Giant Females and Dwarf Males: A Comparative Study of Nuptial Organs in Female Chondracanthidae (Crustacea: Copepoda).

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Abstract. Male chondracanthids attach to specific structures located near the genital apertures of the female. The term nuptial organ is proposed here for these structures. The morphology of the single median nuptial organ in *Blias prionoti* and *Pseudoblias lyrifera*, and of the paired nuptial organs in *Acanthochondria cornuta, A. limandae, Acanthochondrites annulatus, Chondracanthodes deflexus*, *Chondracanthus lophii,* and *Lernentoma asellina* is described. Nuptial organs in *C. lophii* were found to contain glandular tissue, so in addition to providing a site for male attachment it is possible that these glands may either play a role in pheromone production or alternatively may function in supplying nutrients for the male. It is concluded that male chondracanthids are not parasitic, but might be dependent on the female for food, which would be another rare case of nuptial feeding of males by females.

Key words. Parasitic copepod, nuptial organ, morphology, ultrastructure

1. INTRODUCTION

Several families of parasitic copepods show marked sexual dimorphism in body size, including the Lernaeopodidae (order Siphonostomatoida) and Chondracanthidae (order Poecilostomatoida), both of which have been cited as examples of dwarf males (e.g. DE BEER 1951; CLAUS 1861; HEEGAARD 1947; KABATA 1979; NORDMANN 1864; ROUSSET & RAIBAUT 1983; WILSON 1915). These males are many times smaller than the females and, in the Chondracanthidae, the males have sometimes been assumed to be parasitic on the female (e.g. ROUSSET et al. 1978; THOMPSON 1893), although the precise nature of the relationship between female and male has yet to be elucidated.

Male chondracanthids locate and attach to young immature females at the second copepodite stage (HEEGAARD 1947). The male completes its development on the female and remains there until it dies. Preliminary observations on *Chondracanthus lophii* Johnston, 1836 (ØSTERGAARD submitted) revealed that males use the claws of the paired antennae to hook onto the female attaching to specific paired structures located near the genital apertures of the female. Similar structures have been recorded for other chondracanthid species (e.g. HO 1970), but their significance has not been fully understood. This lack of understanding is reflected in the variety of terms used to refer to these structures, e.g. vermiform processes, conical tubercles, flap-like ventral process, knobs, and expansions. The question addressed here is what role does this novel structure play in the relationship between male and female chondracanthids.

The term nuptial organ is proposed here and this paper describes the morphology of the nuptial organ in eight different female chondracanthids: *Acanthochondria cornuta* (Müller, 1776), *A.limandae* (Krøyer, 1863), *Acanthochondrites annulatus* (Olsson, 1868), *Blias prionoti* Krøyer, 1863, *Chondracanthodes deflexus* Wilson, 1932, *Chondracanthus lophii*,

Lernentoma asellina (Linnaeus, 1758) and *Pseudoblias lyrifera* Heegaard, 1962. The internal anatomy of the structure is examined in *C. lophii* using light miscropscopy and transmission electron microscopy.

2. MATERIAL & METHODS

2.1. Material

Material of *Acanthochondria cornuta*, *A.limandae, Chondracanthus lophii* and *Lernentoma asellina* was obtained from gills, gill pouches or oral cavities of *Hippoglossoides platessoides* (Fabricius, 1780), *Limanda limanda* (Linnaeus, 1758), *Lophius piscatorius* (Linnaeus, 1758) and *Chelidonichthys lucerna* (Linnaeus, 1758) respectively. All fish were taken from trawls on R/V *Scotia*, during cruise 0800S (26-29, May 2000) at Bell Rock (56°27'N, 2°15'E) and Fair Isle (59°12'N, 01°26'E). The rest of the material was obtained from the collection at The Natural History Museum, London: *Acanthochondrites annulatus* (BMNH 1976.1225-1228), *Blias prionoti* (BMNH 1979.500-509), *Chondracanthodes deflexus* (BMNH 1985.473) and *Pseudoblias lyrifera* (BMNH 1984.75).

2.2. Scanning Electron Microscopy (SEM)

Specimens from the R/V *Scotia* cruise were fixed as for TEM (see below) and dehydrated through graded acetone series, then critical point dried, mounted and sputter-coated with gold palladium. Material of *A. annulatus, B. prionoti*, *C. deflexus* and *P. lyrifera* which was already stored in alcohol was dehydrated in acetone, then dried following the same protocol as for TEM fixed material. All material was examined in a Hitachi S2500 scanning electron microscope.

2.3. Light Microscopy (LM)

Eight ovigerous females *C. lophii* with males attached were treated using the same protocol as for transmission electron microscopy (see below) and cut in semithin sections (1.0 μm-1.5 μm), which were then stained with toluidine blue and examined in an Olympus Photomicroscope.

2.4. Transmission Electron Microscopy (TEM)

Fresh material of *C. lophii* was fixed in 4.5% glutaraldehyde-formaldehyde mixture (with 0.2M cacodylate buffer, pH 7.4) with added sodium chloride (2.5%) for seawater effect (modified from KARNOVSKY 1965). Secondary fixation was performed in the laboratory using trialdehyde (with added 2.5% sodium chloride and buffered with 0.2M cacodylate, pH 7.4) overnight (modified from LAKE 1973). Finally, the material was postfixed in 1% OsO_4 (buffered with 0.2M cacodylate, pH 7.4). After fixation, the specimens were left in 16% glycerol overnight on a rotating table (after FELGENHAUER 1987) to remove mucus, sand grains and other particles. The specimens were dehydrated through graded alcohol series and embedded in TAAB Resin. Ultra-thin sections (70 nm-90 nm) for TEM were stained with uranyl acetate and lead citrate, and were examined in a Hitachi H7100 transmission electron microscope.

2.5. Abbreviations

a antenna

a1 antennule

c antennal claw

ei epicuticle (inner layer)

eo epicuticle (outer layer)

g genital opening/operculum

i	integument
m	mouth
n	nucleus
o	nuptial organ
p	posterior process
pc	procuticle
p1	leg 1
p2	leg 2
r	caudal ramus
s	egg sac (or remnants of egg sac)
v	secretory vesicle
vc	ventral cord (central nervous system)
♀	female
♂	male

3. RESULTS

3.1. External Morphology

The morphology of the nuptial organ varies in the family, even within genera. It can be paired or can occur as a median unpaired structure. Species with paired nuptial organs include: *Acanthochondrites annulatus* (Olsson, 1868) (Fig. 1G), *Berea ancoralis* (Bere, 1936) (Figs. 1B & F), *Chondracanthus angustatus* Heller, 1865 (cf. ROUSSET et al. 1978: pg. 75), *Heterochondria crassicornis* (Krøyer, 1837) (Fig. 1A), *Lernentoma asellina* (cf. ROUSSET & RAIBAUT 1983: fig. IV), *Mecaderochondria pilgrimi* Ho & Dojiri, 1987 (Fig. 1C) and *Scheherazade scheherazade* Leigh-Sharpe, 1934 (Fig. 1D). Species with a single nuptial

organ include: *Bactrochondria papilla* Ho, Kim & Kumar, 2000 (Fig. 1I), *Protochondracanthus alatus* (Heller, 1868) (Fig. 1H), *Protochondria longicauda* Ho, 1970 (Fig. 1J) and *Pseudoblias lyrifera* Heegaard, 1962 (Fig. 1E).

3.1.1. *Acanthochondria cornuta.* In *A. cornuta* the nuptial organs are paired and located in the ventral groove of the female genital area, on the medial margin at the base of the posterior body processes (Fig. 2A). The two organs differ in shape. The right one, to which the male was attached was clearly expanded and enlarged compared to the left, which did not have a male attached. The nuptial organ (ca. 80 μm high) on the left was produced from a broad base (ca. 30 μm high and 120 μm in diameter at basis) into a tapering tip (ca. 50 μm high), which was apically rounded (ca. 25 μm in diameter) and had a wrinkled surface (Fig. 2B). The right nuptial organ also protruded from a broad base (ca. 30 μm high) (Fig. 2A) however, the tip was inflated (between 70-80 μm) and more flap-like (Fig. 2C), with a smoother surface than on the left. Traces of the detached male antennal claw could be seen embedded in the organ (Fig. 2A).

3.1.2. *Acanthochondria limandae.* In *A. limandae* the nuptial organ was similarly located (Fig. 2D) and also paired. The two nuptial organs differed in shape. The left one, to which the male was attached was larger than the right, which did not have a male attached. The right organ tapered from a broad base (ca. 40 μm high) into an apically rounded tip (ca. 35 μm high) (Fig. 2E). The surface was rippled. The left organ also protruded from a broad base (ca. 35 μm high) (Fig. 2D) had a tip which was inflated (between 60-70 μm) and knob-like (Fig. 2F).

3.1.3. *Acanthochondrites annulatus.* The material of *A. annulatus* was slightly damaged, but we were able to locate the nuptial organs which were positioned on the medial margin at the base of the posterior body processes (Fig. 4A), as in *A. cornuta* and *A. limandae*. The left

organ, which was untouched, was concealed and impossible to study it in detail using SEM, but the right organ, to which the male was attached, was exposed. It was about 50 μm in diameter and 90 μm in length (Fig. 4B) and resembled the left side organ in *A. limnadae* (which had the male attached).

3.1.4. *Blias prionoti.* Females of *B. prionoti* have no posterior processes (Fig. 3A) and the nuptial organ was located midventrally at the posterior end of the female, just anterior to the genital openings (Fig. 3B). The nuptial organ was rather amorphous and appeared more like a bulge formed as a result of the male antennae pinching the integument of the female. Nevertheless, it had a squarish shape and was approximately 25 μm in diameter at the tip and protruded about 40 μm (Fig. 3C). Traces of the male antennal claw could be seen embedded in the organ (Fig. 3C).

3.1.5. *Chondracanthodes deflexus.* The nuptial organ of *C. deflexus* is paired and located on the medial margin at the base of the posterior body processes (Fig. 3D & E). The left and right organs were similar in shape regardless of whether a male was attached or not. They were small, rounded lobes (ca. 80 μm in width and in height) with a smooth surface (Fig. 3E & F).

3.1.6. *Chondracanthus lophii.* Male *C. lophii* were always found attached to the posterior end of the female (Fig. 3G), attached to one of the paired nuptial organs (Fig. 3H) which were located on the medial margin in the ventral groove at the base of the posterior body processes. The nuptial organs were of an unusual shape, each comprising a large process with a dense surface array of smaller protrusions (ca. 7.5 μm in diameter) (Fig. 3H & I), making the structure resemble a pine cone. The left organ which did not have a male attached was about 115 μm high and 100 μm at the base, the right organ, to which the male was attached, had a distorted shape and was about 80-100 μm at the base and 200 μm high.

3.1.7. *Lernentoma asellina.* The nuptial organs of *L. asellina* are paired and found on the medial margin in the ventral groove at the base of the posterior body processes (Fig. 2G).

They were barely visible as small humps on the body surface. The right one where the male was attached protruded slightly more (ca. 40 μm) (Fig. 2I) than the other (ca. 25 μm) (Fig. 2H). They were both about 60 μm in diameter at the base. The surface was rippled.

3.1.8. *Pseudoblias lyrifera.* *P. lyrifera* has no posterior processes and the nuptial organ for the male was located midventrally at the posterior end, anterior to the genital openings (Fig. 4C). The male was attached to the right side of the organ (arrowed in Fig. 4C). However, the organ in *P. lyrifera* looked more like a surface bulge than a well defined organ. The part of the organ to which the male was attached was approximately 20 μm in diamter and 20 μm in height (Fig. 4D). Traces of the detached male antennal claw could be seen embedded in the organ (Fig. 4D).

3.2. Anatomy

Male *Chondracanthus lophii* grasps the nuptial organ using the claws of the paired antennae (Fig. 5A & F). The cuticle of the nuptial organ (Fig. 5B & C) is similar in thickness to the cuticle covering the rest of the body and is of the typical form for free-living and ectoparasitic copepods (cf. BRESCIANI 1986). The cuticle has a multilayered epicuticle (ca. 1.5 μm thick) with a thin, electron-dense outer layer (less than 100 nm) with a dense surface array of rounded cuticular knobs (Fig. 5D). The inner-most layer of the epicuticle is an electron-lucent, homogenous zone (ca. 1.4 μm thick) (Fig. 5E). The procuticle consisted of a single laminated layer (Fig. 5E).

Glandular cells are present within the nuptial organ. These cells are columnar, with a spherical nucleus (Fig. 5B) and numerous secretory vesicles (Fig. 5B & G). The gland contained a reservoir of secretion formed by coalescence of secretory vesicles (Fig. 5F & G). In the material observed we were not able to locate the opening(s) of the gland.

4. DISCUSSION

In the Chondracanthidae only one male is usually found attached to an adult female. Rarely females carry more than one male. For example, HO (1970) described a female *Juanettia cornifera* Wilson, 1921 with one male attached to the posterior end of the female and another, smaller male attached to one of the egg sacs (HO 1970: figs. 175 and 177).

The nuptial organ observed in *Pseudoblias lyrifera* differs from that reported by KABATA (1969, pg 497 & Fig. 1D; our Fig. 1E) who described it as a "small, flap-like ventral process", which he pointed out was the attachment point for the male. Using SEM, the organ of *P. lyrifera* appears more as a bulge in the surface rather than as a flap. Even though the male attached asymmetrically to the structure, we interpret this as a unpaired medial nuptial organ, because of its similarities to the organ present in *Blias prionoti*.

The single median nuptial organ in *B. prionoti* and *P. lyrifera* differs markedly from the paired organs found in the other chondracanthids included in this study. Unfortunately, we were unable to study any of the other species described as having a single median organ. On the basis of *B. prionoti* and *P. lyrifera*, it appears that when a single median organ is present it is more like a bulge in structure, which the male pinches when attaching to the female.

Paired nuptial organs have a better defined morphology. In some cases the shape is affected by male attachment (e.g. *Acanthochondria cornuta*) which causes deformation of the organ, but in other cases no changes were observed that could be correlated with the pressure of an attached male (e.g. *Chondracanthodes deflexus*). The paired nuptial organs in *Chondracanthus lophii* contain glandular tissue, and other chondracanthids may also have glandular nuptial organs, but this has yet to be confirmed. The nuptial organ clearly provides a physical site for male attachment but does it have a dual purpose? What is the function of these glandular structures within the nuptial organ in *C. lophii*?

Adult females of various free-living copepods produce pheromones which are involved in mate location behaviour. DOALL et al. (1998) and WEISSBURG et al. (1998) showed how males of the calanoid *Temora longicornis* (Müller, 1785) could track a potential mate over long distances and YEN et al. (1998) concluded that male *T. longicornis* located the females by using chemoreception. Similarly, males of *Calanus marshallae* Frost, 1974 also find females by following trails of pheromone deposited by females (TSUDA & MILLER 1998). KELLY et al. (1998) found that the pheromones involved in mating in harpacticoid *Tigriopus japonicus* Mori, 1938 were species-specific. Mate location for a parasite is more complex, since males must first successfully locate a host with a female attached, before locating a sexually receptive female. It is possible that the nuptial organ in female *C. lophii* could be responsible for pheromone production. However, there is an alternative possibility.

WILSON (1915) concluded that males of the Lernaeopodidae fed on the host, not on the female, but GRAY (1926) speculated that the paired structure at the junction of the trunk and cephalothorax of the female of the lernaeopodid *Lernaeopoda scyllicola* Leigh-Sharpe, 1916 could function by providing the male with nourishment. He named these structures bromatophores. *L. scyllicola* males were always found near one of the paired bromatophores and the bromatophore with the adjacent or attached male would be large and swollen, whereas, the other bromatophore was hardly developed. This observation on size difference between the bromatophores in *L. scyllicola* is in accord with our observations on differences in some species with paired nuptial organs.

ØSTERGAARD (submitted) concluded that male *C. lophii* continued to feed as an adult because it retained well developed mouth parts and functional oesophagus and midgut. However, she was unable to determine the nature of the food utilised. THOMPSON (1893) claimed that males of *C. lophii* lived as parasites on the female, which would imply some detrimentral effect to the female. Indeed males have often been referred to as dwarf parasitic

males (e.g. CALMAN 1911; NORDMANN 1864; ROUSSET & RAIBAUT 1983; ROUSSET et al. 1978). ROUSSET et al. (1978) found that male *Chondracanthus angustatus* Heller, 1865 always attached to one of the paired nuptial organs and they suggested that they do not only function as a holdfast for the male, but also play a role in providing nutrition for the male. They based this suggestion on SEM images showing that the male was attached to the female in a manner so its mouth opening was within close proximity to the surface of the nuptial organ on the female. The glandular secretions produced by the nuptial organs of female *C. lophii* may represent the food source for the male. This concept of nuptial feeding of males by females is rare, although it has been reported in hexapods (ARNQVIST et al. 2003).

The morphological investment by the female in the production of paired nuptial organs suggests some kind of benefit to the female, presumably in the form of enhanced reproductive success. In addition, the production of glandular secretion by the female, which may be utilised as a food source by the male, represents a further investment of resources by the female. We conclude that chondracanthid males are not parasitic. Instead they may well be dependent upon the female for their nutrient supply, but this is interpreted as a form of nuptial feeding.

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FIGURE CAPTIONS

Fig. 1. A-J. Location of nuptial organs in Chondracanthidae: A) female *Heterochondria crassicornis*, ventral; B) female *Berea ancoralis*, ventral; C) female *Mecaderochondria pilgrimi*, posterior end of trunk, ventral; D) female *Scheherazade scheherazade*, genito-abdomen, lateral; E) female *Pseudoblias lyrifera*, posterior end of trunk, ventral, slightly lateral; F) female *Berea ancoralis*, genito-abdomen, ventral; G) female *Acanthochondrites annulatus*, genito-abdomen, ventral; H) female *Protochondracanthus alatus*, posterior processes and genito-abdomen, ventral; I) female *Bactrochondria papilla*, genito-abdomen, ventral; J) female *Protochondria longicauda*, genito-abdomen, ventral. Arrows point to site of nuptial organs. Scales: 1 mm in (B), 400 μm in (A), 300 μm in (C, G), 100 μm in (D, F, J), 50 μm in (E, H, I). [Adapted from original drawings from HO (1970) (A, B, D, F, G, J) with permission of the editor. HO & DOJIRI (1987) (C) with permission of the publisher. HO et al. (2000) (I, H) and KABATA (1969) (E), with permission of Taylor & Francis Ltd., http://www.tandf.co.uk/journals/tf/00222933.html].

Fig. 2. A-C. Nuptial organs in *Acanthochondria cornuta*: A) ventral aspect of female posterior area showing nuptial organs (large arrows). The male was attached to the right one and traces of the antennal claw can be seen (small arrow). Scale bar: 250 μm. B) detail of untouched nuptial organ. Scale bar: 60 μm. C) detail of nuptial organ to which the male was attached. Scale bar: 15 μm. D-F. Nuptial organ in *Acanthochondria limandae.* D) ventral aspect of female posterior area showing nuptial organs (arrows); the male was attached to the left one. Scale bar: 250μm. E) detail of untouched nuptial organ. Scale bar: 60 μm. F) detail of organ to which the male was attached. Scale bar: 23 μm. G-I. Nuptial organ in *Lernentoma asellina.* G) ventral aspect of female genital area showing nuptial organs (arrows). The male

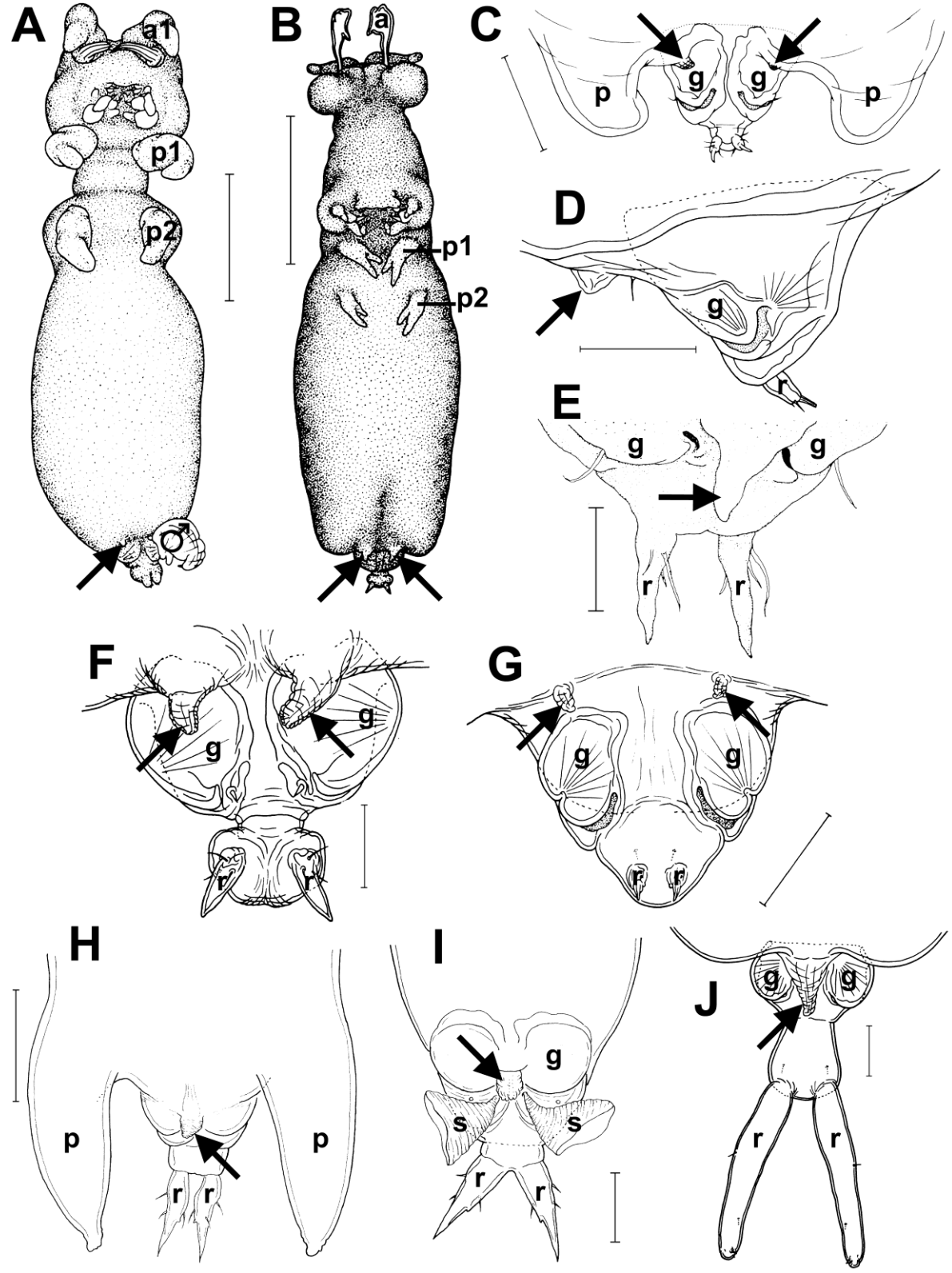
was attached to the right one. Scale bar: 230 μm. H) detail of untouched nuptial organ. Scale bar: 27 μm. I) detail of the nuptial organ to which the male was attached. Scale bar: 30 μm.

Fig. 3. A-C. Nuptial organ in *Blias prionoti.* A) ventral aspect, slightly lateral, of female with male attached. Male is shown in lateral aspect, slightly ventral. Scale bar: 0.75 mm. B) ventral aspect of female posterior area showing single median nuptial organ (arrow). Scale bar: 136μm. C) detail of nuptial organ to which male was attached. Arrow indicates traces of male antennal claw. Scale bar: 25 μm. D-F. Nuptial organ in *Chondracanthodes deflexus.* D) ventral aspect of female with male attached to one of the nuptial organs showing nuptial organ. Scale bar: 1.5 mm. E) ventral aspect of female posterior area showing nuptial organs (arrows). Scale bar: 0.38 mm. F) detail of nuptial organ. Scale bar: 30μm. G-I. Nuptial organ in *Chondracanthus lophii.* G) ventral aspect of female posterior area with male attached. Scale bar: 1.36 mm. H) the two nuptial organs (arrowed).The male was attached to the right one. Scale bar: 100μm. I) detail of the protrusions on the surface of the nuptial organ. Scale bar: 7.5μm.

Fig. 4. A-B. Nuptial organ in *Acanthochondrites annulatus.* A) ventral aspect of female posterior region showing nuptial organ (arrow). The male was attached to the right one and the left one is hidden in a groove. Scale bar 0.3 mm. B) detail of nuptial organ to which the male was attached. Scale bar: 38 μm. C-D. Nuptial organ in *Pseudoblias lyrifera.* C) ventral aspect of female posterior area showing single median nuptial organ (arrow). Scale bar: 75 μm. D) detail of part of the nuptial organ to which the male was attached. Traces of male antennal claws are arrowed. Scale bar: 15 μm.

Fig. 5. A-G. Ultrastructure of nuptial organ in *Chondracanthus lophii*. A) Semithin transverse section of male *C.lophii* attached to nuptial organ. Note the tip of antennal claw in the tissue of the female structure (white circle). Scale bar: 50μm. B) Oblique-transverse semithin section of nuptial organ of adult female *C. lophii* showing multicellular gland. Scale bar: 50μm. C) TEM of superficial section through nuptial organ of *C. lophii* showing the cuticle covering of the numerous protrusions. The epicuticle has a thin outer layer and a thicker inner layer, and a laminated procuticle. Scale bar: 4 μm. D) Higher magnification TEM of the epicuticle shown in Fig. 5C. Note the dense array of cuticular knobs. Scale bar: 400 nm. E) TEM showing detail of the laminated procuticle of the nuptial organ. Scale bar: 5μm. F) TEM of nuptial organ. Notice male antennal claw and reservoir of secretion. Scale bar: 10μm. G) Higher magnification TEM of the reservoir of secretion found in the nuptial organ. Large numbers of secretory vesicles are found. Scale bar: 4 μm.

Figure 1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p1
p2
C
p
g
g
p
D
g
r
E
g
g
r
r
F
g
g
r
r
G
g
g
r
r
H
p
p
r
r
I
g
s
s
r
r
J
g
g
r
r

Figure 2

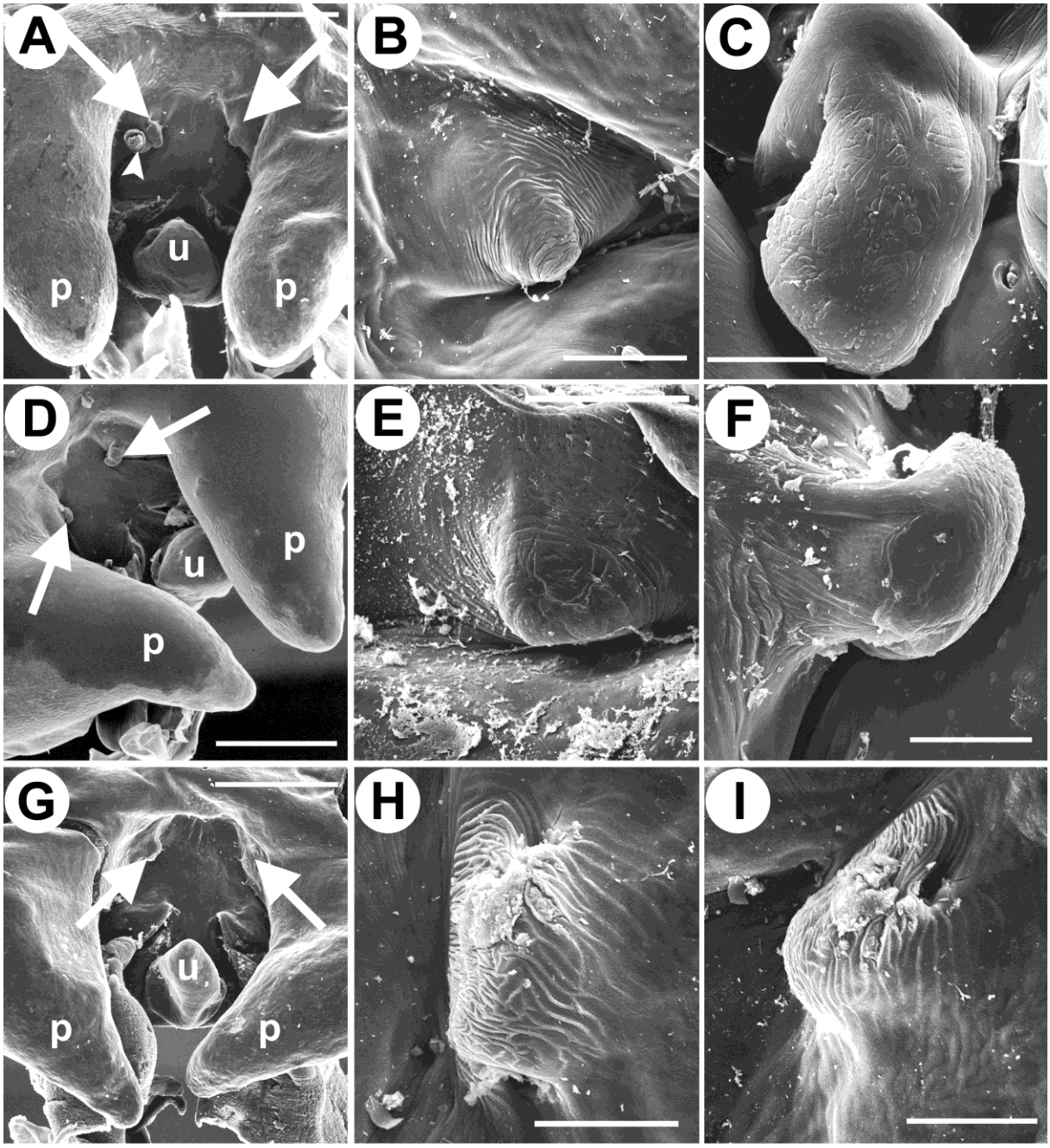

Figure 3

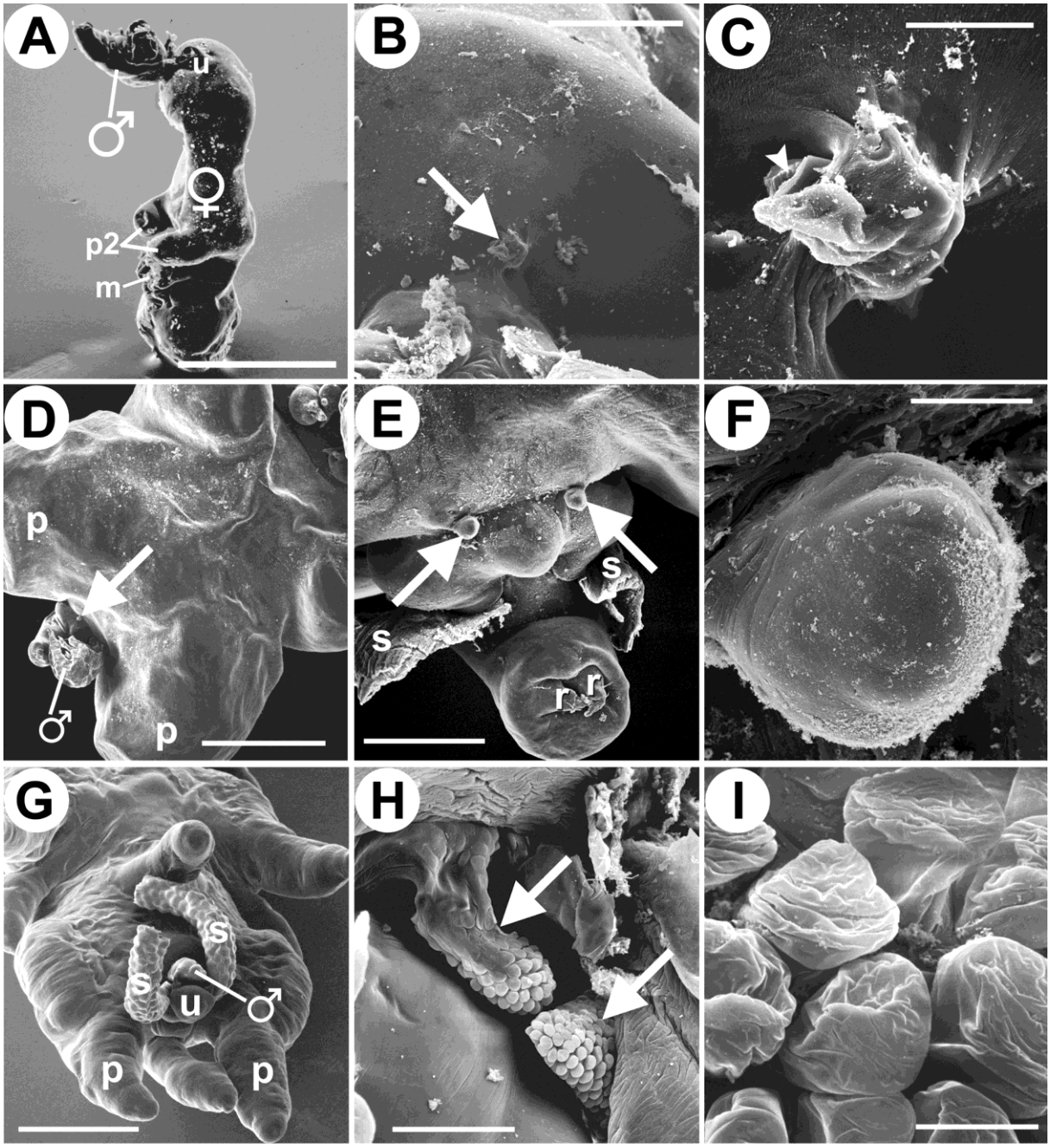

Figure 4

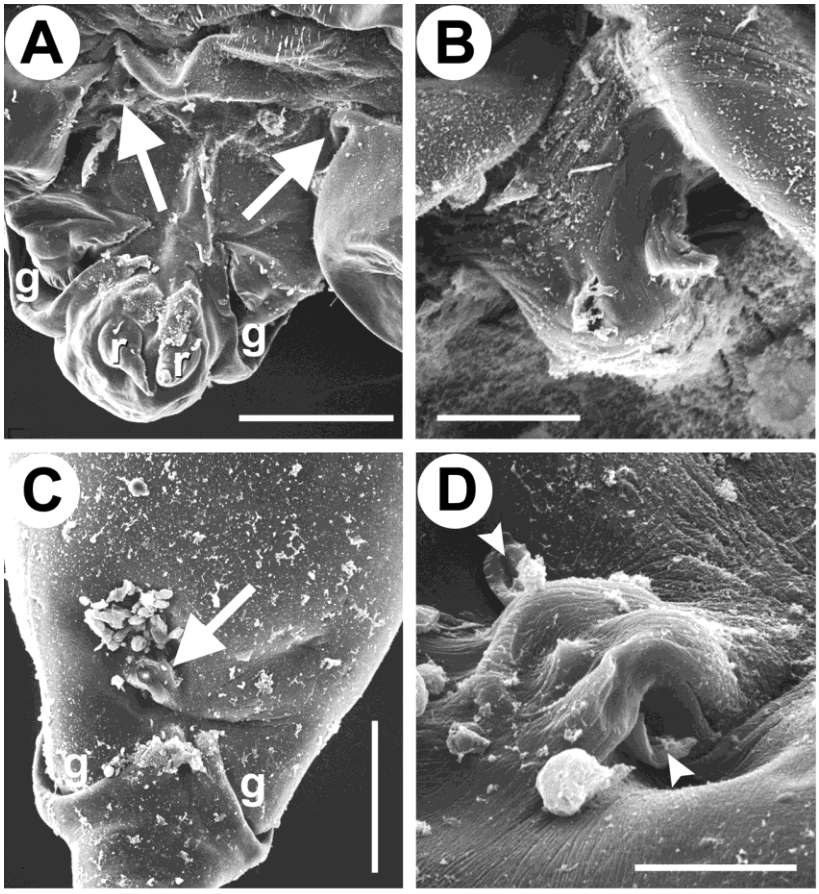

Figure 5