (111 – 222; n = 10)
second right clamp width	173	177 ± 55.7 (127 – 234; n = 3)	176 ± 33.9 (127 – 237; n = 10)
second left clamp length	121	153 ± 68.4 (108 – 232; n = 3)	143 ± 35.3 (108 – 232; n = 9)
second left clamp width	152	222 ± 110.4 (154 – 349; n = 3)	190 ± 62.1 (146 – 349; n =9)
third right clamp length	115	151 ± 56.4 (108 – 215; n = 3)	129 ± 32.1 (103 – 215; n = 10)
third right clamp width	147	179 ± 61.8 (133 – 249; n = 3)	174 ± 33.0 (133 – 249; n = 10)
third left clamp length	104	144 ± 44.1 (116 – 195; n = 3)	126 ± 28.9 (95 – 195; n = 10)
third left clamp width	144	197 ± 89.5 (142 – 301; n = 3)	176 ± 48.1 (137 – 301; n = 10)
posterior right clamp length	79	114 ± 35.1 (90 – 154; n = 3)	99 ± 21.2 (81 – 154; n = 10)
posterior right clamp width	121	140 ± 36.7 (112 – 182; n = 3)	138 ± 22.8 (112 – 182; n = 10)
posterior left clamp length	84	120 ± 54.7 (82 – 183; n = 3)	99 ± 38.5 (79 – 183; n = 10)
posterior left clamp width	115	164 ± 81.3 (112 – 258; n = 3)	146 ± 44.7 (112 – 258; n = 10)
right anchor blade	-	18 ± 1.3 (17 – 19; n = 2)	17 ± 2.0 (15 – 19; n = 3)
right anchor handle	-	32 ± 1.3 (31 – 33; n = 2)	28 ± 7.3 (20 – 33; n = 3)
left anchor blade	-	15 (n = 1)	17 ± 1.5 (15 – 18; n = 2)
left anchor handle	-	32 (n = 1)	28 ± 5.9 (24 – 32; n = 2)

Table 10: Summary morphometrics of the clamps of all species of *Octomacrum*. Data is presented as either type or voucher material when applicable and the mean measurement ± the standard deviation (range of measurements; number of specimens examined).

Morphometric	*O. lanceatum*		*O. microconfibula*		*O. europaeum*	*O. sematilli*		*O. spinum*	*O. mexicanum*	
	type	voucher	type	voucher	voucher	type	voucher	type	type	voucher
	mean ± st dev (range n)		mean ± st dev (range, n)		mean ± st dev (range, n)	mean ± st dev (range, n)		mean (n)	mean ± st dev (range n)	
anterior right clamp length	209 ± 17 7 (189 - 232, n = 4)	251 ± 49.2 (170 - 381 n = 40)	81 (n = 1)	94 ± 18 9 (81 - 107, n = 2)	55 ± 18 4 (42 - 68 n = 2)	86 ± 27 4 (67 - 105, n = 2)	85 ± 19 4 (60 - 120, n = 11)	59 (n = 1)	159 ± 52 3 (128 - 220, n = 3)	140 ± 35 5 (97 - 220 n = 9)
anterior right clamp width	274 ± 10.5 (261 - 284, n = 4)	316 ± 53 8 (224 - 427, n = 40)	99 (n = 1)	128 ± 40 9 (99 - 156, n = 2)	75 ± 19.8 (61 - 89; n = 2)	139 ± 27 7 (119 - 158, n = 2)	132 ± 20 7 (104 - 162, n = 11)	68 (n = 1)	159 ± 53 6 (111 - 217, n = 3)	169 ± 38 8 (111 - 231, n = 9)
anterior left clamp length	189 ± 23.2 (169 - 219; n = 4)	247 ± 54.6 (154 - 403, n = 42)	-	97 ± 24 6 (79 - 114 n = 2)	64 ± 30 5 (42 - 86, n = 2)	75 ± 22.2 (59 - 91, n = 2)	87 ± 19 9 (59 - 115, n = 10)	60 (n = 1)	163 ± 57 5 (119 - 228, n = 3)	140 ± 45 5 (89 - 228, n = 9)
anterior left clamp width	267 ± 12 2 (256 - 285, n = 4)	309 ± 57 1 (209 - 431, n = 42)	-	123 ± 31 1 (101 - 145, n = 2)	77 ± 18 4 (64 - 90, n = 2)	135 ± 28 1 (115 - 155, n = 2)	135 ± 22.3 (101 - 167, n = 10)	72 (n = 1)	216 ± 148 7 (104 - 384, n = 3)	180 ± 81 6 (104 - 384 n = 9)
second right clamp length	183 ± 15 5 (164 - 199, n = 4)	240 ± 46 1 (164 - 343, n = 44)	81 (n = 1)	90 ± 15 1 (81 - 107, n = 3)	61 ± 26 7 (42 - 80, n = 2)	80 ± 30.3 (58 - 101, n = 2)	84 ± 18 9 (58 - 113, n = 12)	60 (n = 1)	157 ± 56 1 (122 - 222 n = 3)	143 ± 31 4 (111 - 222, n = 10)
second right clamp width	277 ± 23 6 (247 - 301, n = 4)	332 ± 62 1 (207 - 452, n = 45)	117 (n = 1)	122 ± 34.5 (90 - 159; n = 3)	82 ± 19.2 (68 - 96 n = 2)	138 ± 32 0 (115 - 160, n = 2)	133 ± 24.2 (105 - 174, n = 12)	74 (n = 1)	177 ± 55 7 (127 - 234, n = 3)	176 ± 33 9 (127 - 237, n = 10)
second left clamp length	197 ± 16 6 (175 - 215, n = 4)	243 ± 46 4 (172 - 341, n = 45)	84 (n = 1)	91 ± 19.2 (76 - 112, n = 3)	62 ± 27 0 (43 - 81, n = 2)	74 ± 23 0 (58 - 90; n = 2)	84 ± 19 0 (58 - 117, n = 12)	50 (n = 1)	153 ± 68 4 (108 - 232, n = 3)	143 ± 35 3 (108 - 232, n = 9)
second left clamp width	277 ± 23.5 (263 - 312; n = 4)	330 ± 60 1 (227 - 453, n = 45)	108 (n = 1)	124 ± 32 6 (103 - 162, n - 3)	78 ± 22 6 (62 - 94, n = 2)	142 ± 39 6 (114 - 170 n = 2)	135 ± 26 1 (105 - 170 n = 12)	74 (n = 1)	222 ± 110 4 (154 - 349; n = 3)	190 ± 62 1 (146 - 349, n =9)
third right clamp length	181 ± 21 7 (158 - 210; n = 4)	236 ± 43 3 (152 - 312 n = 48)	70 (n = 1)	86 ± 23 4 (70 - 113, n = 3)	55 ± 12 6 (46 - 64, n = 2)	74 ± 21 4 (59 - 89; n = 2)	80 ± 18 4 (51 - 113, n = 12)	64 (n = 1)	151 ± 56 4 (108 - 215, n = 3)	129 ± 32 1 (103 - 215, n = 10)
third right clamp width	251 ± 20 9 (231 - 274, n = 4)	316 ± 56 3 (197 - 433 n = 48)	107 (n = 1)	116 ± 32.5 (90 - 153 n = 3)	74 ± 8 9 (68 - 80, n = 2)	138 ± 33 0 (115 - 162, n = 2)	131 ± 24.3 (99 - 166, n = 12)	67 (n = 1)	179 ± 61 8 (133 - 249; n = 3)	174 ± 33 0 (133 - 249, n - 10)
third left clamp length	184 ± 16 6 (167 - 200, n = 3)	234 ± 42 8 (154 - 324, n = 45)	81 (n = 1)	90 ± 13 7 (81 - 105, n = 3)	57 ± 12 9 (48 - 66, n = 2)	74 ± 19.2 (60 - 87, n = 2)	79 ± 15 6 (57 - 100, n = 12)	59 (n - 1)	144 ± 44 1 (116 - 195, n = 3)	126 ± 28 9 (95 - 195, n = 10)
third left clamp width	263 ± 20 8 (245 - 286, n = 3)	314 ± 53 8 (205 - 436, n = 45)	105 (n = 1)	117 ± 31 6 (94 - 153, n = 3)	75 ± 12 7 (66 - 84, n = 2)	134 ± 35.3 (109 - 159; n = 2)	127 ± 22 8 (100 - 159 n = 12)	69 (n = 1)	197 ± 89 5 (142 - 301, n = 3)	176 ± 48 1 (137 - 301, n = 10)
posterior right clamp length	142 ± 5 9 (136 - 148, n = 3)	190 ± 34.2 (133 - 263, n = 46)	60 (n = 1)	76 ± 19.3 (60 - 97, n = 3)	43 ± 5 4 (39 - 47, n = 2)	68 ± 21.2 (53 - 83, n = 2)	76 ± 15 7 (51 - 104, n = 12)	49 (n = 1)	114 ± 35 1 (90 - 154, n = 3)	99 ± 21 2 (81 - 154, n = 10)
posterior right clamp width	194 ± 15 8 (181 - 211, n = 3)	250 ± 40 7 (146 - 327 n = 46)	78 (n = 1)	95 ± 27 0 (78 - 126, n = 3)	61 ± 3 0 (59 - 63, n = 2)	118 ± 38 9 (91 - 146, n = 2)	107 ± 20 9 (78 - 146 n = 12)	55 (n = 1)	140 ± 36 7 (112 - 182, n = 3)	138 ± 22 8 (112 - 182, n = 10)
posterior left clamp length	148 ± 15 0 (127 - 158, n = 4)	187 ± 32 6 (127 - 274, n = 46)	58 (n = 1)	75 ± 25.2 (58 - 93, n = 2)	47 ± 5 7 (43 - 51, n = 2)	66 ± 15 6 (55 - 77, n = 2)	76 ± 13.2 (55 - 97, n = 12)	52 (n = 1)	120 ± 54 7 (82 - 183, n = 3)	99 ± 38 5 (79 - 183, n = 10)
posterior left clamp width	195 ± 17 9 (169 - 208, n = 4)	250 ± 44 0 (169 - 396, n = 46)	79 (n = 1)	102 ± 32 7 (79 - 125, n = 2)	68 ± 8 8 (61 - 74, n = 2)	117 ± 30 6 (95 - 138, n - 2)	109 ± 19 4 (81 - 138, n = 12)	62 (n = 1)	164 ± 81.3 (112 - 258, n = 3)	146 ± 44 7 (112 - 258, n = 10)

Appendix B

Sequence data (18S DNA) of species of *Octomacrum*

Octomacrum lanceatum

AGTCAGAGGTTCGAAGACGATCAGATACCGTCCTAGTTCTGACCATAAACGA
TGCCGACTGACGATCCGTGGGGTAAAATCCTTTTGTCCCCACGGGCAGTCTCC
GGGAAACCTTTAAGTCTTTGGGTTCCGGGGGAAGTATGGTTGCAAAGCTGAA
ACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTA
ATTTGACTCAACACGGGAAAACTCACCCGGCCCGGACACTGTGAGGATTGAC
AGATTGACAGCTCTTTCATGATTCAGTGGTTGGTGGTGCATGGCCGTTCTTAG
TTGGTGGAGCGATTTGTCTGGTTAATTCCGATAACGAACGAGACTCTATTCTG
CTAAATAGTACTTCCGTAATTGGTGGGACTGCTTTGTCCGAGTAATTCTGCAT
CATTTGTAGAGGAACTGGGTTCAGGCGTCCTACTGCGCGGCTGAAACTTCTTA
GAGAGACAAATTGCAATTAACAATACGAAATTGAGCAATAACAGGTCTGTGA
TGCCCTTAGATGTCCGGGGCCGCACGCGCGCTACAATGACGGTACCAGCGAG
TATGACCTCCTGGCCCGAGAGGGTTGGGCAAACTGGTCCA

Octomacrum microconfibula from golden shiner in Nova Scotia

GTCAGAGGTTCGAAGACGATCAGATACCGTCCTAGTTCTGACCATAAACGAT
GCCGACTGACGATCCGTGGGGTAAAATCCTTTTGTCCCCACGGGCAGTCTCC
GGGAAACCTTTAAGTCTTTGGGTTCCGGGGGAAGTATGGTTGCAAAGCTGAA
ACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTA
ATTTGACTCAACACGGGAAAACTCACCCGGCCCGGACACTGTGAGGATTGAC
AGATTGACAGCTCTTTCATGATTCAGTGGTTGGTGGTGCATGGCCGTTCTTAG
TTGGTGGAGCGATTTGTCTGGTTAATTCCGATAACGAACGAGACTCTATTCTG
CTAAATAGTACTCCCGTAATTGGTGGGACTGCTCTGTCCGAGTTGTTCTGCAT
TCTTTGTTGAAGAACTAGGTGCAGGCGTCCTACTGCGCGGGTGAAACTTCTTA
GAGAGACAAATTGCAATTAACAATACGAAATTGAGCAATAACAGGTCTGTGA
TGCCCTTAGATGTCCGGGGCCGCACGCGCGCTACAATGACGGTACCAGCGAG
TATGACCTCCTGGCCCGAGAGGGTTGGGCAAAC

Octomacrum microconfibula from common shiner in Ontario

GTCAGAGGTTCGAAGACGATCAGATACCGTCCTAGTTCTGACCATAAACGAT
GCCGACTGACGATCCGTGGGGTAAAATCCTTTTGTCCCCACGGGCAGTCTCC
GGGAAACCTTTAAGTCTTTGGGTTCCGGGGGAAGTATGGTTGCAAAGCTGAA
ACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTA
ATTTGACTCAACACGGGAAAACTCACCCGGCCCGGACACTGTGAGGATTGAC
AGATTGACAGCTCTTTCATGATTCAGTGGTTGGTGGTGCATGGCCGTTCTTAG
TTGGTGGAGCGATTTGTCTGGTTAATTCCGATAACGAACGAGACTCTATTCTG
CTAAATAGTACTCCCGTAATTGGTGGGACTGCTCTGTCCGAGTTGTTCTGCAT
TATTTGTTGAAGAACTAGGTGCAGGCGTCCTACTGCGCGGGTGAAACTTCTTA

GAGAGACAAATTGCAATTAACAATACGAAATTGAGCAATAACAGGTCTGTGA
TGCCCTTAGATGTCCGGGGCCGCACGCGCGCTACAATGACGGTACCAGCGAG
TATGACCTCCTGGC

Octomacrum europaeum

AGTCAGAGGTTCGAAGACGATCAGATACCGTCCTAGTTCTGACCATAAACGA
TGCCGACTGACGATCCGTGGGGTAAAATTCTTTTGTCCCCACGGGCAGTCTCC
GGGAAACCTTTAAGTCTTTGGGTTCCGGGGGAAGTATGGTTGCAAAGCTGAA
ACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTA
ATTTGACTCAACACGGGAAAACTCACCCGGCCCGGACACTGTGAGGATTGAC
AGATTGACAGCTCTTTCATGATTCAGTGGTTGGTGGTGCATGGCCGTTCTTAG
TTGGTGGAGCGATTTGTCTGGTTAATTCCGATAACGAACGAGACTCTATTCTG
CTAAATAGTACTCCCGTAATTGGTGAGACTGTTCTGTCCTAGTTTTGCTGCAT
CCAATGTGGAAGATCTGGGTTCAGACGTCTTACTACGCGGGTGAAACTTCTTA
GAGAGACAAATTGCAATTAACAATACGAAATTGAGCAATAACAGGTCTGTGA
TGCCCTTAGATGTCCGGGGCCGCACGCGCGCTACAATGACGGTACCAGCGAG
TATGACCTCCTGGCCCGAGAGGGTTGGGCAAACTG

Octomacrum semotili

CTGACCATAAACGATGCCGACTGACGATCCGTGGGGTAAAATCCTTTTGTCCC
CACGGGCAGTCTCCGGGAAACCTTTAAGTCTTTGGGTTCCGGGGGAAGTATG
GTTGCAAAGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTG
GAGCCTGCGGCTTAATTTGACTCAACACGGGAAAACTCACCCGGCCCGGACA
CTGTGAGGATTGACAGATTGACAGCTCTTTCATGATTCAGTGGTTGGTGGTGC
ATGGCCGTTCTTAGTTGGTGGAGCGATTTGTCTGGTTAATTCCGATAACGAAC
GAGACTCTATTCTGCTAAATAGTACTCCCGTAATTGGTGGGACTGCTCTGTCC
GAGTTGTTCTGCATTCTTTGTTGAAGAACTAGGTGCAGGCGTCCTACTGCGCG
GGTGAAACTTCTTAGAGAGACAAATTGCAATTAACAATACGAAATTGAGCAA
TAACAGGTCTGTGATGCCCTTAGATGTCCGGGGCCGCACGCGCGCTACAATG
ACGGTACCAGCGAGTATGACCTCCTGGCCCGAGAGGGT

Octomacrum spinum

GTCAGAGGTTCGAAGACGATCAGATACCGTCCTAGTTCTGACCATAAACGAT
GCCGACTGACGATCCGTGGGGTAAAATCCTTTTGTCCCCACGGGCAGTCTCC
GGGAAACCTTTAAGTCTTTGGGTTCCGGGGGAAGTATGGTTGCAAAGCTGAA
ACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTA
ATTTGACTCAACACGGGAAAACTCACCCGGCCCGGACACTGTGAGGATTGAC
AGATTGACAGCTCTTTCATGATTCAGTGGTTGGTGGTGCATGGCCGTTCTTAG
TTGGTGGAGCGATTTGTCTGGTTAATTCCGATAACGAACGAGACTCTATTCTG
CTAAATAGTACTCCCGTAATTGGTGGGACTGCTCTGTCCGAGTTGTTCTGCAT
TATTTGTTGAAGAACTAGGTGCAGGCGTCCTACTGCGCGGGTGAAACTTCTTA

GAGAGACAAATTGCAATTAACAATACGAAATTGAGCAATAACAGGTCTGTGA
TGCCCTTAGATGTCCGGGGCCGCACGCGCGCTACAATGACGGTACCAGCGAG
TATGACCTCCTGGCCCGAGAGGGT

Octomacrum mexicanum

GTCAGAGGTTCGAAGACGATCAGATACCGTCCTAGTTCTGACCATAAACGAT
GCCGACTGACGATCCGTGGGGTAAAATCCTTTTGTCCCCACGGGCAGTCTCC
GGGAAACCTTTAAGTCTTTGGGTTCCGGGGGAAGTATGGTTGCAAAGCTGAA
ACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTA
ATTTGACTCAACACGGGAAAACTCACCCGGCCCGGACACTGTGAGGATTGAC
AGATTGACAGCTCTTTCATGATTCAGTGGTTGGTGGTGCATGGCCGTTCTTAG
TTGGTGGAGCGATTTGTCTGGTTAATTCCGATAACGAACGAGACTCTATTCTG
CTAAATAGTACTCCCGTAATTGGTGGGACTGCTCTGTCCGAGTTGTTCTGCAT
TATTTGTTGAAGAACTAGGTGCAGGCGTCCTACTGCGCGGGTGAAACTTCTTA
GAGAGACAAATTGCAATTAACAATACGAAATTGAGCAATAACAGGTCTGTGA
TGCCCTTAGATGTCCGGGGCCGCACGCGCGCTACAATGACGGTACCAGCGAG
TATGACCTCCTGGCCCGAGAGGG

Species of *Octomacrum* with corresponding GenBank accession numbers

Species of *Octomacrum*	Host	Accession number
O. europaeum	*Alburnoides bipunctatus*	JN107641
O. lanceatum	*Catostomus commersoni*	JN107642
O. mexicanum	*Algansea lacustris*	JN107643
O. microconfibula	*Notemigonus crysoleucas*	JN107644
O. microconfibula	*Luxilus cornutus*	JN107645
O. spinum	*Campostoma anomalum*	JN107646
O. semotili	*Semotilus atromaculatus*	JN107647
O. semotili	*Phoxinus eos*	JN107648