

Swimming and Cleaning in the Free-Swimming Phase of *Argulus* Larvae (Crustacea, Branchiura)—Appendage Adaptation and Functional Morphology

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ABSTRACT The free-swimming early larval stages of *Argulus foliaceus* (Linnaeus) (Branchiura) are studied using digital video, light microscopy, and SEM. We analyze and document the mode of swimming in the hatching stage of *A. foliaceus* and the subsequent juvenile stages with fully developed thoracopods. We present new observations and an analysis of the functional morphology of a cleaning behavior in the first stage. This stage swims very efficiently using the large exopods of the second antennae in concert with the mandibular palp (naupliar limbs), while the subsequent stages use the now developed thoracopods for propulsion. This posterior shift in propulsion is similar to—but independent from—what is seen in other crustaceans. The hatching stage has previously been referred to as a “metanauplius” but as the first and second maxillae are developed and active, and buds of all four thoracopods are present, it is too advanced to be included in the naupliar phase. The hooks of the first antennae and the distal hooks of the maxillae are demonstrated to function not only as attachment organs (to the host), but also to play a significant role in the cleaning of the naupliar swimming appendages. A digital video-based analysis of the swimming mode is provided. The larval swimming pattern is generally similar to that of other crustaceans such as Branchiopoda and Cirripedia, but autapomorphies of the Branchiura include the following: 1) While actively swimming, the naupliar appendages are almost straight during the recovery stroke and 2) they have a relatively small deflection during movement (~25° or ~35° for mandible and second antenna respectively), 3) the larval mandible has a uniramous palp which is the retained exopod. The morphological implications of the transition from the possibly nonfeeding pelagic, or free-swimming, first larval stage to the feeding, parasitic second stage are discussed and compared with other crustaceans. *J. Morphol.* 268:1–11, 2007. © 2006 Wiley-Liss, Inc.

KEY WORDS: SEM; larval development; ontogeny; larval swimming; parasitism

The majority of investigations on comparative functional morphology within Crustacea focus on adults. In a phylogenetic context, this approach has often proved inadequate because of the great morphological disparity of adult Crustacea. Surprisingly little research has focused on the functional morphology of nauplii considering the importance of the (ortho)nauplius as an autapomorphy of Eucrusta-

cea¹ and, therefore, as one of the links between the major in-group taxa. With regard to this scarcity of research, Branchiura (fish lice) matches the above situation well. The Branchiura is a small group of parasitic Crustacea comprising around 210 species assigned to four genera: *Argulus* Müller, 1785, *Dolops* Audouin, 1837, *Chonopeltis* Thiele, 1900, and the monotypic *Dipteropeltis* Calman, 1912. The vast majority of described species are ectoparasitic on freshwater fish (see e.g., Meehan, 1940; Yamaguti, 1963; Thatcher, 1991; Avenant-Oldewage and Knight, 1994). Approximately 10 species are marine/estuarine forms, but some of these species also occur in freshwater (see e.g., Thorell, 1864; Avenant-Oldewage and Oldewage, 1995; van As et al., 1999; Schram et al., 2005). Free-swimming hatching larvae are found in the species of *Argulus*, *Dolops*, and *Chonopeltis*, while the ontogeny in *Dipteropeltis hirundo* is still unknown (Wilson, 1902; Fryer, 1956, 1961, 1964, 1965). The larval stages of *A. japonicus* are well-known and have been described by various authors (Tokioka, 1936; Lutsch and Avenant-Oldewage, 1995). The developmental sequence of *A. foliaceus* was described in detail by Rushton-Mellor and Boxshall (1994), but the first descriptions date back to Claus (1875). Martin (1932) presented details of the mouth cone morphology (including ontogeny) of *A. viridis*. More recently, Gretszy et al. (1993) provided

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¹In this article we briefly compare Branchiura to various ‘Orsten’ crustaceans, including some placed on the crustacean stem lineage. We employ the term Eucrustacea when specifically referring to the Crustacea crown group = Eucrustacea (*sensu* Walossek, 1999; see also Waloszek, 2003a,b).

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morphological details of the adult cephalic appendages of *A. japonicus*. Gretsny et al. (1993) also presented one of the only reconstructions of the larval mouth cone, but generally detailed descriptions of the functional morphology of the larval *Argulus* are scarce.

We analyze and document the functional morphology of swimming in the hatching stage (Stage I) and the (first) juvenile Stage II of *Argulus foliaceus*. We present new observations and analyses of a cleaning-behavior in the earliest stage. The general similarities between the “metanauplius” of *Argulus* and nauplii or metanauplii of other Eucrustacea make this stage in the development of the Branchiura the most promising to study in a general crustacean comparative and phylogenetic framework.

MATERIALS AND METHODS

Live *Argulus foliaceus* (Linné, 1758) larvae were collected using a plankton net (mesh size 63 μm) in an exhibition tank at the Danish National Aquarium, Charlottenlund. Egg strings were scraped off the front glass of the same tank using a razor blade. Larvae were kept in aerated glass aquaria in the lab. Larval Stages I, II, and III were observed in a standard Leica dissection microscope (Leica MZ95) using variable magnifications. Identification of stages followed Rushton-Mellor and Boxshall (1994). Video sequences were recorded on the microscope with a color Sony 3CCD camera ExwaveHAD DXC-C33P on miniDV media using a Panasonic NV-DV2000 recorder. The single image sequences were extracted using VirtualDub software (freely available from <http://www.virtualdub.org>) and color-corrected using image manipulation software.

All specimens were transferred to clean water (duration minimum 30 min) before fixing in standard aldehyde fixatives (2.5% glutaraldehyde/5% formalin). Before observing in LM, some specimens were made translucent using lactophenol. The light microscope used was a Leica DMRXA, fitted with an Evolution MP digital camera using ImagePro software, including the EDF-stack (Enhanced Depth of Field) algorithm. Specimens for SEM, some dissected, were brought through a graded alcohol series and were critical-point-dried in acetone on a Bal-Tec 030 CPD. The dry specimens were mounted on SEM stubs and observed in a JEOL JSM-6335-F SEM. All images were saved and processed digitally.

RESULTS

General

In *Argulus foliaceus*, the swimming and cleaning of Stage I and II are fundamentally different from each other. Stage I, the hatching stage, uses the exopod of the second antenna (antennal exopod in the following) and the mandibular palp for propulsion (i.e., the second and third naupliar appendages) as in the nauplii and metanauplii of eucrustaceans in general (Figs. 1A–C and 2A,C,F–J). The first thoracic appendage is an advanced limb bud capable of slight movements though not contributing in any way to the propulsion (Fig. 2A). By Stage II (juvenile) propulsion has been transferred to the thoracic appendages, as in the adult (Fig. 2B,D,E).

Propulsion, Structure, and Function of Stage I

The second antenna is biramous with a small, three-segmented endopod, and a much longer,

unsegmented exopod equipped with four long and one short terminal setae (Figs. 1C and 2A,F,I,J). The long setae reach considerably beyond the margins of the head-shield. They are plumose with long setules, most likely increasing the grip in the water (Figs. 1C and 2J). The sockets of the long terminal setae are clearly separated from each other (Fig. 2F). The only externally visible part of the mandible is the palp. The palp consists of (at least) two segments, as is indicated by the capability of angular flexion (Figs. 2H,I and 6B). Three long, plumose setae arise terminally from the palp in a manner similar to that of the antennal exopod (compare Fig. 2F,G), suggesting that the mandibular palp is the retained exopod. Also similar to the antennal exopod, the terminal setae of the mandibular palp reach well beyond the shield margins (Figs. 1C and 2A). A mandibular coxal process is present, but concealed within the mouth tube. It is elongate, flat, and sickle-shaped with distal spines (dissected free in Fig. 6D). The remaining part of the mandible has not been examined.

Stage I swimming. The short power stroke of the mandibular palp initiates the swimming cycle (Fig. 3A). The power stroke of the antennal exopod follows shortly, while the mandibular palp (exopod) remains at rest (Fig. 3B,C). When viewed anteriorly, the long terminal setae on the antennal exopod fan out the setules in a paddle-like outline (not illustrated). The recovery strokes—which finish the cycle—are simultaneous in both pairs of appendages, causing the larva to stop or even move slightly backwards (Fig. 3D). The angles of limb deflection are quite small. As illustrated in stick-models (see Fig. 3), the antennal exopods only swing through $\sim 35^\circ$ of a full circle during the cycle. The mandibular palps have an even smaller deflection, only $\sim 25^\circ$, and it is executed faster than the swing of the antennal exopod (Fig. 3A–D) and supplementary materials. Also characteristically, the limbs are not greatly bent but appear rigid, during both the power and the recovery strokes. Thus, the forward movement can be dissected into a small thrust by the mandibular palps (exopods), and a larger, more powerful main thrust by the antennal exopods. The cycle in Figure 3A–D lasts 0.16 s (4 frames of 1/25 s), and illustrates a typical swimming speed of the larvae. This speed can be varied considerably depending on the circumstances. With increased speed, the movements and angles of the swimming appendages remain similar to the already described, only with an increase in frequency.

Propulsion, Structure, and Function Stage II

In Stage II, the four pairs of thoracopods are all well-developed biramous appendages resembling the adult state in most details (Fig. 1D–F). Characteristically, the thoracopods are oriented later-

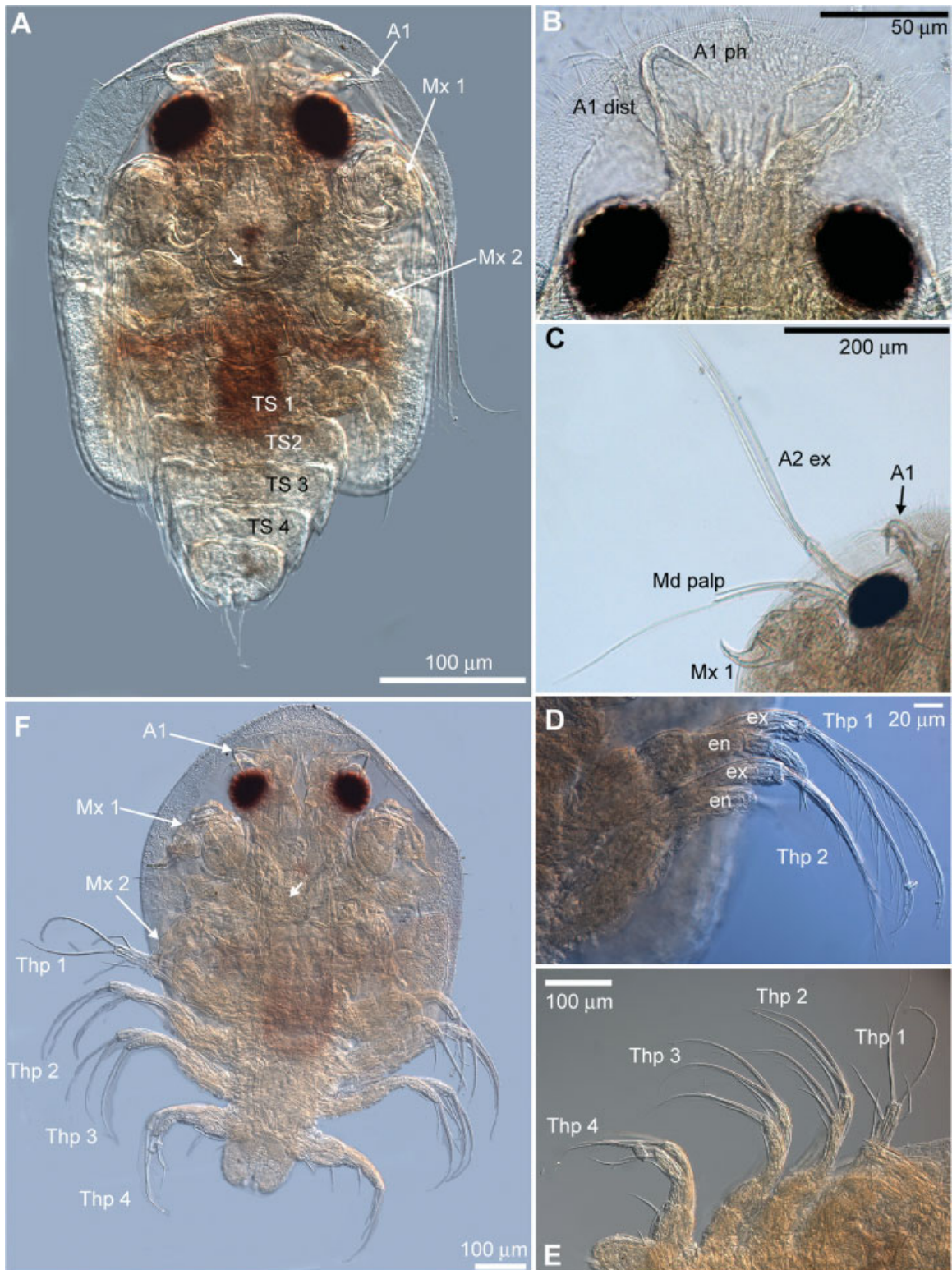


Fig. 1. **A–C:** *Argulus foliaceus*, Stage I. **D–F:** Stage II. LM. **A:** Ventral view. **B:** First antennae, ventral view. **C:** Swimming appendages (second antennae, mandibles) full length, ventral view. **D:** Thoracopods 1 and 2, ventral view (fused EDF-stack). **E:** All four thoracopods, ventral view (fused EDF-stack), same specimen as E. Arrow indicates mouth opening. *A1*, first antenna; *A1 dist*, first antenna distal part; *A1 ph*, first antenna proximal hook; *A2 ex*, second antenna exopod; *en*, endopod; *ex*, exopod; *Md palp*, mandibular palp; *Mx 1*, first maxilla; *Mx 2*, second maxilla; *Thp 1–4*, thoracopods 1–4; *TS 1–4*, thoracic segments 1–4.

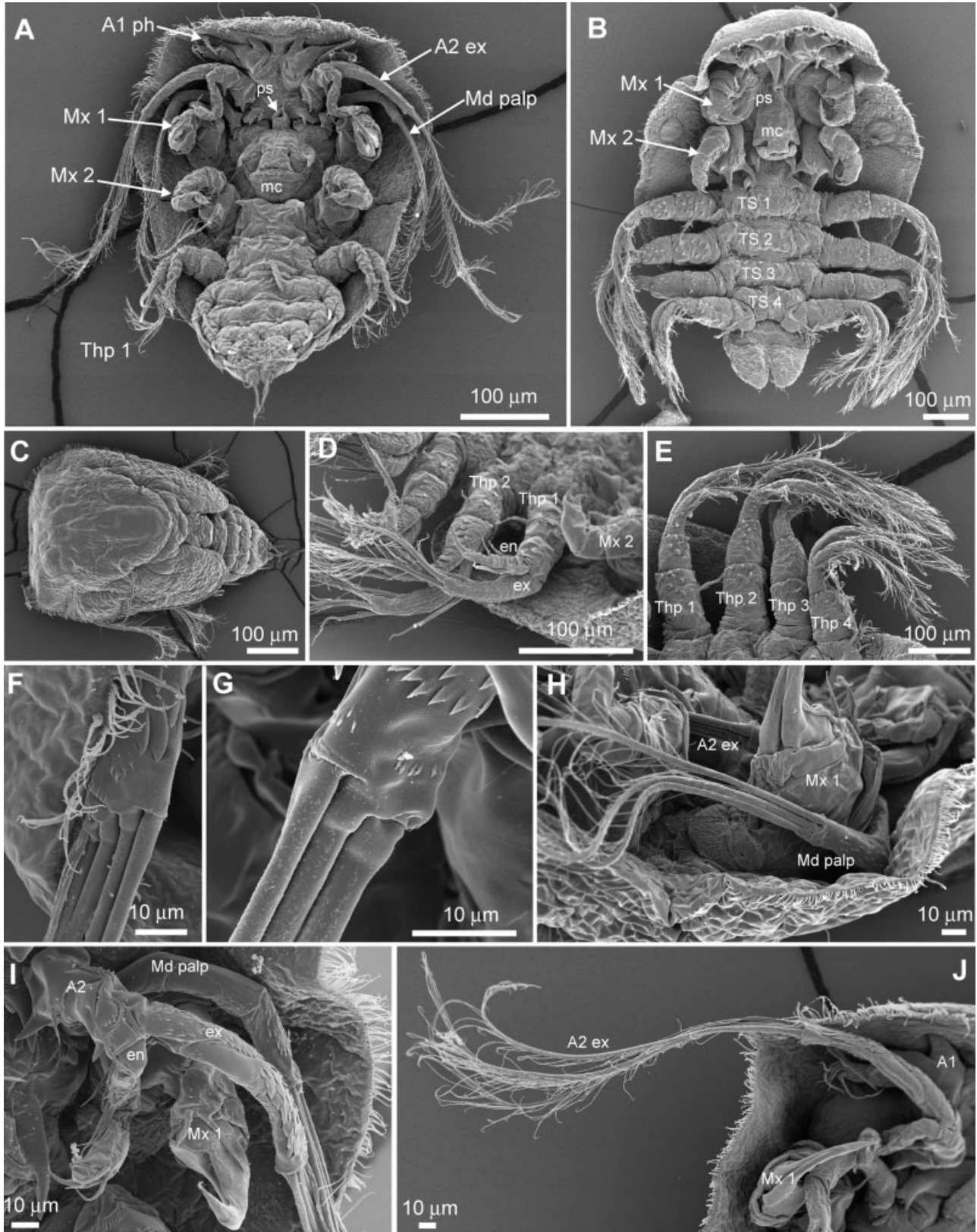


Fig. 2. **A,C–J:** *Argulus foliaceus* Stage I. **B,D–E:** Stage II. SEM. **A:** Ventral view. **B:** Ventral view. **C:** Dorsal view. **D:** Thoracopods, antero-lateral view. **E:** Thoracopods, dorsal view. **F:** Second antenna exopod, socket of terminal setae. **G:** Mandibular palp, sockets of terminal setae. **H:** Swimming appendages and first maxilla, lateral view. **I:** Second antenna, mandibular palp, and first maxilla, dorsal view. **J:** Full length of second antenna exopod, ventral view. *A1*, first antenna; *A1 ph*, first antenna proximal hook; *A2 ex*, second antenna exopod; *en*, endopod; *ex*, exopod; *mc*, mouth cone; *md palp*, mandibular palp; *Mx 1*, first maxilla; *Mx 2*, second maxilla; *ps*, preoral stylet; *Thp 1–4*, thoracopods 1–4; *TS 1–4*, thoracic segments 1–4.

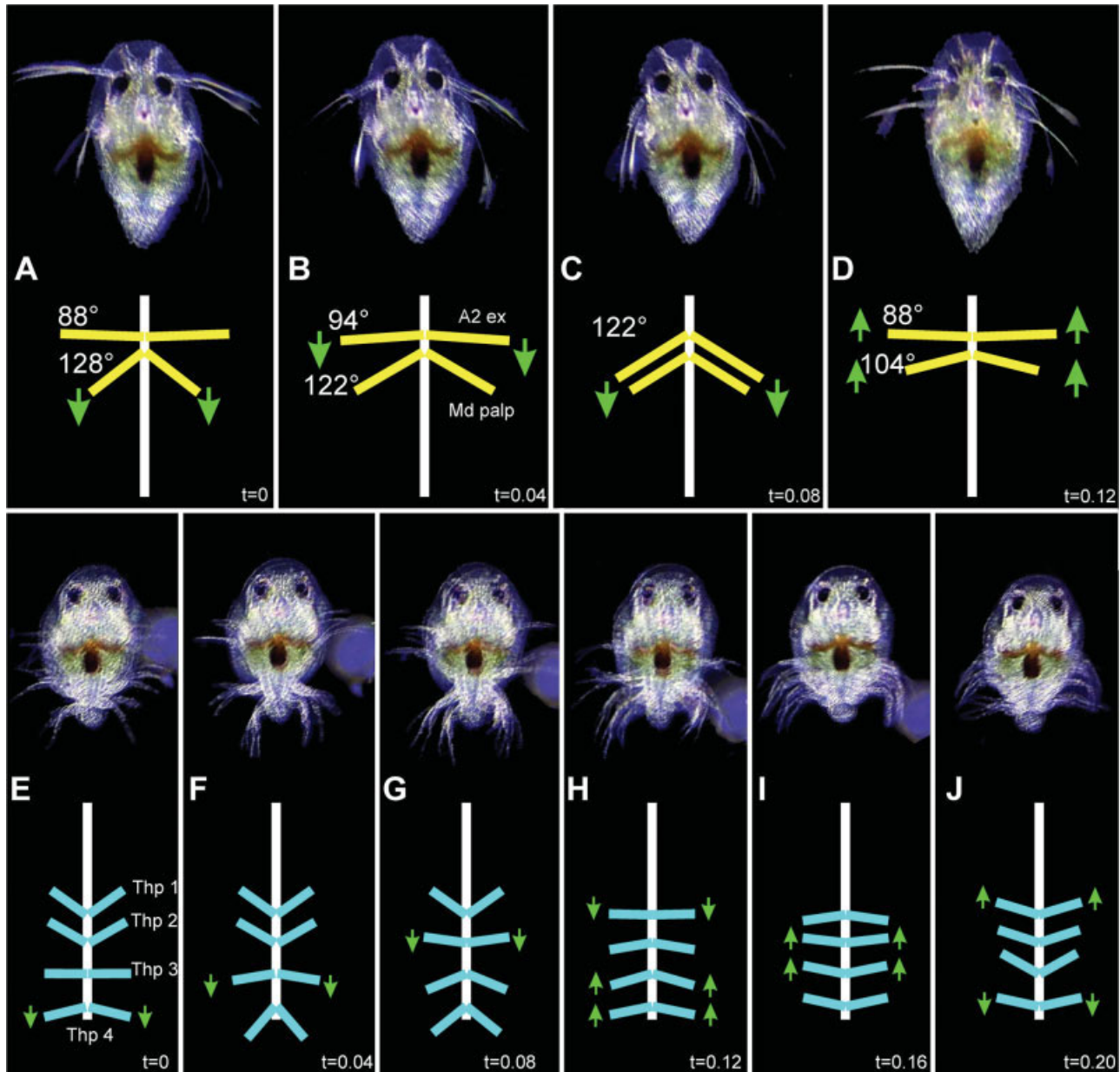


Fig. 3. *Argulus foliaceus* Stage I (A–D), and Stage II (E–J). Frame-by-frame sequence showing (in ventral view) motion of swimming appendages in a single specimen. 0.04 s between frames (25 fps). Stick models: Only long axis and swimming appendages (second antennae, mandibles) depicted. Arrows indicate movement of appendages, angles measured as deflection from long axis (anterior). A–D: Full cycle of swimming motion in Stage I. Note the small deflection of the appendages. Sequence shown lasts 0.16 s. E–J: Full cycle of the swimming motion in Stage II. This mode of swimming is similar to the adult. Sequence shown lasts 0.24 s. *A2 ex*, second antenna exopod; *Md palp*, mandibular palp; *Thp 1–4*, thoracopods 1–4. Also see supplementary materials.

ally as in the adults. The main difference between the Stage II larva and the adult is the lack of a flagellum—a much elongate and setose basal lobe of the exopod situated dorsally—present on the first and second thoracopods in adults. Another difference is that the thoracopods of the adult carry a higher number of setae on both rami increasing the efficiency of swimming (Figs. 1D–F and 2B,D,E). However, already in Stage II, all

thoracopodal exopods carry two long terminal setae and two subterminal setae (Figs. 1D,E and 2D,E). The endopods of Thoracopods 2–4 all carry a single large terminal and at least two subterminal setae. The long subterminal and terminal plumose setae of the rami considerably enhance the functional width of the thoracopods and are the main effectors in propulsion (Figs. 1F and 2B,E). Observations on the swimming of later juvenile

stages and adults of *Argulus foliaceus* suggest that the swimming pattern is the same as in Stage II, even given the differences in thoracopod morphology mentioned earlier.

Stage II swimming. The laterally held thoracopod 4 initiates the power stroke (Fig. 3E), with thoracopod 3 following shortly after. Thoracopod 4 completes the stroke first, while thoracopod 3 is still moving (Fig. 3F). The power strokes of thoracopod 2 and 1 follow successively (Fig. 3G,H), always with a small overlap of successive appendages as thoracopod 2 initiates its stroke while thoracopod 3 is still moving. The recovery strokes of thoracopods 3 and 4 are initiated shortly before the power stroke of the thoracopod 1 is completed (Fig. 3H,I). The recovery strokes of thoracopod 2 and 3 are then completed (Fig. 3I,J). The recovery stroke of thoracopod 1 completes the cycle and overlaps slightly with the onset of thoracopod 4 power stroke (Fig. 3J,E) and supplementary materials.

Cleaning in Stage I

The first antenna, the endopod of the second antenna, as well as the first and second maxillae are all very characteristically equipped with hooks and their function in the subsequent stages is, most likely, as hold-fast organs (marked by arrows, Fig. 6). However, the video sequences reveal that the free-swimming first larval stage utilizes these structures for other purposes as well: The larva stops its forward movement and exhibits a behavior that we interpret as a cleaning of the swimming appendages. To understand the sequence of movements given below, a more detailed description of the structures involved is needed and thus is presented first.

Stage I cleaning structures. The first antenna is three-segmented (articulation between distal two segments only weak) and the enlarged proximal segment forms a prominent hook (Fig. 6A,I). The hook is curved posteriorly and medially at an oblique angle at rest. The two remaining segments are small and flat, ending in a group of 7–8 setae (Fig. 6I).

The endopod of the second antenna is three-segmented and twisted in a characteristic question-mark shape. It terminates in a sclerotized inwards curved claw with three setae arising laterally from the penultimate portion (Fig. 6C). The first maxilla is a large uniramous appendage carrying at its tip a substantial hook (Fig. 6A,E,F). The hook has a well-defined tip equipped with barbs, but is apparently bifid in origin, consisting of two hooks sleeved together (Fig. 6F). The second maxilla is six-segmented and carries two terminal hooks (Fig. 6G,H,J–L). Several scale-like setules arise from the medial and the ventral surfaces of the appendage and the distal segments are recurved medially (Fig. 6L).

Stage I cleaning movements. During a cleaning cycle, the larva stops swimming and swings

the antennal exopods anteriorly, greatly exceeding the normal rotation of the appendage in the swimming mode (Fig. 4A–D). The first antennae rotate anteriorly toward each other and the proximal segment hooks catch the setae of the swimming appendages (A1 ph on Fig. 1). This apparent insertion is evident in Figures 4D and 5A, showing a frame from the video sequence (also see supplementary materials). The antennal exopods are pulled through the hooks and swung medially and ventrally (perpendicular to the long axis of the larva). This movement causes the antennal exopods to deflect and positions them ventral to the body, obscuring them in the dorsal view (Figs. 4F–H and 5B). The setae are then released from the hooks and can be seen unfolding while the rami swing back to their “normal” lateral swimming position (Figs. 4I–K and 5C). At the same time, the mandibular palps (sometimes also the antennal exopods) are caught by the hooks of the first and the second maxillae (Figs. 4I and 6B,G,H). After the mandibular palps are released, the larva continues swimming (Figs. 4L–N and 5B). The cleaning behavior described earlier was observed numerous times.

Occasionally, at a point in the cycle corresponding to Figure 4H,I, distinct, rapid anterior/posterior movement of both the first and the second maxillae occurs. At this point, the antennal exopods and the mandibular palps are in direct contact with the distal portion of the first and the second maxillae and the movements are therefore interpreted as further cleaning processes.

The cleaning processes described are often accompanied by a strong anterior flexion of the thorax and abdomen, a “tail-flip.” The flipping of the trunk is initiated just after the extreme forward swing of the antennal exopods (corresponding to Figs. 4D,E and 5A), and is completed just as the mandibular

Fig. 4. **A–N:** *Argulus foliaceus* Stage I cleaning behavior. Frame-by-frame sequence showing the cleaning behavior in a single specimen. 0.04 s between frames (25 fps), *t* indicates time elapsed from first frame. **A–N:** The full sequence lasting 0.52 s. *A1 ph*, first antenna proximal hook; *A2 ex*, second antenna exopod; *Md palp*, mandibular palp; *Mx 1*, first maxilla. Also see supplementary materials.

Fig. 5. *Argulus foliaceus* Stage I cleaning behavior; selected frames of interest from Figure 4. Stick model coloration: Yellow indicates swimming appendages (second antennae, mandibles), blue indicates appendages involved in cleaning (first antennae, first and second maxillae). **A:** The second antenna exopod is flexed anteriorly and inserted into the hook of the A1 (corresponds to D in Fig. 4). **B:** The second antenna exopods are pulled through the hooks and can be seen bent at an angle (corresponds to G in Fig. 4). **C:** The second antenna exopod is released and unfolded, while the mandibular palp is pulled through the hooks of the first and possibly also the second maxilla (corresponds to I in Fig. 4). **D:** Both pairs of swimming appendages are released and the larva continues swimming (corresponds to M in Fig. 4). *A1 ph*, first antenna proximal hook; *A2 ex*, second antenna exopod; *Md palp*, mandibular palp; *Mx 1*, first maxilla; *Mx 2*, second maxilla.

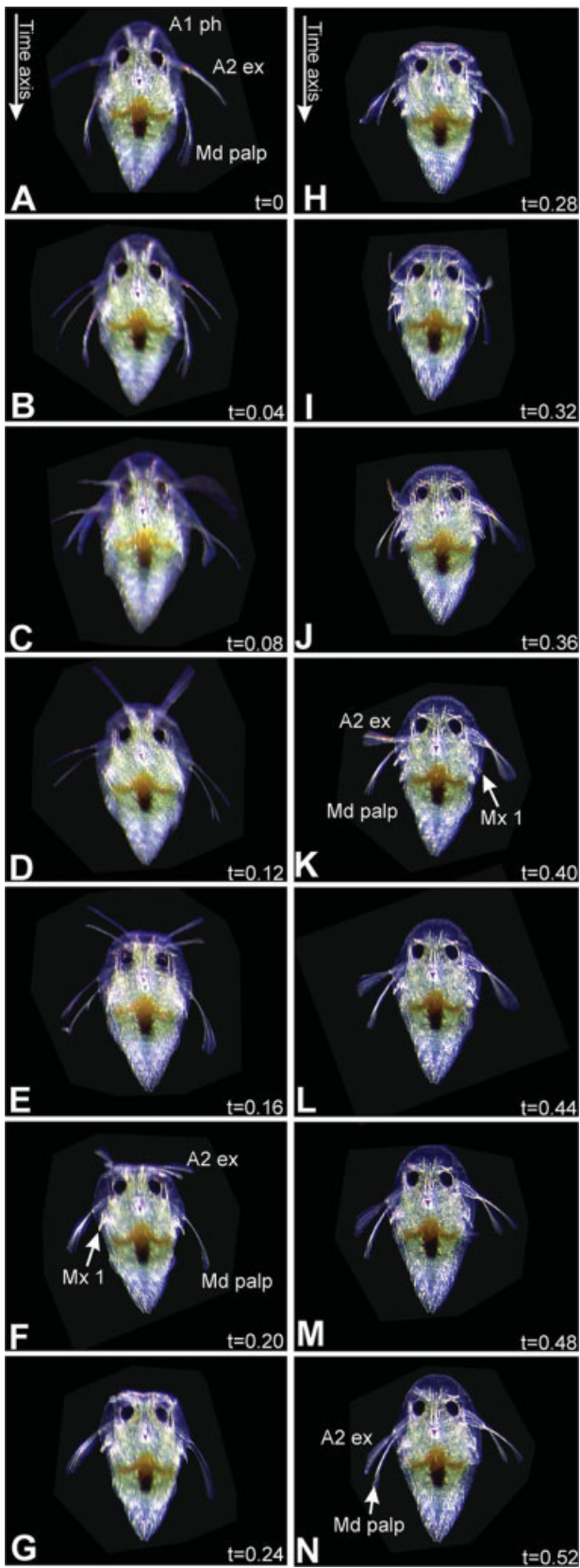


Figure 4

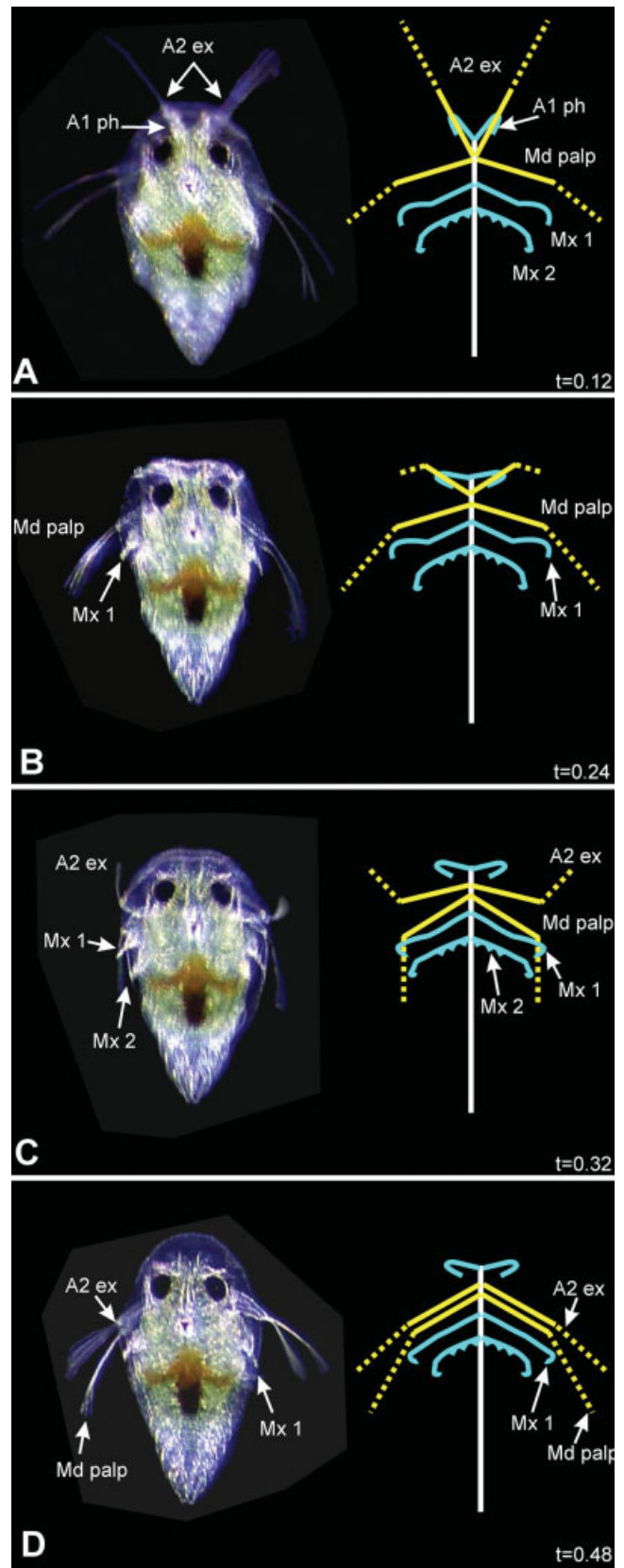


Figure 5

palps are caught by the hooks of the first and the second maxillae. The precise function of this flexion is unknown as yet.

DISCUSSION

Swimming in *Argulus* Stage-I Larvae

This study shows that newly hatched larvae of *Argulus foliaceus* are able to overcome the challenge of a preparasitic free-swimming phase. Stage I larvae are capable swimmers using the second and third naupliar appendages: the antennal exopod and the mandibular palp (the latter of which is interpreted as the retained exopod, see below). Only nine swimming setae are involved, but they are long and quite heavily setulated on the distal third (Figs. 1 and 2). The setules “fan out” during the power stroke making the limb functionally paddle-shaped, similar to the situation in *Branchinecta ferox* (Fryer, 1983). Despite many similarities to early larvae of the Branchiopoda (for details see Barlow and Sleight, 1980; Fryer, 1983, 1988; Walossek, 1993; Møller et al., 2003) or Cirripedia (e.g., Moyse, 1984), a few details are quite different in *A. foliaceus* and some may even be considered autapomorphies of the Branchiura (details of swimming pattern are lacking for other genera than *Argulus*). During swimming, the swing or flexion of at least the second antenna is smaller than in other crustacean larvae. In addition, the second antennae show no characteristic bend during the recovery stroke as that seen in e.g., *Branchinecta ferox* (Fryer, 1983), *Artemia* sp. (Barlow and Sleight, 1980) or *Lynceus brachyurus* (Olesen, 2005). Instead, the swimming appendages are held relatively stiff during both the power and recovery stroke. Collectively these details of the swimming result in a very characteristic appearance of the *Argulus* larvae.

Evolutionary Aspects of Naupliar/Early Larval Appendage Morphology

In the antennal exopod of *Argulus foliaceus*, the bases of the terminal swimming setae are arranged in a “step-like” manner with each of the four (or five) setae set off on its own level (Fig. 2F). This indicates a strongly effaced segmentation of the exopod, with traces of the original segmentation still recognizable. The terminal setae of the mandibular palp are arranged in exactly the same way, and we therefore interpret the palp as the retained exopod. A similarly reduced segmentation is present in the exopod of the larval antenna of *Euphausia superba* (Maas and Waloszek, 2001), i.e., with visible traces of the originally multisegmented antennal exopod having a single seta per well-defined annulus. This plesiomorphic state is present not only in many (recent) eucrustacean early larvae such as those of Copepoda or Cirripedia, but has also been shown for Cambrian ‘Orsten’ eucrustaceans such as *Rehbachella kinnekullensis*

(Walossek, 1993), *Bredocaris admirabilis* (Müller and Walossek, 1988) or the phosphatocopines (interpreted as the sister taxon of the Eucrustacea, see Maas et al., 2003; Siveter et al., 2003). As it is present in Crustacea stem group taxa as well, this character (state) can be considered autapomorphic for the first postantennular limb in the Crustacea ground-plan (Walossek and Müller, 1990; Waloszek, 2003a; Stein et al., 2005; see also Boxshall, 2004). Consequently, within two not closely related eucrustacean taxa—Branchiura and Euphausiacea—an antennal exopod with distally placed setae and effaced segmentation has evolved independently.

“Metanauplius” (Stage I) and First Juvenile (Stage II)

The hatching stage found in *Argulus japonicus* and *A. foliaceus* has been called a “metanauplius” by several authors (e.g., Shafir and van As, 1986; Rushton-Mellor and Boxshall, 1994). In his classical textbook, Kaestner (1967) defined a metanauplius as a larval stage in which only the first and second antennae and the mandibles are active, but with more segments developed, sometimes with *anlagen* to limbs as well (rephrased from German). Since the first stage of *A. foliaceus* already has fully active first and second maxillae, and even buds of all four thoracopods, it is, by this definition, too advanced to be called a metanauplius. Walossek (1993) has established a more precise way to compare larval sequences within the Eucrustacea. Based on his description of the very gradual (anamorphic) development in the “Orsten” fossil *Rehbachella kinnekullensis*, Walossek (1993) suggested that various eucrustacean larval series have evolved either by skipping parts of an anamorphic *Rehbachella*-like sequence, or by developing special types of larvae as those seen in (eu)crustacean subgroups. Using Walossek’s (1993) scheme for comparison, the hatching stage of *Argulus* corresponds, at least, to the 10th or 11th stage in the sequence of *Rehbachella* (to a TS4 [=trunk segment 4] stage). Thus, this approach also shows that the hatching stage of *Argulus foliaceus* (“metanauplius”) is too advanced to be included in a naupliar phase. It is even more advanced than, e.g., the first two copepodid stages of copepods, the hatching metanauplius of the cephalocarid *Hutchinsoniella macracantha* (Sanders, 1963), or the sixth larval stage of penaeid malacostracans (Walossek, 1993 and references therein).

After the first molt in *Argulus foliaceus*, the antennal exopod and mandibular palp are strongly reduced or missing (see also Gretszy et al., 1993). Stage II can be termed “juvenile” as it is generally similar to the adult, apart from size. Also as in the adult, the juvenile uses its thoracopods as the only means of propulsion (Figs. 1, 2, and 3E–J). This posterior shift of propulsion is closely coupled with the earlier presence of a naupliar swimming appen-

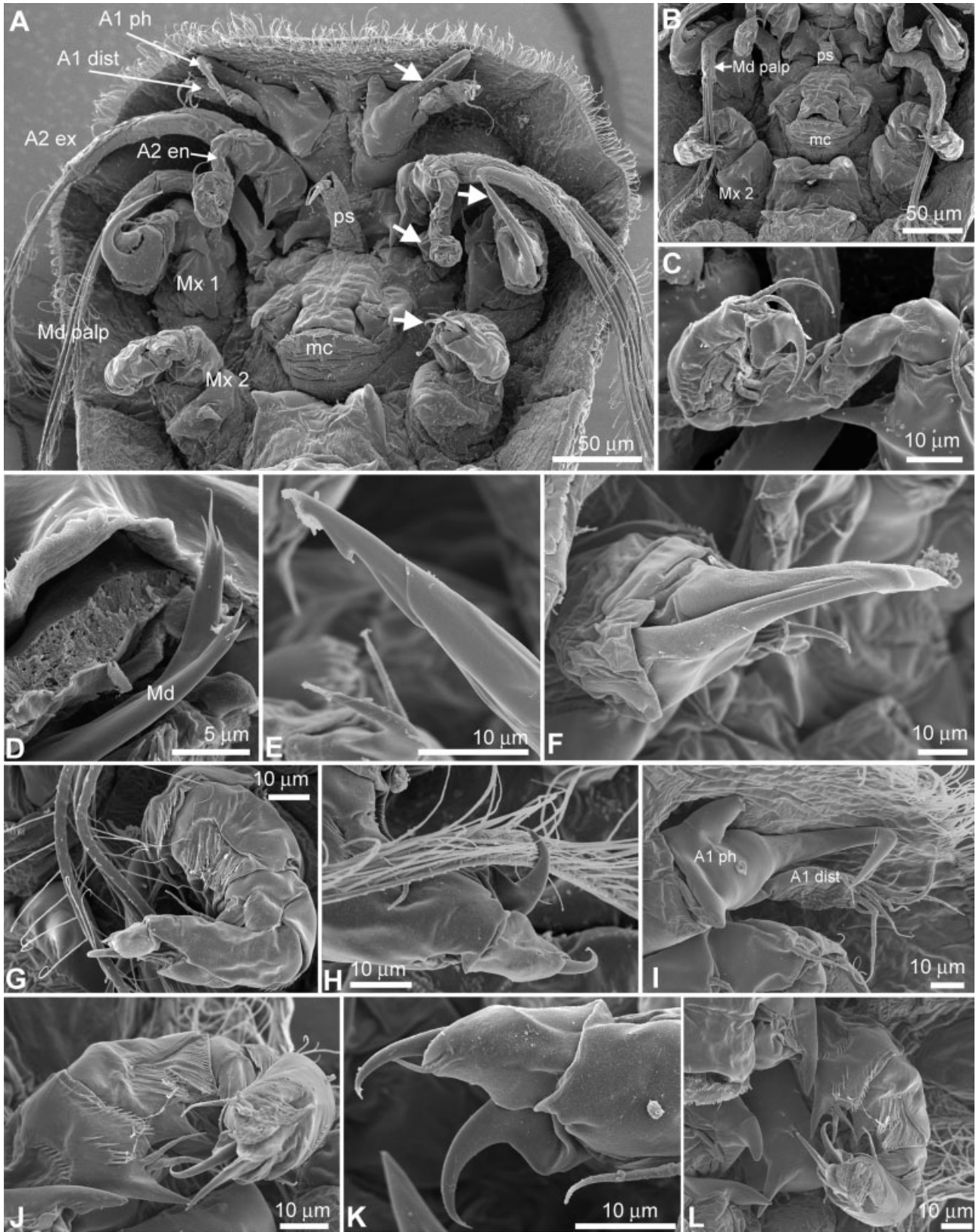


Fig. 6. **A-L:** *Argulus foliaceus* Stage I head appendages. SEM. **A:** The larval head (ventral view). Note the preoral stylet. Arrows indicate position of hooks on appendages. **B:** Larval head, ventral view. Note the long setae of the mandibular palp caught by the second maxillae. **C:** Endopod of the second antenna, ventral view. **D:** The blade-like mandible dissected from the mouth cone. **E:** Barbed tip of the large hook on the first maxilla. **F:** Hook on first maxilla showing its bifid nature, ventro-lateral view. **G:** Second maxilla with setae from swimming appendages caught on distal hooks, ventral view. **H:** As G, different specimen. **I:** Detail of first antenna showing proximal hook and distal part, mid-line view. Note the numerous comb-like setules. **K:** Detail of distal part of second maxilla showing two hooks. **L:** As J, different angle, ventral view. *A1 dist*, first antenna distal part; *A1 ph*, first antenna proximal hook; *A2 en*, second antenna endopod; *A2 ex*, second antenna exopod; *mc*, mouth cone; *md*, mandible; *Md palp*, mandibular palp; *Mx 1*, first maxilla; *Mx 2*, second maxilla; *ps*, preoral stylet.

dages, and is seen in several other taxa of the Eucrustacea (e.g., Branchiopoda: see Barlow and Sleigh, 1980; Walossek, 1993; Møller et al., 2003, 2004; Olesen and Grygier, 2003; Olesen, 2004—Cirripedia: see Walley, 1969; Moyse, 1984; Lagersson and Høeg, 2002). The setal armature of both thoracopodal rami is well developed and functional already at the Stage II larva, and thus these larvae are generally as effective swimmers as the adults. With the successive molts, the setal armature of the thoracopods increases considerably (not shown). The thoracopod-based mode of swimming—explained in detail in “Results” (see particularly Fig. 3E,J) is used throughout life—whenever the animals leave their host. This very effective type of swimming has also been observed in adult *Dolops*, while adult *Chonopeltis* are known to be virtually unable to swim (Wilson, 1902; Fryer, 1956, 1961).

Parasitic or Pelagic

The length of the free-swimming phase of *Argulus* larvae has not yet been studied in any detail. Obviously the length is variable and depends on many ecological variables such as host density, predator density, etc. Temperature is most probably important in determining the time of ecdysis, as it has been shown to strongly influence the hatching time (Shafir and van As, 1986). The feeding structures are already specialized for the parasitic mode at Stage I, most evidently seen in the long and slender blade-like mandibles precluding anything but parasitism (Fig. 6D) (see Lutsch and Avenant-Oldewage, 1995 for a much similar condition in *A. japonicus*). The presence of morphological adaptations for parasitism already in the “metanauplius” does not necessarily indicate *active* parasitism. Accordingly, reports vary on the presence of parasitism in the earliest larvae: Wilson (1902) reported that first stage larvae of *A. catostomi* did not attach to fish, even if presented with several different choices of hosts. In contrast, in an experiment with the first stage larvae of *A. coregoni*, Shimura (1981) showed that newly hatched larvae would attack a cyprinid host before molting, and that the molt only occurred three to four days after hatching. Rushton-Mellor and Boxshall (1994) reported the Stage I larvae of *A. foliaceus* to last up to six days. Wilson (1902) presented a drawing of a Stage II larva, noting that the first molt took place five days after hatching. Based on the information summarized earlier, it thus seems plausible that the first molt can occur even if the larvae were never attached to a host. If attachment to a host occurs in Stage I, no doubt the large hooks of the first maxilla would be the primary hold-fast structures. As shown, these hooks are also used during cleaning, but lose their function as the appendage is transformed into the adult suction disc. So, whether parasitic or pelagic in the first stage, the first maxilla is a key element both in the larval as well as in the adult phase in *A. foliaceus*.

The free-swimming phase is a significant part of the larval life in *Argulus foliaceus*. The behavioral pattern described herein and interpreted as a cleaning of the larval swimming appendages fits well into the general picture of *A. foliaceus* as having a “semi-pelagic” early phase of larval life. Obviously, the problem of getting the swimming appendages clogged with debris and particles presents a major challenge to the larvae. This would heavily impair swimming and, likewise, also the subsequent location of a host fish. An effective system for cleaning is a clear evolutionary advantage. While the naupliar swimming appendages are only found in the Stage I larva, the structures in the larva undertaking the actual cleaning are mostly retained in the adult. The proximal hooks on the first antenna and the distal hooks on the second maxilla show the adult morphology and do not change significantly during ontogeny/morphogenesis (see Fig. 6).

Retention of a free-swimming “metanaupliar” phase is not found throughout the genus *Argulus*. In fact, several species such as *A. megalops*, *A. funduli* and *A. africanus* hatch as juveniles morphologically similar to Stage II or III in *A. foliaceus* (Wilson, 1907; Rushton-Mellor and Boxshall, 1994). This could be seen as a phylogenetically more advanced situation, while the retention of a “metanaupliar” phase evidently involves a greater risk for predation on the larvae, representing the plesiomorphic character state. Among the species of the genus *Argulus*, though, the most widespread species *A. foliaceus* and *A. japonicus* still retain such a phase with great success.

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