DEVELOPMENTAL STAGES OF *ACHTHERES PERCARUM* (CRUSTACEA: COPEPODA), PARASITIC ON EUROPEAN PERCH, *PERCA FLUVIATILIS* (ACTINOPTERYGII: PERCIFORMES)

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Background. *Achtheres percarum* is an important copepod parasite (Crustacea: Copepoda) of European perch. Adult females permanently attach to the gill arches, roof of the mouth, tongue, and gill filaments. Attachment, at the latter site, may result in necrosis and epithelial hypertrophy, both compromising fish respiration during oxygen deficiencies. Adult males can move freely on gills of perch. To date there has been no published record of the complete set of developmental stages of this fish parasite. Provision of such may have practical implications for freshwater ichthyopathology, for example helping to monitor the dynamics of the parasite's populations. It may also provide useful information regarding copepod phylogenetics.
Materials and Methods. Early developmental stages (nauplius and copepodid) of *A. percarum* were acquired through incubation of eggs within egg sacs of females collected from European perch, *Perca fluviatilis* L., caught commercially in 1994 in Lake Dąbie, Szczecin, Poland. All subsequent larval stages were collected from gills of perch caught in the same lake, in 1990. All copepods were fixed and preserved in 75% ethanol. A modified "wooden slide" method was used to observe the collected developmental stages in a suspended drop of lactic acid, using a compound microscope. Specimens were stained in lignin pink and morphologic details of were drawn using a drawing tube.
Results. The life cycle of *A. percarum* consists of 7 developmental stages, separated by moults (nauplius, copepodid, chalimus I, chalimus II, chalimus III, chalimus IV, and adult). The nauplius hatches from the egg and quickly moults into the copepodid. Both stages are free swimming and the copepodid is the infective stage, attaching to the host's gill filaments, through the frontal filament. The subsequent chalimus stages (I through IV) "inherit" the copepodid's frontal filament, modifying its proximal end, such that the structure of the proximal end of the frontal filament explicitly identifies the stage of a chalimus. Two adult males were found attaching, by means of claws of its maxillipeds, to the frontal filament, left over by previous stages.
Conclusion. The number of developmental stages of *A. percarum* determined within the presently reported study is consistent with that hitherto found in the life cycles of other lernaeopodids.

Keywords: morphology, developmental stages, *Achtheres percarum*, fish parasite, copepod, perch, *Perca fluviatilis*

INTRODUCTION

Achtheres percarum von Nordmann, 1832 is a parasitic copepod that infects European perch, *Perca fluviatilis* L. The parasite shows distinct sexual dimorphism. The adult female attaches to the host using bulla and elongate arm-like second maxillae (Kozikowska et al. 1956). Females attach to gill arches, roof of the mouth, tongue, and rarely to gill filaments. The adult male is distinctly smaller than the adult female and can move about the surface of fish gills (Piasecki et al. 2006). Unlike in the majority of other lernaeopodids (Lernaeopodidae), the male of *A. percarum* is capable of independent living on its host (Piasecki, unpublished). Kozikowska et al. (1956) described and illustrated lesions associated with *A. percarum* infections on perch, said lesions (necrotic changes and tissue erosion) attributed to only those female copepods, found on gill filaments. Epithelium of neighbouring gill filaments, disturbed by these copepods, showed signs of hyperplasia, fusion of gill filaments, and substantial thickening of gill lamellae. *Achtheres sandrae* Gadd, 1901, infecting zander, *Sander lucioperca* (L.) have recently been declared a valid species by Kempter et al. (2006).

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The morphology of adult females of *A. percarum* was described by Kabata (1979) while the morphology of males of this copepod—by Piasecki et al. (2006).

The developmental stages of *Achtheres percarum* have not been described, except for its free-swimming stages (von Nordmann 1832, Claus 1861) and chalimus I stage (Claus 1861).

The presently reported study constitutes the first account on a complete set of developmental stages of *Achtheres percarum*, parasitizing European perch.

MATERIALS AND METHODS

To obtain nauplii and copepodids of *A. percarum*, naturally infected perch were purchased (autumn 1994, spring 1995) from commercial fishermen stationed at Stołczyn and operating on Lake Dąbie, Szczecin, Poland. Ovigerous females of *A. percarum* were collected from the perch and those with pigmented eggs were placed in 100-mL glass beakers filled with water. The water was replaced twice a day. The processes of hatching, as well as the first moulting, were periodically monitored under a dissecting microscope. All subsequent larval stages of *A. percarum*, representing the parasitic phase of development, were collected in April 1990 and in April 1995 directly from commercially caught perch captured in Lake Dąbie. Copepods were fixed and preserved in 75% ethanol, stained in lignin pink and observed in a suspended drop of lactic acid, a modification of the "wooden slide method" of Humes and Gooding (1964) was used to study the morphology of all larval stages, using a compound microscope (Olympus BX50). Details of morphology were drawn using a drawing tube of the microscope.

Terminology used to describe the morphology of larval stages follows that of Kabata (1979) (with the exception of caudal rami).

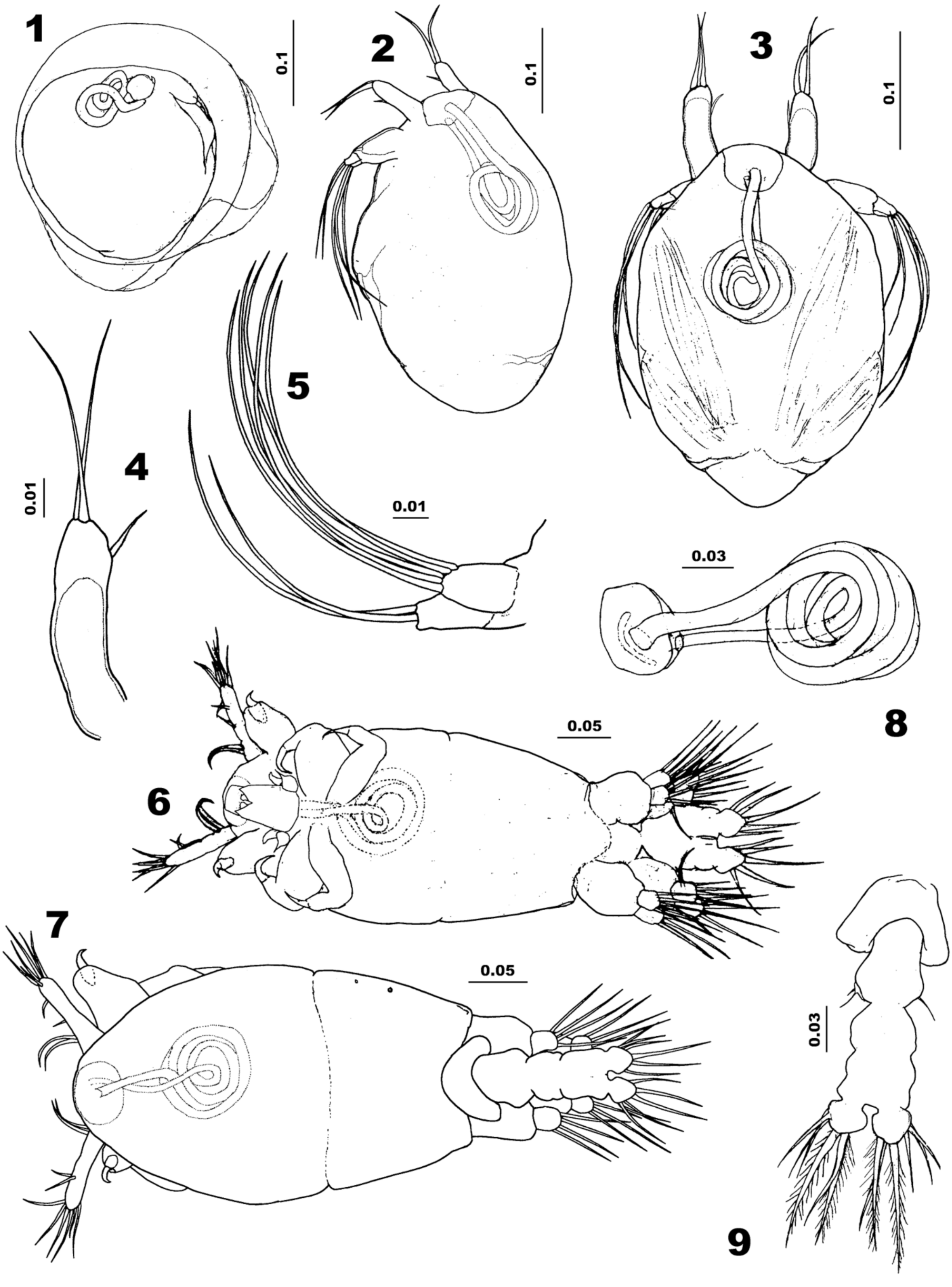
RESULTS

The life cycle of *Achtheres percarum* consists of the following stages: nauplius, copepodid, chalimus I, chalimus II, chalimus III, chalimus IV and adult. The nauplius (Figs. 1–5) and copepodid (Figs. 6–17) are free swimming. The copepodid is infective and all subsequent larval stages (each referred to as a chalimus) are attached to the gill filaments of the host by means of a long, partly coiled frontal filament. The adult female is permanently attached to the host at one location by means of a mushroom-shaped bulla. The adult male does not attach itself permanently at one location on the host.

All larval stages collected from the gills of perch were identified as chalimus I, chalimus II, chalimus III, chalimus IV (Figs. 18–67, 69, 70). They were attached by means of the frontal filament to the gill filaments. The structure of the proximal part of the filament permitted identification of respective chalimus stages. Each stage modifies the structure of the proximal end of the frontal filament, by adding a rounded "plug", secreted by the frontal filament of the larva (Figs. 63, 64, 66). The newly secreted plug consists of soft, poorly-stained "glue" lump and a harder, well stained "hard plug" with pocket-like canals for hosting the claws of the next stage's second maxillae (Fig. 65). The glue lump is attached to the original "plug" at the proximal end of the filament. All pre-moult chalimi (except chalimus IV female) featured a distinctly stained (in lignin pink) "plug" in their frontal area. Post-moult specimens din not have "plugs" and the "age" of respective stages was marked by the advancement of the plug formation. Thus, the blunt claws of the second maxillae of chalimus I were inserted into the original "plug" of the proximal filament's end, unchanged since its extrusion by the copepodid (Figs. 18, 23, 64). The filament of chalimus II featured two "plugs" (Figs. 28, 33), chalimus III—three "plugs" (Figs. 39, 44, 66), and chalimus IV—four "plugs" (Figs. 49, 55, 60, 67). The frontal organs of female chalimus IV produced no plug. No signs of the bulla production, needed by the female parasite at adult stage, were observed in the collected specimens of chalimus IV female. The sexual dimorphism was first visible at the stage of chalimus IV (Figs. 49, 60). Frontal organs of some male chalimus IV secreted new plug (Fig. 67), but no chalimus V was observed, holding the frontal filament featuring 5 plugs. Instead, two cases of the adult male were observed, holding the four-plug filament end by means of its maxillipeds (not second maxillae!) (Fig. 68). Only the tips of mandibles in all chalimus stages were inserted between labium and labium into the mouth cone. They could be easily withdrawn during moulting.

Nauplius. Females of *A. percarum* bear prominent, long, multiseriate egg sacs. The embryonic development of larvae within the egg sacs is marked by progressive pigmentation of these eggs. Shortly before hatching, the nauplius (first larval stage) is well visible inside the egg shell (Fig. 1). At the moment of hatching the nauplius is ready to moult, such that inside the cuticle of nauplius a fully formed copepodid can be seen (Figs. 2, 3). The copepodid, crammed into the tight semispherical confinement of the nauplius cuticle, is twisted into an s-shape with its abdomen bent dorsally (Fig. 2). The copepodid promptly started to emerge form the nauplius cuticle after hatching, however, the moulting process was delayed (indefinitely in some cases) under conditions in some of our study beakers.

Morphology of nauplius. Oval, unsegmented body at pre-moult stage. Body wider in dorsal view than in lateral view (cf. Figs. 2, 3) (Total length 319 ± 6 µm; 313–328 µm; $n = 6$). Fully formed frontal filament visible inside body (Figs. 2, 3). Distinct pigment pattern visible inside body (not illustrated). Anterior part of body with two pairs of setose, swimming appendages: first antenna and second antenna. Posterior end of body without balancers. First antenna (Fig. 4), uniramous, unsegmented, subcylindrical, digitiform, surmounted with two large, equal in length setae. Third, small seta located subterminally, on medial side. Second antenna biramous, subcylindrical (Fig. 5). Sympod long, unarmed. Rami relatively short, unsegmented. Endopod slightly longer than exopod. Exopod with 5 strong, long apical setae. Endopod with two long apical setae (shorter than those of exopod). Mandible absent.



Figs. 1–9. *Achtheres percarum*; nauplius (Figs. 1–5) and copepodid (Figs. 6–9); **Fig. 1.** Nauplius, in toto, inside egg; **Fig. 2.** Nauplius, in toto, dorso-lateral; note that the thin inner contour marks the copepodid, twisted in an s-shape, inside the nauplius cuticle (thicker, outer contour) **Fig. 3.** Nauplius, in toto, dorsal; **Fig. 4.** First antenna; **Fig. 5.** Second antenna; **Fig. 6.** Copepodid in toto, ventral; **Fig. 7.** Copepodid, in toto, dorsal; **Fig. 8.** Frontal filament; **Fig. 9.** Urosome, dorsal; scale bars in mm

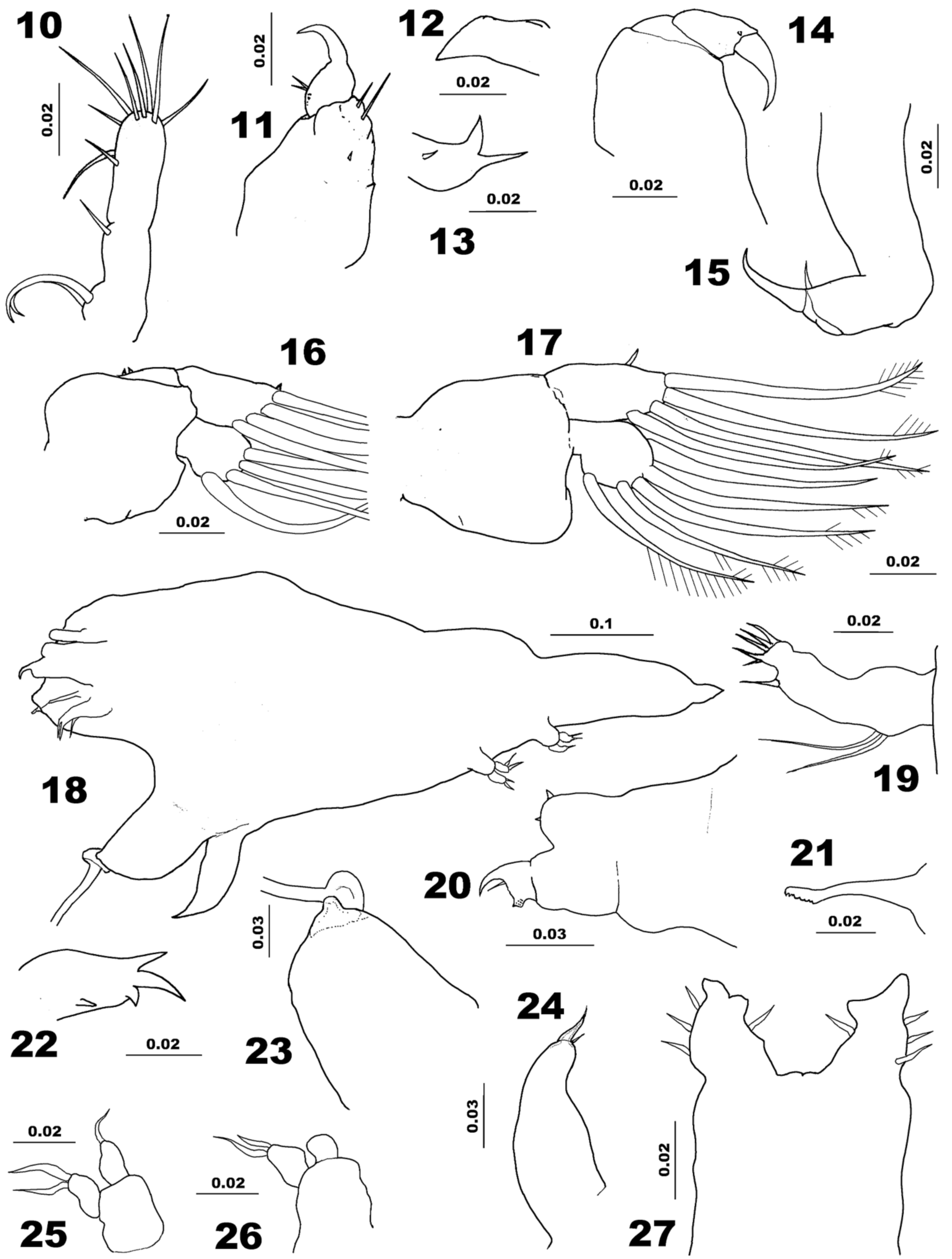
Copepodid (Figs. 6–17) is well adapted to its role as the infective stage. The frontal filament (larval attachment organ) with its distal attachment disc and proximal "plug" is visible inside the cephalosome (Figs. 6–8).

Morphology of copepodid. Body elongate, consisting of large prosome and small urosome (Figs. 6–7) (Total length without furcal setae 490 ± 13 µm; 478–503 µm; *n* = 3). Prosome subdivided by shallow constriction into slightly larger cephalosome (anterior) and less prominent metasome. Cephalosome, unsegmented, bullet shaped with antero-ventrally located mouth siphon, neighbouring 6 pairs of appendages: first antenna, second antenna, mandible, first maxilla, second maxilla, and maxilliped (Fig. 6). Posterior part of metasome with one pair of thoracopods (leg 1). Second pair of thoracopods (leg 2) on first free thoracic somite. Third, vestigial, pair of thoracopods (leg 3) located on first somite of urosome (Fig. 9). Well developed frontal filament visible through semi-transparent cuticle inside anterior part of body (Figs. 6, 7). Distinct pigment pattern visible inside body (not illustrated). Single, centrally located pigment patch on dorsal side associated with copepodid's eye, located under cuticle (not illustrated). Frontal filament consisting of proximal plug, distal attachment plug, and regularly coiled cylindrical filament proper (Fig. 8). Distal and proximal stretches of filament straight, central portion with 4–5 coils. Length of uncoiled filament exceeding length of copepodid's body (Fig. 38). Urosome elongate, consisting of unsegmented genital complex (bearing third pair of thoracopods) and two somites of abdomen without appendages. Last abdominal segment terminating as well developed furca (caudal rami) (Fig. 9).

First antenna (Fig. 10) elongate, cylindrical, indistinctly three segmented. Basal segment with two long setae, medial segment with short seta, and distal segment with 7 terminal and 2 subterminal setae. Second antenna prehensile, biramous (Fig. 11); sympod elongate, robust; exopod short, unsegmented, bulbous, armed apically with two small setae of different length and single minute setule at base. Endopod longer than exopod, armed with strong claw 1, two setae 5, and denticulate pad 4. Mandible (Fig. 12) short, uniramous, digitiform with triangular spatulate tip; appendage apparently non-functional (for feeding) and situated lateral to mouth cone. First maxilla (Fig. 13) short, unsegmented, biramous, dominated by endopod tipped by two conical setiferous processes. Minute exopod at base of endopod in form of small seta. Second maxilla (Fig. 14) prehensile, robust, subchelate. Sympod wider than long, unarmed. Subchela consisting of large, curved claw and shaft with small seta at base of claw. Maxilliped (Fig. 15) prehensile, subchelate. Sympod elongate, cylindrical, slender. Subchela large, well delimited, consisting of large, slim, curved claw and shaft with small seta at base of claw. Leg 1 (Fig. 16) biramous with flattened unsegmented sympod and unsegmented, spatulate rami. Exopod with 4 stout and long pinnate setae, located terminally and setule laterally. Endopod with 4 terminally located large pinnate setae. Leg 2 (Fig. 17) similar to leg 1 of this stage, with exception of medially extended lobe of sympod and five pinnate setae of endopod. Leg 3 (Fig. 9) reduced to inconspicuous tubercle tipped with 2 setules. Caudal ramus (Fig. 9) well developed with 3 lateral setae, two large plumose setae located terminally and 1 smaller medial seta.

Chalimus I. Body elongate, bulbous, unsegmented, but still divisible into prominent, wide prosome and narrower urosome (Fig. 18) (Total length 593 ± 55 µm; 506–673 µm; *n* = 10). Body with same appendages as copepodid (though without thoracopod 3), but mostly reduced in size and structure. Robust second maxillae permanently attached to frontal filament. Filament retaining tendency to coil back to its original configuration, thus possibly serving as spring shock absorber (also in subsequent stages). Siphonostome mouth cone well developed (Fig. 63). First antenna (Fig. 19) elongate and divided into three indistinct segments. First segments with 2 long medial setae. Second segment with small medial seta, near junction with terminal segment. Terminal segment very small, armed with 6 apical setae reduced in size. Second antenna (Fig. 20) similar in overall shape to previous stage, except for armature transformed and reduced in size. Tip of unsegmented, round exopod with single, small conical seta and small denticle. Two-segmented endopod; terminal segment with claw 1, seta 2, denticulate pad 4, and seta 5. Mandible (Fig. 21) uniramous, typical siphonostome (dental formula un-determined). First maxilla (Fig. 22) similar in shape and structure to that of previous stage; endopod tipped with three conical setiferous processes of unequal length (middle longest); exopod at base on endopod in form of single small seta. Second maxilla (Fig. 23), indistinctly subchelate, unsegmented, subcylindrical; blunt barbed claw inserted into plug of frontal filament base. Maxilliped (Fig. 24), subchelate, indistinctly segmented, subcylindrical. Claw very small, almost straight, with single small seta at base of claw. Leg 1 (Fig. 25) dramatically reduced in size and structure, biramous with flattened unsegmented sympod and unsegmented rami. Exopod small, tipped with 2 small setiferous outgrowths. Endopod with 1 setiferous outgrowth. Leg 2 (Fig. 26) similar to leg 1 of this stage, with exception to unarmed endopod. Caudal ramus (Fig. 27) with armament dramatically reduced in size (structure retained) and consisting of 3 lateral setules, 2 terminal, ill delimited digitiform processes, and medial seta.

Chalimus II. Body, bulbous, unsegmented, moderately elongate; more compact than chalimus I; not divisible into prosome and urosome (Fig. 28) (Total length 767 ± 71 µm; 648–883 µm; *n* = 13). Body with same appendages as chalimus I, though second maxillae being relatively longer and other appendages further reduced. First antenna (Fig. 29) subcylindrical, unsegmented, with single small seta (whip?) medially, near base and 4 apical setae (2 peripheral setae larger than those in centre). Second antenna (Fig. 30) as in chalimus I, but with reduced rami. Bulbous exopod with two small setae. Endopod two-segmented; terminal segment with claw 1,



Figs. 10–27. *Achtheres percarum*; copepodid (Figs. 10–17) and chalimus I (Figs. 18–27); **Fig. 10.** First antenna; **Fig. 11.** Second antenna; **Fig. 12.** Mandible; **Fig. 13.** First maxilla; **Fig. 14.** Second maxilla; **Fig. 15.** Maxilliped; **Fig. 16.** First leg; **Fig. 17.** Second leg; **Fig. 18.** Chalimus I, whole, lateral; **Fig. 19.** First antenna; **Fig. 20.** Second antenna; **Fig. 21.** Mandible; **Fig. 22.** First maxilla; **Fig. 23.** Second maxilla; **Fig. 24.** Maxilliped; **Fig. 25.** First leg; **Fig. 26.** Second leg; **Fig. 27.** Caudal rami; scale bars in mm

seta 2, and denticulate pad 4. Mandible (Fig. 29) uniramous, typical siphonostome, similar in shape and proportion to that of previous stage (dental formula un-determined). First maxilla (Fig. 32) similar in shape and structure to that of previous stage; endopod tipped with three conical setiferous processes, almost equal in length (middle longest); exopod at base on endopod in form of small bulbous outgrowth, tipped with single small seta. Second maxillae (Fig. 33) similar in shape and proportions to that of chalimus I, although differing in structure of frontal filament end, they hold to. Maxilliped (Fig. 34) unsegemented with conical tip (poorly delimited claw) and minute auxiliary spine below claw. Leg 1 (Fig. 35) papilla-like, surmounted with single small seta. Leg 2 (Fig. 36) papilla-like surmounted with 2 small setae. Caudal ramus (Fig. 37) digitiform and unarmed.

Chalimus III. Body elongate, slender, unsegmented. Distinct constriction dividing body into two major, almost equal parts: cephalosome and genital trunk. (Fig. 39) (Total length 1062 ± 61 µm; 969–1167 µm; *n* = 12). Body with same appendages as previous stage, with further reduction of all appendages except second maxillae. First antenna (Fig. 40) short (length comparable with that of this appendage in previous stage) and stout, unsegmented with single small seta (whip) medially near base. Apical armament consisting of 4 small setae of unequal length. Second antenna (Fig. 41) biramous, with better developed rami than those in chalimus II. Bulbous exopod with two small setae. Endopod two-segmented; terminal segment with large claw 1, seta 2, pad 4, and minute, conical seta 5. Mandible (Fig. 42) uniramous, typical siphonostome, similar in shape and proportion to that of previous stage (dental formula un-determined). First maxilla (Fig. 43) similar in shape and structure to that of previous stage. Second maxillae (Fig. 44) similar in shape and proportions to that of chalimus I, although differing in structure of frontal filament end, they hold to. Maxilliped (Fig. 45) similar to that of previous stage; minute seta at base of claw. Leg 1 (Fig. 46) reduced to small seta. Leg 2 (Fig. 47), reduced to 2 small setae. Caudal ramus (Fig. 48), digitiform and unarmed.

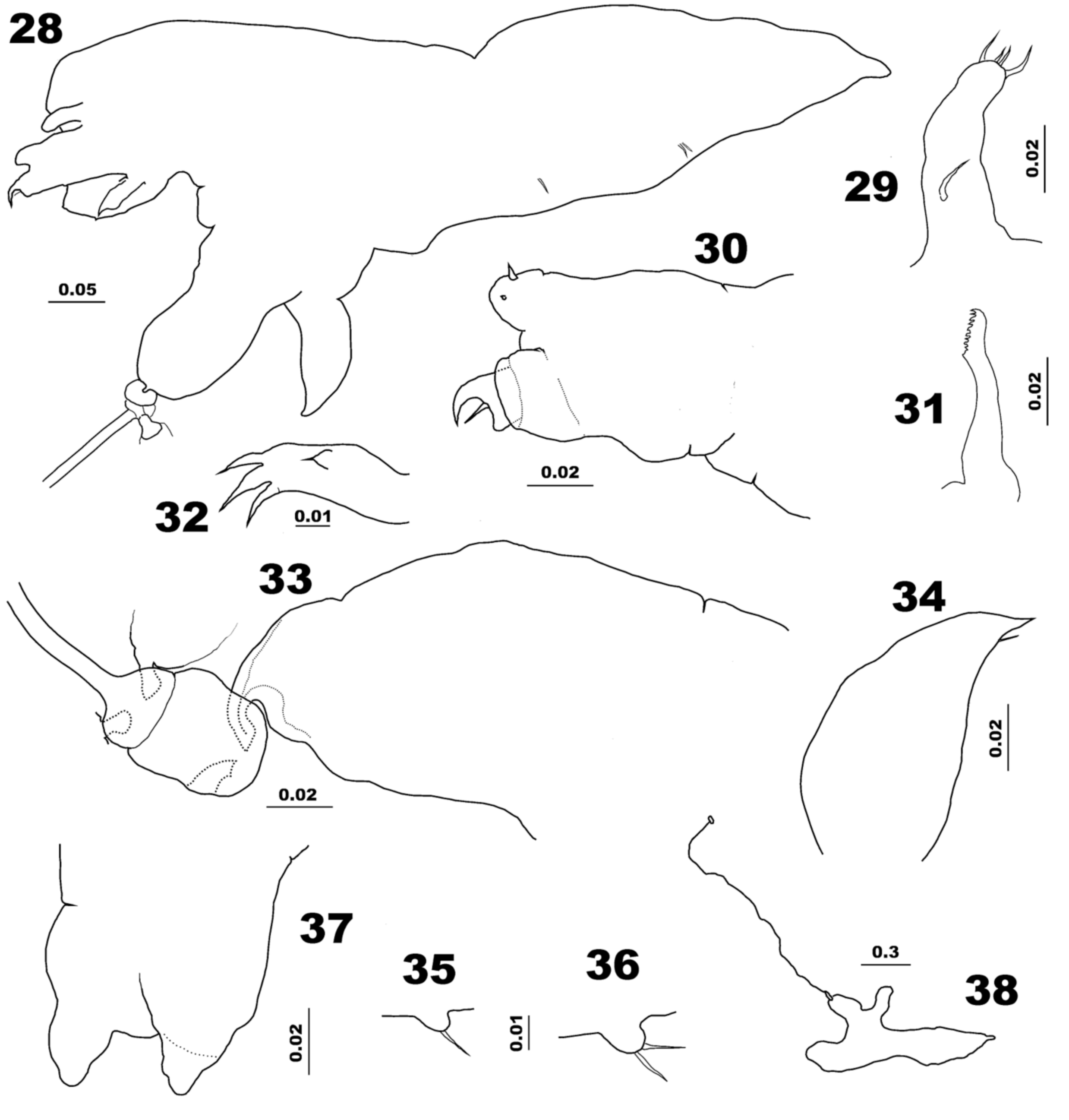
Chalimus IV. Body similar to chalimus III (Figs. 49, 60), but relatively more elongate and with different proportions of second maxilla and maxilliped. Relative proportions of those appendages and body proper differentiating male and female. Attachment to host through second maxillae and frontal filament. Female distinctly elongate (Fig. 49) (Total length 1521 ± 112 µm; 1296–1654 µm; *n* = 8) with very long second maxillae. Maxillary canals of bulla attachment system (of next stage) with string-like structure (Fig. 69) visible inside pre-moult females. Male specimens more compact, with short second maxillae (Fig. 60) (Total length 1318 ± 185 µm; 1148–1580 µm; *n* = 4). Their frontal organ capable of secreting new plug for frontal filament (Fig. 67). Both sexes with distinctly developed dorsal shield (male: Fig. 67; female: not illustrated). First antenna of female (Fig. 50) unsegmented, surmounted with 3 small setae. First antenna of male (Fig. 60a) more stout, with 4 apical setae. Reduced whip at base of appendage. Second antenna (Fig. 51) biramous with well developed rami. Bulbous exopod with two distinct small setae. Endopod two-segmented; terminal segment with large claw 1, seta 2, denticle 3, pad 4, and small seta 5. Mandible (Figs. 52, 53) uniramous, typical siphonostome, similar in shape and proportion to that of previous stage (dental formula: P2, S1, B5). First maxilla (Fig. 54) similar in structure to that of previous stages, but with distinctly larger setiferous processes of endopod and relatively smaller exopod. Second maxillae (Fig. 55) similar in shape and proportions to that of chalimus I, although differing in structure of frontal filament end, they hold to. Second maxilla in female (Figs. 49, 55), strongly elongate, arm-like. That of male short and stout (Fig. 60). Maxilliped of respective sexes differing in shape and structure. Maxilliped in female (Fig. 56) prehensile, heavily sclerotized, functionally and structurally subchelate, distinctly divided into sympod and subchela. Subchela elongate, cylindrical with seta on medial margin, divided into larger shaft and smaller claw; shaft with two small setae one at base of claw and one near junction with subchela. Base of maxilliped with poorly developed internal sclerite linking it to second maxilla base. (Figs. 67, 70). Maxilliped of male (Fig. 61) similar to that of previous stage but differing in having prominent bulbous outgrowth medially. Base of maxilliped with internal sclerite linking it to second maxilla base. (Fig. 67). Leg 1 (Fig. 57) reduced to, small seta. Leg 2 (Fig. 58) reduced to 2 small setae. Caudal ramus digitiform; in female (Fig. 59) with fine apical denticulation; in male (Fig. 62) with 3 small apical tubercles.

Adult male: Total length of specimens attached to frontal filament: 1593 and 1630 µm.

DISCUSSION

Copepods of the order Siphonostomatoida are quite consistent regarding two features of their development, namely the shortening of the nauplius phase and the conservation of the copepodid number. The number of nauplius stages ranges from two in *Caligus elongatus* (Caligidae)(cf. Piasecki and MacKinnon 1995) to one (at the pre-moult stage) in *Tracheliastes maculatus* (Lernaeopodidae)(cf. Piasecki 1989), or oven total absence (nauplius in egg only) in *Salmincola californiensis* (Lernaeopodidae) (cf. Kabata and Cousens 1973). Larval stages of many of siphonostomatoid families use the frontal filament as an organ of permanent attachment to the hosts. The filament is absent in *Dichelesthium oblongum* (Abildgaard, 1794) (Dichelestheidae)(cf. Kabata and Hodorevskij 1977) and *Lernanthropus kroyeri* van Beneden, 1851 (Lernanthropidae)(cf. Cabral et al. 1984). In siphonostome copepods using the frontal filament the first (infective) copepodid is free-swimming, while the following four copepodids are attached by means of the frontal filament. The attached larval stages of siphonostome copepod are referred to as chalimus stages. Consequently: copepodid II = chalimus I, copepodid III = chalimus II, copepodid IV = chalimus III, and copepodid V = chalimus IV. The notable example of the number of "copepodid" stages exceeding five was observed in *Lepeophtheirus salmonis* (Krøyer, 1838) (Caligidae) (cf. Johnson and Albright 1991). The latter species features one copepodid, four chalimi and two preadults.

There have been few successful attempts to describe complete life cycles of lernaeopodid copepods representing the "freshwater branch". Zandt (1935) studied the life cycle of *Salmincola coregonorum* (Kessler, 1868). Kabata and Cousens (1973) described the life cycle of *Salmincola californiensis* (Dana, 1852), while Piasecki (1989)—the life cycle of *Tracheliastes maculatus* Kollar, 1835. The life cycles of the following marine lernaeopodids were described: *Alella macrotrachelus* (Brian, 1906)(cf. Caillet 1979, Kawatow 1980); and *Parabrachiella lata* (Song et Chen, 1976)(cf. Ho et al. 2007). Partial descriptions of lernaeopodid life cycles were published by: Heegaard (1947)—studying *Clavella adunca* (Strøm, 1762); and Kabata (1964)—studying *Vanbenedenia chimerae* (Heegaard, 1962). Early, free-swimming stages of lernaeopodid copepods were studied by: von Nordmann (1832), Kollar (1835),



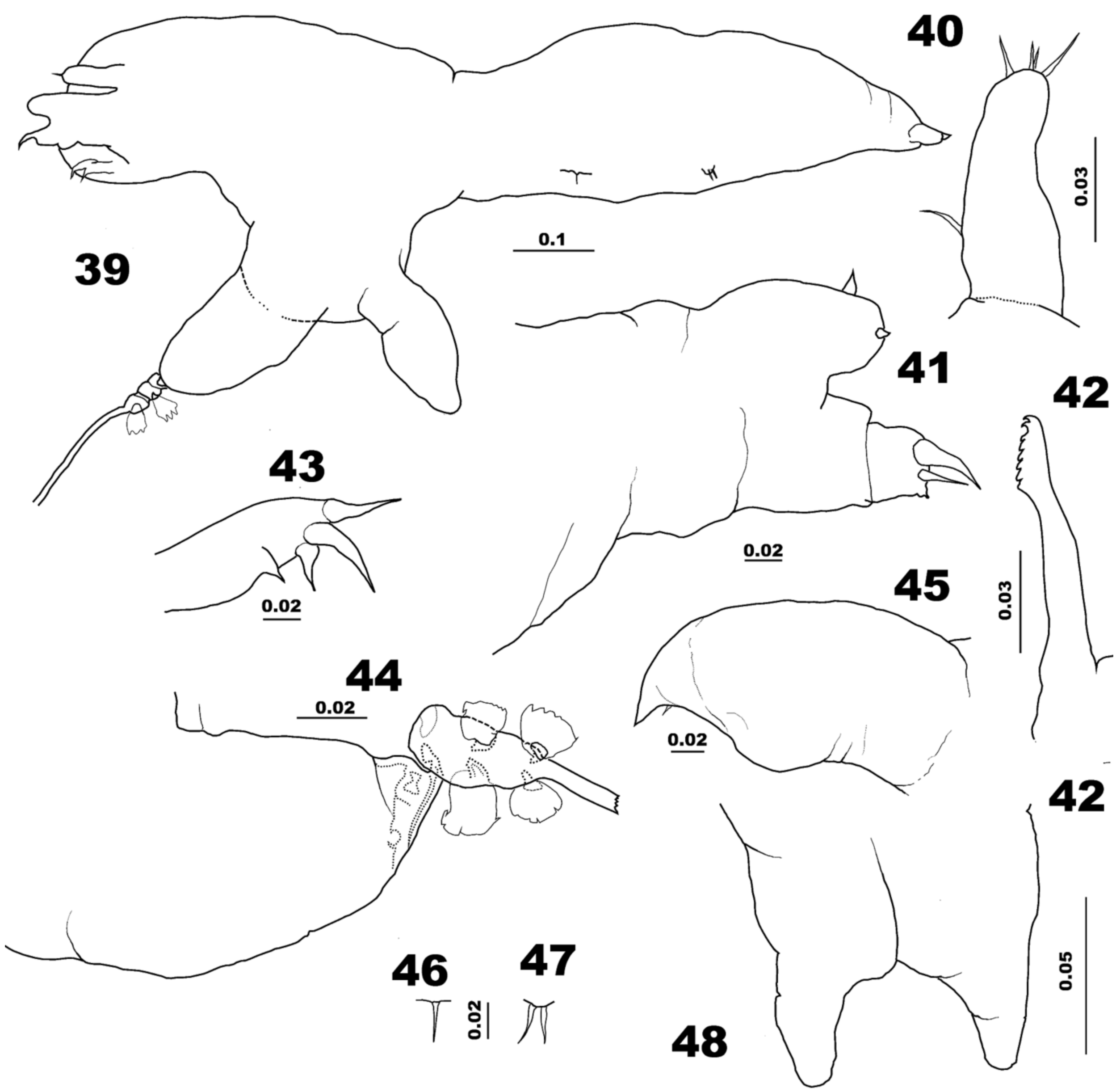
Figs. 28–38. *Achtheres percarum*; chalimus II (Figs. 28–37) and chalimus III (Fig. 38); **Fig. 28.** Chalimus II, in toto, lateral; **Fig. 29.** First antenna; **Fig. 30.** Second antenna; **Fig. 31.** Mandible; **Fig. 32.** First maxilla; **Fig. 33.** Second maxilla; **Fig. 34.** Maxilliped; **Fig. 35.** First leg; **Fig. 36.** Second leg; **Fig. 37.** Caudal rami; **Fig 38.** Silhouette of chalimus III and full length of frontal filament; scale bars in mm

Turner and Wilson 1862, van Beneden (1870), Wilson (1911), Fasten (1919), Fasten (1921), Gurney (1934), Savage (1935), Dedie (1940), Friend (1941), Wilkes (1966), Shotter (1971), Kabata (1976), Chandran and Balakrishnan Nair (1980), and Kabata (1987).

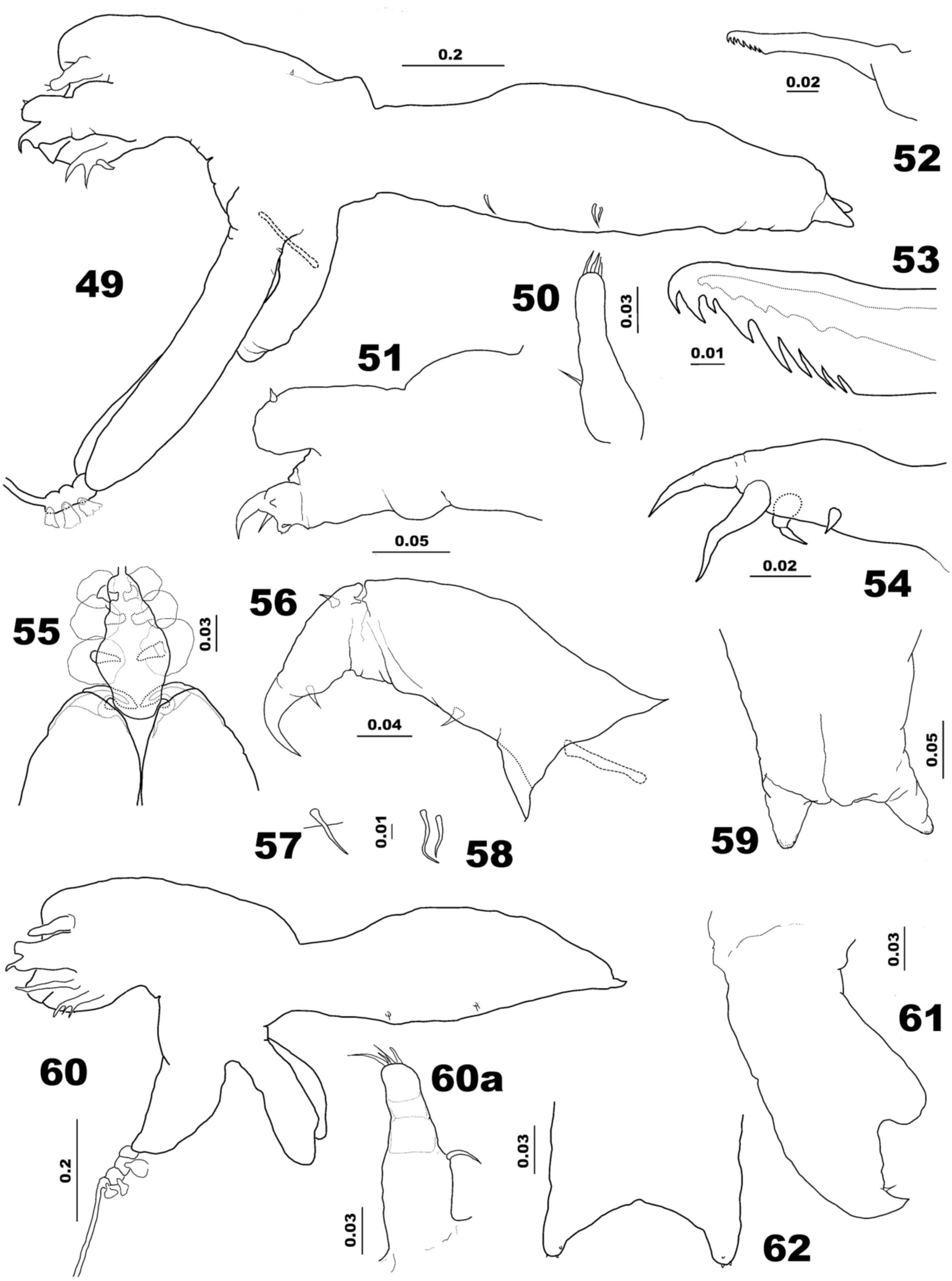
A quite puzzling life cycle of *Clavella adunca* (Strøm, 1762) (Lernaeopodidae) was reported by Heegaard (1947). He insisted that the chalimus phase in that species was replaced by a "pupa". The Heegaard's "pupa" is attached by the frontal filament and it looks like a chalimus enclosed inside a shed cuticle of the previous stage. Other life cycles of the copepods representing the *Clavella* branch were described by Kawatow et al. 1980 (*Alella macrotrachelus*) and by Ho et al. (2007) (*Parabrachiella lata*). They both reported a copepodid followed by four chalimus stages. Therefore the findings of Heegaard (1947) should be revised.

Two nauplius stages observed by Zandt (1935) provide an interesting exception among lerneaopodid life cycles and as such should be verified .

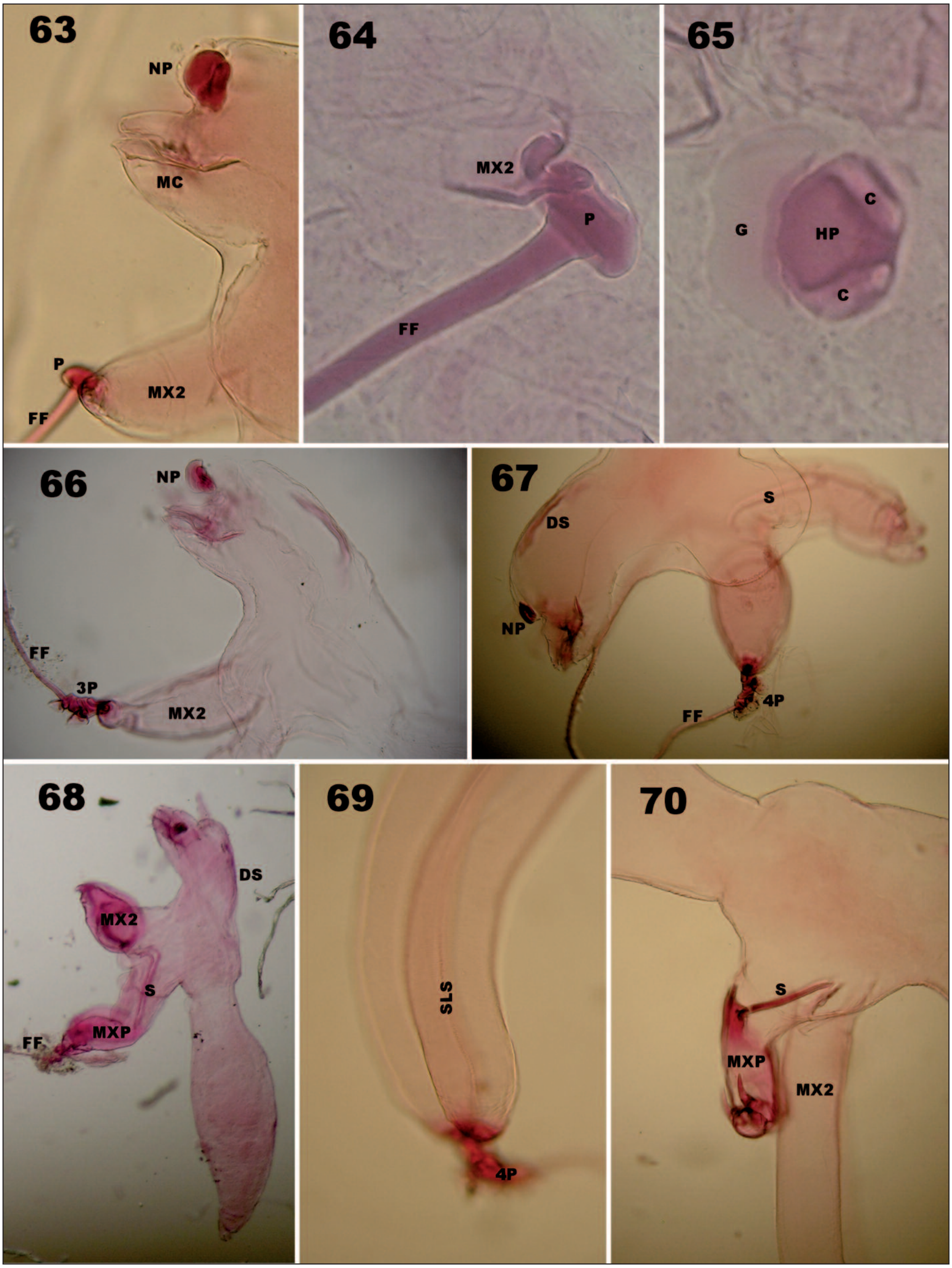
The observed life cycle of *Achtheres percarum* features a pre-moult nauplius stage. On the other hand Kabata and Cousens (1973), studying the life cycle of a closely related *Salmincola californiensis*, observed that



Figs. 39–48. *Achtheres percarum*; chalimus III; **Fig. 39.** In toto, lateral; **Fig. 40.** First antenna; **Fig. 41.** Second antenna; **Fig. 42.** Mandible; **Fig. 43.** First maxilla; **Fig. 44.** Second maxilla; **Fig. 45.** Maxilliped; **Fig. 46.** First leg; **Fig. 47.** Second leg; **Fig. 48.** Caudal rami; scale bars in mm



Figs. 49–62. *Achtheres percarum*; chalimus IV female (Figs. 49–59) and chalimus IV male (Figs. 60–62); **Fig. 49.** Chalimus IV female, in toto, lateral; **Fig. 50.** First antenna; **Fig. 51.** Second antenna; **Fig. 52.** Mandible, in toto; **Fig. 53.** Mandible, denticulate blade; **Fig. 54.** First maxilla; **Fig. 55.** Second maxilla; **Fig. 56.** Maxilliped; **Fig. 57.** First leg; **Fig. 58.** Second leg; **Fig. 59.** Caudal rami; **Fig 60.** Chalimus IV male, in toto, lateral; **Fig. 60a.** First antenna; **Fig. 61.** Maxilliped; **Fig. 62.** Caudal rami; scale bars in mm



Figs. 63–70. *Achtheres percarum*; selected structural elements of chalimi and adult male; **Fig. 63.** Chalimus I; anterior part lateral; new plug visible at frontal area; **Fig. 64.** Chalimus I; claw of second maxilla inserted into the plug of proximal part of frontal filament; **Fig. 65.** New plug formed by chalimus I for chalimus II; ; **Fig. 66.** Pre-moult Chalimus III; anterior part lateral; **Fig. 67.** Pre-moult Chalimus IV male; anterior part lateral; **Fig. 68.** Adult male; whole lateral; note maxillipeds holding frontal filament; **Fig. 69.** Chalimus IV female; Second maxillae attached to frontal filament; **Fig. 70.** Second antenna; **Fig. 52.** Chalimus IV female; Second maxillae and maxillipeds; abbreviations: NP, new plug; MC, mouth cone; P, plug; MX2, second maxilla; FF, frontal filament; G, "glue lump"; HP, "hard plug"; C, canals; 3P, 3-element filament end; 4P, 4-element filament end; DS, dorsal shield; SLS, string-like structure

the larva hatching from the egg is the copepodid. The apparent difference may result from different egg incubation regimes in both experiments. In the presently reported study, the eggs were incubated in water-filled beakers. Even though the water was changed periodically, we suspect that the gas exchange between the water and the eggs might have been inadequate. Among the factors contributing to the gas exchange during egg incubation, an important factor could be the speed of water flowing over the eggs. Under natural conditions, the respiratory movements of the fish subject the gill-attached females of *A. percarum* to a substantial water flow. Therefore, we suspect that the gas exchange on the gill location is much more intensive than that in the still water of an experimental beaker. Consequently, an inadequate oxygenation during embryonic development may probably delay the normal course of events, producing nauplii in the life cycles where under natural conditions, hatching copepodids would be otherwise observed (leaving nauplius exuvium inside the egg shell). The above reasoning constitutes an attempt to explain contradicting reports of lernaeopodids hatching as a nauplius or as a copepodid.

In all four life cycles (including the presently described *Achtheres percarum*) the nauplii have only two appendages (first antenna and second antenna) and they lack balancers on their posterior ends. The occasional reports about alleged third pair of appendages in lernaeopodid nauplii were probably appendages of the copepodid, visible through the transparent cuticle of pre-moult nauplii (Fig. 2).

The presently observed "string-like structure" inside maxillary canals was reported earlier for chalimus IV female of *Tracheliastes maculatus* by Piasecki (1989). They may pleas some role in the moulting process, temporarily securing the link between newly emerged adult female and the shed cuticle of chalimus IV.

The structure of frontal organ in adult females of *Achtheres peracarum* was described by Piasecki (1993). Stages of bulla formation in *Tracheliastes maculatus* (Lernaeopodidaee) were described by Piasecki (1989).

Another structure worth attention was the internal sclerite linking the maxilliped base to second maxilla base, observed in both sexes of chalimus IV (Figs. 67, 70). It was earlier reported for adult males of *A. percarum* by Piasecki et al. (2006).

The overall structure and arrangement of larval appendages were similar among the four species described. Also the structure of the frontal filament is a common feature of all four species studied. It enables explicit identification of individual chalimus stages, the feature observed also in the genus *Caligus* (cf. Piasecki and MacKinnon 1993).

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