NOTE TO USERS

Taxonomy and ecology of *Ergasilus* sp. and *Thersitina gasterostei* (Copepoda) parasitizing gasterosteiforms along the coasts of the Atlantic Canadian Provinces.

By

Victoria Lynn Savoie

Submitted in partial fulfillment of the requirements for the degree of
Master of Science in Applied Sciences

Biology Department
Saint Mary's University
Halifax, Nova Scotia
Submitted: November 1, 2004

Your file Votre référence
ISBN: 0-612-98944-5
Our file Notre référence
ISBN: 0-612-98944-5

Table of Contents

Abstract

Taxonomy and ecology of *Ergasilus* sp. and *Thersitina gasterostei* (Copepoda) parasitizing gasterosteiforms along the coasts of the Atlantic Canadian Provinces.

Victoria Savoie
November 1, 2004

An *Ergasilus* sp. and *Thersitina gasterostei* infected *Gasterosteus aculeatus* (threespine stickleback), *Apeltes quadracus* (fourspine stickleback), *Gasterosteus wheatlandi* (black-spotted stickleback), and *Pungitius pungitius* (ninespine sticklebacks) along coastal Atlantic Canada. The taxonomic position of *Ergasilus* sp. was under question due to its resembling both *T. gasterostei* and *Ergasilus* spp.. While the greatly inflated cephalothorax of *Ergasilus* sp. was similar to that of *T. gasterostei,* morphology of the antennule and antennae, mouthparts and legs were more similar with that of representatives of *Ergasilus. Ergasilus* sp. infected *A. quadracus* and *G. wheatlandi,* both endemic to the area, at significantly higher prevalences than on *G. aculeatus*. In contrast, *T. gasterostei* occurred primarily on *G. aculeatus*, which shares the parasite's circumpolar distribution. Mixed species infections were recorded most often on *G. aculeatus*, supporting host specificity displayed by *T. gasterostei* for this stickleback host. On *G. aculeatus*, *T. gasterostei* attached most frequently to the inner surface of the operculum. At low intensities on *A. quadracus*, *Ergasilus* sp. also attached to this site. However, when intensity was higher, the prevalence of *Ergasilus* sp. increased on the gills. Comparison of attachment of *Ergasilus* sp. on the inner surface of the opercula to that on the gills of *A. quadracus* resulted in a slight, but significant, difference in individual egg size but a marked difference in both egg sac length and the number of eggs. Histological sections of infection associated with *Ergasilus* sp. showed tissue proliferation and hyperplasia at sites of attachment and feeding. Molecular sequences of 28S rDNA of *Ergasilus* sp., *T. gasterostei* and *Ergasilus manicatus* supported the classification of this undescribed copepod as *Ergasilus* sp.. By investigating the taxonomy, ecology, and molecular taxonomy of the previously undescribed species, this study establishes it as an *Ergasilus* which looks and lives like *T. gasterostei.*

Acknowledgements

My supervisor, Dr. David Cone, provided so many opportunities throughout our work together. His unfaltering belief in his students and their abilities is something I have witnessed and experience first hand. He challenged me throughout my university education and encouraged me to become a better student and researcher. I thank him for his patience, support, advice and friendship. I am also very thankful for the support of my supervisory committee: Dr. George Benz whose knowledge of copepods helped me to understand and appreciate these amazing organisms; Dr. Stewart Johnson who helped to develop this understanding and appreciation in me; and Dr. Genlou Sun who gave me both the use of his laboratory and his expertise to help in the completion of my molecular analysis. Also thanks to Dr. Strongman for the use of the SMU taxonomy laboratory.

I would also like to thank both Ken Meade from Nova Scotia Power, and Dr. Neil Ross from the National Research Council Institute for Marine Biosciences for their employment in fulfillment of my co-operative education requirement; both experiences were an invaluable part of my education. Dr. Neil Ross also deserves my most sincere gratitude for offering his help in trying to get my molecular work off and running.

Many people have helped with the many collections of sticklebacks in the region: Troy Swan, Gerald Savoie, Sherri Savoie, Steve MacIsaac, Caroline Picot, Marc Skinner, Russell Easy, and Dr. David Marcogliese. I would also like to the thank Jing Dong, Rusell Easy, Denise Muise, and Mark Fast for all their help with my molecular analyses.

Finally, I have a wonderful support system of family and friends who I am so very grateful for. Troy, you have been so much to me throughout this experience, my partner, my motivator, my confidante, and so much more. Thank you for helping me through the rough spots and celebrating with me when I succeed – I love you. To my parents and sisters you are all so supportive and understanding. Thanks for listening and acting excited when I call you with "big news" about my bugs, I would be lost without all of you. Tessa, my best friend through all our adventures over the years, thank you so much for your unconditional love and for making each day bright. To my lab mate Chantelle Cormier, to my dear friends and my incredible family, thanks for listening and being so supportive throughout my education.

List of Figures

List of Tables

Introduction

Ergasilids (Ergasilidae Nordmann, 1832; Copepoda: Poecilostomatoida) comprise about 260 nominal species belonging to 25 genera (Boxshall & Montu 1997), of which 19 genera are considered valid (Amado *et al.* 1995). They occur worldwide and predominantly on bony freshwater fishes, with few occurring on estuarine and marine fishes (Kabata 1979). Ergasilids are much more widely distributed geographically than most other lineages of parasitic copepod because many of them retain a partially free-living lifestyle (Kabata 1979). Thus, ergasilids have the distinction of being a morphological middle ground between the most prominent groups of free-living copepods and the highly modified parasitic forms (Huys & Boxshall 1991).

The ergasilid habitus consists of four major regions: the cephalothorax, thorax, genital complex, and abdomen. Appendages include two pairs of antennae, one pair of mandibles, two pairs of maxillae, one pair of maxillipeds (found only in the males of some species) and five pairs of swimming legs (the fifth pair is vestigial in most species). Adult females develop large paired egg sacs that extend from the genital complex. The morphology of parasitic females resembles that of their free-living counterparts, with subtle adaptations of their appendages used in attachment and feeding suited for the parasitic lifestyle. Female ergasilids typically infect the gill filaments or gill chambers of their hosts, with attachment being facilitated by the antennae, which are enlarged and pierce host tissue (Gurney 1933).

Nordmann (1832) established the Family Ergasilidae when describing *Ergasilus sieboldi*, the type species, and *Ergasilus gibbus*. Wilson revised the family diagnosis, defining the basic morphology as, having a cylindrical and elongate body, with its ventral

surface projecting. The first antennae are small with slender setae, the second antennae are elongated into a muscular organ used in attachment to the host and often found to be half or three-quarters of the entire body length and ending with a stout claw structure. The mouthparts are found at the centre of the cephalothorax and are somewhat projected from the ventral surface. They consist of a labium, mandible, mandibular palp, as well as first and second maxilla. The first four pairs of swimming legs are similar with the fifth pair being more simple and one-jointed. Most species measure between 0.6 and 1 mm in length (Wilson 1911).

Taxa within Ergasilidae are typically differentiated on the basis of morphology of the antennae, mouthparts, and swimming legs. The antennae vary intraspecifically in shape and size, as do the shape and armature of the terminal joints of the mandibles and maxillae (Margolis & Kabata 1988).

The life cycles of few ergasilids have been studied. Development appears to include six naupliar and five copepodid stages. Free-living adult males and females copulate in the water column and females then seek a host. Eggs develop and pass from the internal environment of the female to her egg sacs as the sacs are extruded from the genital complex. From the membranous egg sacs protruding from the genital complex, nauplii hatch in the water column and mature (Wilson 1911).

Ergasilus Nordmann, 1832 is the most speciose genus (160 species) within the Ergasilidae. *Ergasilus* spp. infect a wide range of hosts and show varying degrees of host specificity (Hudson *et al.* 1994).

Six *Ergasilus* spp. have been reported within the Atlantic Provinces of Canada (Margolis & Kabata 1988) (Table I). These records stem from parasite community

studies or from surveys of host species and hence they document important distribution and host patterns within the region. However, apart from Hogans (1993) and Hanek and Threlfall (1970a), the remainder of reports citing *Ergasilus* spp. from Atlantic Canadian provinces do not specifically focus on the taxonomy of these parasites.

Relevant to the present thesis, only *Ergasilus auritus* and *Ergasilus manicatus* have been reported from *Gasterosteus aculeatus* Linnaeus, 1758 (threespine sticklebacks) and *Gasterosteus wheatlandi* Putnam, 1867 (black spotted sticklebacks) while none has been reported from *Apeltes quadracus* Linnaeus, 1758 (fourspine sticklebacks) (Arai 1969, Hanek & Threlfall 1970a, 1970b, Lester 1974, Roberts 1970, Margolis & Kabata 1988). *Ergasilus auritus* has most often been reported to infect *G. aculeatus* (Hanek & Molnar 1974, Hanek & Threlfall 1970a, 1970b, Arai 1969), and can be distinguished by an antennary region of the cephalothorax that projects anteriorly, along with a third pedigerous somite that overlaps the fourth somite, making it difficult to observe. Also distinct in *E. auritus* is a near spherical inflation between first and second segments of the antennae, a claw equipped with a knob-like growth as well as a protuberance on the second segment. The total length of *E. auritus* adult females is 0.7 mm (Margolis & Kabata 1988). *Ergasilus manicatus* (syn. *E. funduli* Wilson, 1911) has been reported from *G. aculeatus* and *G. wheatlandi* (Roberts 1970). Its distinguishing characters include an ovoid cephalothorax that is truncated posteriorly. The antennae have a cone-shaped growth found on the inner margin, and the total length of adult females is 1.0 mm (Margolis & Kabata 1988).

Thersitina Norman, 1905, is a monotypic genus within the Ergasilidae that is represented in Atlantic Canada. *Thersitina gasterostei* Pagenstecher, 1861, is a parasite

of *G. aculeatus* (Hanek & Threlfall 1969, Zander *et al.* 1999 and 2002). Zander *et al.* (2000) identified *T. gasterostei* as a specialist in Northwest Mecklenberg, Baltic Sea, with high degrees of prevalence on their main host (*G. aculeatus*) and lower degrees in one or two other hosts in the region (*Pungitius pungitius* Linnaeus, 1758 ninespine sticklebacks and *Spinachia spinachia* Linnaeus, 1758 fifteen spine sticklebacks). The antennules of *T. gasterostei* are five-segmented rather than six-segmented as in *Ergasilus* spp.. *Thersitina gasterostei* has antennae each with an accessory claw, and a second pair of legs incorporated into the cephalothorax, further inflating this structure (Kabata 1979). *Thersitina gasterostei* attaches to the inner surfaces of the host's opercula (Walkey *et al.* 1970).

Taxonomy of the Ergasilidae has almost exclusively employed the use of light and scanning electron microscopy. Molecular techniques have recently been used to aid the identification and classification of free-living copepod species (Kiesling *et al.* 2002) but have yet to be applied to ergasilids.

Research question

Apeltes quadracus from coastal south central Nova Scotia harbour infections of adult females of an undescribed ergasilid. Initially I assumed these copepods to be *T. gasterostei.* This assumption was based on both the greatly inflated cephalothorax of specimens, as well as their attachment to the inner surfaces of opercula. However, upon closer examination, it was established that the copepod collected shares common morphological and ecological characteristics with representatives of *Ergasilus* and *Thersitina.* The species studied differs from *T. gasterostei* in that it possesses a proximal bump in place of an accessory claw on the inner margin of the fourth joint of the

antennae, as well as its possessing a cephalothorax with only the first pair of swimming legs incorporated rather than legs 1 and 2 as in *T. gasterostei*. The incorporation of only one pair of swimming legs in the cephalothorax is found in members of *Ergasilus*. Finally, the parasite most often infects the inner surface of the operculum, an area widely reported to be infected by *T. gasterostei* and rare for *Ergasilus* spp..

This study sets out to describe the morphology of the previously undescribed ergasilid collected, using traditional morphological techniques, as well as scanning electron microscopy. The latter serves to complement the former in elucidation of fine details not readily discernible with light microscopy. A detailed taxonomic description will identify the most appropriate genus in which to group this copepod and the parasite can be named*. The thesis will then describe the most frequent site of attachment on the host and the histology associated with infection at the site of attachment. It will also document the geographical distribution of the parasite on gasterosteiforms in the region and determine whether or not the parasite's range overlaps with that of *Thersitina gasterostei*, a parasite that seems to have a preference for the same microhabitat on the host stickleback. Finally, molecular analyses will be undertaken to describe further the taxonomic placement of this *Ergasilus* sp.

* While Article 9 (What does not constitute published work), Chapter 3 (Criteria of Publication) of the International Code of Zoological Nomenclature Fourth Edition (Effective 1 January 2000) does not specifically omit theses, as Chapter 3, Article 9.11 of the third edition (1985) does, "Article 9. What does not constitute publication....9.11. deposit of a document (e.g., a thesis) in a collection of documents, a library, or other archive.", it is this author's belief that a thesis does not meet the provisions set out in Chapter 3; Article 8 (What constitutes published work) of the most recent edition which states, "Article 8.1 Criteria to be met...8.1.3 it must have been produced in an edition containing simultaneously obtainable copies by a method that assures numerous identical and durable copies." As such, the undescribed species dealt with in the present work shall be named in a subsequent publication meeting the above criterion and will simply be referred to as *Ergasilus* sp. for the purposes of this thesis.

Materials and Methods

Sampling Localities and Collection Methods

Gasterosteus aculeatus (Fig. 1A), *Apeltes quadracus* (Fig. 1B), *Gasterosteus wheatlandi* (Fig. 1C), *Pungitius pungitius* (Fig. 1D), *Syngnathus fuscus* Storer, 1839 (northern pipefish), *Fundulus heteroclitus* Linnaeus, 1766 (mummichog), *Osmerus mordax* Mitchill, 1814 (rainbow smelt) and *Anguilla rostrata* Lesueur, 1817 (American eel) were collected at 25 coastal localities in eastern New Brunswick, north eastern and southern Nova Scotia, and eastern Newfoundland (Fig. 2). Geographic coordinates and salinity levels were recorded for each site using a Garmin eTrex ® global positioning unit and a Sper Scientific Ltd. salt refractometer, respectively (Table II). A beach seine (approximately 1 x 5 m, with a 1 cm^2 mesh) drawn through 1 m deep water was used to collect fishes from most estuarine localities, whereas a baited minnow trap was used to collect fishes at Little River, New Brunswick, and Quidi Vidi Lake and Gallow's Cove Pond, Newfoundland. Collections were made from early spring to late summer of 2002-2003 during which gasterosteiforms are typically found close to shore. Two additional collections from Todd's Island, Nova Scotia were collected from the spring of 1992 and fall of 1996. All sticklebacks were anesthetized using MS-222 (1:2000 solution) and preserved in either a 10% formalin-seawater solution or 95% ethanol. In collections containing fish species other than gasterosteiforms a representative sample of these were anesthetized and preserved in a 10% formalin solution. Samples of copepod parasites from *G. aculeatus* from Sable Island, Nova Scotia were collected in 1990, preserved in 95% ethanol, and supplied for study by Dr. David Marcogliese. (Environment Canada, St. Lawrence Centre, Montreal)

Dissection and Parasite Preparation

Total length and sex were recorded for each fish. Left and right opercula and gill arches were excised and examined microscopically, and the buccal cavity adjacent to the gill arches was examined for parasites. Copepods were carefully removed with fine forceps so as not to damage the parasite's antennae, which were often deeply embedded in host tissue. Specimens collected from individual hosts were stored separately in either 95% ethanol for molecular analysis or 10% formalin for morphological study.

Copepod Taxonomy

Approximately 25 formalin-fixed copepod specimens were cleared and stained in a 10% solution of lignin pink dye and lactic acid (Reid 2000). These were subsequently dissected with the aid of mounted acupuncture needles and insect pins. Ventral, dorsal, and lateral body views as well as dissected mouthparts, antennae, and swimming legs were illustrated with the aid of a camera lucida mounted on a Leitz compound microscope. Nomarsky differential interference contrast microscopy was used to study the fine details of the structures and when acquiring digital images (Zeiss Axioplan 2 Imaging). The shape and size of the cephalothorax, configuration of setae on swimming legs and antennules, body length (determined using an ocular graticule) as well as mouthpart and antennae claw structure were compared to information contained in Fryer (1982), Kabata (1979, 1992), Margolis and Kabata (1988), and Huys and Boxshall (1991). Swimming leg armature was compiled and presented using the convention as laid in Huys and Boxshall (1991).

Scanning Electron Microscopy

Seventeen specimens, 15 of the undescribed ergasilid collected from *A. quadracus* at Todd's Island, Nova Scotia, and 2 of *T. gasterostei* collected from *G. aculeatus* at Sable Island, Nova Scotia, were examined using a scanning electron microscope. Thirteen of the *Ergasilus* sp. specimens were dehydrated in graded ethanol solutions (70%, 95% 30 min each; 3 x 10 min 100%) and then transferred in 100% ethanol to a critical point dryer. The central chamber was flooded with liquid CO_2 under high pressure and lowered temperature (10-15 °C). The CO_2 was then vented slowly. Flooding and venting was repeated several times to complete substitution with CO_2. The temperature was raised to 42 °C over a period of about 10 minutes and to a pressure of 1200 psi for critical point drying. The chamber was slowly vented and the specimens retrieved with fine forceps for mounting (Laforsch and Tollrian 2000). The remaining four specimens, two *Ergasilus* sp. and two *T. gasterostei*, were dehydrated in a graded ethanol series (70%, 80%, 90%, 2 x 98% and 2 x 100% for 10 min each). Samples were immersed in 1.5 ml HMDS (1,1,1,3,3,3 hexamethyldisilazane) in 20 ml glass vials in a fume hood. After 30 minutes about 90% of the HMDS was removed and the vials transferred to a desiccator whose base was covered with silica gel beads. The dessicator was evacuated and sealed to prevent water contamination. The remaining HMDS was allowed to evaporate overnight under anhydrous conditions (Laforsch and Tollrian 2000).

Following dehydration, samples were mounted on aluminum stubs with double sided sticky tabs under a dissecting scope using a fine-tipped paintbrush. Specimens were then sputter coated with gold for 2 minutes. The samples were examined with a Hitachi scanning electron microscope at 5 kV to 20 kV.

Histological Sectioning at Attachment Sites

Six fish infected with *Ergasilus* sp. (infections determined by lifting the operculum slightly and visually identifying the presence of parasites) were cut in cross section immediately in front of the eye and immediately behind the operculum. Tissue blocks were individually decalcified overnight in 10% formic acid, dehydrated in ethanol, cleared in xylene, and embedded in Paraplast Plus (Ted Pella Inc.). Sections were prepared at a thickness of 7 μm and were stained with hematoxylin and eosin. Slides were examined using a compound light microscope and details of attachment to the host noted. Uninfected tissue on the opposite side of the body of infected fish served as controls.

Site Selection and Orientation on the Host

The number, specific site of attachment (operculum, specific gill arch, wall of buccal chamber) and body orientation of 4,500 parasites was noted and sketched during necropsy of 2,000 fish. In the case of multiple species infections (of *Ergasilus* sp. and *T. gasterostei*) of a single host, the relative position and orientation of the parasites to one another was also noted. Digital images of parasite attachment were made using a Zeiss Stemi SV-11 Apo dissection microscope.

Statistical Analysis – Ecological Component

Prevalence (percentage of fish infected in a sample) was determined for both *Ergasilus* sp. and *T. gasterostei* for each host species at each locality. Prevalence of infection was also determined for each of four different attachment sites (operculum, gills, wall of buccal chamber, pectoral fin). The distribution among these attachment sites was compared with a χ^2 test ($P = 0.05$) among four different sampling localities for

each parasite species. In the case of *Ergasilus* sp., differences in the prevalence of infection among the different gill arches were also tested. The prevalence of mixed species infections was calculated for each host species and all localities sampled were combined for statistical comparison using a χ^2 test ($P = 0.05$). Mean intensity was also calculated for these mixed species infections and compared for significance ($P = 0.05$) using a non-parametric Kruskall-Wallis test. A frequency distribution was plotted of intensity of infection among *A. quadracus* at all sampling localities and this plot was used to determine low, medium, and high intensity ranges (Fig. 3). Fifteen *A. quadracus* were then randomly chosen (using MSOffice Excel's random number generator) from each intensity level and the prevalence of infection among different attachment sites calculated for each group. Prevalence at each attachment site was compared statistically using a χ^2 test ($P = 0.05$). Finally, approximately 60 *A. quadracus* from three localities, all collected within two days of one another and harbouring low intensity infections of only gravid female *Ergasilus* sp., were isolated from either the gills or inner surface of the operculum of hosts. The number of eggs, length of egg sacs, as well as egg size (to nearest 0.1 mm), were determined for each parasite and the means compared between the two attachment sites using a non-parametric Mann Whitney test for significance ($P = 0.05$).

Molecular Taxonomy

Samples of each parasite species were obtained through dissection of fish hosts preserved in 95% ethanol at time of collection. Specimens of *Ergasilus* sp. were isolated from *A. quadracus* and *T. gasterostei* from *G. aculeatus* from Bouctouche, NB. Specimens of *E. manicatus* were from a collection of mummichogs obtained by donation

from the field station of the Virginia Institute of Marine Sciences. After necropsy, individual parasites were stored in 95% ethanol until nucleic acid extraction.

For *Ergasilus* sp., *T. gasterostei* and *E. manicatus* a single copepod was removed from ethanol and placed into a 500 µl microcentrifuge tube containing 10 µl of ice-cold PCR buffer (10X, 500 mM KCl, 100 mM Tris-HCl, pH 8.3, 15 mM $MgCl_2$ and 0.01% gelatine; Sigma). The tube was centrifuged briefly to bring both the parasite and solution to the bottom of the tube, 1 µl of a 1 mg/ml proteinase K solution was added and mixed by pipetting. This solution was transferred to a water bath and incubated at 55 °C for 3 h (mixing solution every h). Following incubation, tubes were placed in a 100 °C heating block for 5 minutes to inactivate completely the proteinase-K. Subsequently, 10 µl of "GeneReleaser" (BioVentures, Inc.) was added and cycling protocol run as per the manufacture's instructions. Afterward, tubes were centrifuged for 1 min at 13,000 g and the clear supernatant containing nucleic acids, was transferred to a new 500 µl tube (Shizas *et al.* 1997).

Primers were designed using Primer 3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) default parameters from an alignment of sequence data for the 28S region rRNA from *Oithona nana* (AF385457), *Oithona simplex* (AF385458), *Cyclopidae* sp. (AY210813), *Pseudocalanus mimus* (AF385472), *Metridia lucens* (AF385468), *Metridia pacifica (*AF385469), and *Euterpina acutifrons* (AF385454). Oligonucleotide primers were prepared by IDT, Inc. and upon receipt were resuspended in ddH_2O at a concentration of 100 µM, then diluted to a working concentration of 10 µM.

The 5' end region of the large subunit (28S) rRNA was targeted for analysis. For a 50 µl reaction, 4 µl of supernatant (isolated nucleic acid from above) was used in mixture with 5 µl of 10X PCR buffer, 5 µl dNTPs (10 mM; Sigma), 5 µl each forward and reverse primers and 1 unit *Taq* DNA polymerase (5 units/µl in 20 mM Tris-HCl, pH 8.0, 100 mM KCl 0.1 mM EDTA, 1 mM DTT, 0.5% Tween 20, 0.5% Igepal CA-630, 50% glycerol; Sigma).

Thermal cycling consisted of 5 min at 94 °C, followed by 35 cycles of 45 sec at 94 °C, 45 sec at 55 °C and 180 sec at 72 °C, and a final extension time of 10 min at 72 °C. Using electrophoresis, target PCR products were run on 1% agarose gels in 1X TAE buffer. Single bands of product were excised from gels and placed into a 1.5 mL microfuge tube.

DNA was extracted from gel using a UltraClean 15 DNA gel purification kit (Mo Bio Laoratories, Inc.). Once purified, 4 µl of the PCR product was used for cloning and transformation reactions using a TOPO TA Cloning Kit (Invitrogen). Cloning transformants were streaked onto LB plates containing ampicillin + x-gal and grown overnight at 37 °C. Five colonies from each plate containing inserts were transferred into 3 ml LB broth + ampicillin and grown at 37 °C at 300 rpm overnight. A total volume of 2.5 ml of overnight cultures was transferred to a 1.5 ml microcentrifuge tube and centrifuged twice for five minutes at 13,000 g, discarding the supernatant after each spin. Plasmid DNA was isolated using a QIA prep Spin Miniprep Kit (Qiagen) and stored at -20 °C. Four µl of plasmid DNA yielded from isolation was then digested using EcoRI restriction enzyme to confirm the presence of inserts in cloned products.

A portion of these overnight cultures was also used to make glycerol stocks. Each stock colony was prepared by mixing 500 µl of culture with 500 µl of a 50% glycerol and water solution. The stock cultures were then stored at –80 °C

Plasmid DNA or whole clones were sent for sequencing at either the Sequencing Lab of the National Research Council Institute for Marine Biosciences or DNA Landmarks, Inc.

Nucleotide sequences were analyzed using Sequencher under default parameters while alignments were carried out using Genomatix DiAlign software (http://www.genomatix.de/cgi-bin/dialign/dialign.pl 2004). Resulting sequence data for all copepod species will be submitted to Genbank.

Results

Taxonomy

***Ergasilus* sp.**
(Figs. 4-8)

Description of adult female. Body comprises a cephalothorax, four pedigerous somites, genital complex, and 3-segmented abdomen (Fig. 4A). Free pedigerous somites narrower than cephalothorax, gradually narrowing posteriorly; fourth somite shorter in width and length than preceding two (Fig. 4B). Cephalothorax globose, nearly ovoid, with a slight lateral constriction associated with the inclusion of first pair of swimming legs, and rostral bump in centre of anterior dorsal surface (Figs. 4B and 7A). Cephalothorax compressed dorsoventrally, with small oral projection in centre of ventral surface of cephalothorax posterior to antennary region (Figs. 5A and 8A). Genital complex orbicular, with dorsolateral oviduct orifices and several transverse, complete or incomplete rows of spinules on ventral surface (Fig. 5B). Abdomen 3-segmented, similar in length to genital complex, slightly tapered distally; second somite shortest; third somite deeply incised in centre of posterior margin, its border with caudal rami denticulated (Figs. 5B and 8B). First and second abdominal somites with rows of spinules along posterior margin, with several small groupings of spinules along ventral surface of first somite. Caudal rami similar in length and width, approximately same length as anal somite, with small spinules along posterior margin, apical armature of four setae (Figs 5B and 8C). Medial distal region of caudal rami apex with thick seta more than twice length of abdomen, lateral margin with two thin setae longer than caudal ramus and, ventral to these, one thinner and shorter seta, similar in length to caudal ramus (Fig 5B). Mean body length from anterior margin of body to posterior margin of caudal

rami (not including setae) 728 μm (± 99.7 μm N=25). Mean width of cephalothorax at level of mouthparts 420.8 μm (± 56.8 μm N= 25).

Antennule 6-segmented, segments tapering distally, about equal length, with apical segment shortest (Figs. 5C and 7C). First segment bearing five setae, second with eleven, third with five, fourth with three, fifth with two and sixth with six; terminal segment bearing two setae of equal length at apex, two short setae at anterior side of apex, a short seta at posterior side of apex, and single longer seta near mid-dorsal margin (Fig. 5C). Antennae four segmented with short, robust coxobasis lacking armature, bearing large inflated process extending laterally around first endopodal segment. First endopodal segment long, width thickened at articulation between first and second segments and somewhat inflated and rounded; second segment tapered at base, thick toward fourth joint claw; claw curved with blunt protuberance on inner margin (Figs. 5D and 7B). Mandible short anterior blade with teeth; long distal and posterior blades with stout teeth along posterior margin. Maxillule with small medial process. Maxilla indisinctly segmented, with robust syncoxa and tapering, falciform distal process covered with spinules on dorsal surface (Fig. 5E).

Four pairs of biramous swimming legs with segmented sympods (Figs. 6A-E and 8C). Sympods with spinules on posterior margins and ventral surfaces. Rami 3-segmented, except for 2-segmented fourth exopod (Fig. 6E). Lateral margins of both rami partially or completely covered by rows of spinules (Fig 8C). Fifth leg vestigial. Armature of rami:

	Coxa	Basis	Exopod			Endopod		
			1	2	3	1	2	3
Leg 1	0	1-0	I-0	0-1	2,1,4	0-1	0-1	1,2,1
Leg 2	0	0	I-0	0-1	1,3,3	0-1	0-1	0,2,3
Leg 3	0	0	0	0-1	2,2,2	0-1	0-1	0,0,4
Leg 4	0	0	0	0,4,1	-	0-1	I-2	0,3,1

Geographical distribution and host specificity of Ergasilus *sp.*

Ergasilus sp. was widespread geographically occurring at 18 of 24 localities from Little River in northern New Brunswick to Medway on the south shore of Nova Scotia (Table III). The parasite was absent in samples of *Gasterosteus aculeatus* from Newfoundland and Sable Island, Nova Scotia. It occurred on all four species of sticklebacks examined (Table III), but was absent from *Fundulus heteroclitus, Osmerus mordax, Syngnathus fuscus,* and *Anguilla rostrata.* Infections of two or more host species were found at 16 of 23 collection localities (Table III). Numbers of *Apeltes quadracus, G. aculeatus, Gasterosteus wheatlandi,* and *Pungitius pungitius* collected at these localities were usually so unequal, that it was not possible to compare prevalence statistically among host species at all localities (Table III). However, with the relatively large and equal sample sizes obtained at Todd's Island (2 and 3), prevalence was significantly higher on *A. quadracus* than on *G. aculeatus* ($\chi^2 = 13.7, 8.76$, respectively, $P < 0.05$). When combining total collections from all samples, prevalence differed significantly among host species ($\chi^2 = 96.9$, $P < 0.05$) with prevalence on *G. aculeatus* less than on *A. quadracus, G. wheatlandi,* and *P. pungitius* (Table III).

Attachment of Ergasilus *sp. to the host*

Ergasilus sp. attached to the inner surface along the ventral edge of the operculum, as well as the gill lamellae, the wall of the buccal chamber adjacent to the gills, and on a rare occasion, to the base of a pectoral fin. Body orientation on the

operculum and buccal chamber wall did not produce any noticeable patterns, while those copepods found on the gills attached to the base of lamellae. Incidence of attachment at the three main attachment sites (inner surfaces of opercula, gills and buccal chamber wall) differed significantly (Table IV: $\chi^2 = 299.4, 258, 111.9, 487, P < 0.05$), with gills and opercula being used most often by the parasite at Cap Pélé, NB; Cheticamp, NS; Todd's Island, NS #2 and Tracadie, NS. Prevalence of attachment was highest on gills in all but the Todd's Island #2 locality, at which the parasite was attached more frequently to opercula (Fig 9). When comparing the prevalences of two attachment sites (operculum vs. gills, operculum vs. buccal chamber, gills vs. buccal chamber) each analysis yielded a significant difference at all three localities (Cap Pélé NB: $\chi^2 = 152.1, 18.93, 249.8, P < 0.05$; Cheticamp, NS: $\chi^2 = 387.4, 389.9, 460.7, P < 0.05$; Todd's Island, NS #2: $\chi^2 = 105.4, 41.0, 18.4, P < 0.05$; Tracadie, NS: $\chi^2 = 300.8, 4.7, 355.1, P < 0.05$, Table IV). The distribution of *Ergasilus* sp. among gill arches differed significantly (Table V: $\chi^2 = 53.7, 34.4, 32, P < 0.05$), with a consistently low occurrence on the fourth and smallest gill arch. Comparisons among individual gill arches, regarding number of copepods, showed no significant differences in prevalence among arches 1, 2, and 3 at Cheticamp and Tracadie, NS ($\chi^2 = 2.8, 0.71, P > 0.05$) while at Cap Pélé, NB prevalence between arches 1 and 3 ($\chi^2 = 2.1, P > 0.05$) as well as prevalence between arches 2 and 4 ($\chi^2 = 2.4, P > 0.05$) did not differ significantly (Table V).

Prevalence of infection at different attachment sites (operculum, gills, buccal chamber, and fin) differed significantly within different intensity levels for *Ergasilus* sp. infecting *A. quadracus* (Table VI: $\chi^2 = 12.6, 129.7, 812.3, P < 0.05$). Prevalence of attachment to the operculum was notably higher in single infections although with

medium and high intensity infections (defined by plotting a frequency distribution of infections with *Ergasilus* sp. on *A. quadracus*) the parasites attached most often to gills. Prevalences differed significantly between intensity levels when dealing with attachment to the operculum and gills ($\chi^2 = 27.1, 20.4, P < 0.05$) but stayed consistent as intensity increased on the buccal cavity and body ($\chi^2 = 0.162, 0.63, P > 0.05$) (Table VI).

Egg size, number of eggs and egg-sac length differed significantly for *Ergasilus* sp. attached to the operculum versus those attached to the gills of *A. quadracus* (Table VII: $P < 0.05$) in all but one case, namely egg size at Tracadie, NS. Differences seen in egg size, though significant, varied by approximately 5 μm, while egg-sac length differed by approximately 150 μm. Attachment to the operculum at all three localities, Cheticamp, NS, Cap Pélé, NB, and Tracadie, NS, yielded double or nearly three times the numbers of eggs as those found on the gills (Table VII).

Tissue sections revealed infection associated with hyperplasia, within the epidermis and underlying loose connective tissue of the dermis (Fig. 10A). Epithelial debris was noted at the site of attachment (Fig. 10B) and was thought to be a result of parasite feeding. The hyperplasia of these tissues was particularly evident when comparing an infected operculum to the uninfected one on the host fish (Fig. 11). In some cases, penetration of the host's epidermal and dermal layers by the copepod's antennae claws was evident (Fig. 12A). The host's gill filament was similarly penetrated by the antennae (Fig. 12B), causing displasia.

Taxonomy

Thersitina gasterostei

(Fig. 13 A,B)

Body comprises a cephalothorax, four pedigerous somites, genital complex, and 3-segmented abdomen (Fig. 13A). Cephalothorax inflated, rounded and projects laterally. First and second pedigerous somites incorporated into cephalothorax, with remaining pedigerous somites extending at a right angle from cephalothoracic region when viewed laterally. Fifth pedigerous somite small. Genital complex orbicular, with dorsolateral oviduct orifices. Abdomen 3-segmented, about length of genital complex, slightly tapered. Body length approximately 500 μm.

Antennary region projecting forward on the lateral plane of cephalothorax. Antennule 5-segmented; segments not clearly separated posteriorly, tapered distally. Setal formula; 15 setae on first segment, 5 on the second, 4 on the third, 2 on the fourth and 7 on the fifth. Antennae 4-segmented, with coxobasis and 3-segmented endopod, about half length of cephalothorax. First segment long and thick, second and third segments short, claw curved with slender accessory claw at the base of inner margin (Fig. 13B).

Geographical distribution and host specificity of T. gasterostei

The geographical distribution of *T. gasterostei* was similar to that reported above for *Ergasilus* sp., occurring at 18 of 24 localities sampled (Table VIII). Likewise, the parasite was absent in samples of *G. aculeatus* from Newfoundland but occurred on all four species of sticklebacks sampled on the mainland (Table VIII). *Thersitina gasterostei* was not encountered on *F. heteroclitus, O. mordax, S. fuscus,* and *A. rostrata.* Infections of two or more host species at any one location were found at 6 of 23 collection localities

(Table VIII). The numbers of *A. quadracus, G. aculeatus, G. wheatlandi,* and *P. pungitius* collected at these localities were usually so unequal, that it was not possible to compare prevalence statistically between host species at all localities (Table VIII). However, with the relatively large sample size obtained at Medway, NS, prevalence was significantly higher on *G. aculeatus* then on *A. quadracus* ($\chi^2 = 13.0$ $P < 0.05$, Table VIII). It was also noted that despite high numbers of *A. quadracus* collected at Cap Pélé NB, prevalence of infection on the low numbers of *G. aculeatus* and *G. wheatlandi* collected was significantly higher ($\chi^2 = 59.9$ $P < 0.05$, Table VIII). When combining the totals of all samples (Table VIII), prevalence differed significantly among host species ($\chi^2 = 113.8$, $P < 0.05$) with infection rates similar for the parasites on *G. aculeatus, G. wheatlandi,* and *P. pungitius* but lower prevalence on *A. quadracus.* Overall, prevalence of infection of *T. gasterostei* (1.4 to 16.1%, Table VII) was much lower than for *Ergasilus* sp. (29.5% to 57.5%, Table III).

Attachment of T. gasterostei *to the host*

Thersitina gasterostei attached to the ventral inner surface of the operculum , gill lamellae, wall of the buccal chamber and the body at the base of the pectoral fin (Figs. 14A, 14B, 14C). However, prevalence of attachment to these sites differed significantly (Table IX: $\chi^2 = 509.3, 100.6, 20.2$, $P < 0.05$) on *G. aculeatus,* with most infections occurring on the inner surface of the operculum (Table IX).

When attaching to the host's opercula, specimens of *T. gasterostei* typically aggregated (Fig. 14A) but body orientation was not consistent among individuals or samples. In some cases, attachment to the gills produced epithelial nodules (Fig 14B).

Co-occurrence of Ergasilus *sp. and* T. gasterostei

In mixed species infections, prevalence of infection with either parasite or both differed significantly on all four host species (Table X: $\chi^2 = 1628, 113.2, 12.83, 11.7, P < 0.05$). *Gasterosteus aculeatus* and *G. wheatlandi* harboured mixed infections most often with both parasites rarely occurring together on *A. quadracus.* Mean intensity also differed significantly in all host species except *A. quadracus*, with the differences observed on *G. aculeatus, G. wheatlandi* and *P. pungitius* reflecting higher intensity levels for mixed infections when compared to those intensities with either *Ergasilus* sp. or *T. gasterostei* (Table XI: $P = 1.00, 0.00, 0.00, 0.091$, respectively).

Molecular Taxonomy

A 230 base-pair product of 28S rDNA was successfully cloned and sequenced for three individual *Ergasilus* sp., and *T. gasterostei,* and two individual *E. manicatus.* An alignment of all sequences demonstrated nucleotide differences at seven bases between the cloned sequences (Appendix I). Percent similarities differed between pairwise comparisons of species with *Ergasilus* sp. and *T. gasterostei* being most dissimilar (97% similarity) while, *E. manicatus* and *Ergasilus* sp. were most similar (99% similarity) (Table XII).

Discussion

This study revealed that gasterosteiforms inhabiting coastal marine waters of the Atlantic Canadian Provinces host two common and widespread parasitic copepods. One species is the circumpolar *Thersitina gasterostei*, which has been reported previously on *Gasterosteus aculeatus* and *Gasterosteus wheatlandi* in Newfoundland (Hanek & Threlfall 1969), *G. aculeatus, G. wheatlandi,* and *Pungitius pungitius* in neighbouring Quebec (Poulin & Fitzgerald 1987) and on *G. aculeatus* on Sable Island, Nova Scotia (Marcogliese 1992). The second species is a previously undescribed species of *Ergasilus*.

Morphology of *T. gasterostei* collected in the present study was consistent with that reported in the original species description (Pagenstecher 1861) as well as subsequent taxonomic studies (Gurney 1913, Hanek & Threlfall 1969, Kabata 1979, Ohtsuka *et al.* 2004). The parasite is apparent during necropsy due to its enlarged cephalothorax which most notably extends along the lateral plane. In members of the Ergasilidae, this enlarged cephalothorax is a character found only in the genera *Teredophilus* Rancurel, 1954 and *Thersitina* Norman, 1905 (Amado *et al.* 1995). The presence of a 5-segmented antennule is exhibited by members of *Thersitina* as well as species of 11 other genera belonging to Ergasilidae (*Acusicola* Cressey, 1970, *Amplexibranchius* Thatcher & Paredes, 1985, *Diergasilus* Do, 1981, *Gamispatulus* Thatcher & Boeger, 1984, *Gamispinus* Thatcher & Boeger, 1984, *Mugilicola* Tripathi, 1960, *Paeonodes* Wilson, 1944, *Paraergasilus* Markevich, 1937, *Prehendorastrus* Boeger & Thatcher, 1990, *Teredophilus,* and *Therodamas* Krøyer, 1863). Of these genera, only representatives of *Diergasilus, Gamispatulus* and *Gamispinus* have a

terminal armature of two claws on the antennae, a diagnostic feature of species of *Thersitina*. However, none of the representatives of these three groups, *Diergasilus, Gamispatulus,* and *Gamispinus,* has an inflated cephalothorax. Despite this difference, Ohtsuka *et al.* (2004) determined the monotypic genus *Diergasilus* a junior synonym of *Thersitina* based on the identical armature of the swimming legs. Percival (1937), proposed a new species of *Thersitina, T. inopinata,* however the structure of leg 4 is not consistent with *Thersitina* having a 1-segmented exopod and 2-segmented endopod as seen in *Vaigamus*. Based on this Ohtsuka *et al.* (2004) considered *T. inopinata* as *incertae sedis.*

Ergasilus is distinguishable from the most closely aligned genera *Dermoergasilus* Ho & Do, 1982 and *Sinergasilus* Yin, 1949 (Amado *et al.* 1995) due to variations of the number of antennal segments in the former and metasomal somites in the latter. The metasomal somites of *Ergasilus* spp. are well defined as seen in the majority of genera represented in the Ergasilidae while *Sinergasilus* spp. have fused metasomes, also found in representatives of *Mugilicola, Paeondes,* and *Pseudergasilus* Yamaguti, 1936. Species of *Dermoergasilus* display a terminal digitiform process on the caudal ramus, antennal segments that are enveloped within a cuticular membrane (as seen in *Acusicola* and *Amplexibranchius* spp.) along with a seta on the second segment of the second leg endopod. This latter is also seen in representatives of *Abergasilus* Hewitt, 1978 whose species are distinguishable in having only three pairs of legs. The *Ergasilus* sp. under study shares a single seta on the middle segment of the second leg endopod, rather than the two occurring on other *Ergasilus* spp.. However, Gussev (1987) questioned the validity of *Dermoergasilus* based on some *Ergasilus* spp. (namely *Ergasilus tumidus*

Markevich, 1941, *Ergasilus briani* Markevich, 1933, *Ergasilus gibbus* Nordmann, 1832, and *Ergasilus gobiorum* Markevich & Sukhnenk, 1967) having only one seta (instead of two) on the medial surface of the second segments of the second and third legs. In response, Ho *et al.* (1992) argued that the absence of the other characters typical of *Dermoergasilus* spp. (digitiform process on caudal rami and cuticular membrane surrounding antennae) within these four species of *Ergasilus* did not support the rejection of *Dermoergasilus.* However, this does lend insight into the proper placement of the currently studied *Ergasilus* sp.. Due to its lacking an antennal membrane and digitiform process, and despite the presence of only one seta on the medial second segment of leg 2, the species under study most closely resembles members of *Ergasilus*.

Ergasilus sp. collected in this study is most similar to: *E. auritus* Markevich, 1940, *Ergasilus cotti* Kellicott, 1879, *Ergasilus cyprinaceus* Rogers, 1969, *Ergasilus luciopercarum* Henderson, 1927, *Ergasilus manicatus* Wilson, 1911, *Ergasilus orientalis* Yamaguti, 1939, *Ergasilus turgidus* Frazer, 1920, *Ergasilus wareaglei* Johnson, 1971, and *Ergasilus wilsoni* Markevich, 1933, all of which form a distinct group within the genus *Ergasilus* typically characterized by a swollen cephalothorax, an antennal area projecting from the cephalothorax, and an inflated process of the antenna that tends to overwhelm the other segments of this structure. While *Ergasilus* sp. and *E. auritus* both have an inflated antennal process, and globose cephalothorax, Roberts (1970) noted that, depending on the expansion of the uterine process, the typically rectangular trunk of *E. auritus* may be reduced and can therefore be intraspecifically variable. *Ergasilus auritus* has two knob-like processes on the medial margins of the third and fourth antennal segments, characteristics also seen in *E. turgidus* and *E. luciopercarum. Ergasilus* sp.

has a single tooth present only on the inner surface of the fourth antennal claw segment. The length of the third segment of the antennae of *E. auritus* differs from that of *Ergasilus* sp. in that is shorter, equal in length to the claw (Roberts 1970) and similar to those third antennal segments seen in *E. cotti*, and *E. turgidus*. *Ergasilus* sp. has a slender and elongate third antennal segment measuring double the length of the fourth segment. Although the antennary area of *E. cyprinaceus* projects from the cephalothorax with a distinct lateral suture on the dorsal surface (Roberts 1970), similar to that of *Ergasilus* sp., the antennae have no knobs or teeth associated with them. The antennae of *E. wareaglei* also contain no processes along the inner margins, and, while its stout cephalothorax is reminiscent of that of *Ergasilus* sp., its leg armature is not consistent with *Ergasilus* sp., with *E. wareaglei* displaying the typical *Ergasilus* armature of two setae on the second endopodal segment of the second leg (Johnson 1971). This feature also occurs in *E. luciopercarum* (Henderson 1927; Davis 1969) as well as *E. orientalis* (Yamaguti 1963) which also has a narrower cephalothorax. The antennae of *E. manicatus* most closely resemble those of *Ergasilus* sp., however, *E. manicatus* is distinguishable from *Ergasilus* sp. in having small dorsolateral processes on its first pedigerous somite (Roberts 1970) as well as by its more elongate body.

Ergasilus sp. is taxonomically distinct from the six *Ergasilus* spp. reported from Atlantic Canada. *Ergasilus caeruleus, E. lizae* and *E. manicatus* all have a slender and more elongate body not consistent with specimens collected in this study. The second antennae of *E. manicatus,* however, resemble closely those of *Ergasilus* sp. having a single process on the inner claw. The antennae of *E. labracis* have two such knob processes on the medial margin of the fourth segment, along with a third process located

distally on the third segment. With the exception of *E. auritus,* reported as being specific to *G. aculeatus*, most of these species have been rarely reported to infect stickleback hosts (Margolis & Kabata 1988).

Collections from estuarine sites in this study reflected a widespread geographic distribution for both *Ergasilus* sp. and *T. gasterostei.* Both species infected four species of stickleback (i.e., *G. aculeatus, A. quadracus, G. wheatlandi,* and *P. pungitius*) but none of the other species sampled (i.e., *Syngnathus fuscus*, *Fundulus heteroclitus, Anguilla rostrata*). Both parasitic species appear to have specificity towards gasterosteiforms.

Multiple host species were represented in many of the field samples, and the parasites occured on the hosts at different prevalences. *Thersitina gasterostei* infected *G. aculeatus* at notably higher prevalences throughout the region, despite the larger number of *A. quadracus* collected at many of these localities. This is consistent with the literature in that, although *T. gasterostei* has been reported to infect a range of gasterosteids (Gurney 1913, Walkey *et al.* 1970, Hanek and Threlfall 1969, Zander *et al.* 2002), it has often been reported to infect *G. aculeatus* more often than other species (Walkey *et al.* 1970, Zander *et al.* 1999 and 2000). Host and parasite have a circumpolar distribution in the Northern Hemisphere (Scott & Scott 1988; Amado *et al.* 1995). It has been noted (Walkey *et al.* 1970) that in estuaries in which *P. pungitius* far outnumber *G. aculeatus*, *T. gasterostei* attaches more frequently to *G. aculeatus*. However, Poulin (1999) noted that *P. pungitius* lives in vegetation and doesn't usually shoal with *G. aculeatus* and *G. wheatlandi,* therefore transmission of *T. gasterostei* and other parasitic copepods may be limited in *P. pungitius*. Poulin (1999) found that in the case of fish (*G.*

aculeatus and *G. wheatlandi*) infected with *T. gasterostei,* those in larger shoals incur more severe infections than those in smaller shoals. When comparing the host species Poulin (1999) also found that the smaller *G. wheatlandi* harboured more *T. gasterostei* than *G. aculeatus* from the same shoals, but that *G. aculeatus* was consistently infected with a greater proportion of egg bearing *T. gasterostei.* Walkey *et al.* (1970) also noted that at lower salinities, *T. gasterostei* didn't infect *G. aculeatus*. However, the large sampling localities in the present study were all coastal saline waters so this observation could not be substantiated.

Ergasilus sp. infected *A. quadracus* and *G. wheatlandi*, both endemic to the Atlantic region of Canada (Scott & Scott 1988), at significantly higher prevalences than *G. aculeatus* or *P. pungitius*. Many authors have suggested that *G. aculeatus* is infected most often due to its larger size and greater susceptibility to invading copepodids (Gurney 1913, Walkey *et al.* 1970). In the present study, *Ergasilus* sp. attached most frequently on the smaller host, *A. quadracus,* thus discounting the notion of differential susceptibility due to host size.

Mixed species infections occurred most often on *G. wheatlandi* and *G. aculeatus* and such infections rarely occurred on *A. quadracus* despite similar sympatric distributions throughout the region (Scott & Scott 1988). Host specificity is displayed by *T. gasterostei* for *G. aculeatus* with mixed infections involving *T. gasterostei* rarely occurring on *A. quadracus*. However, *Ergasilus* sp. infected a more diverse number of hosts that included *A. quadracus, G. aculeatus, G. wheatlandi,* and *P. pungitius*. Ergasilids are often cited to infect a wide range of hosts due to their lack of morphological development specific to any one host environment (Oldewage 1987).

Walkey (1970) cited the over-dispersed distribution of *T. gasterostei* as a factor influencing both parasite burden and distribution over the left and right sides of the host. In the present study, parasite aggregation was noted when examining fish individually. It was a common occurrence to find an abundance of *T. gasterostei* and *Ergasilus* sp. under one operculum (left or right side) only to discover the opposite operculum to be without a single infection. Fryer (1966) documented uneven distributions of crustacean parasites on the left and right sides of some African fresh-water fishes, noting that these parasites tended to attach to particular sites in groups. The reasons underlying this unequal distribution on the host are not known.

Many studies of *Ergasilus* spp. report these parasites from the gills of their hosts however, rarely is the particular site of attachment described in detail. The present study identifies three primary attachment sites, all found within the host's opercular region; the ventral inner surface of the operculum, the gill lamellae, and the wall of the buccal chamber adjacent to the gills. The most frequently noted attachment site of *T. gasterostei* in the present study is the inner surface of the operculum which is consistent with reports in the literature (Pagenstecher 1861, Gurney 1913, Walkey *et al.* 1970) and was noted for all host species studied. It is puzzling then that, Hanek and Threlfall (1969) described *T. gasterostei* from samples collected in Newfoundland and indicated the parasite's location to be only the gills.

Ergasilus sp. was found attached most frequently to gills or the inner surfaces of the opercula of hosts. In infections of *A. quadracus* involving a single *Ergasilus* sp., the parasite attached at most frequently to the operculum, although with medium and high intensities the parasites attached to the gills most often. This means that as intensities of

infection increase to medium and high levels, there is a shift in colonization away from the operculum onto the gills. It is possible that density-dependent restraints are coming into play within the spatially limited microhabitat of the inner surfaces of the opercula. Interestingly, the slender body of most ergasilids is thought to be most conducive to life on the gills (Oldewage 1987) where the parasites attach to the base of individual gill filaments by using their elongated antennae to wrap and clutch the filament. These parasites then graze over the length of the filament feeding on host tissue and blood (Wilson 1911, Oldewage & van As 1987). The stout body of *Ergasilus* sp. is reminiscent of the gross morphology of *T. gasterostei,* suggesting that it has evolved to exploit the same specific niche as *T. gasterostei* (the operculum) within its common host species, *A. quadracus.*

When comparing the operculum to the gills as a successful attachment site for *Ergasilus* sp. fecundity may reflect the parasite's apparent initial, and possibly preferred, colonization of the operculum. Fecundity is of major importance to parasitic species and their proliferation within an environment and can therefore be a good indicator of overall success (Tedla & Fernando 1969, 1970). In this study, ovigerous female parasites under similar conditions (occurring on the same host species, collected from the same sampling season, and from low intensity infections) possessed both longer eggs sacs and greater egg numbers when attaching to the operculum. Compared to the gills, egg size also differed significantly, being marginally larger on those females attached to the operculum. There are many factors influencing reproductive success in parasitic copepods, and the host environment is not the sole influence on the development of eggs (Tedla & Fernando 1969). The host's external environment most likely also plays an

important role in this development, and in all three localities used in this study the same patterns were noted with egg numbers and size being greater when the parasites attached to the opercula of hosts. Tedla and Fernando (1970) observed similar variations in fecundity of *Ergasilus confuses* Bere, 1931 that was attributable to the external environment (i.e., the water system in which both host and parasite lived), with overwintering females producing more and larger eggs than those seen in the broods of summer generations. However, in a previous study, Tedla and Fernando (1969) found that *Ergasilus centrarchidarum* Wright, 1882 produced significantly higher numbers of eggs on rock bass than on smallmouth bass, which supports the idea that the host also contributes to reproductive success in parasitic copepods. Poulin (1999) used the proportion of egg-bearing females found on different host species as an indicator of reproductive success and found that *T. gasterostei* developed more egg bearing females on *G. aculeatus* than on *G. wheatlandi* in estuaries near Isle Verte, Quebec.

Differences seen in fecundity at these two sites of attachment (gills versus opercula) may be due to features of the microhabitat environment. The histological sections show that in the case of attachment to the inner surfaces of the opercula, the deeply embedded antennae cause hyperplasia. This thickened tissue likely provides a renewable food source that allows the parasite to graze with its mouthparts, which rest adjacent to the epithelial proliferation. Gill hyperplasia was also observed in this study and has been reported for several other ergasilids (Oldewage & van As 1987, Roubal 1989, and Paperna & Thurston 1968). However, unlike many slender ergasilids, the more globose body may be at a disadvantage energetically when attaching to the gills, in that it may limit the ability to move along the length of the lamellae and feed. The

parasite's presence also causes the lamellae to become disorganized and hinder its ability to graze, grow, and produce eggs. No other morphological differences were noted during examination of these parasites among the two attachment sites.

Sequence data of the 28S rDNA region differed (7 nucleotides) between the species examined in the present study. *Ergasilus* sp. and *E. manicatus* aligned most closely to one another (99% similar) and *T. gasterostei* was most dissimilar to *Ergasilus* sp. (97%). This initial analysis supports the morphological data in indicating that *Ergasilus* sp. should be classified as *Ergasilus* sp. rather than *Thersitina* sp.. In this instance, the data reflect that two species whose antennae are extremely comparable do indeed resemble one another closely at the molecular level. The sequences obtained in this study are limited in relative base pair length, and as such would be much more informative if more of this region could be sequenced and analyzed. Future analyses would also benefit from the use of more species for comparison. The work presented herein is valuable in that it can be used as a stepping stone for future molecular research of this group particularly with the design of primers.

Literature Cited

Amado, M.A.P.M., J.S. Ho, and C.E.F. Rocha. 1995. Phylogeny and biogeography of the Ergasilidae (Copepoda, Poecilostomatoida), with reconsideration of the taxonomic status of the Vaigamidae. *Contributions to Zoology*, 65: 233-243.

Arai, H. P. 1969. Preliminary report on the parasites of certain marine fishes of British Columbia. *Journal of Fisheries Research Board of Canada*, 26: 2319-2337.

Bere, R. 1930. The Parasitic copepods of the fish of the Passamaquoddy Region. *Contributions to Canadian Biology and Fisheries*, 5: 423-430.

Boxshall, G. A. and M. A. Montu. 1997. Copepods parasitic on Brazilian coastal fishes. A handbook. *Nauplius*, 5: 1-225.

Davis, C.C. 1969. *Ergasilus luciopercarum* males in an unusual collection from Newfoundland. *Canadian Journal of Zoology*, 47: 1249-1252.

Fryer, G. 1966. Habitat selection and gregarious behaviour in parasitic Crustaceans. *Crustaceana*, 10: 199-209.

Fryer, G. 1982. The parasitic Copepoda and Branchiura of British freshwater fishes: a handbook and key. *Scientific Publications of the Freshwater Biological Association, Ambleside,* 46: 1-87.

Gurney, R. 1913. Some notes on the parasitic copepod *Thersitina gasterostei*, Pagenstecher. *The Annals and Magazine of Natural History*, 12 (8): 415-424.

Gurney, R. 1933. British Freshwater Copepoda, 3. The Ray Society, London.

Gussev, A.V. 1987. Phylum Athropoda. In O.N. Bauer (ed.), Key to the Parasites of the Freshwater Fishes of the USSR (Leningrad: Nauka), 3: 378-524.

Hanek, G. and W. Threlfall. 1969. *Thersitina gasterostei* (Pagenstecher, 1861) (Copepoda: Ergasilidae) from *Gasterosteus wheatlandi* Putnam 1867. *Canadian Journal of Zoology*, 47: 627-629.

Hanek, G. and W. Threlfall. 1970a. *Ergasilus auritus* Markewitsch, 1940 (Copepoda: Ergasilidae) from *Gasterosteus aculeatus* Linnaeus, 1758 in Newfoundland. *Canadian Journal of Zoology*, 48: 185-187.

Hanek, G. & W. Threlfall. 1970b. Parasites of the threespine stickeback (*Gasterosteus aculeatus*) in Newfoundland and Labrador. *Journal of the Fisheries Research Board of Canada*, 27: 901-907.

Henderson, J. T. 1927. Description of a copepod gill-parasite of pike-perches in lakes of northern Quebec, including an account of the free-swimming male and of some developmental stages. *Contributions to Canadian Biology and Fisheries*, 3: 235-245.

Hicks, F. J. and W. Threlfall. 1973. Metazoan parasites of salmonids and coregonids from coastal Labrador. *Journal of Fish Biology*, 5: 399-415.

Ho, J.S., P. Jayarajan, and S. Radhakrishnan. 1992. Copepods of the family Ergasilidae (Poecilostomatoida) parasitic on coastal fishes of Kerala, India. *Journal of Natural History, London*, 26 (6) :1227-1241.

Hogans, W.E. 1993. ARC Species ID Leaflet #93-01-INV. Guide to Common External Parasites of Fishes of the Canadian Atlantic: I. Crustacea: Isopoda, Amphipoda, Brachiura & Copepoda.

Hudson, P. L., C. A. Bowen, and R. M. Stedman. 1994. New records of *Ergasilus* (Copepoda: Ergasilidae) in the Laurentian Great Lakes, including a lakewide review of records and host associations. *Canadian Journal of Zoology*, 72: 1002-1009.

Huys, R. and G. A. Boxshall. 1991. Copepod Evolution. The Ray Society, London.

Johnson, S.K. 1971. *Ergasilus wareaglei* sp. n. (Copepoda: Cyclopoida) from North American catostomid fishes. *Journal of the Alabama Academy of Science*, 42: 243-247.

Kabata, Z. 1979. Parasitic Copepoda of British Fishes. The Ray Society, London.

Kabata, Z. 1992. Copepods Parasitic on Fishes. The Linnean Society, London.

Kiesling, T., E. Wilkinson, J. Rabalais, P. Ortner, M. McCabe, and J. Fell. 2002. Rapid identification of adult and naupliar stages of copepods using DNA hybridization methodology. *Marine Biotechnology*, 4: 30-39.

Laforsch, C. and R. Tollrian. 2000. An new preparation technique of daphnids for Scanning Electron Microscopy using hexamethyldisilazane. *Archiv fur Hydrobiologie*, 149: 587-596.

Lester, R.J.G. 1974. Parasites of *Gasterosteus aculeatus* near Vancouver, British Columbia. Syesis, 7: 195-200.

Marcogliese, D.J. 1992. Metazoan parasites of sticklebacks on Sable Island, Northwest Atlantic Ocean: biogeographic considerations. *Journal of Fish Biology*, 41: 399-407.

Margolis, L. and Z. Kabata. 1988. Guide to the parasites of fishes of Canada. Part II Crustacea. Canadian Department of Fisheries and Oceans, Ottawa.

Nordmann, A. Von. 1832. Mikrographische Beitrage zur Naturgeschichte der Wirbellosen Thiere. *G. Reimer, Berlin*, 1: 1-150.

Ohtsuka, S., J. Ho, K. Nagasawa, J. Morozinska-Gogol, and W. Piasecki. 2004. The identity of *Limnoncaea diuncata* Kokubo, 1914 (Copepoda: Poecilostomatoida) from Hokkaido, Japan, with the relegation of *Diergasilus* Do, 1981 to a junior synonym of *Thersitina* Norman, 1905. *Systematic Parasitology*, 57: 35-44.

Oldewage, W.H. 1987. *Ergasilus*: captivating copepod. *Ichthos Newsletter*, 14: 12-13.

Oldewage, W.H. and J.G. van As. 1987. Observations on the attachment of a piscine gill parasitic ergasilid (Crustacea: Copepoda). *South African Journal of Zoology*, 22: 313-317.

Pagenstecher, A.H. 1861. *Thersites gasterostei*, eine neue gattung parasitischer Crustaceen. *Weigmann's Arch. Naturg,* 27: 118-126.

Paperna, I. and J.P. Thurston. 1968. Report on ectoparasitic infections of fresh water fish in Africa. *Bulletin de l'Office International des Epizooties*, 69: 1197-1206.

Percival, E. 1937. New species of Copepoda from New Zealand lakes. *Records of the Cantebury Museum,*4:169-175.

Poulin, R. 1999. Parasitism and shoal size in juvenile sticklebacks: conflicting selection pressures from different ectoparasites? *Ethology*, 105: 959-968.

Poulin, R. and G.J. Fitzgerald. 1987. The potential of parasitism in the structuring of a salt marsh stickleback community. *Canadian Journal of Zoology*, 65: 2793-2798.

Reid, J. 2000. Workshop on Taxonomic Techniques for Copepods. http://www.nmh.si.edu/iz/copepod/tachniques.htm.

Roberts, L. S. 1970. *Ergasilus* (Copepoda: Cyclopoida): revision and key to species in North America. *Transactions of the American Microscopy Society*, 89: 134-161.

Roubal, F.R. 1989. Pathological changes in the gill filaments of *Acanthopagrus australis* (family Sparidae) associated with post-settlement growth of a lernaeopodid copepod, *Alella macrotrachelus*. *Journal of Fish Biology*, 34: 333-342.

Sandeman, I. M. and J. H. C. Pippy. 1967. Parasites of freshwater fishes (Salmonidae and Coregonidae) of insular Newfoundland. *Journal of the Fisheries Research Board of Canada*, 24: 1911-1943.

Scott, W.B. and M.G. Scott. 1988. Atlantic Fishes of Canada. Canadian Bulletin of Fisheries and Aquatic Sciences, 219.

Shizas, N.V., G.T. Street, B.C. Coull, G.T. Chandler, and J.M. Quattro. 1997. An efficient DNA extraction method for small metazoans. *Molecular Marine Biology and Biotechnology*, 6: 381-383.

Tedla, S. and C. H. Fernando. 1969. Observations on the biology of *Ergasilus* spp. (Cyclopoidea: Copepoda) infesting North American freshwater fishes. *Canadian Journal of Zoology*, 47: 405-408.

Tedla, S. and C. H. Fernando. 1970. On the biology of *Ergasilus confuses* Bere, 1931 (Copepoda) infesting yellow perch, *Perca fluviatilis* L., in the bay of Quinte, Lake Ontario, Canada. *Crustaceana*, 19: 1-14.

Threlfall, W. and G. Hanek. 1970. Metazoan parasites, excluding Mongenea, from longnose and white suckers. *Journal of the Fisheries Research Board of Canada*, 27: 1317-1319.

Walkey, M., D.B. Lewis, and H.J.G. Dartnall. 1970. Observations on the host parasite relations of *Thersitina gasterostei* (Crustacea: Copepoda). *Journal of Zoology*, 162: 371-381.

Wiles, M. 1975. Parasites of *Fundulus diaphanous* (LeSueur) (Pisces: Cyprinodontidae) in certain Nova Scotian freshwaters. *Canadian Journal of Zoology*, 53: 1578-1580.

Wilson, C. B. 1911. North American parasitic copepods belonging to the family Ergasilidae. *Proceedings of the United States National Museum*, 39: 263-400.

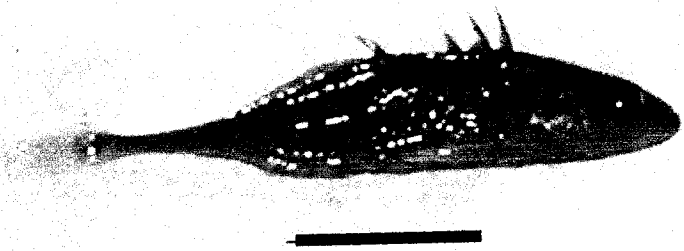
Yamaguti, S. 1963. Parasitic Copepoda and Branchiura of fishes. John Wiley and Sons, Interscience, New York.

Zander, C.D., O. Kocoglu, M. Skroblies, and U. Strohbach. 2002. Parasite populations and communities from the shallow littoral of the Orther Bright (Fehmarn, SW Baltic Sea). *Parasitology Research,* 88: 734-744.

Zander, C.D., L.W. Reimer, and K. Barz. 1999. Parasite communities of the Shalzhaff (Northwest Mecklenburg, Baltic Sea). I. Structure and dynamics of communities of littoral fish, especially small-sized fish. *Parasitology Research,* 85: 356-372.

Zander, C.D., L.W. Reimer, K. Barz, G. Dietel, and U. Stronbach. 2000. Parasite communities of the Shalzhaff (Northwest Mecklenburg, Baltic Sea). II. Guild communities, with special regard to snails, benthic crustaceans, and small-size fish. *Parasitology Research,* 86: 359-372.

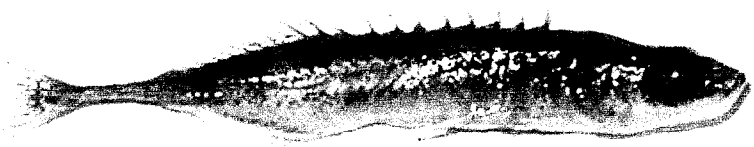
Figure 1: *Apeltes quadracus*, fourspine stickleback (A). Scale bar = 1 cm. *Gasterosteus aculeatus*, threespine stickleback (B). Scale bar = 1 cm. *Gasterosteus wheatlandi*, blackspotted stickleback (C). Scale bar = 1 cm. *Pungitius pungitius*, ninespine stickleback (D). Scale bar = 1 cm.



A

B

C



D

Figure 2: Twenty-four collection localities in New Brunswick, Nova Scotia and Newfoundland. 1-Bouctouche, NB; 2-Cap Pélé, NB; 3-Cocagne, NB; 4-Little River, NB; 5-Nelson, NB; 6-Newcastle, NB; 7-Richibucto, NB; 8-Cheticamp, NS; 9-Clam Harbour, NS; 10-Hwy 105, NS; 11-Hwy 331, NS; 12-Johnstown, NS; 13-Lawrencetown, NS; 14-Mabou, NS; 15-Medway, NS; 16-Merigonish, NS; 17-Porter's Lake, NS; 18-Rainbow Haven, NS; 19-Rear Monroe's Point, NS; 20-Todd's Island, NS; 21-Tracadie, NS; 22-Gallow's Cove, NF; 23-Hopeall River, NF; 24-Quidi Vidi Lake, NF. See Table II for collection locality information.

24
23
Newfoundland
19
8
14
21
PEI
16
9
Nova Scotia
13
18
20
11
15
7
1
3
2
4

Figure 3: Frequency distribution of parasite intensity with low medium and high intensity classes outlined. ■ Low □ Medium ■ High

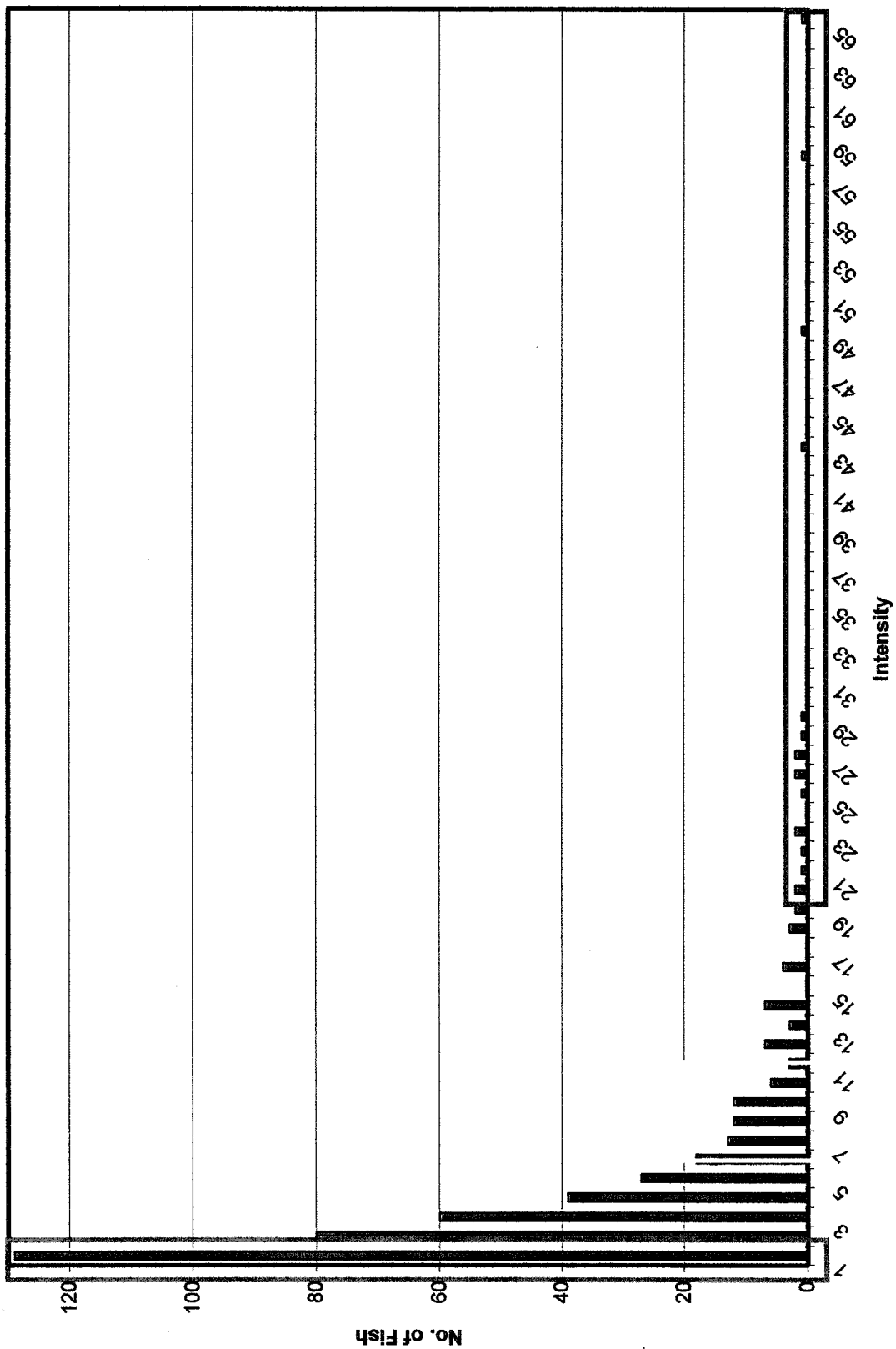

No. of Fish
0
20
40
60
80
100
120
Intensity
1
3
5
7
9
11
13
15
17
19
21
23
25
27
29
31
33
35
37
39
41
43
45
47
49
51
53
55
57
59
61
63
65

Figure 4: *Ergasilus* sp. from *Apeltes quadracus* collected from off Todd's Island, NS. Adult female. Ventral (A) and dorsal (B) view of body based on drawings of stained specimens. Scale bar = 100 μm.

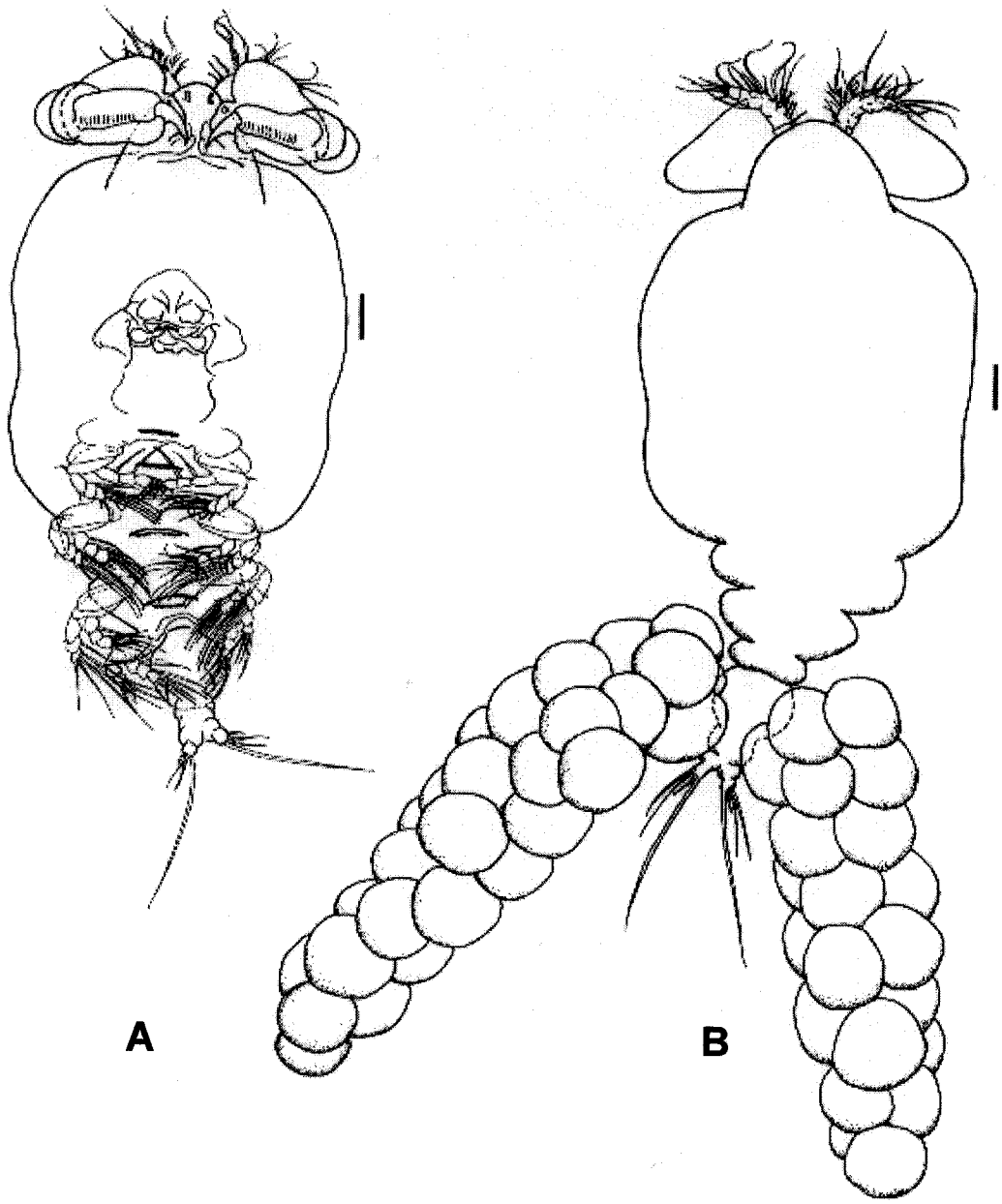

A
B

Figure 5: *Ergasilus* sp. from *Apeltes quadracus* collected from off Todd's Island, NS. Lateral view of body (A). Scale bar = 100 μm. Ventral view of genital complex and abdomen (B). Dorsal view of antennule (C). Scale bar = 10 μm. Antennae claws, ventral (D). Scale bar = 100 μm. Dorsal view of maxilla (E). Scale bar = 10 μm. Based on drawings of stained specimens. Scale bar = 25 μm.

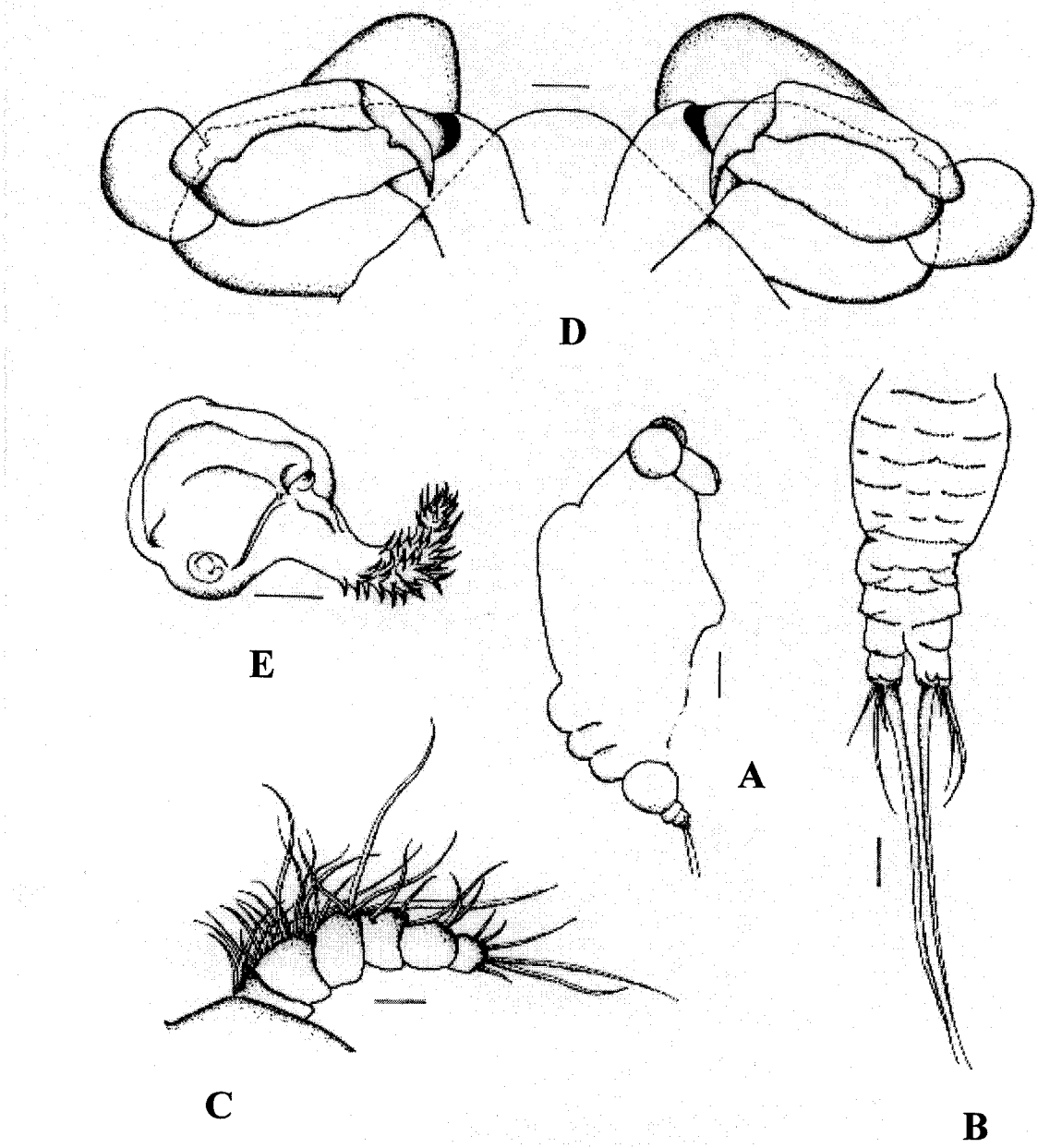

D
E
A
B
C

Figure 6: *Ergasilus* sp. from *Apeltes quadracus* collected from off Todd's Island, NS. First leg (A). Scale bar = 25 μm. Second leg (B). Scale bar = 25 μm. Third leg (C). Scale bar = 25 μm. Fourth leg endopod (D). Scale bar = 10 μm. Fourth leg exopod (E). Scale bar = 10 μm.

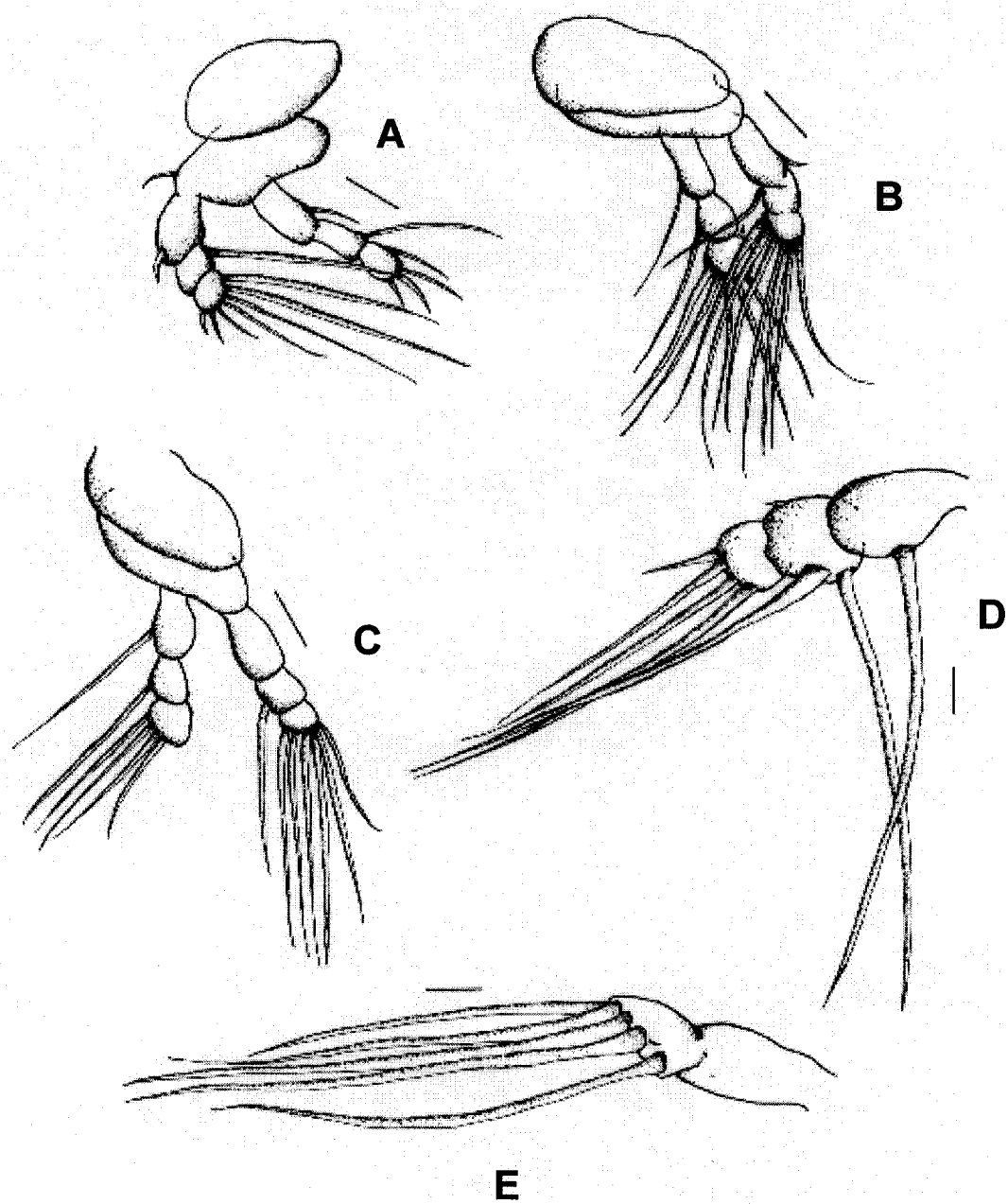

A
B
C
D
E

Figure 7: Photomicrographs of *Ergasilus* sp. from *Apeltes quadracus* collected from off Todd's Island, NS. Body in ventral view (A). Scale bar = 200 μm. Antenna in lateral view (B). Note the small bump on inner margin of claw (arrow). Scale bar = 20 μm. Antennule in ventral view (C). Scale bar = 50 μm.

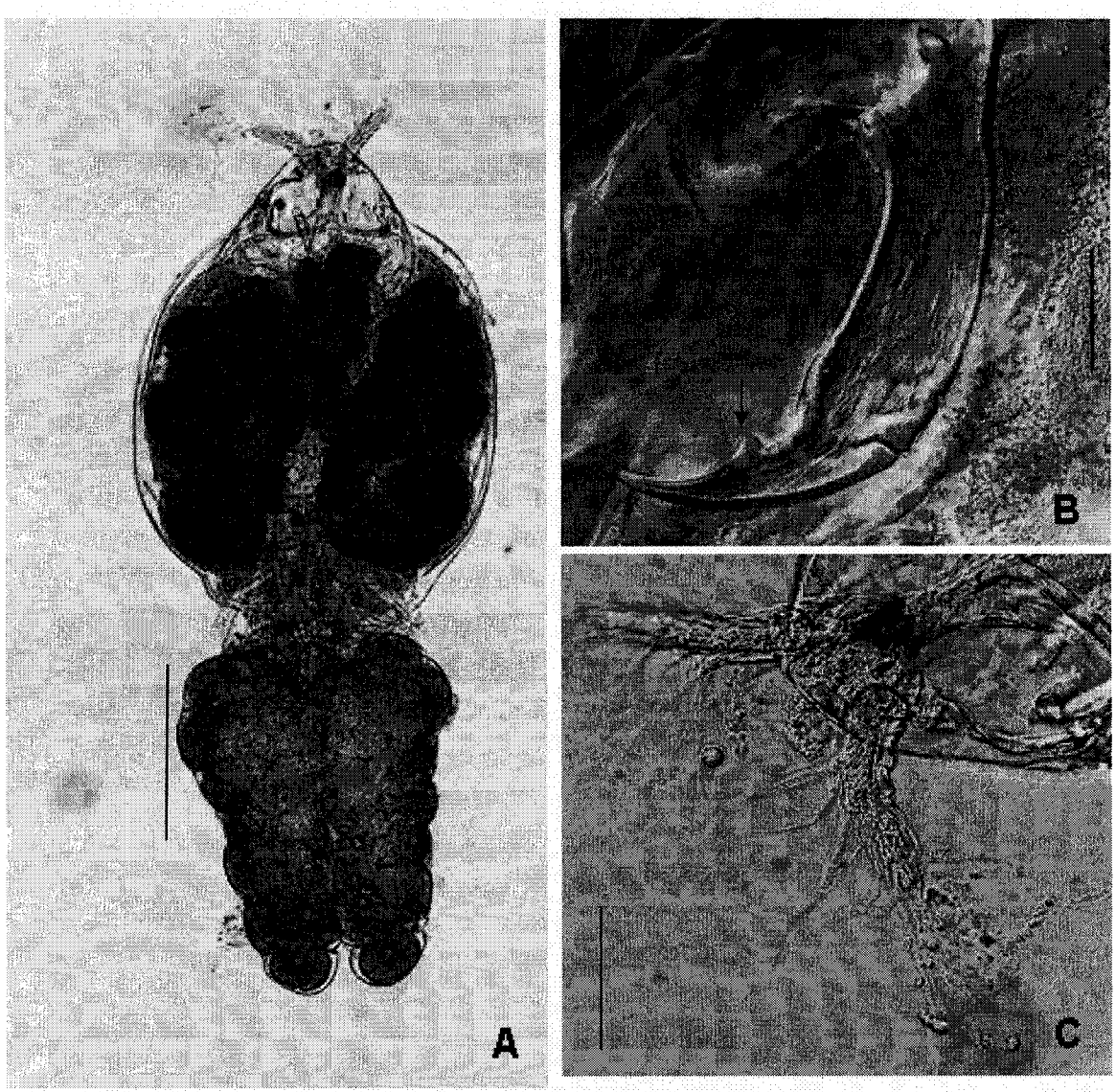

A
B
C

Figure 8: SEM images of *Ergasilus* sp. from *Apeltes quadracus* collected from off Todd's Island, NS. Ventral view of body, note the incorporation of the first pair of legs into the cephalothorax. (A, Scale bar = 250 μm), abdomen with denticles (B, Scale bar = 10 μm), arrangement of legs one through four (C, Scale bar = 100 μm).

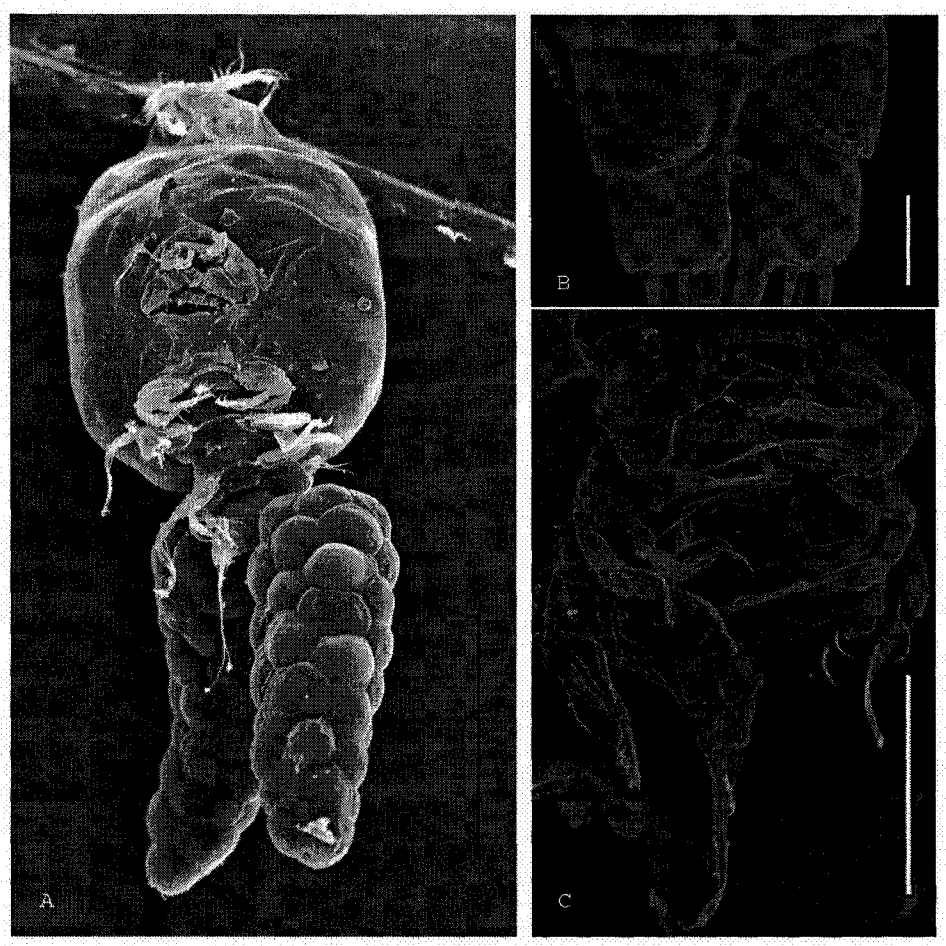

A
B
C

Figure 9: *Ergasilus sp.* attached to the operculum of *Apeltes quadracus.* Scale bar = 2mm.

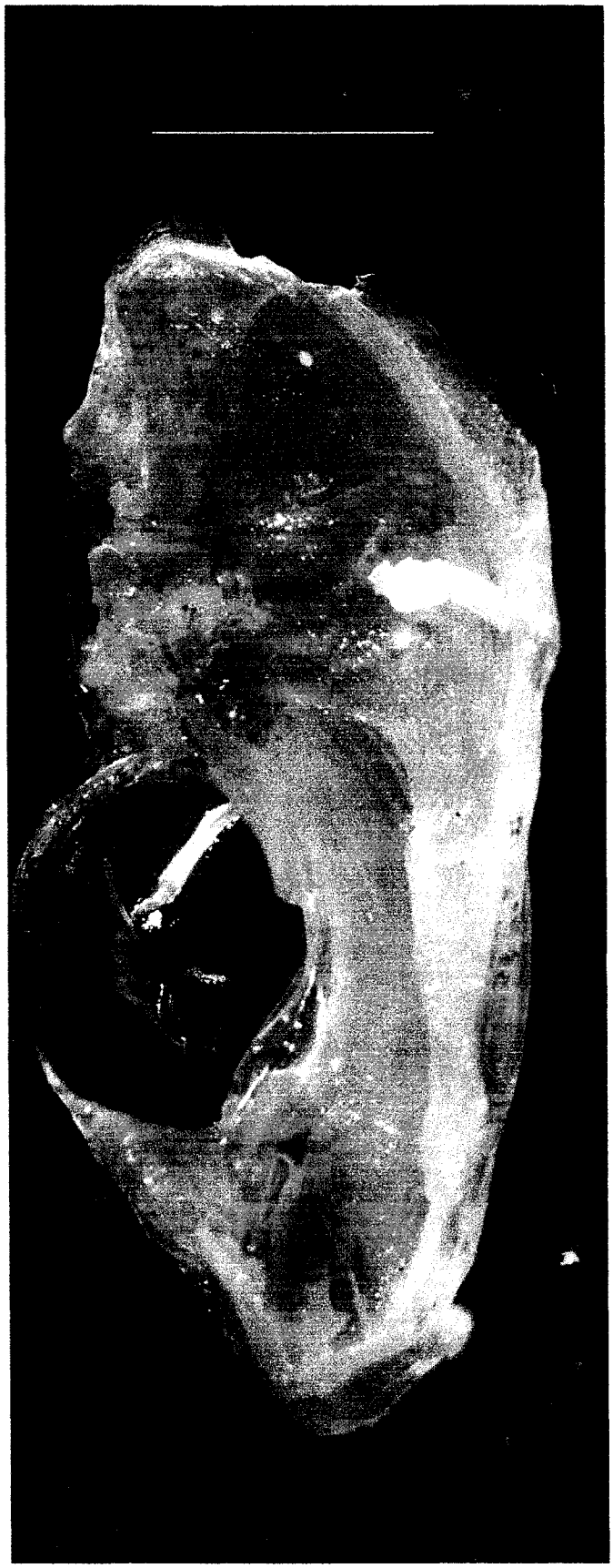


Figure 10: Tissue sections of *Ergasilus* sp. attached to the inner surface of the operculum of *Apeltes quadracus*. Penetration of host by parasite's antennae (A, scale bar = 50 μm) and cellular debris at the attachment site (B, scale bar = 20 μm). Es = *Ergasilus* sp., H = hyperplasia, A= antenna, HT = host, M = mouthparts, E = epidermis, and D = dermis.

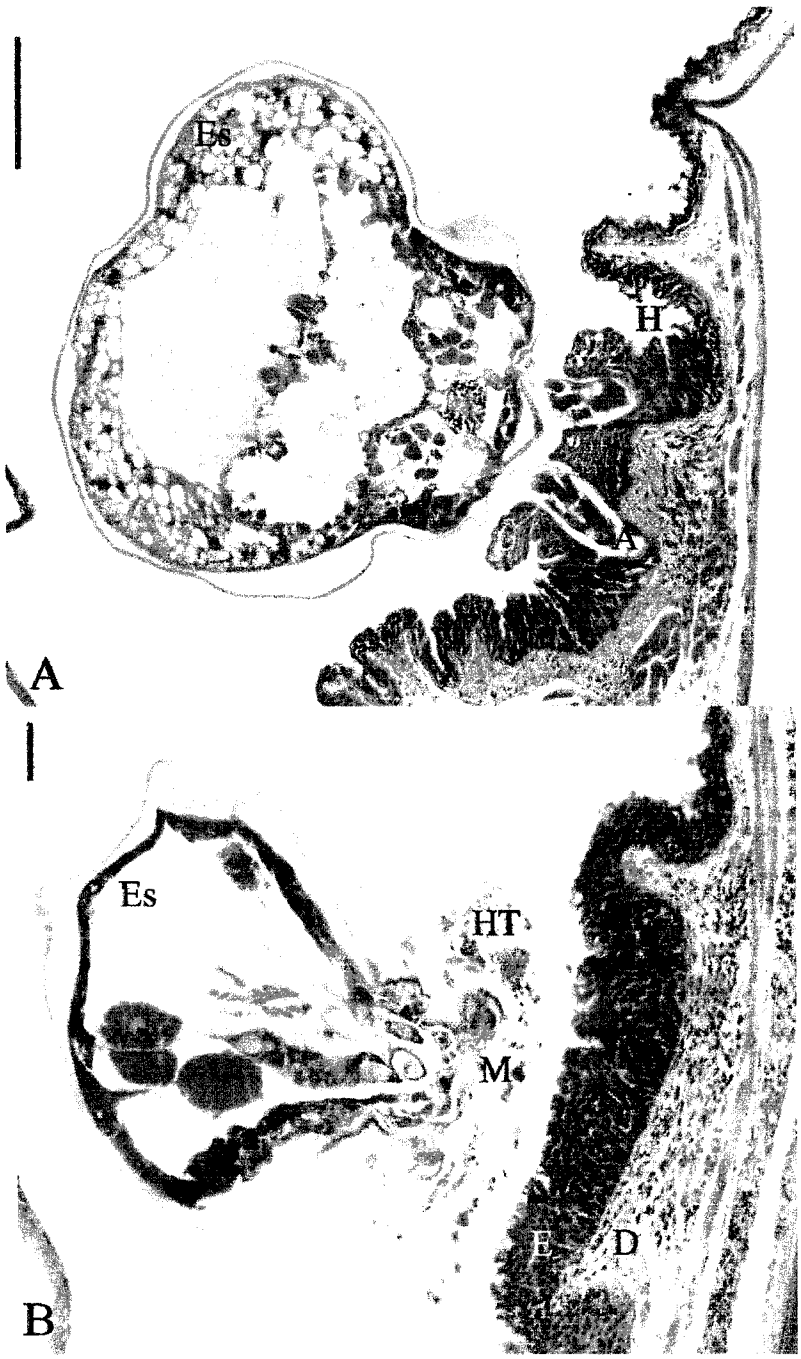

Es
H
A
A
Es
HT
M
E
D
B

Figure 11: *Ergasilus* sp. attached to the inner surface of the operculum of *Apeltes quadracus.* Hyperplasia at attachment site compared to uninfected tissue on opposite operculum. G = gills, IO = infected operculum, Es = *Ergasilus* sp., and UO = uninfected operculum.

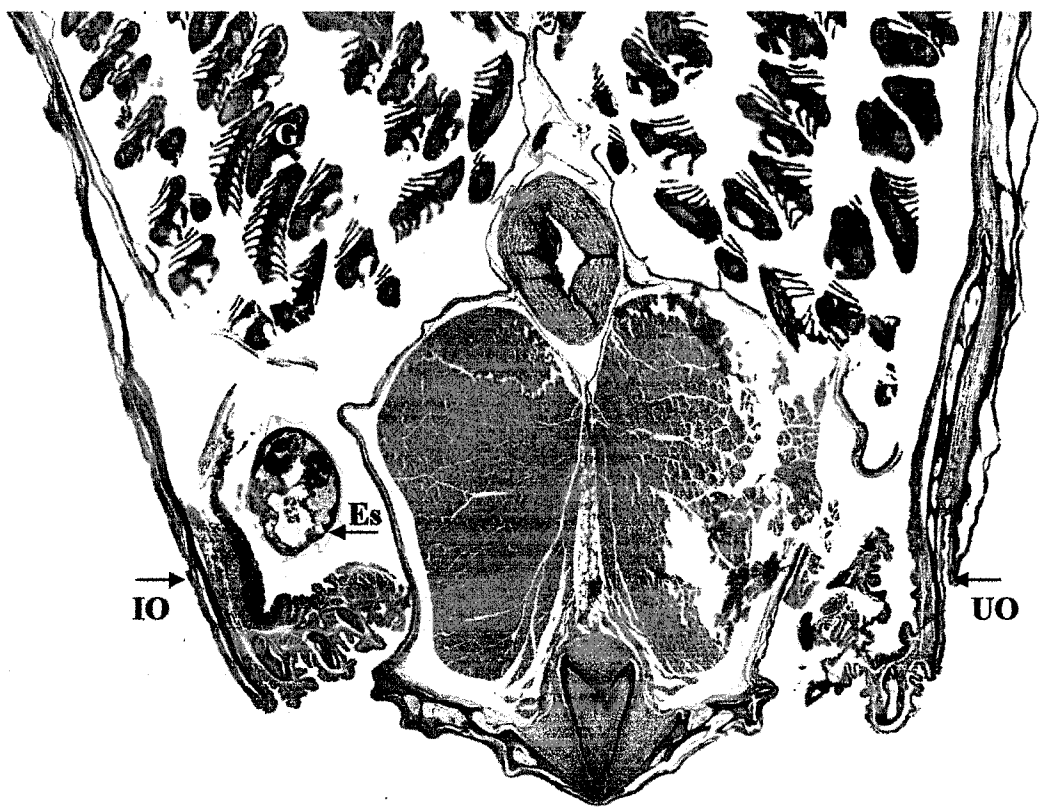

G
Es
IO
UO

Figure 12: Antennae of *Ergasilus* sp. embedded within host (*Apeltes quadracus*) epidermal , dermal and loose connective tissues (A), gill tissue also penetrated by antennae (B). Scale bar = 20 μm. A = antenna, D = dermis, E = epidermis, LC = loose connective tissue, Es = *Ergasilus* sp. and G = gill filament.

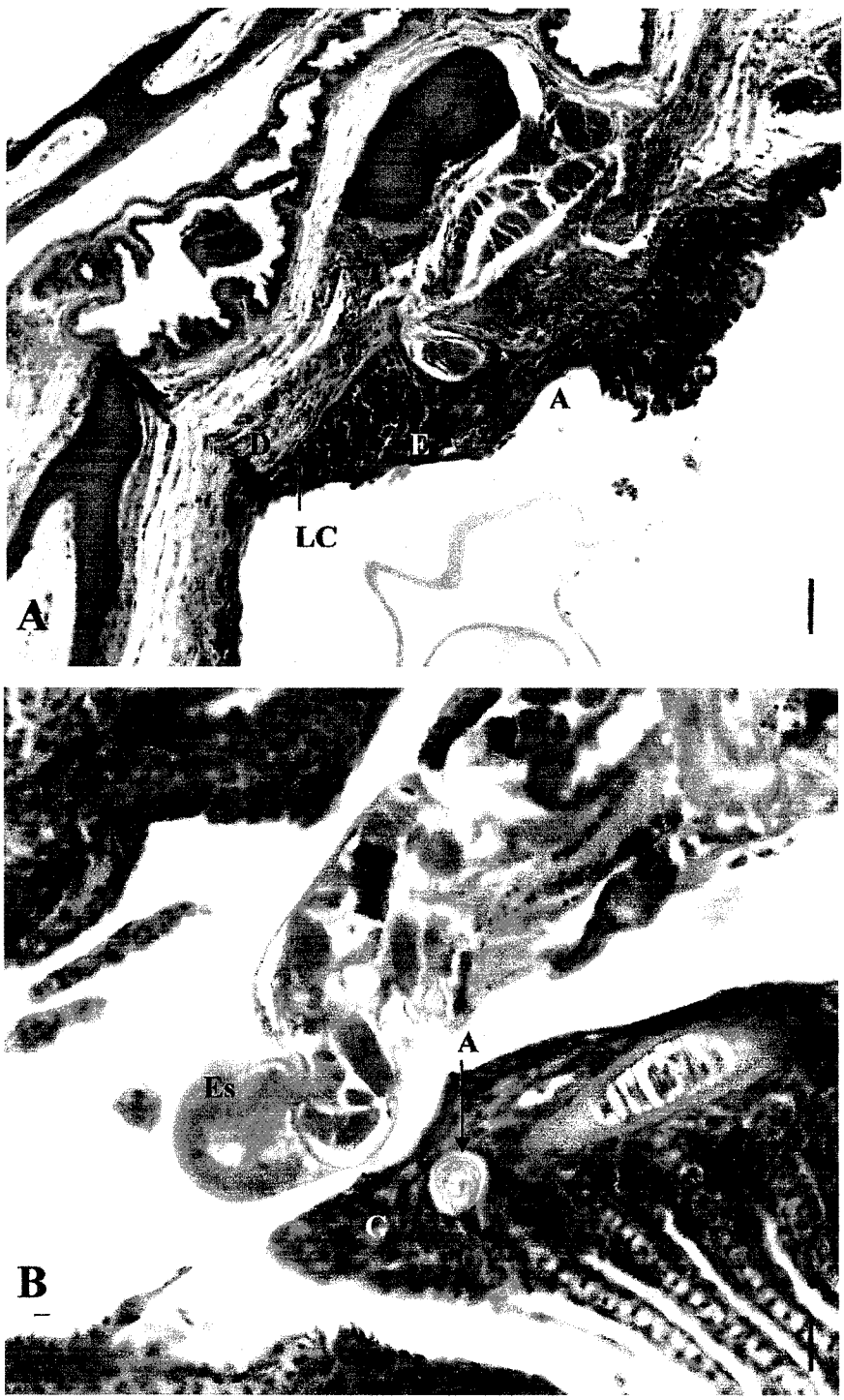

A
D
E
LC
A
B
Es
A
C

Figure13: Photomicrographs of *Thersitina gasterostei* from *Gasterosteus aculeatus* collected from off Bouctouche, NB. Ventral view of body (A). Scale bar = 100 μm. Antenna claw, with accessory claw on inner margin of fourth joint (B). Scale bar = 20 μm. AC = accessory claw.

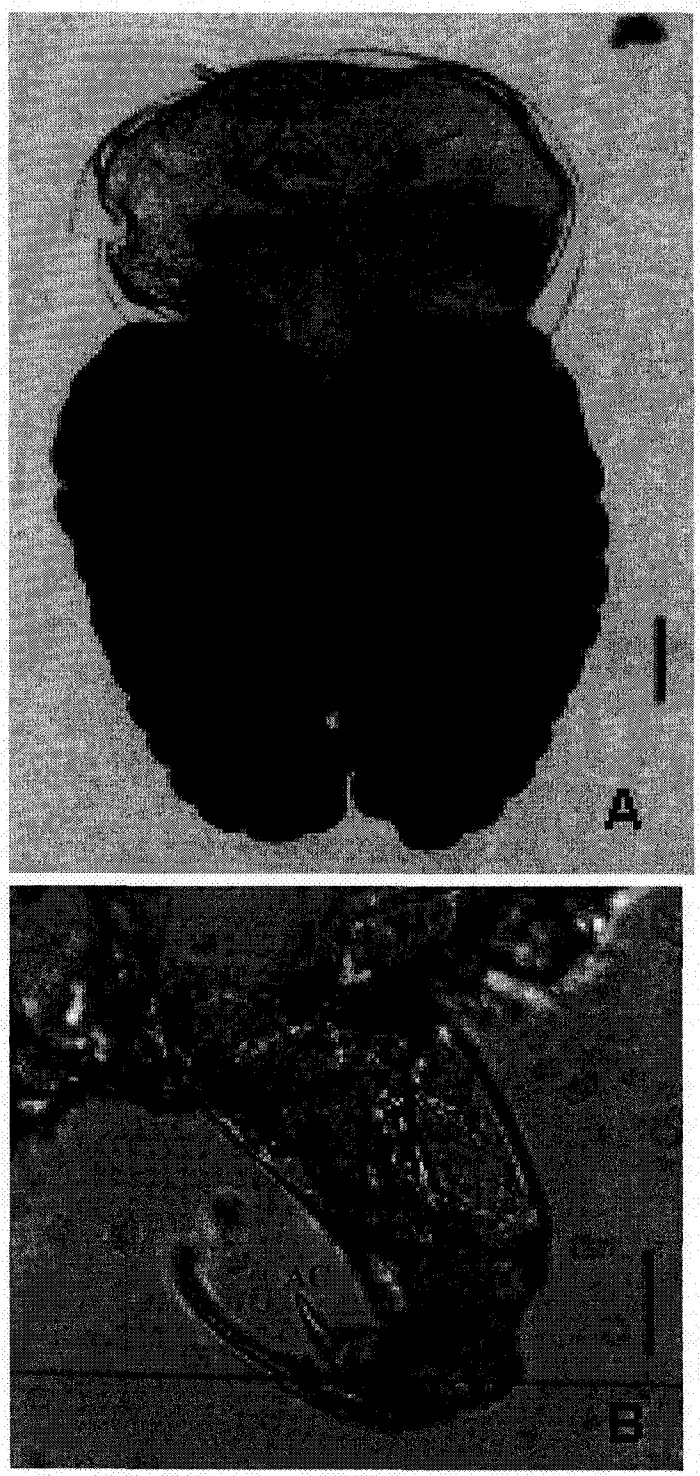

A
B

Figure 14: *Thersitina gasterostei* attached to *Gasterosteus aculeatus* and *Pungitius pungitius*. Cluster of *T. gasterostei* on operculum of *G. aculeatus* (A). Scale bar = 5 mm. Hyperplastic nodule at the attachment site of *T. gasterostei* on gill of *P. pungitius* (B). Scale bar = 5 mm. *Thersitina gasterostei* attached at base of *G. aculeatus* pectoral fin.

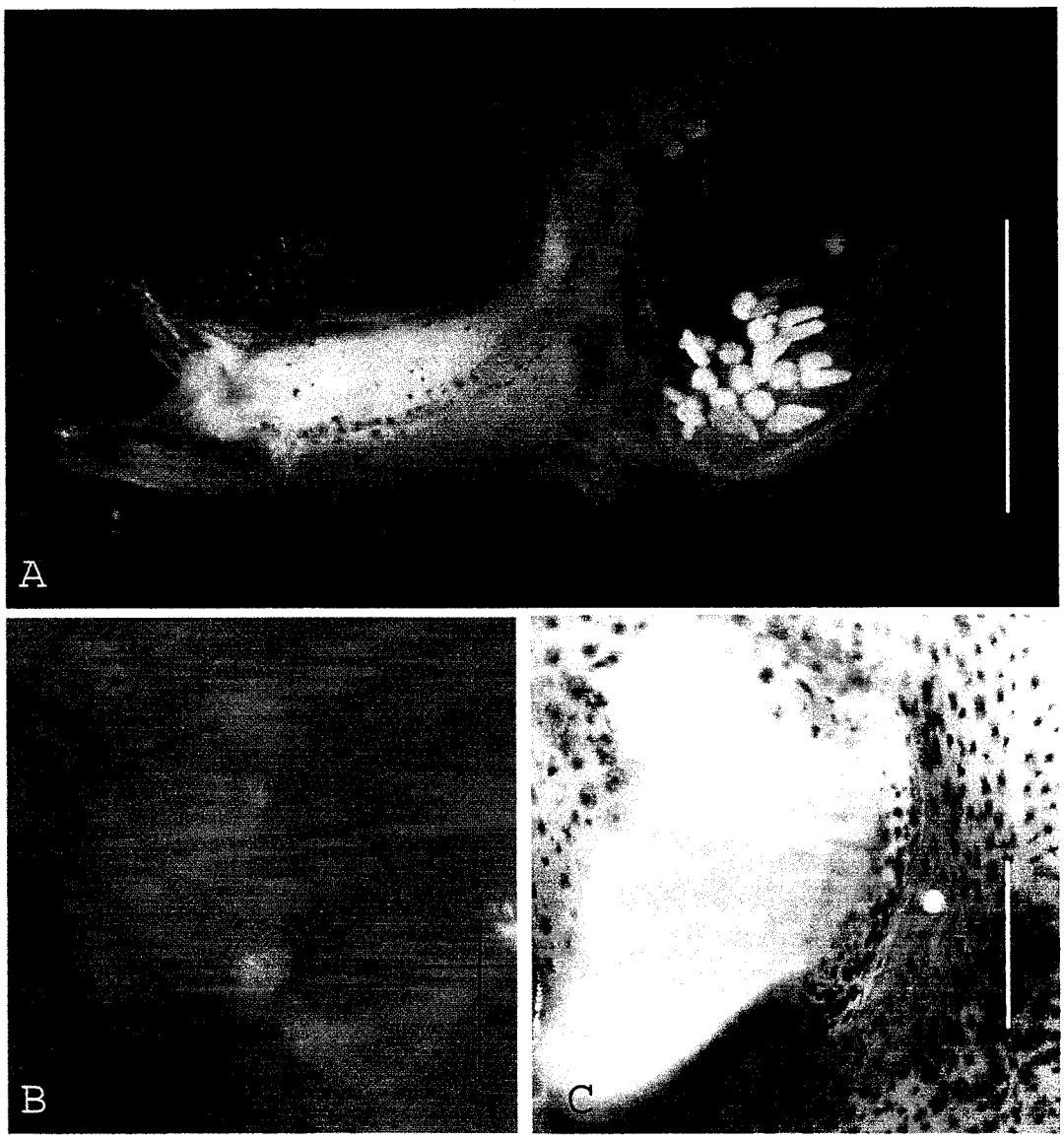

A
B
C

Table I: Summary of host and distribution records for *Ergasilus* spp. off the Eastern Atlantic Canadian Provinces.

Species	Author	Host(s)	Reported From
Ergasilus auritus	Hanek & Threlfall (1970a)	*Gasterosteus aculeatus*	Long Pond, Saint John's NF
	Hanek & Threllfall (1970b)	*Gasterosteus aculeatus*	Newfoundland & Laborador
	Margolis & Kabata (1988)	*Gasterosteus aculeatus*	Pacific Coast, British Columbia, Labrador, Newfoundland
Ergasilus caeruleus	Threlfall & Hanek (1970)	*Catostomus catostomus*	Eagle River, NF; Grand Lake, NF
	Margolis & Kabata (1988)	*Ambloplites rupestris, Anguilla rostrata, Carpiodes cyprinus, Catostomus catostomus, Catostomus commersoni, Coregonus artedii, Lepomis gibbosus, Lota lota*	Ontario, Labrador, British Columbia
Ergasilus labracis	Margolis & Kabata (1988)	*Morone saxatilis, Osmerus mordax*	Newfoundland, New Brunswick
Ergasilus lizae	Wiles (1975)	*Fundulus diaphanus*	Shubenacadie Lake, NS; Ponhook Lake, NS; Five Islands Lake, NS
	Margolis & Kabata (1988)	*Fundulus diaphanous*	Nova Scotia
Ergasilus luciopercarum	Davis (1969)	*Salvelinus fontinalis*	Barachois Pond, NF
	Hicks & Threlfall (1973)	*Prosopium cylindraceum*	Grand Lake, NF
	Sandeman &Pippy (1967)	*Salvelinus fontinalis*	Raft Pond, NF
	Margolis & Kabata (1988)	*Morone americana, Perca flavescens, Prosopium cylindraceum, Salvelinus fontinalis, Stizostedion, vitreum*	Labrador, Newfoundland, Ontario, Quebec
Ergasilus manicatus	Bere (1930)	*Menidia notata, Osmerus mordax*	Waweig, NB; St. Croix, NB; Magaguadavic Rivers, NB; St. Andrews Harbour, NB; Head Harbour, NB; Birch Cove, NB
	Margolis & Kabata (1988)	*Menidia menidia, Osmerus mordax*	Atlantic Provinces

Table II: Collection localities – geographical co-ordinates and salinities.

Collection Locality	N Co-ordinates	W Co-ordinates	Salinity (ppt)
1-Bouctouche, NB	46°27.668'	064°43.565'	30
2-Cap Pélé, NB	46°12.696'	064°24.112'	32
3-Cocagne, NB	46°20.127'	064°36.918'	35
4-Little River, NB	47°36.061'	065°40.283'	36
5-Nelson, NB	46°50'00"	65°37'00"	N/A
6-Newcastle, NB	47°10'00"	65°35'00"	N/A
7-Richibucto, NB	46°41.839'	064°57.236'	33
8-Cheticamp, NS	46°35.914'	061°01.337'	35
9-Clam Harbour, NS	44°44.030'	062°54.401'	32
10-Hwy 105, NS	46°12.669'	060°35.511'	35
11-Hwy 331, NS	44°09.539'	064°33.782'	21
12-Johnstown, NS	45°47.970'	060°43.898'	23
13-Lawrencetown, NS	44°39'00"	63°20'00"	31
14-Mabou, NS	46°04.172'	061°23.720'	30
15-Medway, NS	44°09.539'	064°33.782'	28
16-Merigonish, NS	45°38'00"	62°27'00"	32
17-Porter's Lake, NS	44°43.598'	063°17.942'	11
18-Rainbow Haven, NS	44°39.178'	063°25.466'	35
19-Rear Monroe's Point, NS	46°14.860'	060°36.244'	22
20-Todd's Island, NS	44°41.044'	063°54.528'	32
21-Tracadie, NS	45°36.942'	061°37.557'	31
22-Gallow's Cove Pond, NF	47°05'00"	52°54'00"	N/A
23-Hopeall River, NF	47°36'30"	53°30'55"	N/A
24-Quidi Vidi Lake, NF	47°35'00"	52°41'00"	N/A

Table III: Prevalence of infection (number infected/number examined; expressed as a percentage) followed in parentheses by number infected/sample size (X/N) with *Ergasilus* sp. on gasterosteiforms from collection localities off the Eastern Atlantic Canadian Provinces.

Location	***Host***			
	A. quadracus	***G.aculeatus***	***G. wheatlandi***	***P. pungitius***
Bouctouche, NB	77.3 (68/88)	n/a	33.3 (1/3)	n/a
Cap Pélé, NB	85.9 (79/92)	100 (6/6)	42.9 (3/7)	n/a
Cocagne, NB	75 (12/16)	n/a	50 (1/2)	50 (1/2)
Little River, NB	n/a	58.3 (7/12)	n/a	n./a
Nelson, NB	21.7 (5/23)	n/a	n/a	33.3 (1/3)
Newcastle, NB	8.2 (5/61)	n/a	n/a	0 (0/1)
Richibucto, NB	15.4 (2/13)	20 (1/5)	0 (0/7)	0 (0/1)
Cheticamp, NS	70.3 (78/111)	28.6 (2/7)	40.8 (20/49)	n/a
Clam Harbour, NS	84.6 (11/13)	n/a	n/a	0 (0/1)
Hwy 105, NS	85.7 (6/7)	0 (0/2)	n/a	n/a
Hwy 331, NS	43.8 (14/32)	42.9 (3/7)	0 (0/1)	n/a
Johnstown, NS	n/a	n/a	93.8 (15/16)	n/a
Lawrencetown, NS	42.9 (3/7)	33.3 (10/30)	42.9 (12/28)	n/a
Mabou, NS	87.5 (63/72)	66.7 (4/6)	n/a	50 (1/2)
Medway, NS	3.2 (3/95)	5.2 (6/116)	0 (0/3)	0 (0/3)
Merigonish, NS	100 (9/9)	41.7 (5/12)	n/a	n/a
Rear Monroe's Point, NS	n/a	n/a	0 (0/5)	n/a
Todd's Island, NS1	96.6 (28/29)	n/a	n/a	n/a
Todd's Island, NS2	46.9 (90/194)	27.6 (43/156)	n/a	n/a
Todd's Island, NS3	93 (14/15)	57.1 (24/42)	0 (0/1)	75 (6/8)
Todd's Island, NS4	61.5 (8/13)	33.3 (1/3)	100 (1/1)	n/a
Todd's Island, NS5	100 (1/1)	85.7 (6/7)	n/a	n/a
Tracadie, NS	69 (77/112)	100 (2/2)	100 (1/1)	0 (0/1)
Total	57.5 (576/1003)	29.1 (120/413)	43.5 (54/124)	40.9 (9/22)

Table IV: Percentage of infection of *Ergasilus* sp. among the different attachment sites on *Apeltes quadracus* (followed in parentheses by number of *Ergasilus* sp. at attachment site/total number of *Ergasilus* sp. recovered (X/N)).

Location	**Attachment Site**			χ^2	***P***
	Operculum	Gills	Buccal Chamber		
Cap Pélé NB	21.5 (73/338)	68.5 (233/338)	9.4 (32/338)	299.4	0.000
Cheticamp NS	20.6 (59/285)	69 (198/285)	9.8 (28/285)	258.0	0.000
Todd's Island NS – 2	57.8 (133/226)	12.2 (28/226)	28.3 (65/226)	111.9	0.000
Tracadie NS	13.9 (53/378)	76.5 (291/378)	8.9 (34/378)	487.0	0.000

Table V: Percentage of infection of *Ergasilus* sp. among the different gill arches on *Apeltes quadracus* (followed in parentheses by number of *Ergasilus* sp. attached to specific gill arch/total number of *Ergasilus* sp. recovered from gills (X/N)).

Location	**Attachment Site**				χ^2	***P***
	Gill 1	Gill 2	Gill 3	Gill 4		
Cap Pélé NB	26.2 (89/233)	12.1 (41/233)	21.8 (74/233)	8.5 (29/233)	53.7	0.000
Cheticamp NS	17.8 (51/198)	21.3 (61/198)	23 (66/198)	7 (20/198)	34.4	0.000
Tracadie NS	21.6 (82/291)	21.6 (82/291)	23.7 (90/291)	9.7 (37/291)	32.0	0.000

Table VI: Percentage of infection of *Ergasilus* sp. among the different attachment sites of *Apeltes quadracus* within different intensity levels.

Attachment Site	Operculum	Gills	Buccal Cavity	Other	χ^2	***P* value**
Low (1)	66.7	26.7	6.7	-	12.6	0.002
Medium (7-10)	31.0	58.5	9.8	0.81	129.7	0.000
High (>20)	18.3	72.0	9.2	0.61	812.3	0.000
χ^2	27.1	20.4	0.162	0.63		
***P* value**	0.000	0.000	0.922	0.802		

Table VII: Mean number of eggs and mean egg size of *Ergasilus* sp. from the operculum and gills of *Apeltes quadracus* at three sampling localities.

	No. of Eggs			Egg Size (μm)			Egg Sac Length (μm)		
	Operc.	Gills	*P*	Operc.	Gills	*P*	Operc.	Gills	*P*
Cheticamp, NS	62.5 ± 22 n=13 (33-106)	26.7 ± 9.48 n=12 (10-47)	0.0001	25.4 ± 2.25 n=13 (22.5-30)	22.3 ± 1.98 n=12 (20-25)	0.0044	706.15 ± 95.70 n=13 (550-870)	542.5 ± 120.24 n=12 (320-840)	0.0003
Cap Pele, NB	65.9 ± 16.64 n=10 (36-98)	22.0 ± 7.93 n=10 (11-37)	0.0001	24.25 ± 3.74 n=10 (17.5-30)	19.75 ± 3.62 n=10 (15-25)	0.0229	755 ± 99.8 n=10 (640-1000)	488 ± 90.8 n=10 (370-670)	0.0001
Tracadie, NS	43.8 ± 12.7 n=10 (26-61)	26.1 ± 7.9 n=10 (16-43)	0.0052	20.5 ± 2.84 n=10 (15-25)	17.75 ± 3.22 n=10 (15-25)	0.0529	548 ± 99.9 n=10 (360-680)	411 ± 107.2 n=10 (210-540)	0.0113

Table VIII: Prevalence of infection (number infected/number examined; expressed as a percentage) followed in parentheses by number infected/sample size (X/N) with *Thersitina gasterostei* on gasterosteiforms from collection localities off the Eastern Atlantic Canadian Provinces.

Location	***Host***			
	A. quadracus	***G. aculeatus***	***G. wheatlandi***	***P. pungitius***
Bouctouche, NB	2.3 (2/88)	n/a	0 (0/3)	n/a
Cap Pélé, NB	4.3 (4/92)	83 (5/6)	85.7 (6/7)	n/a
Cocagne, NB	0 (0/16)	n/a	100 (2/2)	0 (0/2)
Little River, NB	n/a	91.7 (11/12)	n/a	n/a
Nelson, NB	0 (0/23)	n/a	n/a	66.7 (2/3)
Newcastle, NB	9.8 (6/61)	n/a	n/a	0 (0/1)
Richibucto, NB	0 (0/13)	60 (3/5)	28.6 (2/7)	0 (0/1)
Cheticamp, NS	0 (0/111)	14.3 (1/7)	2.04 (1/49)	n/a
Clam Harbour, NS	7.7 (1/13)	n/a	n/a	0 (0/1)
Hwy 105, NS	14.3 (1/7)	100 (2/2)	n/a	n/a
Hwy 331, NS	0 (0/32)	14.3 (1/7)	0 (0/1)	n/a
Johnstown, NS	n/a	n/a	75 (12/16)	n/a
Lawrencetown, NS	0 (0/7)	13.3 (4/30)	39.3 (11/28)	n/a
Mabou, NS	0 (0/72)	33.3 (2/6)	n/a	50 (1/2)
Medway, NS	0 (0/95)	12.1 (14/116)	0 (0/3)	0 (0/3)
Merigonish, NS	0 (0/9)	50 (6/12)	n/a	n/a
Rear Monroe's Point, NS	n/a	n/a	80 (4/5)	n/a
Todd's Island, NS1	0 (0/29)	n/a	n/a	n/a
Todd's Island, NS2	0 (0/194)	0 (0/156)	n/a	n/a
Todd's Island, NS3	0 (0/15)	0 (0/42)	100 (1/1)	0 (0/8)
Todd's Island, NS4	0 (0/13)	0 (0/3)	0 (0/1)	n/a
Todd's Island, NS5	0 (0/1)	0 (0/7)	n/a	n/a
Tracadie, NS	0 (0/112)	0 (0/2)	0 (0/1)	0 (0/1)
Total	1.4 (14/1003)	14.8 (61/413)	16.1 (20/124)	13.6 (3/22)

Table IX: Percentage of infection of *T. gasterostei* among the different attachment sites on *Gasterosteus aculeatus* (followed in parentheses by number of *T. gasterostei* at attachment site/total number of *T. gasterostei* recovered (X/N)).

Location	**Attachment Site**				χ^2	***P***
	Operculum	Gills	Buccal Chamber	Pectoral Fin		
Little River NB	79.8 (178/214)	4 (9/214)	11.7 (26/214)	0.45 (1/214)	509.3	0.000
Lawrencetown NS	71.4 (5/7)	n/a (0)	28.6 (2/7)	n/a (0)	2.57	0.109
Medway NS	85.4 (47/55)	3.6 (2/55)	10.9 (6/55)	n/a (0)	100.6	0.000
Merigonish NS	73.9 (17/23)	n/a (0)	8.7 (2/23)	17.4 (4/23)	20.2	0.000

Table X: Percentage of mixed species infections of *Ergasilus* sp. and *Thersitina gasterostei* on hosts (expressed as a percentage with number of mixed species infections/sample size in parentheses (X/N)).

Host	*Ergasilus* sp.	Mixed Infection	*T. gasterostei*	χ^2	*P*
Apeltes quadracus (584)	97.6 (570/584)	1.2 (7/584)	1.2 (7/584)	1628	0.000
Gasterosteus aculeatus (148)	66.9 (99/148)	14.2 (21/148)	18.9 (28/148)	113.2	0.000
Gasterosteus wheatlandi (76)	48.7 (37/76)	22.4 (17/76)	28.9 (22/76)	12.83	0.002
Pungitius pungitius (11)	72.7 (8/11)	9.1 (1/11)	18.2 (2/11)	11.7	0.003

Table XI: Mean intensity of mixed species infections of *Ergasilus* sp. and *Thersitina gasterostei* on hosts.

Host Species	*Ergasilus*	Mixed Infection	*T. gasterostei*	*P*
Apeltes quadracus	5.4 ± 7.4 1-65, n = 570	5.4 ± 11.7 1-34, n = 7	1.1 ± 0.38 1-2, n = 7	1.000
Gasterosteus aculeatus	4.8 ± 9.6 1-76, n = 99	9.4 ± 13.8 1-61, n = 21	3.6 ± 11.1 1-60, n = 28	0.000
Gasterosteus wheatlandi	2.7 ± 2.4 1-12, n = 37	4.7 ± 5.3 1-26, n = 17	3.8 ± 6.5 1-24, n = 22	0.000
Pungitius pungitius	4.3 ± 3.3 1-11, n = 8	36 ± n/a 36, n = 1	1.0 ± 0 1, n = 2	0.091

Table XII: Percent similarity between sequences for alignments of *Ergasilus* sp., *T. gasterostei,* and *E. manicatus*.

Species	*E. manicatus*	*Ergasilus* sp.	*T. gasterostei*
E. manicatus	-	99% (228/230)	97% (224/230)
Ergasilus sp.		-	98% (226/230)
T. gasterostei			-

Appendix

```
E.manicatus-1             1   TTCCCTCACG GTACTTGTTC GCTATCGGTC TCGGGGTCAT ATTTAGCCTT
E.manicatus-2             1   TTCCCTCACG GTACTTGTTC GCTATCGGTC TCGGGGTCAT ATTTAGCCTT
Ergasilussp-1             1   TTCCCTCACG GTACTTGTTC GCTATCGGTC TCGGGGTCAT ATTTAGCCTT
Ergasilussp-2             1   TTCCCTCACG GTACTTGTTC GCTATCGGTC TCGGGGTCAT ATTTAGCCTT
Ergasilussp-3             1   TTCCCTCACG GTACTTGTTC GCTATCGGTC TCGGGGTCAT ATTTAGCCTT
T.gasterostei-1           1   TTCCCTCACG GTACTTGTTC GCTATCGGTC TCGGGGTCAT ATTTAGCCTT
T.gasterostei-2           1   TTCCCTCACG GTACTTGTTC GCTATCGGTC TCGGGGTCAT ATTTAGCCTT
T.gasterostei-3           1   TTCCCTCACG GTACTTGTTC GCTATCGGTC TCGGGGTCAT ATTTAGCCTT

E.manicatus-1            51   ACGTGGAGTT TACCACGCAC TTTGAGCTGC ACTCCCAAGC AACTCGACTC
E.manicatus-2            51   ACGTGGAGTT TACCACGCAC TTTGAGCTGC ACTCCCAAGC AACTCGACTC
Ergasilussp-1            51   ACGTGGAGTT TACCACACAC TTTGAGCTGC ACTCCCAAGC AACTCGACTC
Ergasilussp-2            51   ACGTGGAGTT TACCACACAC TTTGAGCTGC ACTCCCAAGC AACTCGACTC
Ergasilussp-3            51   ACGTGGAGTT TACCACACAC TTTGAGCTGC ACTCCCAAGC AACTCGACTC
T.gasterostei-1          51   ACGTGGAGTT TACCACGCAC TTTGAGCTGC ACTCCCAAGC AACTCGACTC
T.gasterostei-2          51   ACGTGGAGTT TACCACGCAC TTTGAGCTGC ACTCCCAAGC AACTCGACTC
T.gasterostei-3          51   ACGTGGAGTT TACCACGCAC TTTGAGCTGC ACTCCCAAGC AACTCGACTC

E.manicatus-1           101   TGGGGAAAAG CCACCTCGGC GAGCCGACCG TCCTACGGGC CTATCACCCT
E.manicatus-2           101   TGGGGAAAAG CCACCTCGGC GAGCCCACCG TCCTACGGGC CTATCACCCT
Ergasilussp-1           101   TGGGGAAAAG CCACCTCGGC GAGCCGACCG TCCTACGGGC CTATCACCCT
Ergasilussp-2           101   TGGGGAAAAG CCACCTCGGC GAGCCGACCG TCCTACGGGC CTATCACCCT
Ergasilussp-3           101   TGGGGAAAAG CCACCTCGGC GAGCCGACCG TCCTACGGGC CTATCACCCT
T.gasterostei-1         101   TGGGGAAAAG CCACCTCGGC AAGCCGACCG TCCTACGGGC CTATCACCCT
T.gasterostei-2         101   TGGGGAAAAG CCACCTCGGC AAGCCGACCG TCCTACGGGC CTATCACCCT
T.gasterostei-3         101   TGGGGAAAAG CCACCTCGGC AAGCCGACCG TCCTACGGGC CTATCACCCT

E.manicatus-1           151   CTCTGAGCAA TGGCCCCTGT CAAGATGGAC TTGGACAGGC ACTCTCACCT
E.manicatus-2           151   CTCTGAGCAA TGGCCCCTGT CAAGATGGAC TTGGACAGGC ACTCTCACCT
Ergasilussp-1           151   CTCTGAGCAA TGGCCCCTGT CAAGATGGAC TTGGACAGGC ACTCTCACCT
Ergasilussp-2           151   CTCTGAGCAA TGGCCCCTGT CAAGATGGAC TTGGACAGGC ACTCTCACCT
Ergasilussp-3           151   CTCTGAGCAA TGGCCCCTGT CAAGATGGAC TTGGACAGGC ACTCTCACCT
T.gasterostei-1         151   CTCTGAGCAA TGGCCCCTGT CAAGATGGAC TTGGACAGTC ACACTCGCCT
T.gasterostei-2         151   CTCTGAGCAA TGGCCCCTGT CAAGATGGAC TTGGACAGTC ACACTCGCCT
T.gasterostei-3         151   CTCTGAGCAA TGGCCCCTGT CAAGATGGAC TTGGACAGTC ACACTCGCCT

E.manicatus-1           201   CGATGAGGCT CTCCTAAACA CCACATTCCG
E.manicatus-2           201   CGATGAGGCT CTCCTAAACA CCACATTCCG
Ergasilussp-1           201   CGATAAGGCT CTCCTAAACA CCACATTCCG
Ergasilussp-2           201   CGATAAGGCT CTCCTAAACA CCACATTCCG
Ergasilussp-3           201   CGATAAGGCT CTCCTAAACA CCACATTCCG
T.gasterostei-1         201   CGATGAGGCT CTCCTAAACA CCACATTCCG
T.gasterostei-2         201   CGATGAGGCT CTCCTAAACA CCACATTCCG
T.gasterostei-3         201   CGATGAGGCT CTCCTAAACA CCACATTCCG
```

Sequence alignment of *E. manicatus*, *Ergasilus* sp. and *T. gasterostei*.

```
Ergasilussp: 1   ttccctcacggtacttgttcgctatcggtctcggggtcatatttagccttacgtggagtt 60
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Emanicatus: 1    ttccctcacggtacttgttcgctatcggtctcggggtcatatttagccttacgtggagtt 60

Ergasilussp: 61  taccacacactttgagctgcactcccaagcaactcgactctggggaaaagccacctcggc 120
                 ||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||
Emanicatus: 61   taccacgcactttgagctgcactcccaagcaactcgactctggggaaaagccacctcggc 120

Ergasilussp: 121 gagccgaccgtcctacgggcctatcaccctctctgagcaatggcccctgtcaagatggac 180
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Emanicatus: 121  gagccgaccgtcctacgggcctatcaccctctctgagcaatggcccctgtcaagatggac 180

Ergasilussp: 181 ttggacaggcactctcacctcgataaggctctcctaaacaccacattccg 230
                 ||||||||||||||||||||||| |||||||||||||||||||||||
Emanicatus: 181  ttggacaggcactctcacctcgatgaggctctcctaaacaccacattccg 230
```

Paired comparison of *Ergasilus* sp. versus *E. manicatus*, 228 of 230 identities identical, 99% similarity.

Paired comparison of *T. gasterostei* versus *Ergasilus* sp., 224 of 230 identities identical, 97% similarity.

```
Tgasterostei:1    ttccctcacggtacttgttcgctatcggtctcggggtcatatttagccttacgtggagtt 60
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Emanicatus: 1     ttccctcacggtacttgttcgctatcggtctcggggtcatatttagccttacgtggagtt 60

Tgasterostei:61   taccacgcactttgagctgcactcccaagcaactcgactctggggaaaagccacctcggc 120
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Emanicatus: 61    taccacgcactttgagctgcactcccaagcaactcgactctggggaaaagccacctcggc 120

Tgasterostei:121  aagccgaccgtcctacgggcctatcaccctctctgagcaatggcccctgtcaagatggac 180
                   ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Emanicatus: 121   gagccgaccgtcctacgggcctatcaccctctctgagcaatggcccctgtcaagatggac 180

Tgasterostei:181  ttggacagtcacactcgcctcgatgaggctctcctaaacaccacattccg 230
                  |||||||| ||| ||| ||||||||||||||||||||||||||||||||
Emanicatus: 181   ttggacaggcactctcacctcgatgaggctctcctaaacaccacattccg 230
```

Paired comparison of *T. gasterostei* versus *E. manicatus*, 226 of 230 identities identical, 98% similarity.