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First maxillae suction discs in Branchiura (Crustacea): Development and evolution in light of the first molecular phylogeny of Branchiura, Pentastomida, and other ''Maxillopoda''

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Abstract

The fish ectoparasites Branchiura (Crustacea) display two different ways of attachment to the fish surface as adults: the first maxillae are either hooks (*Dolops*) or suction discs (*Argulus*, *Chonopeltis*, and *Dipteropeltis*). In larval *Argulus foliaceus* the first maxillae are hooks. With the first molecular phylogeny of the Branchiura as a background, the present paper discusses the evolutionary scenarios leading to hooks *versus* suction discs. Specific homologies exist between larval *Argulus foliaceus* hooks and adult *Dolops ranarum* hooks. These include the presence of a comparable number of segments/portions and a distal segment terminating in a double structure: a distal two-part hook (in *Argulus*) or one hook and an associate spine-like structure (in *Dolops*). In the phylogenetic reconstruction based on three molecular markers (mitochondrial 16S rRNA, nuclear 18S and 28S rRNA), *Dolops ranarum* is found to be in a sister group position to all other Branchiura, which in this analysis include six *Argulus* and one *Chonopeltis* sequences. Based on the molecular phylogeny a likely evolutionary scenario is that the ancestral branchiuran used hooks (on the first maxilla) for attachment, as seen in *Dolops*, of which the proximal part was subsequently modified into suction discs in *Argulus* and *Chonopeltis* (and *Dipteropeltis*). The sister group relationship of the Branchiura and Pentastomida is confirmed based on the most comprehensive taxon sampling until now. No evidence was found for a branchiuran in-group position of the Pentastomida.
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1. Introduction

Modifications for different kinds of attachment are common among parasitic crustaceans, but the suction discs seen in most species of the Branchiura Thorell, 1864, are among the most elaborate and fascinating of such structures. The term 'suction disc' is well-chosen since they operate by muscle-facilitated suction (Gresty et al., 1993). Suction discs are found in three of the four currently recognized genera of the Branchiura, in *Argulus* Müller, 1785, *Chonopeltis* Thiele, 1900, and in the monotypic *Dipteropeltis* Calman, 1912, and it is well known that these structures are modified first maxillae. In the early larvae of *Argulus* and *Chonopeltis* no such discs are present, but the hatching stage is known only from five species of *Argulus*, four species of *Chonopeltis*, and unknown in *Dipteropeltis* (Claus, 1875; Wilson, 1902; Thiele, 1904; Tokioka, 1936; Meehan, 1940; Fryer, 1956, 1961, 1964; Shimura, 1981; Shafir and Van As, 1986; van Niekerk and Kok, 1989; Avenant-Oldewage and Knight, 1994; Van As and Van As, 1996). The described larvae all have a pair of segmented appendages, each with a distal hook that probably also serves

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a prehensile purpose (Fryer, 1956, 1961; Shimura, 1981; Møller et al., 2007). During larval development, the proximal segment of each first maxilla gradually transforms into a suction disc, while the more distal segments degenerate (see e.g., Fryer, 1956: *C inermis*, 1961: *C. brevis*, 1977: *C. minutus*, Lutsch and Avenant-Oldewage, 1995: *A. japonicus*, Rushton-Mellor and Boxshall, 1994: *A. foliaceus*, Wilson, 1902: *A. megalops*).

The species of the genus *Dolops* Audouin, 1837, are notable exceptions to this pattern of suction discs in Branchiura (see e.g., Wilson, 1902; Fryer, 1969; Avenant et al., 1989). In all known species of *Dolops* the adults lack suction discs and have segmented first maxillae, each ending in a distal hook apparently of a morphology comparable to the hooked first maxillae of larval *Argulus* and *Chonopeltis* (Krøyer, 1863; Bouvier, 1897, 1898, 1899a; Thiele, 1904; Maidl, 1912; Brian, 1940; Fryer, 1961). Larvae of *Dolops* have only been described from *D. ranarum* by Fryer (1964) and Avenant et al. (1989), and, as do some species of *Argulus*, *D. ranarum* hatch as a juvenile resembling the adult in most respects. As in the adult of *D. ranarum*, the first maxillae of the hatching stage are equipped with hooks (for details, see Avenant et al., 1989). The ontogeny of *Dipteropeltis hirundo* is unknown and only very few individuals of this species have been described (Calman, 1912; Ringuelet, 1943, 1948).

We are interested in the evolutionary implications of the occurrence of first maxillar suction discs *versus* distal hooks within the Branchiura. A simple—but important—question remains to be addressed: is it an ancestral feature of the Branchiura to have the first maxillae modified as suction discs, or did this modification take place later in the course of branchiuran evolution? We are aware of essentially no discussion of this point in existing literature, but Overstreet et al. (1992) do refer to the hooked condition in adults of *Dolops* as being neotenic. Following this, the hooks in *Dolops* would have evolved secondarily by retention of the larval character state from ancestors that possessed suction discs as adults. However, if the direction of development during ontogeny in *Argulus* and *Chonopeltis* (first maxillae with hooks in larvae developing into suction discs in adults) is taken as an indicator of possible evolutionary scenario, it points to the opposite conclusion: the suction discs of the first maxillae have evolved from hooks in the adults of a common ancestor. The fact that no phylogenetic analyses of the internal branchiuran relationship are available or have even been attempted makes the question completely open to speculation (but see Fryer, 1956, 1977). A classical outgroup comparison is of no help since all possible close relatives to the Branchiura neither have suction discs nor hooks. Furthermore, there is no consensus on what group actually is the closest relative of the Branchiura (not considering the Pentastomida). Martin (1932) convincingly argued that the Branchiura should be separated from the Copepoda, and since Dahl (1956) the group has been considered a "maxillopod" by most authors (e.g., Schram, 1986; Walossek and Müller, 1998; Martin and Davis, 2001). Here we maintain the maxillopodan affinity of the Branchiura as a starting point for the analysis and selection of included taxa.

In this paper we address the question of suction disc evolution within the Branchiura based on the first molecular phylogeny for the group (three molecular loci and representatives from three genera: *Argulus, Chonopeltis,* and *Dolops*). As a basis for identifying homologies, we describe and compare the segmentation of the first maxilla hooks in immature *Argulus foliaceus* and adult *Dolops ranarum*. As material of species of the enigmatic Pentastomida (arthropod parasites of debated phylogenetic affinity living in respiratory tracts of various vertebrates, see e.g., Waloszek et al., 2006) was available to the authors, we take this chance to present the first analysis with in-group sampling of both Branchiura and Pentastomida in order to test a possible branchiuran in-group position of the Pentastomida.

2. Material and methods

2.1. Light- and scanning electron microscopy

2.1.1. Specimens

Live *Argulus foliaceus* (Linné) larvae were collected using a plankton net (mesh size 63 μm) in an exhibition tank at the Danish National Aquarium, Charlottenlund, Denmark. Egg strings were scraped off the front glass of the same tank by using a razor blade. Larvae were kept in aerated glass aquaria in the lab. Larval stages were observed in a standard Leica dissection microscope (Leica MZ95). Identification of stages followed Rushton-Mellor and Boxshall (1994). All specimens were transferred to clean water for at least 30 min before being fixed in standard aldehyde fixatives (2.5% Glutaraldehyde or 5% formalin). *Dolops ranarum* (Stuhlmann, 1891): specimens were collected using gill-nets in the lake behind the Tzaneen Dam in November 2004 near Tzaneen, Limpopo Province, South Africa, where they were found on *Clarias gariepinus* (Burchell, 1822) known as "sharptooth catfish" (Skelton, 2001). Specimens were fixed in either Davidson's AFA (acetic acid, formalin, ethanol) (Kiernan, 1990) or 5% formalin.

2.1.2. Common procedures

All specimens were transferred to 70% ethanol before storage. In preparation for LM observation, specimens were made translucent using lactophenol. LM used was a Leica DMRXA, equipped with an Evolution MP digital camera, using oil-immersion and the ImagePro© software, including the EDF-stack ("Enhanced Depth of Field") algorithm. Specimens for SEM, some dissected wet, were dehydrated through a graded alcohol series and then critical-point-dried in acetone in a Bal-Tec 030 CPD. The dry specimens were mounted on SEM stubs and observed in a JEOL JSM-6335-F. All images were saved and processed digitally.

2.2. Molecular techniques

Table 1 lists the data of collection origin and GenBank data from the taxa included in this analysis. Specimens of the pentastomid *Reighardia lomviae* Dyck, 1975 were collected from the Common Guillemot (*Uria aalge* (Pontoppidan, 1763)) on

Table 1
A list of included taxa. The classification generally follows Martin and Davies (2001)

Taxon	Collection details	Sequence length			GenBank Acc. numbers (if available)		
		18S	28S	16S	18S	28S	16S
Crustacea Brünnich, 1772							
Branchiura Thorell, 1864							
(Arguloida Yamaguti 1963)							
Argulidae Leach, 1819							
Argulus Müller, 1785							
Argulus japonicus Thiele, 1900	9/11-2004, Boskop Dam N. of Potchefstroom, NW Province, Rep. South Africa	1833	620	539			
Argulus foliaceus (L)	11/6-2006 Utterslev Mose, N. of Copenhagen, Denmark	1796	709	576			
Argulus nobilis Thiele, 1904		1843			M27187		
Argulus monody Fryer, 1959		529			DQ813452		
Argulus sp. 1 (OC_2001)			764			AF363322	
Argulus sp. 2 (JMM 2003)			3790			AY210804	
Chonopeltis Thiele, 1900							
Chonopeltis australis Fryer, 1977	30/11-2004, Maselspoort Dam, outside Bloemfontain, Free State, Rep. South Africa	1741	713	582			
Dolops Audouin, 1837							
Dolops ranarum Stuhlmann, 1891	17/11-2004, Tzaneen Dam, Tzaneen, Limpopo Province, Rep. South Africa	1822	662	461			
Pentastomida Diesing, 1836							
Cephalobaenida Heymons, 1935							
Cephalobaenidae Fain, 1961							
Raillietiella sp. Sambon, 1910		1785	1984		AY744887	AY744894	
Reighardiidae Heymons, 1935							
Reighardia lomviae Dyck, 1975	3/7-2004, Nólsoy, Faerer Islands. Donation by J.-K. Jensen	1804		588			
Porocephalida Heymons, 1935							
Porocephalidae Fain, 1961							
Porocephalus crotali (Humboldt, 1808)		1830			M29931		
Malacostraca Latreille, 1802							
Euphausiacea Dana, 1852							
Meganyctiphanes norvegica (M Sars, 1857)		1807	606	517	AY744892	AF296700	AY744910
Stomatopoda Latreille, 1817							
Squilla empusa Say, 1818		1818	3914	475	L81946	AY210842	AF107617
Branchiopoda Latreille, 1817							
Anostraca Sars, 1867							
Artemia salina (Linné, 1758)		1810	589		AJ238061	X90461	
Branchipus schaefferi Fischer, 1834		1806	633		AJ238068		
Eubranchipus grubii (Dybowski, 1860)		1713	735	445		DQ470652	DQ470610
Ostracoda Latreille, 1802							
Myodocopida Sars, 1866							
Cypridinidae Baird, 1850							
Vargula hilgendorfi (Müller, 1890)		1907	761	943	AB076654	AF363317	AY624737
Melavargula japonica Poulsen, 1962		1868	753	947	AF363300	AF363318	AY624733
Thecostraca Gruvel, 1905							
Cirripedia Burmeister, 1834							
Iblidae Leach, 1825							
Ibla quadrivalvis (Cuvier, 1817)		1817	1794	526	AY520655	AY520612	AY520755
Balanidae Leach 1817							
Semibalanus balanoides (Linné, 1758)		1821	1775		AY520626	AY520592	

Nólsoy, Faeroe Islands and kindly donated to the authors by Jens-Kjeld Jensen. For the specimens collected by the authors (primarily Branchiura), the following methods were applied. Total genomic DNA was extracted from tissue-samples of individuals using the Qiagen DNeasy® Tissue Kit following the Protocol for Animal Tissues 03/2004. PCR products for the 28S rRNA fragment (ca. 600 bp) was amplified using the following primers: 1274 (5′ GAC CCG TCT TGA AAC ACG GA 3′) (Markmann and Tautz, 2005) and 1276 (5′ CTA GTT GCT TCG GCA GGT GAG 3′) designed for this study. The 18S rRNA fragment (nearly complete fragment) was amplified using the following primers in combinations: 9R (5′
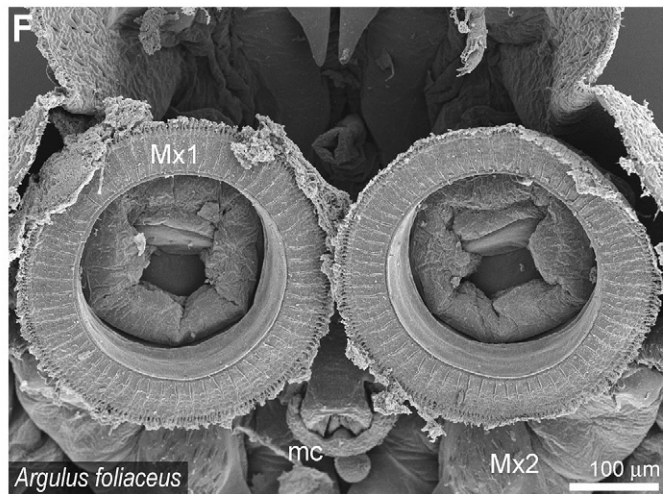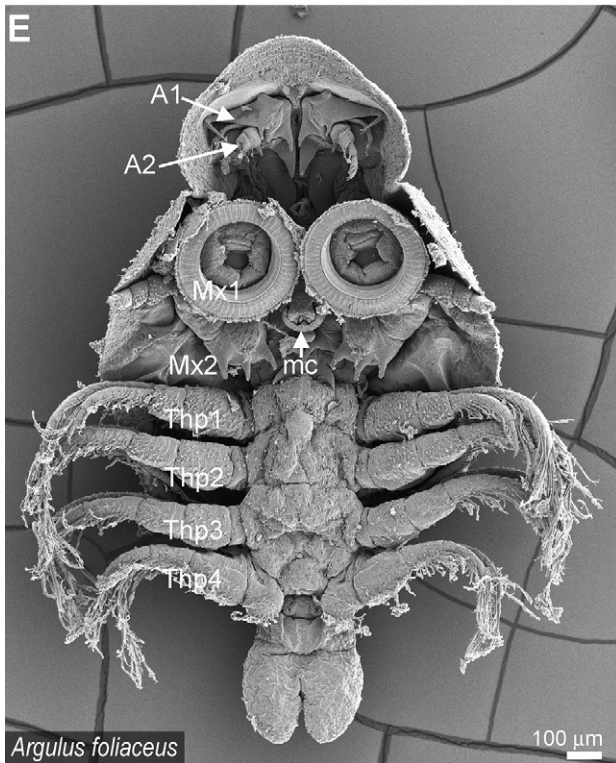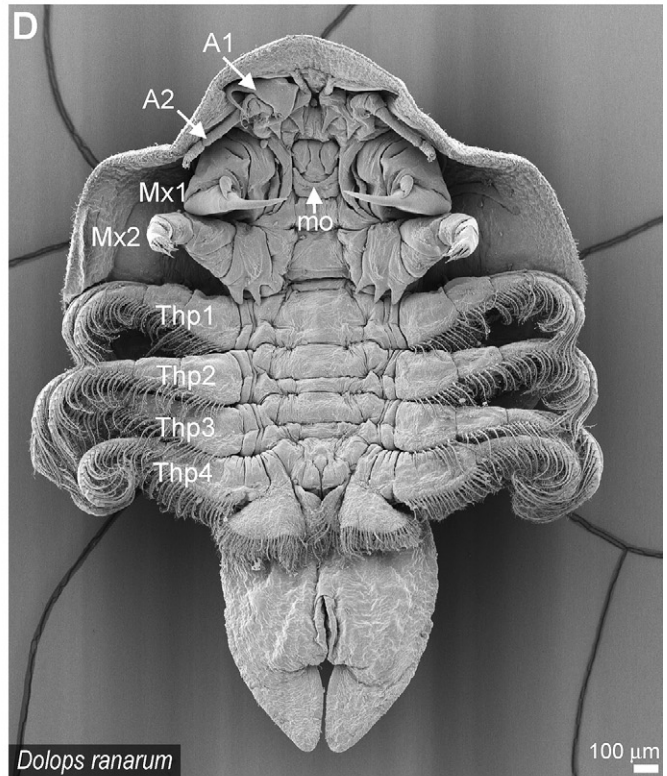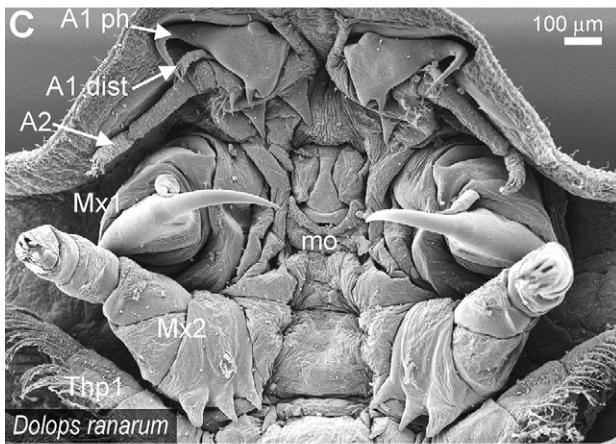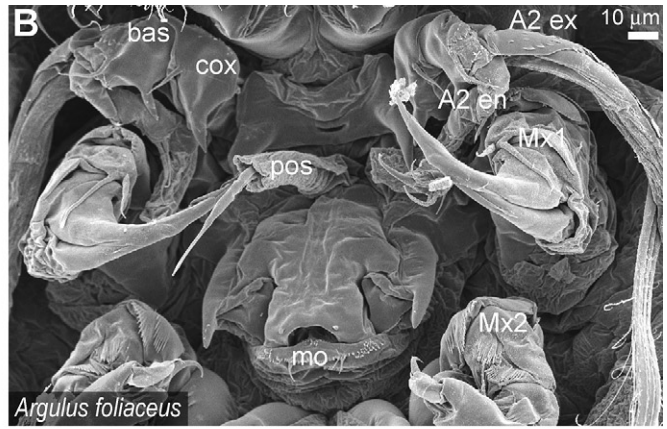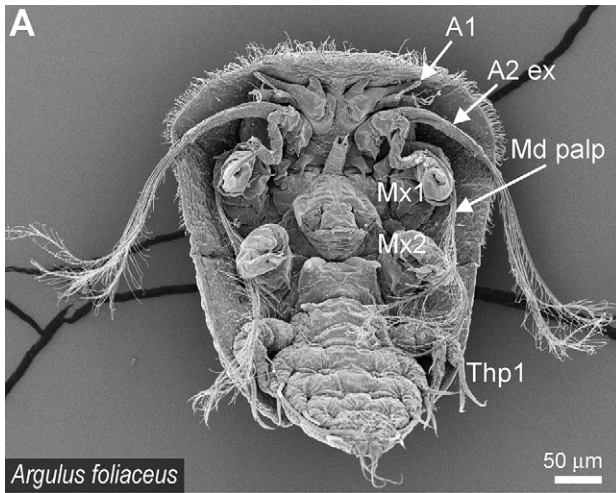
GAT CCT TCC GCA GGT TCA CCT AC 3′) and 5f (5′ GCG AAA GCA TTT GCC AAG AA 3′), 5R (5′ CTT GGC AAA TGC TTT CGC 3′) and 1f (5′ TAC CTG GTT GAT CCT GCC AGT AG 3′), 18SintR (5′ GCG GTT AAA AAG CTC GTA G 3′) and 18SintL (5′ TGC AAC CAT ACT TCC CCC GG 3′). All primers for 18S rRNA were designed for this study.

A total of 50 μl doublestranded PCR mix was prepared from 5 μl PCR-buffer, 5 μl of each primer at 10 μM, 14 μl ddH$_2$O, 20 μl (0.5 μM) GATC-mix, and 0.25 μl 5 U/μl Amplitaq® DNA Polymerase. Genomic DNA (1 μl) was used as template. Conditions for all amplifications were 2 min initial denaturing at 94 °C followed by 35 cycles with 94 °C/30 s denaturing, 50 °C/30 s annealing, and 72 °C/40 s extension, with an additional 10 min extension at 72 °C. Reactions were performed using Stratagene RoboCycler® Gradient 96. Purification was made using Qiagen QiaQuick Spin Columns following the protocol. The purified PCR products were stored at −20 °C.

Ten microliters sequencing-mix was made from 3 μl purified PCR products, 1.2 μl of 10 μM primer, 2 μl BigDye® Terminator v1.1 Cycle Sequencing, 1 μl BigDye® Terminator v1.1 5× buffer and 2.8 μl ddH2O. Conditions for the cyclic sequencing reaction was 35 cycles of: 96 °C/10 s denaturing, 50 °C/5 s annealing and 60 °C/4 min extension. Reactions were performed using Stratagene RoboCycler® Gradient 96.

Sequencing products were precipitated using Ethanol and analyzed on ABI PRISM™ 377 DNA Sequencer or ABI 3130xl Genetic Analyzer.

Each sample was sequenced in both directions, in order to improve accuracy. Forward and reverse sequences were aligned and checked for base ambiguity in Sequencher™ 3.1.1 and consensus sequences were compiled. Aligning of sequences was performed in Clustal_X (Thompson et al., 1997) using default parameters.

2.3. Sequence analysis

The 18S, 28S and 16S sequences were analyzed separately and combined. The sequence analysis and phylogenetic reconstructions were carried out using Bayesian Inference of phylogeny as implemented in the MrBayes v3.1.2 software in multi-processor (MPI) mode (Ronquist and Huelsenbeck, 2003; Altekar et al., 2004). Maximum Parsimony (MP) analyses were conducted in the T.N.T. (Tree analysis using New Technology) software (Goloboff et al., 2003), and PAUP*4b10 (Swofford, 2002) was used to run MrModeltest2.2 (Nylander et al., 2004; see also Posada and Crandall, 1998) to select an appropriate substitution model for the MrBayes analyses. The model and parameters for both the combined and separate loci analyses were selected according to the Akaike Information Criterion (AIC) and implemented in the analyses

(Sullivan and Joyce, 2005; Kelchner and Thomas, 2006). The parsimony analyses' trees were obtained using traditional search, TBR branch swapping, "Rule 1" collapsing, random seed 0, 500 random addition sequences holding 50 trees pr. replication. Before the analysis the "tree space" (maxtrees) was increased to 10,000. A strict-consensus (Nelsen) tree was constructed from the retained most parsimonious trees. A total of 5000 bootstrap (standard bootstrap) replicates were conducted using the same settings as above.

All MrBayes analyses were run on the free computing resource "BioPortal" situated at the University of Oslo, Norway (official webpage: http://www.bioportal.uio.no). All the MCMC analyses were run for six million generations, six independent runs with five Markov chains sampled every 1000th generation. For all Bayesian analyses, the taxon *Squilla empusa* was defined as outgroup. The combined three-loci dataset was analyzed as a partitioned dataset using loci as partitions. The partitions were unlinked allowing the model parameters and rates to vary independently (unlink and prset ratepr = variable commands) (see e.g., Nylander et al., 2004; Glenner et al., 2004). The first 10% (600,000) of the generations were regarded as "burnin" generations, and the sampled trees (i.e. 600) were discarded in the summarizing of the .p and .t files. The resulting 50% majority-rule tree's posterior probability values are based on 5401 trees from each of the six runs (summaries and probabilities based on a total of 36,406 trees).

2.4. Outgroup selection

The mantis shrimp *Squilla empusa* (Malacostraca: Stomatopoda) was defined *a priori* as outgroup taxon in all analyses. Other crustacean taxa (Branchiopoda, Cirripedia, Ostracoda, and Malacostraca) were included to test the monophyly of the Branchiura and its relation to the Pentastomida.

3. Results

3.1. Light- and scanning electron microscopy

3.1.1. Maxilla 1

The development of the larval hooks into the adult suction discs is described for *Argulus foliaceus* (Figs. 1–3 and 4A). The adult condition of the hooked first maxillae of *Dolops ranarum,* which resemble the larval hooks of *Argulus* (Figs. 1, 2 and 4B), is described for comparison. The ontogenetic series presented here for *A. foliaceus* is not complete but represents important stages in the development. A description of its larval development was given by Rushton-Mellor and Boxshall (1994). The larvae of *Dolops ranarum* have not been treated here but have previously been described by Fryer (1964) and Avenant et al. (1989).

Fig. 1. Attachment structures (hooks and suction discs) of first maxillae in larval and adult Branchiura (Crustacea). A–B, E–F: *Argulus foliaceus,* Linné, 1758. C–D: *Dolops ranarum* (Stuhlmann, 1891). SEM. A: Larval stage 1, median view. B: Stage 1, close-up of mouth region, anterior view (different specimen from A). C: Cephalic region, median view. D: Ventral view (different specimen from C). E: Adult, ventral view. F: Close-up of suction discs (Mx1) from E. Abbr. *A1* first antenna, *A1 dist* first antenna distal part, *A1 ph* first antenna proximal hook, *A2* second antenna, *A2 en* second antenna endopod, *A2 ex* second antenna exopod, *bas* basipod, *cox* coxa, *md palp* mandibular palp, *Mc* mouth cone, *mo* mouth opening, *Mx1* first maxilla, *Mx2* second maxilla, *pos* pre-oral spine, *Thp1–4* Thoracopods 1 to 4.
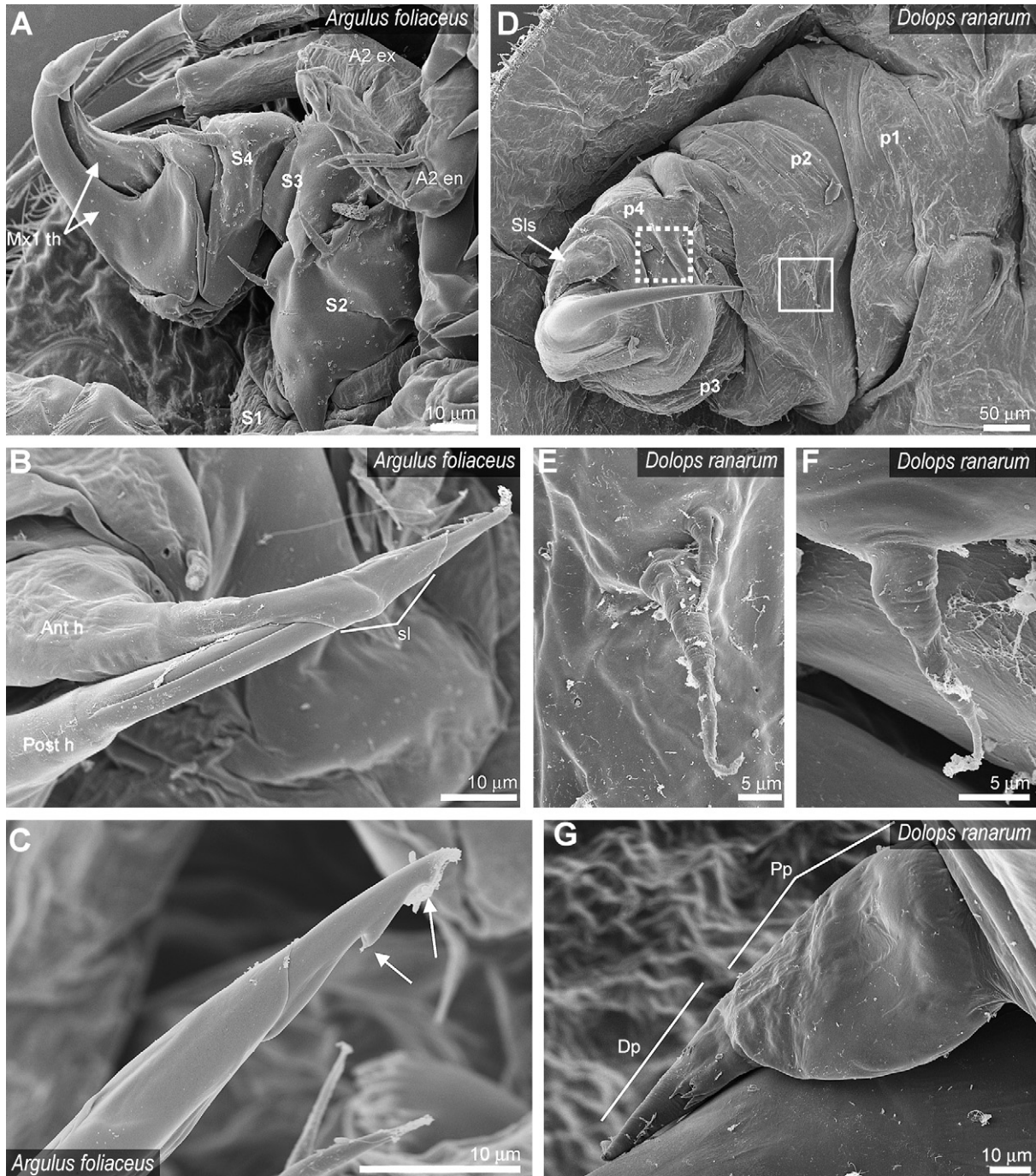
Fig. 2. First maxillae hooks of larval stage 1 in *Argulus foliaceus* and adult *Dolops ranarum*. A–C: *Argulus foliaceus*, larval stage 1, SEM. D–G: *Dolops ranarum*, adult, SEM. A: First maxilla, median view. B: First maxilla, detail of the two-part hook showing sleeve. C: First maxilla, close-up, tip of hook. Arrows indicate barbs. D: First maxilla, anterior view. E: Close-up from D, right-hand box, full outline. F: Close-up from D, left-hand box, dotted outline, rotated image. G: First maxilla, detail of spine-like structure, anterior view. Abbr. *A2 en* second antenna endopod, *A2 ex* second antenna exopod, *Ant h* anterior hook element, *Dp* distal portion, *Mx1 th* first maxilla two-part hook, *p1–4* portions 1 to 4, *Post h* posterior hook element, *Pp* proximal portion, *S1–4* segments 1 to 4, *sl* sleeve, *Sls* spine-like structure.

3.2. Argulus foliaceus

3.2.1. Morphology of Maxilla 1: stage 1 (Figs. 1A,B, 2A–C, 3 and 4A)

The first maxilla is uniramous and four-segmented and carries a large, two-part hook at the tip (Figs. 1B, 2A–C and 4A). The proximal segment is cylindrical and its shrinkage during

SEM preparations implies that it is not as sclerotized as the more distal segments (''S1'' in Figs. 2A and 3A). S1's precise insertion on the body is not revealed by SEM, but it is slightly anterior to the mouth cone basis. The second segment (S2) is sub-cylindrical and carries a large, posteriorly directed spine proximally and two setae distally, one on either side of the spine projecting from the third segment. The third segment
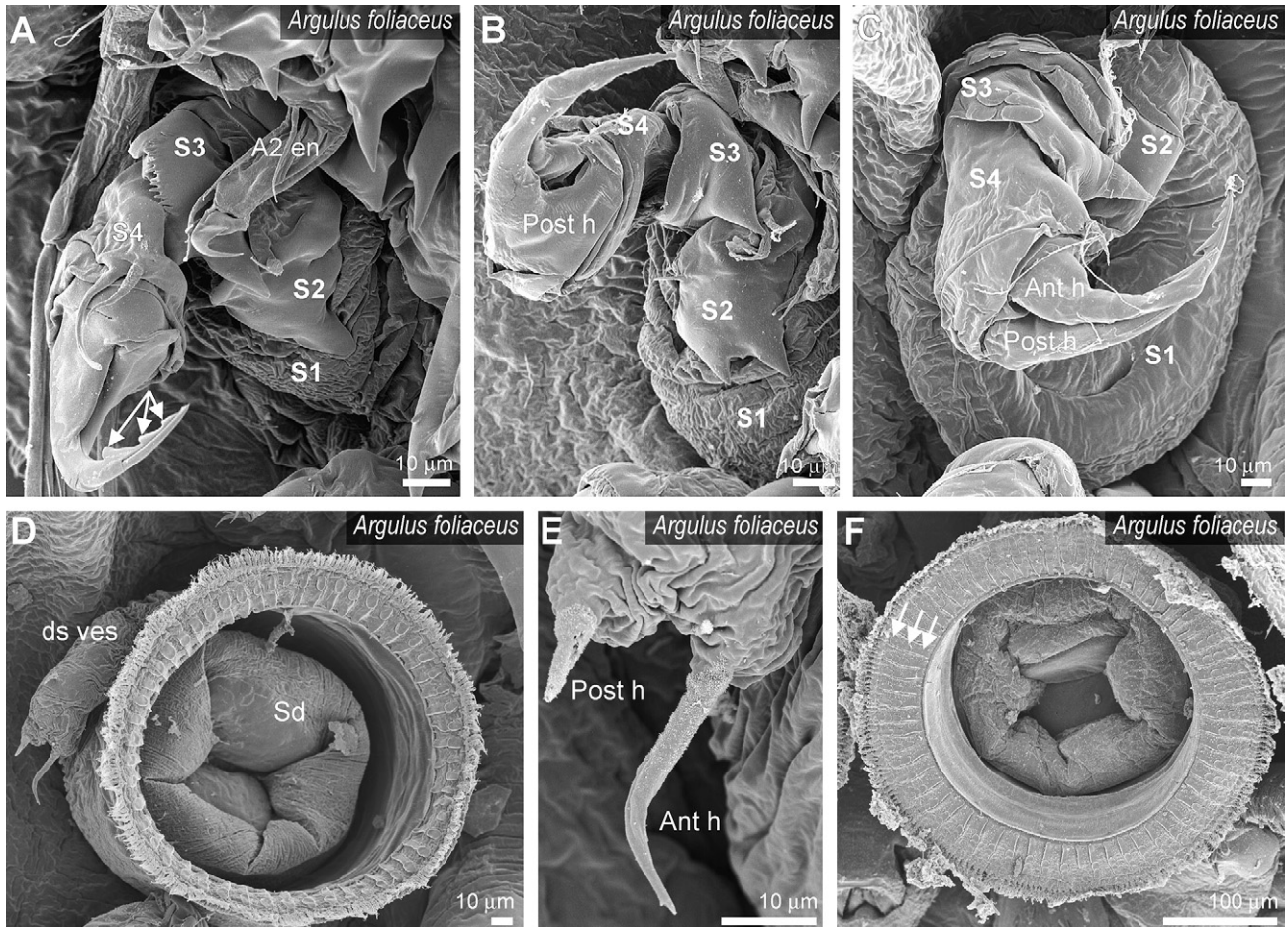
Fig. 3. Ontogeny of first maxillae in *Argulus foliaceus* showing development from larval morphology with hooks, to adult morphology with suction disc, SEM. A: First maxilla, larval stage 1, median view, arrows indicate barbs. B: First maxilla, larval stage 3. C: First maxilla, larval stage 5. D: First maxilla, larval stage 6. Vestige of distal segments on suction disc. E: Close-up of D showing vestiges of the posterior and anterior hook elements. F: First maxilla, fully developed adult suction disc, arrows indicate rim support rods. Abbr. *A2 en* second antenna endopod, *Ant h* anterior hook element, *ds ves* distal segments vestige, *Post h* posterior hook element, *S1–4* segments 1 to 4, *Sd* suction disc.

(S3) inserts on the second segment at an oblique angle, making a clear bend in the axis of the appendage. The third segment is also generally sub-cylindrical, but shorter than the second segment. It carries a prominent, postero-medially directed spine on the ventral face. The anterior edge is characteristically armed with small teeth, and overlaps the joint of the third and fourth segment somewhat. The fourth segment (S4) is irregularly wedge-shaped. Two setae are found on the distal edge of its anterior face. The fourth segment carries a substantial two-part hook with barbs (Figs. 2A–C, 3A–E and 4A). This hook has a well-defined tip (Figs. 2B,C) but clearly consists of two separate hook-like structures that are sleeved together distally (Figs. 2A–C, ''sl'' in 2B). The more posterior of the two hook-components is the larger and comprises with the actual tip of the hook, and has a conspicuous furrow or channel on its outer face (''Post h'' on Figs. 2B and 4C). The more anterior hook-component forms a sleeve around the posterior one near the tip of the latter (''sl'' on Fig. 2B). The two distinct hook-components are not fused in the ''sleeve'', which can lead them to separate during preparation for LM; see examples in drawings by Claus (1875) or

Rushton-Mellor and Boxshall (1994). Our SEM observations indicate that the two parts of the hook function as one unit, and most probably it is directed anterior-medially when relaxed, as indicated by the articulation angles of the proximal first maxilla segments.

3.3. Argulus foliaceus

3.3.1. Morphology of Maxilla 1: later stages and adult (Figs. 1 and 3)

With further development, the first maxilla changes its shape completely. In stage 3 (Fig. 3B), the proximal segment (S1) is enlarged and the second segment now bears two distinct spines, as well as the two distal spines that were already present in stage 1. The third segment still carries a single spine, now directed more medially. The fourth segment is still irregularly wedge-shaped, although it has become more elongate. The two-part hook is still very prominent.

In stage 5 (Fig. 3C), the first segment has enlarged significantly and is now clearly disc-shaped. The support rods in the disc margin are already visible. The second segment is more
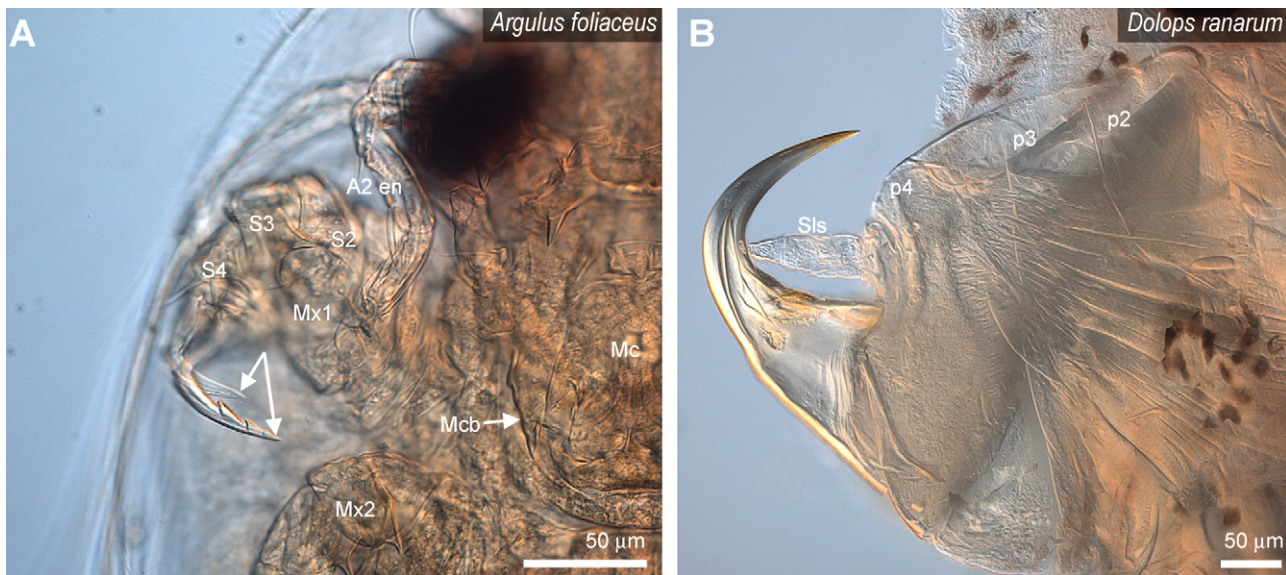
Fig. 4. Light microscopy of hooked first maxilla in first stage larva of *Argulus foliaceus* (A) and in adult *Dolops ranarum* (B). A: Posterior cephalic appendages and mouth cone, ventral view. B: First maxilla (partly dissected), median view, (Fused EDF-stack). Abbr. *A2 en* second antenna endopod, *Mc* mouth cone, *Mcb* mouth cone lateral bars, *Mx1* first maxilla two-part hook, *Mx2* second maxilla, *p2–4* portions 2 to 4, *S2–4* segments 2 to 4, *Sls* spine-like structure.

compressed than before and still carries two setae (not shown). The third segment is also compressed somewhat and retains the medially directed spine. The fourth segment is wedge-shaped, and the two-part distal hook is still prominent.

In stage 6 (Figs. 3D,E), the first segment has become fully developed into a suction disc with a highly specialized morphology. Only a vestige remains of the distal three segments. Very characteristically, the fourth segment still carries two small, setal-like structures: the remainder of the posterior and anterior components of the two-part hook.

In the adult (Figs. 1E,F and 3F) no trace of the distal part of the larval first maxilla remains. For more details of the specific morphology and function of the suction disc, see Gresty et al. (1993).

3.4. Dolops ranarum

3.4.1. Morphology of Maxilla 1: adult
(Figs. 1C,D and 2D–G)

The first maxilla in the adult is uniramous and subdivided into four parts (Figs. 2D and 4B). We hesitate to use the term "segment" for these parts because their precise external delimitations are difficult to trace using SEM, as the appendage is generally compressed and shortened. No clear segmental patterns could be seen in the musculature using conventional light microscopy and the muscles are therefore not useful for tracing segmentation (Fig. 4B). Generally, the four parts of the limb are slightly conical, decreasing in size distally. A few scattered setae are carried medially: two on the second part (Fig. 2E) and a single seta on the border of the third and fourth parts (Fig. 2F). The fourth part carries a large hook distally as well as a smaller spine-like structure anteriorly to this (Fig. 2G). This spine-like structure consists of a flattened and wide proximal part and a more slender distal part, separated by a slight constriction ("Pp" and "Dp" in Fig. 2G). On the

narrow distal part, a small cluster (4–5) of spines is present medially. Sub-apically, a short row of tooth-like projections is found. The spine-like structure anterior to the hook has also been described from the first-stage larva by earlier authors (Avenant et al., 1989; Fryer, 1964). Its function is unknown.

3.4.2. Phylogenetic analyses, Bayesian inference

The Bayesian Inference analyses were performed under the $GTR + I + \Gamma$ model (general time reversible model with correction for invariant characters (I) and Γ-distributed rate heterogeneity, *in casu*: inverse Γ-distribution) as selected by MrModeltest using the AIC criterion (see Section 2 for references).

The results of the analysis of the combined dataset (18S, 28S and 16S) are summarized in the 50% majority-rule tree in Fig. 6A. The mean (harmonic) marginal likelihood for the six runs was −33273.10. The monophyly of the Branchiura is supported by this analysis with a bpp = 1, and likewise the position of *Dolops ranarum* as the sister group to the remaining *Argulus* species and *Chonopeltis australis*. The genus *Argulus* is paraphyletic with respect to the included *Chonopeltis* species, with high posterior probability (bpp = 0.94) for a clade containing *C. australis*, *A. monodi*, and two additional *Argulus* species. A clade containing the Branchiura and Pentastomida (Ichthyostraca *sensu* Zrzavý, 2001) is strongly supported (bpp = 1), as well as *Porocephalus crotali* being the sister group to a monophyletic Cephalobaenidae (containing *Reighardia lomviae* and *Raillietiella* sp.) within the Pentastomida.

3.4.3. Phylogenetic analyses, maximum parsimony

The maximum parsimony analysis (MP) of the combined dataset resulted in 30 most parsimonious trees (mpt) with a length of 6518 steps. Fig. 6B shows the strict-consensus (Nelsen) compromise of these trees. The monophyly of the Branchiura is highly supported (bss = 95) with *Dolops ranarum* as
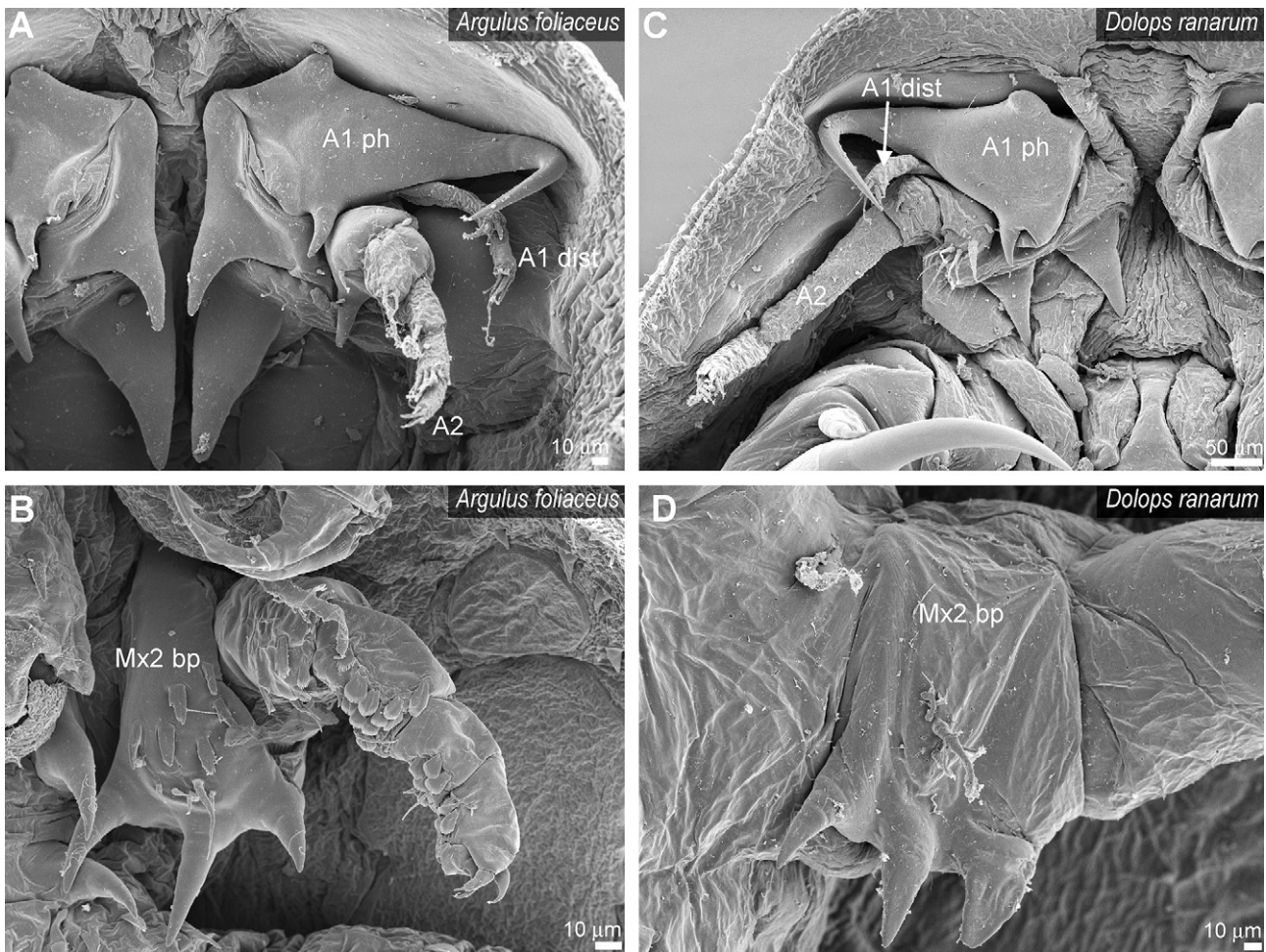
Fig. 5. Similarities between adult *Argulus* and *Dolops* in the morphology of the two pairs of antennae and second maxillae. A–B: *Argulus foliaceus,* C–D: *Dolops ranarum*. A: First and second antennae, adult, median view. B: Second maxilla with teeth on basal plate, adult, median view. C: First and second antennae, adult, anterior view. D: Second maxilla with teeth on basal plate, adult, median view. Abbr. *A1 dist* first antenna distal part, *A1 ph* first antenna proximal hook, *A2* second antenna, *Mx2 bp* second maxilla basal plate.

a sister group to a well-supported clade containing the included *Argulus* species and *Chonopeltis australis* (bss = 79). As *C. australis* is included in this unresolved node, there is no unambiguous support for the genus *Argulus*. A clade containing Branchiura and Pentastomida is highly supported (bss = 97). Within the Pentastomida clade (bss = 99), the *Porocephalus crotali* species is the sister group to a monophyletic Cephalobaenida with *Reighardia lomviae* and *Raillietiella* sp. (bss = 99). The clade containing (Ostracoda + Cirripedia), Branchiopoda, and (Pentastomida + Branchiura) is not resolved further, but has a high bootstrap support of 99. Finally, *Meganyctiphanes norvegica* is the sister group to the clade containing all the non-malacostracans in the analysis.

4. Discussion

4.1. First maxillae: are hooks or suction discs ancestral in the Branchiura?

With the exception of the genus *Dolops*, the first maxillae of all adult branchiurans are modified as suction discs for temporary attachment to their fish hosts. In *Dolops*, the first maxillae terminate in stout hooks already in the hatching stage and remain so during ontogeny (e.g., Wilson, 1904; Avenant et al., 1989; Gresty et al., 1993, and references above). So, is the hooked condition in *Dolops* ancestral for adult Branchiura or did it appear later in branchiuran evolution, retained from the larval condition in suction disc-bearing ancestors? Unfortunately the knowledge of branchiuran larvae is rather limited, but all known ontogenies of *Argulus* and *Chonopeltis* species (*Dipteropeltis* unknown) show larval first maxilla with distal hooks and a subsequent development of the proximal segments into suction discs. This condition is found in the species hatching as advanced metanauplii (e.g., *A. foliaceus* and *A. japonicus*) as well as in those hatching as juveniles e.g., *A. funduli* (Wilson, 1904, 1907; Shimura, 1981; Avenant et al., 1989. Hence, this is a classic case of adult structures in one taxon (hooks in adult *Dolops*) being similar to larval structures in other closely related taxa (hooks in larval *Argulus* and *Chonopeltis*).

To address this evolutionary question in detail we have followed two lines of research: (1) morphological comparisons

A

Squilla empusa
Meganyctiphanes norvegica

Dolops ranarum
Argulus nobilis
Argulus foliaceus
Argulus japonicus
Chonopeltis australis
Argulus monodi
Argulus sp. 1
Argulus sp. 2 — **Branchiura**

Porocephalus crotali
Reighardia lomviae
Raillietiella sp. — **Pentastomida**

Vargula hilgendorfi
Melavargula japonica — Ostracoda

Artemia salina
Eubranchipus grubii
Branchipus schaefferi — Branchiopoda

Semibalanus balanoides
Ibla quadrivalvis — Cirripedia

B

Squilla empusa
Meganyctiphanes norvegica

Vargula hilgendorfi
Melavargula japonica — Ostracoda
Semibalanus balanoides
Ibla quadrivalvis — Cirripedia

Eubranchipus grubii
Branchipus schaefferi
Artemia salina — Branchiopoda

Porocephalus crotali
Reighardia lomviae
Raillietiella sp. — **Pentastomida**

Dolops ranarum
Argulus sp. 1
Argulus sp. 2
Argulus japonicus
Argulus foliaceus
Argulus nobilis
Chonopeltis australis
Argulus monodi — **Branchiura**

between the adult first maxillar hooks in *Dolops* and the larval hooks in *Argulus* to examine the question of homology; and (2) a cladistic analysis including representatives of three branchiuran genera, several ''Maxillopoda'' taxa, and the Pentastomida, based on sequences from the small and large ribosomal subunits 18S rRNA, 28S rRNA and 16S mtDNA (see Table 1).

4.2. Morphological comparison of the first maxilla: hooks in adult Dolops and larval Argulus

A simple way of comparing arthropod limbs is by number of segments, although this approach is rarely sufficient to establish homologies. The first maxilla of larval *Argulus* has traditionally described as being four-segmented (Claus, 1875; Rushton-Mellor and Boxshall, 1994), and using the term ''segment'' *sensu* Boxshall (2004), this is also the number identified by us (see Figs. 2A, 3A–C and 4A). In *Dolops ranarum* four portions can be identified (Fig. 2D), as did Avenant et al. (1989). However, these subdivisions are not as easily identified as segments. Furthermore, the presence of specialized heavy musculature running through the entire limb complicates a precise identification of the subdivions, even when combining both light and scanning electron microscopic data (Fig. 4B).

Another way to identify homologies between limbs of various arthropod taxa is to look for specific 'markers', such as the presence or position of setae. Only a few setae can be found on the hooked first maxillae of adult *Dolops* and larval *Argulus* but the ones present are found in the same number on approximately the same portions in both taxa: portion 2 in both has two small setae (precise position of at least one of them is shared) and portion 4 of both has one seta (position also in common for this: cf. Figs. 2A,D).

When comparing the first maxilla distal hooks of larval *Argulus* with distal hooks of adult *Dolops*, they display approximately the same curvature and relative size. Furthermore in both taxa the first maxillae terminate in a double structure. In larval *Argulus* this takes the form of a two-part hook, where one hook-component is sleeved around the other near the tip, together forming in effect a single functional hook (Figs. 1B, 2A–C, 3A–C and 4A). In adult *Dolops* the first maxillae also end in a double structure (Figs. 2D and 4B). The hook itself is formed by only one element, but next to it is a characteristic spine-like structure (Figs. 2D,G) (see also Avenant et al., 1989). We consider these two terminal structures (hook and spine) of the first maxillae in adult *Dolops* as a homologue of the two hook components in larval *Argulus*. All these structures have the same distal position on the limb; furthermore, the distal part of the two-part hook in stage three *Argulus* -immediately before the segmented part of the limb degenerates- looks somewhat similar to the condition in adult *Dolops*

(i.e., the hooks are no longer intersleeved; cf. Figs. 2D, 3D,E and 4B). We conclude that there is good evidence for a homology between the first maxilla hooks of larval *Argulus* and the adult first maxilla hooks of *Dolops*. However, this does not clarify how these structures evolved within the Branchiura. Both an ancestral (i.e., symplesiomorphic) and a neotenic status of the maxillary hooks in *Dolops* are congruent with the homologies outlined above. As pointed out by Fink (1982) a question such as neotenic development versus retained ancestral condition of the maxillar hooks in *Dolops* can only be addressed in a phylogenetic context.

4.3. Maxillary hooks of Dolops ancestral for Branchiura

The phylogenetic reconstructions based on three loci place *Dolops ranarum* as a sister group to the remaining Branchiura with a very high support, both in the parsimony and Bayesian analysis (Fig. 6). But with regard to the first maxillae, it is impossible to decide whether the hooks or suction discs represent the plesiomorphic condition, as all possible outgroups have a different morphology. With our present knowledge and no additional hypotheses, it is equally parsimonious to assume suction discs as present in the last common ancestor to the Branchiura (and lost once in *Dolops*), as it is to assume hooks present in the last common ancestor and then lost once in the *Argulus* + *Chonopeltis* clade (Fig. 7). However, following Patterson (1996) in adding ontogeny data for character polarization, we suggest that the first maxilla development as seen in *Argulus* approximately recapitulates what took place during evolution (for further discussion of the ontogenetic criterion in character polarization and examples see e.g., Meier, 1997; Olesen, 2004). Hence, if this is accepted, the elaborate suction discs seen in *Argulus* (and other genera) have evolved from a *Dolops*-like hooked precursor. Boxshall (1998) pointed out that the first maxilla of *Argulus* maintains its functional continuity during a remarkable ontogenetic change from a distally located (claw) to a proximally located (suction disc) attachment structure (see also Rushton-Mellor and Boxshall, 1994). Even if the first maxillae and other hooked structures are recruited for cleaning purposes in the first larval stage in *A. foliaceus* (Møller et al., 2007), the first maxillae are at all times during development the primary attachment organ in *Argulus*. Thus, it is likely that the evolution from distal hooks to proximal suctions discs did not happen abruptly. There must have been ancestral intermediate forms with distal hooks and proximal suctions discs existing concomitantly as in the larvae of *Argulus*. The plausibility of such transitional morphology also has some support outside *Argulus*. Van As and Van As (1996) described the first maxillae of ''sub-adults'' of *Chonopeltis lisikili* Van As and Van As, 1996, as equipped with both a large (two-part) hook as well as fully formed suction discs. Being an extant species, this offers no direct proof

Fig. 6. Phylogenetic reconstruction of Branchiura, Pentastomida, and selected ''Maxillopoda''. A: Phylogeny as suggested by Bayesian Inference of combined 18S, 28S and 16S mtRNA data. 50% majority-rule consensus tree showing posterior probability values at bisections. B: Phylogeny as suggested by Maximum Parsimony analysis of the combined data (same dataset as A). Strict consensus (Nelsen) tree from 30 equally short trees. Bootstrap support values based on 5000 replicates, and only clades with bss > 50% are shown.
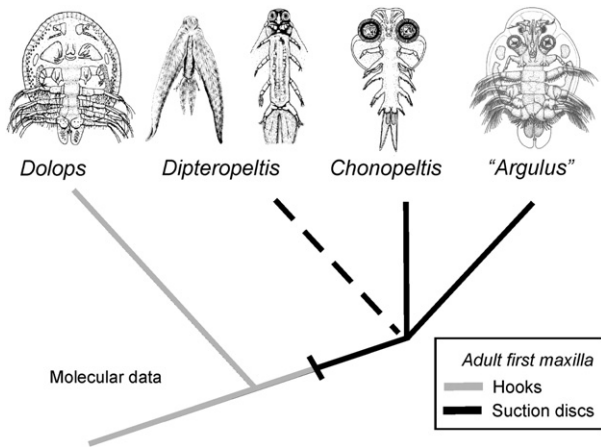
Fig. 7. Simplified summary of the phylogenies presented in Fig. 6. *Dolops* is the sister group of the remaining Branchiura suggesting that the first maxilla hooks of *Dolops* are ancestral in origin (plesiomorphic), and that suction discs have appeared later during the evolution of Branchiura (apomorphic). Note: In this phylogeny *Dipteropeltis hirundo* is included only for the sake of clarity (no material available). The position is speculative and thus marked by a dashed line. The possible paraphyletic status of *Argulus* is not illustrated.

of the ancestral condition, but it speaks in favour of the tenability of such morphology.

4.4. Morphological similarities between Argulus and Dolops

Some obvious morphological similarities are shared by *Argulus* and *Dolops* and not found in *Chonopeltis* or the monotypic South American *Dipteropeltis hirundo* (although only poor and incompatible data exist for this species: Calman, 1912; Ringuelet, 1943, 1948; Weibezahn and Cobo, 1964). As pointed out by, e.g., Maidl (1912) especially two groups of similarities between *Argulus* and *Dolops* are significant: the first and second antennae (Figs. 5A,C) and the second maxillae (Figs. 5B,D). In adult members of both taxa, the first antenna's first segment is a characteristic hook, bearing two smaller distal segments (A1 ph in Fig. 5). The small second antennae are situated just below the first and have the same number and proportions of the segments in both taxa (A2 in Fig. 5). Similarly, in both taxa the relative size and proportions of the second maxilla segments are the same, and the most basal segment has three characteristic "teeth" along the posterior margin (only two in some *Argulus* species) (Figs. 5B,D) (*Dolops*: Bouvier, 1897, 1898, 1899a,b; Thiele, 1904; Fryer, 1969; Avenant et al., 1989; *Argulus*: Claus, 1875; Wilson, 1902; Meehan, 1940; Fryer, 1956, 1959; Cressey, 1972; Gresty et al., 1993). The mentioned similarities between *Argulus* and *Dolops* could support a close relationship between these two genera, but this speaks against the results from the molecular based phylogenies presented above, and consequently we consider them symplesiomorphies at this point. A more comprehensive sampling of morphological characters is needed to reach a conclusive morphology-based

phylogeny and especially *Dipteropeltis hirundo* is in need of a re-study.

4.5. Non-monophyly of Argulus

Analyses of the molecular data reveal no support for the monophyly of the genus *Argulus*. The parsimony analysis places all included species of *Argulus* in an unresolved clade together with *Chonopeltis australis*. The result of the Bayesian analysis places *Chonopeltis australis* inside *Argulus* as sister group to three out of six *Argulus* species with high support values (Fig. 6).

Despite the limited number of included taxa (only one species of *Chonopeltis*) molecular evidence strongly favour that *Argulus* is paraphyletic with respect to *Chonopeltis*. The lack of a detailed morphology-based matrix makes it premature to evaluate this non-monophyly from a morphological point of view, but the following comments deserve to be made: no apparent morphological characters are shared between *Chonopeltis* and any grouping of *Argulus* species included here, leaving no obvious morphological support for *Argulus* being paraphyletic with respect to *Chonopeltis*. On the other hand, it has proved surprisingly difficult to locate solid synapomorphies for *Argulus* in our morphological character set for *Argulus* (e.g., a pre-oral stylet is also found in *Dipteropeltis hirundo*, see Ringuelet, 1943, 1948) leaving the monophyly of *Argulus* hard to corroborate at present.

4.6. Branchiura and Pentastomida relationship

Our analyses confirm the Branchiura + Pentastomida (=Ichthyostraca *sensu* Zrzavý, 2001) clade with a very high support. This relationship was suggested by Wingstrand (1972) based on sperm morphology, and has been fervently debated since. The Pentastomida are parasitic animals living in the respiratory tracts of vertebrates (see reviews by e.g., Osche, 1963; Self, 1969; Storch and Jamieson, 1992; Walossek and Müller, 1994; Almeida and Christoffersen, 1999; Waloszek et al., 2006). Apart from the spermatological evidence presented by Wingstrand (1972) and later corroborated by, e.g., Storch and Jamieson (1992), most morphological data point towards a position of the Pentastomida far from the Crustacea, possibly as a sister group to the Euarthropoda (Maas and Waloszek, 2001; Waloszek et al., 2006). Conversely, using molecular data, a very strong case has been made for the inclusion of Pentastomida in the Crustacea (e.g., Abele et al., 1989; Peterson and Eernisse, 2001; Zrzavý, 2001; Lavrov et al., 2004, reviewed in Waloszek et al., 2006). However, none of these molecular analyses have employed a true in-group sampling of the Branchiura, so until now it has not been tested whether the Branchiura and Pentastomida are true sister groups, or whether the Pentastomida possibly form an in-group of the Branchiura, a possibility mentioned already by Wingstrand (1972). In the present analyses of three gene loci, with representatives from three genera of Branchiura and both Porocephalidae and Cephalobaenidae

within the Pentastomida, we have a very strong confirmation of the close relation between the Branchiura and Pentastomida. Our in-group relationship within the Pentastomida differs from that suggested by Almeida and Christoffersen (1999), but both the Pentastomida and Branchiura remain monophyletic and appear as sister groups. Hence, there is no evidence of the Pentastomida being in-group of the Branchiura, but this does not exclude that the common ancestor to the two groups were closer to branchiurans than to pentastomids in morphology, given the extreme degree of specialization to parasitism shown by the latter.

4.7. Conclusions

- Molecular data (three loci: 16S, 18S and 28S) suggest *Dolops ranarum* as sister group to the remaining Branchiura (Figs. 6 and 7).
- The first maxilla hooks of adult *Dolops* are homologous to first maxillae hooks of larvae of *Argulus*.
- Based on this homologisation and on the presented phylogeny, we suggest that the ontogenetic sequence of first maxilla in *Argulus* (hooks at first, with subsequent development into suction discs) recapitulates the evolutionary pathway. A previous theory—the hooks of *Dolops* as being neotenic—is rendered unlikely.
- The early branch-off of *Dolops* suggests that the ancestral mode of host attachment in the Branchiura was by the use of first maxilla hooks (as in *Dolops*), and that the specialized first maxilla suction discs (e.g., in *Argulus* and *Chonopeltis*) developed later (Fig. 6).
- Molecular data suggest that *Argulus* is paraphyletic with respect to *Chonopeltis australis* (Fig. 6).
- Molecular data with in-group sampling suggest the Branchiura and Pentastomida as sister groups; the Pentastomida are not nested within the Branchiura.

References

Abele, L.G., Kim, W., Felgenhauer, B.E., 1989. Molecular evidence for inclusion of the phylum Pentastomida in the Crustacea. Molecular Biology and Evolution 6, 685−691.

Almeida, W.O., Christoffersen, M.L., 1999. A cladistic approach to relationships in Pentastomida. Journal of Parasitology 85, 695−704.

Altekar, G., Dwarkadas, S., Huelsenbeck, J.P., Ronquist, F., 2004. Parallel metropolis coupled Markov chain Monte Carlo for Bayesian phylogenetic inference. Bioinformatics 20, 407−415.

Avenant, A., Van As, J.G., Loots, G.C., 1989. On the hatching and morphology of *Dolops ranarum* larvae (Crustacea: Branchiura). Journal of Zoology (London) 217, 511−519.

Avenant-Oldewage, A., Knight, E., 1994. A diagnostic species compendium of the genus *Chonopeltis* Thiele, 1900 (Crustacea: Branchiura) with notes in its geographical distribution. Koedoe 37, 41−56.

Bouvier, M.E.L., 1897. Observations sur les Argulidés du genre *Gyropeltis* recueillis par M. Geay au Vénézuela. Bulletin Du Muséum D'Histoire Naturelle Paris, Section A 3, 13−19.

Bouvier, M.E.L., 1898. Les crustacés parasites du genre *Dolops* Audouin (premiere partie). Bulletin de Sciences de la Société Philomatique de Paris, Sér. 8 (10), 53−81.

Bouvier, M.E.L., 1899a. Les crustacés parasites du genre *Dolops* Audouin (seconde partie). Bulletin de Sciences de la Société Philomatique de Paris, Sér. 9 (1), 12−40.

Bouvier, M.E.L., 1899b. Sur les Argulidés du genre *Gyropeltis*, recueillis récement par M. Geay dans la Guyane. Bulletin Du Muséum D'Histoire Naturelle Paris, Section A 5, 39−41.

Boxshall, G.A., 1998. Comparative limb morphology in major crustacean groups: the coxa-basis joint in postmandibular limbs. In: Fortey, R.A., Thomas, M.M. (Eds.), Arthropod Relationships. Chapman and Hall, London, pp. 155−167.

Boxshall, G.A., 2004. The evolution of arthropod limbs. Biological Reviews 79, 253−300.

Brian, A., 1940. Sur quelques argulidés d'Afrique. Revue de Zoologie et de Botanique Africaines 33, 77−98.

Calman, W.T., 1912. On *Dipteropeltis*, a new genus of the crustacean order Branchiura. Proceedings of the Zoological Society of London 3, 763−766.

Claus, C., 1875. Über die Entwicklung, Organisation und systematische Stellung der Arguliden. Zeitschrift für wissenschaftliche Zoologie 15, 1−68.

Cressey, R.F., 1972. The Genus *Argulus* (Crustacea: Branchiura) of the United States. The Environmental Protection Agency, Washington D.C. Biota of Freshwater Ecosystems Identification Manual no. 2, 15.

Dahl, E., 1956. Some crustacean relationships. In: Wingstrand, K.G. (Ed.), Bertil Hanström. Zoological Papers in Honour of his Sixty-fifth birthday Nov. 20th, 1956. Zoological Institute, Lund, pp. 138−147.

Fink, W.L., 1982. The conceptual relationship between ontogeny and phylogeny. Paleobiology 8, 254−264.

Fryer, G., 1956. A report on the parasitic Copepoda and Branchiura of the fishes of Lake Nyasa. Proceedings of the Zoological Society of London 127, 293−344.

Fryer, G., 1959. A report on the parasitic Copepoda and Branchiura of the fishes of the Lake Bangweulu (Northern Rhodesia). Proceedings of the Zoological Society of London 132, 517−550.

Fryer, G., 1961. Larval development in the genus *Chonopeltis* (Crustacea: Branchiura). Proceedings of the Zoological Society of London 137, 61−69.

Fryer, G., 1964. Further studies on the parasitic Crustacea of the African freshwater fishes. Proceedings of the Zoological Society of London 143, 79−102.

Fryer, G., 1969. A new freshwater species of the genus *Dolops* (Crustacea: Branchiura) parasitic on a galaxiid fish of Tasmania − with comments on disjunct distribution patterns in the southern hemisphere. Australian Journal of Zoology 17, 49−64.

Fryer, G., 1977. On some species of *Chonopeltis* (Crustacea: Branchiura) from the rivers of the extreme South West Cape region of Africa. Journal of Zoology (London) 182, 441−455.

Glenner, H., Hansen, A.J., Sørensen, M.V., Ronquist, F., Huelsenbeck, J.P., Willerslev, E., 2004. Bayesian inference of the metazoan phylogeny: a combined molecular and morphological approach. Current Biology 14, 1644–1649.

Goloboff, P.A., Farris, J.S., Nixon, K.C., 2003. T.N.T.: Tree Analysis Using New Technology. Ver. 1.0. Program and Documentation. Available from the Authors, and at: www.zmuc.ku.dk/public/phylogeny.

Gresty, K.A., Boxshall, G.A., Nagasawa, K., 1993. The fine structure and function of the cephalic appendages of the branchiuran parasite, *Argulus japonicus* Thiele. Philosophical Transactions: Biological Sciences 339, 119–135.

Kelchner, S.A., Thomas, M.A., 2006. Model use in phylogenetics: nine key questions. In: Trends in Ecology and Evolution, 22. 87–94.

Kiernan, J.A., 1990. Histological & Histochemical Methods: Theory & Practice. Pergamon Press, Oxford, 433 pp.

Krøyer, H., 1863. Bidrag til kundskab om Snyltekrebsene. Naturhistorisk Tidsskrift 3, 1–352.

Lavrov, D.V., Brown, W.M., Boore, J.L., 2004. Phylogenetic position of the Pentastomida and (pan)crustacean relationships. Proceedings of the Royal Society B: Biological Sciences 271, 537–544.

Lutsch, E., Avenant-Oldewage, A., 1995. The ultrastructure of the newly hatched *Argulus japonicus* Thiele, 1900 larvae (Branchiura). Crustaceana 68, 329–340.

Maas, A., Waloszek, D., 2001. Cambrian derivatives of early arthropod stem lineage, Pentastomides, Tardigrades and Lobopodians - an "Orsten" perspective. Zoologischer Anzeiger 240, 451–459.

Maidl, F., 1912. Beiträge zur Kenntnis des anatomischen Baues der Branchi-urengattung *Dolops*, 19. Arbeiten aus dem Zoologischen Institut der Universität Wien. 317–346.

Markmann, M., Tautz, D., 2005. Reverse taxonomy: an approach towards determining the diversity of meiobenthic organisms based on ribosomal RNA signature sequences. Philosophical Transactions: Biological Sciences 360, 1917–1924.

Martin, M.F., 1932. On the morphology and classification of *Argulus* (Crustacea). Proceedings of the Zoological Society of London, 771–806.

Martin, J.W., Davis, G.E., 2001. An updated classification of the recent Crustacea. Natural History Museum of Los Angeles County Science Series 39, 1–124.

Meehan, O.L., 1940. A review of the parasitic Crustacea of the genus *Argulus* in the collections of the United States National Museum. Proceedings of the U.S. National Museum 88, 459–522.

Meier, R., 1997. A test and review of the empirical performance of the ontogenetic criterion. Systematic Biology 46, 699–721.

Møller, O.S., Olesen, J., Waloszek, D., 2007. Swimming and cleaning in the free-swimming phase of *Argulus* larvae (Crustacea, Branchiura) - appendage adaptation and functional morphology. Journal of Morphology 268, 1–11.

Nylander, J.A.A., Ronquist, F., Huelsenbeck, J.P., Nieves-Aldrey, J.L., 2004. Bayesian phylogenetic analysis of combined data. Systematic Biology 53, 47–67.

Olesen, J., 2004. On the ontogeny of the Branchiopoda (Crustacea): contribution of development to phylogeny and classification. In: Scholtz, G. (Ed.), Evolutionary Developmental Biology of Crustacea. Swets & Zeitlinger B.V., Lisse, The Netherlands, pp. 217–271.

Osche, G., 1963. Die systematische Stellung und Phylogenie der Pentastomida. Zeitschrift für Morphologie und Ökologie der Tiere 52, 487–596.

Overstreet, R.M., Dyková, I., Hawkins, W.E., 1992. Branchiura. In: Harrison, F.W., Humes, A.G. (Eds.), Microscopic Anatomy of Inverte-brates. Crustacea, Vol. 9. Wiley-Liss, Inc., New York, pp. 385–413.

Patterson, C., 1996. Comments on Mabee's "Empirical rejection of the onto-genetic polarity criterion". Cladistics 12, 147–167.

Peterson, K.J., Eernisse, D.J., 2001. Animal phylogeny and the ancestry of bilaterians: inferences from morphology and 18S rDNA gene sequences. Evolution and Development 3, 170–205.

Posada, D., Crandal, K.A., 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Ringuelet, R., 1943. Revision de los argúlidos argentinos (Crustácea, Branchiura) con el catálogo de las especies neotropicales. Revista del Museo de La Plata (Nueva Serie) 3, 43–99.

Ringuelet, R., 1948. Argúlidos del Mueso de La Plata. Revista del Museo de La Plata (Nueva Serie) 5, 281–296.

Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.

Rushton-Mellor, S.K., Boxshall, G.A., 1994. The developmental sequence of *Argulus foliaceus* (Crustacea: Branchiura). Journal of Natural History 28, 763–785.

Schram, F.R., 1986. Crustacea. Oxford University Press, Oxford, 606 pp.

Self, J.T., 1969. Biological relationships of the Pentastomida; a bibliography on the Pentastomida. Experimental Parasitology 24, 63–119.

Shafir, A., Van As, J.G., 1986. Laying, development and hatching of eggs of the fish ectoparasite *Argulus japonicus* (Crustacea: Branchiura). Journal of Zoology 210, 401–414.

Shimura, S., 1981. The larval development of *Argulus coregoni* Thorell (Crustacea: Branchiura). Journal of Natural History 15, 331–348.

Skelton, P., 2001. A Complete Guide to the Freshwater Fishes of Southern Africa. Struik Publishers, Cape Town, 395 pp.

Storch, V., Jamieson, B.G.M., 1992. Further spermatological evidence for including the Pentastomida (tongue worms) in the Crustacea. International Journal of Parasitology 22, 95–108.

Sullivan, J., Joyce, P., 2005. Model selection in phylogenetics. Annual Review of Ecology, Evolution and Systematics 36, 445–466.

Swofford, D.L., 2002. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4.0 beta 10. Sinauer Associates, Sunderland, MA.

Thiele, J., 1904. Beiträge zur Morphologie der Arguliden. Mitteilungen aus dem Zoologischen Museum zu Berlin 2, 5–51.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 25, 4876–4882.

Tokioka, T., 1936. Larval development and metamorphosis of *Argulus japonicus*. Memoirs of the College of Science, Kyoto Imperial University, Series B 12, 93–114.

Van As, L.L., Van As, J.G., 1996. A new species of *Chonopeltis* (Crustacea: Branchiura) from the southern Rift Valley, with notes on larval development. Systematic Parasitology 35, 69–77.

van Niekerk, J.P., Kok, D.J., 1989. *Chonopeltis australis* (Branchiura): Structural, developmental and functional aspects of the trophic appendages. Crustaceana 57, 51–56.

Waloszek, D., Müller, K.J., 1994. Pentastomid parasites from the Lower palaeozoic of Sweden. Transactions of the Royal Society of Edinburgh 85, 1–37.

Waloszek, D., Müller, K.J., 1998. Early arthropod phylogeny in light of the Cambrian "Orsten" fossils. In: Edgecombe, G.D. (Ed.), Arthropod Fossils and Phylogeny. Columbia University Press, New York, pp. 185–231.

Waloszek, D., Repetski, J.E., Maas, A., 2006. A new Late Cambrian pentastomid and a review of the relationships of this parasitic group. Trans-actions of the Royal Society of Edinburgh, Earth Sciences 96, 163–176.

Weibezahn, F.H., Cobo, T., 1964. Seis argulidos (Crustacea: Branchiura) parasitos de peces dulce-acuicolas en Venezuela, con descripcion de una nueva especie del genero *Argulus*. Acta Biologica Venezuelica 4, 119–144.

Wilson, C.B., 1902. North American parasitic copepods of the family Argulidae, with a bibliography of the group and a systematic review of all known species. Proceedings of the U.S. National Museum 25, 635–742.

Wilson, C.B., 1904. A new species of *Argulus*, with a more complete account of two species already described. Proceedings of the U.S. National Museum 27, 627–655.

Wilson, C.B., 1907. Additional notes on the development of the Argulidae, with description of a new species. Proceedings of the U.S. National Museum 32, 411–424.

Wingstrand, K.G., 1972. Comparative spermatology of a pentastomid, *Raillie-tiella hemidactyli*, and a branchiuran crustacean, *Argulus foliaceus*, with a discussion of pentastomid relationships. Det Kongelige Danske Videnskabernes Selskab, Biologiske Skrifter 19, 1–72.

Zrzavý, J., 2001. The interrelationships of metazoan parasites: a review of phylum- and higher-level hypotheses from recent morphological and molecular phylogenetic analyses. Folia Parasitologica 48, 81–103.