Biological Journal of the Linnean Society, 2002, **75**, 187–217. With 16 figures

Brooding in cocculiniform limpets (Gastropoda) and familial distinctiveness of the Nucellicolidae (Copepoda): misconceptions reviewed from a chitonophilid perspective

RONY HUYS FLS[1]*, PABLO J. LÓPEZ-GONZÁLEZ[2], ELISA ROLDÁN[3] and ÁNGEL A. LUQUE[3]

[1]*Department of Zoology, The Natural History Museum, Cromwell Road, London SW7 5BD, UK*
[2]*Departamento de Fisiología y Zoología, Facultad de Biología, Universidad de Sevilla, Reina Mercedes, 6,41012 Sevilla, Spain*
[3]*Laboratorio de Biología Marina, Departamento de Biología, Universidad Autónoma, 28049 Madrid, Spain*

Received 21 January 2001; accepted for publication 16 August 2001

Nauplii, copepodids and adults of a new mesoparasitic genus and species of Chitonophilidae, *Lepetellicola brescianii*, are described from the pallial cavity of a deepwater cocculiniform limpet, *Lepetella sierrai*, collected in the Bay of Biscay and Gulf of Cádiz. Re-examination of the type material of the recently established Nucellicolidae revealed several important observational errors in the original description, such as the oversight of the rootlet system in the adult female and misinterpretations of the tagmosis and antennulary segmentation in the late copepodid. Lamb *et al.*'s (1996) criteria used to justify the familial distinctiveness of the Nucellicolidae are all invalid. The family is relegated to a junior synonym of the Chitonophilidae on the basis of overwhelming support provided by copepodid and adult morphology. The impact of heterochrony on the body plan of adults and developmental stages is discussed. Phylogenetic analysis supports a basal dichotomy dividing the Chitonophilidae into a mesoparasitic clade, utilizing exclusively polyplacophoran hosts, and a sisterclade grouping genera associated with chitons, prosobranch gastropods and cocculiniform limpets. The presence of maxillipeds and postmaxillipedal apodemes in the adult males of the latter clade is considered as apomorphic rather than plesiomorphic, being the result of incomplete moulting and correlated with the ventral position of the genital apertures. *Nucellicola* is identified as the sistergroup of the only other endoparasitic genus, *Tesonesma*, found in the body cavity of chitons. The inferred relationships indicate that host switching has occurred twice in the evolution of the Chitonophilidae.
Examination of the antennulary segmentation and setation patterns of copepodids in *Lepetellicola* and *Nucellicola* unequivocally refutes both the current placement of the Chitonophilidae in the Poecilostomatoida and its alternative assignment to the Siphonostomatoida. Exclusion from the Poecilostomatoida is reinforced by the absence of a coxo-basis in the antenna. The family is placed with the cyclopoids, providing further evidence for the crown-group status of the Poecilostomatoida within the currently paraphyletic Cyclopoida. A critical review of the published reports of brooding in cocculiniform limpets demonstrated that there is, as yet, no tangible evidence for this phenomenon in either the Lepetelloidea or Cocculinoidea. *Biological Journal of the Linnean Society,* 2002, **75**, 187–217.

ADDITIONAL KEYWORDS: Chitonophilidae – heterochrony – *Lepetella* – ***Lepetellicola* gen. nov.** – molluscan hosts – phylogeny.

*Corresponding author. E-mail: rjh@nhm.ac.uk

INTRODUCTION

Copepods, together with nematodes and insects, are the most abundant multicellular organisms on Earth. Current evidence suggests that copepods originated from the marine hyperbenthic habitat (Huys & Boxshall, 1991) and subsequently spread out in sediments across the entire salinity spectrum. Their current position of world predominance, however, can be attributed to two principal, recurrent, radiation events, i.e. their major habitat shift into the marine plankton, and the evolution of parasitism. Given their moderately high host specificity in conjunction with the dazzling spectrum of potential marine hosts, it is highly conceivable that parasitic copepods significantly outnumber their free-living counterparts in species diversity. It would not be extravagant to assume that every other kind of marine macroinvertebrate on earth has at least one copepod species acting as its own personalized parasite. This successful colonization or utilization of virtually every metazoan phylum has generated a great diversity in copepod body morphology, which is arguably unparalleled among the Crustacea. Regrettably, this variety in body plan has also given rise to unfortunate consequences for phylogenetic analysis. More specifically, the highly transformed forms frequently lie at the root of two important misconceptions.

Failure to recognize the extreme modification of certain copepods has repeatedly resulted in them being misinterpreted as part of the host. Garstang (1890) recorded 'pieces of spawn' attached to the dendronotid nudibranch *Lomanotus genei* (Vérany, 1846) but these were later identified by Scott & Scott (1895) as egg-sacs of the splanchnotrophid copepod *Lomanoticola insolens* Scott & Scott (1895), the urosome of which is typically protruding through the host integument. Another example of such observational error is shown by Muñoz *et al.* (1996) who figured an unusual penis with '. . . a row of hooks on each side' in their redescription of the goniodoridid nudibranch *Okenia luna* (Millen *et al.,* 1994). The offset lobate anterior end, the traces of segmentation in the posterior part, the number of lateral processes and the presence of an unpaired median outgrowth leave no doubt that the supposed penis is an adult female of *Ismaila* Bergh, 1867, a genus of endoparasitic copepods (Splanchnotrophidae) typically associated with the kidney, pericardium or the digestive gland of nudibranch and sacoglossan opisthobranchs (Huys, 2001).

Secondly, failure to place strange copepods in existing classifications, often compounded by imperfect dissection and observation, has recently led to the proposal of a number of monogeneric or monotypic families. An example of such undesirable inflation of higher categories is illustrated by the families Vaigamidae and Amazonicopeidae (Thatcher & Robertson, 1984; Thatcher, 1986). These families were proposed for highly modified fish parasitic copepods but in reality are merely specialized lineages of the Ergasilidae (Abdelhalim *et al.*, 1993; Amado *et al.*, 1995). The uncritical acceptance of such familial identity, without considering the possibility of the taxon being at a terminal node in a larger encompassing clade, is potentially naive and unscientific, particularly when no attempt has been made to identify the hypothetical outgroup.

We have used mollusc-infesting copepods as an example to demonstrate these misconceptions. The discovery of a new genus of Chitonophilidae prompted us to challenge the widely assumed phenomenon of brooding in cocculiniform limpets, an anatomically and biologically very diverse group currently classified as an order or suborder of the streptoneurous Gastropoda (Haszprunar, 1998). It has also provided new morphological information of high phylogenetic significance, causing serious implications for the taxonomic status of the Nucellicolidae, a recently proposed family of highly transformed endoparasitic copepods based solely on autapomorphies.

MATERIAL AND METHODS

Museum collections of *Lepetella sierrai* Dantart & Luque (1994) (Mollusca, Cocculiniformia, Lepetelloidea), deposited in the Naturhistoriska Riksmuseet in Stockholm (NRS) and the Museo Nacional de Ciencias Naturales in Madrid (MNCN), were examined for parasitic copepods. This material was collected in the Iberian-Moroccan Gulf (BALGIM expedition, 1984, coordinated by the Muséum National d'Histoire Naturelle, Paris) and in the Bay of Biscay (Fauna Ibérica II expedition, coordinated by MNCN). Host material was obtained from empty or occasionally inhabited, corneous tubes of the sedentary polychaete *Hyalinoecia tubicola* (O.F. Müller, 1776), on which *Lepetella sierrai* lives and feeds. Copepods were cleared and dissected in lactic acid; the dissected parts were mounted on slides in lactophenol mounting medium. Preparations were sealed with Glyceel (BDH Laboratory Supplies, Poole, UK) or transparent nail varnish. All drawings have been prepared using a camera lucida on an Olympus BH (Hamburg, Germany) or a Leica DMR (Wetzlar, Germany) differential interference contrast microscope. Both host and parasite specimens were examined with a Philips XL 30 scanning electron microscope (Eindhoven, The Netherlands). Specimens were prepared by dehydration through graded acetone, critical point dried, mounted on stubs and sputter-coated with gold or palladium.

The descriptive terminology is adopted from Huys &

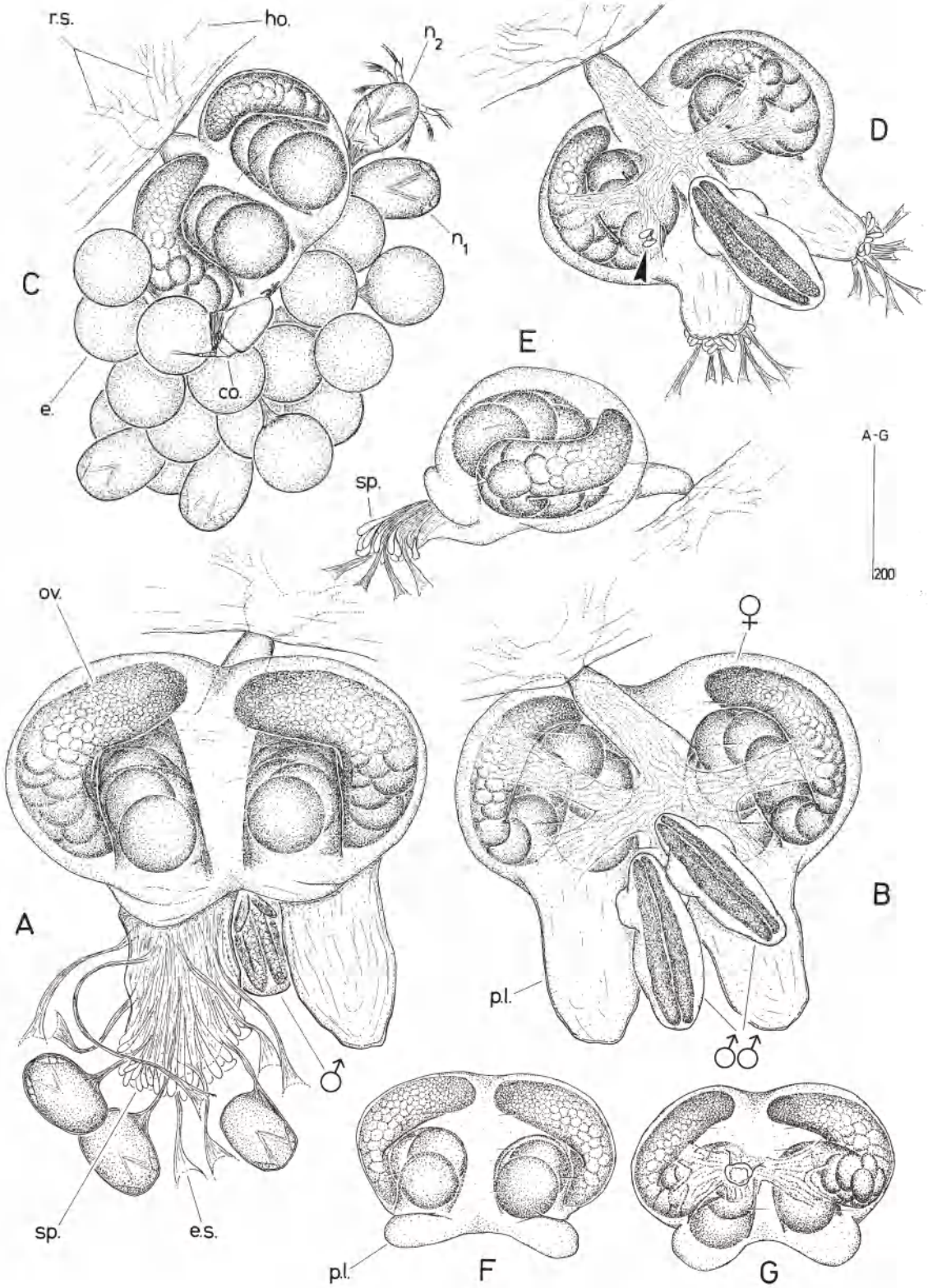

Figure 1. ***Lepetellicola brescianii*** **gen.** et **sp. nov.** A, paratype ♀ attached to host, dorsal view showing attached dwarf male (♂), eggs and spermatophores [e.s., remaining egg string of hatched egg; sp., spermatophore; ov., ovary]; B, same, ventral view showing two dwarf males (♂♂), another two being removed and not illustrated [p.l., posterior lobe]; C, paratype ♀ attached to host with offspring at different stages of development, dorsal view [co., copepodid; e., egg; ho., host's integument; n_1, young nauplius enclosed in egg membrane; n_2, fully developed nauplius in process of eclosion; r.s., rootlet system]; D, paratype ♀ attached to host, ventral view showing single dwarf male attached medially and maxillipeds of second dislodged male (arrowed); posterior lobes with attached spermatophores and remaining egg-strings of hatched eggs; E, paratype ♀ attached to host, lateral view showing spermatophores (sp.) and remaining egg-strings of hatched eggs; F, young ♀, showing weakly developed posterior lobes (p.l), dorsal view (two dwarf males originally attached having been removed); G, same, ventral view.

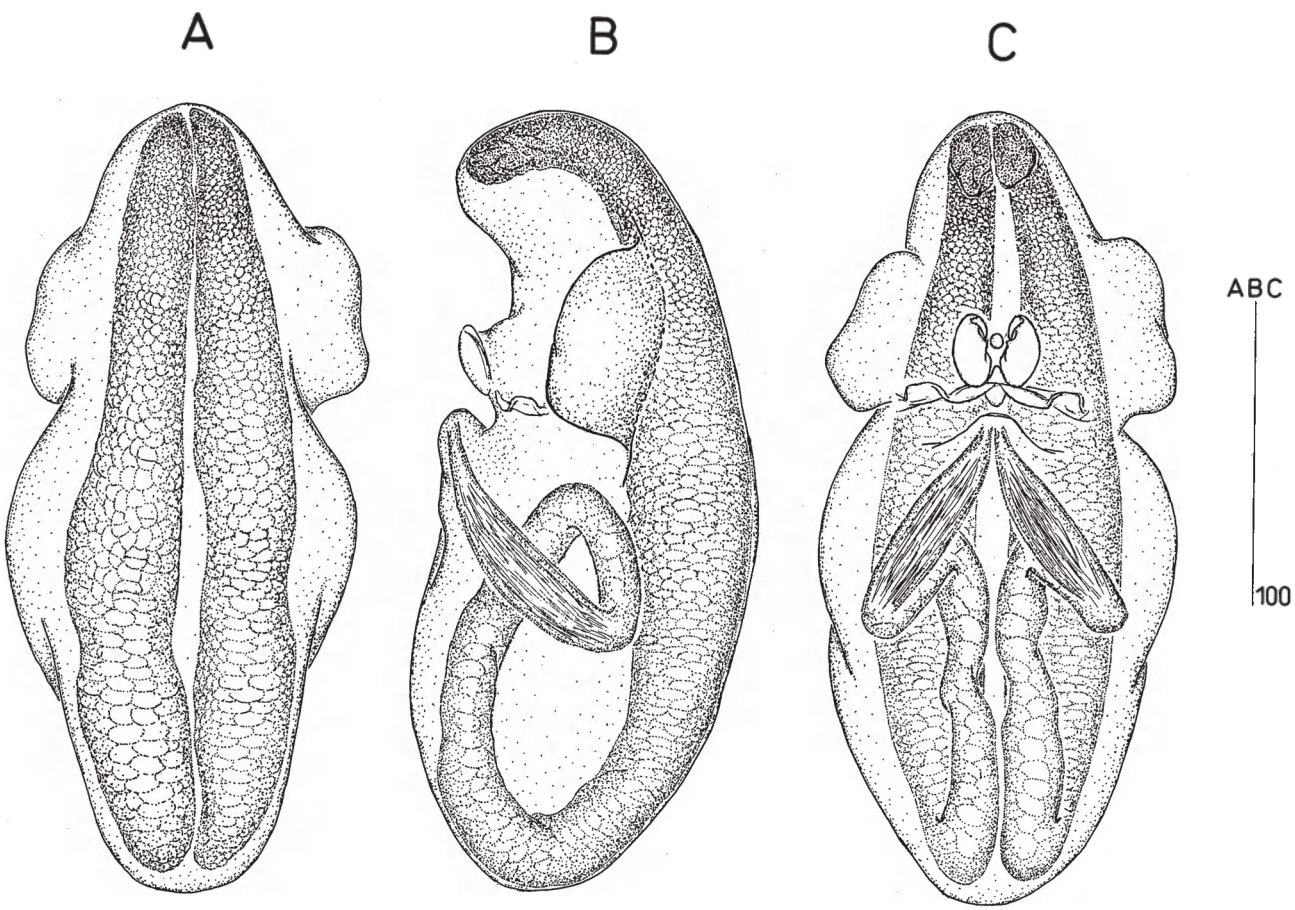

Figure 2. ***Lepetellicola brescianii*** **gen.** et **sp. nov.** Adult ♂. A, habitus, dorsal view; B, same, lateral view; C, same, ventral view.

Boxshall (1991). Abbreviations used in the text are: ae, aesthetasc; P1-P4, first to fourth thoracopod. Scale bars in Figures 1–13 are in μm.

Type material examined in the present paper has been deposited in the MNCN, NRS and the Natural History Museum in London (NHM).

In order to study the effect of the copepod parasite on the host, 51 specimens of *L. sierrai* (ranging between 0.6 and 2.0 mm), 26 of which were infested, were examined for gonad development and maturation. Material for this histological study comes from the Iberian-Moroccan Gulf (BALGIM expedition). Specimens were preserved in 70% ethanol and their external anatomy was examined using a stereomicroscope. Material for histological studies was dehydrated, embedded in paraplast, serially sectioned at 7 μm and stained with Mayer's haematoxylin-eosin.

The phylogenetic software package PAUP 3.1.1, written by David Swofford of the Laboratory of Molecular Systematics, Smithsonian Institution (Swofford, 1993), was used to analyse phylogenetic relationships within the Chitonophilidae.

RESULTS

MORPHOLOGICAL OBSERVATIONS

Family Chitonophilidae Avdeev & Sirenko, 1991
Syn. Nucellicolidae Lamb *et al.*, 1996.

History

All genera currently allocated to the Chitonophilidae have been discovered in the last 15 years. Jones & Marshall (1986) described ovigerous females of *Cocculinika myzorama* from the wood-associated cocculinid limpet *Coccopigya hispida* Marshall, 1986 in New Zealand bathyal waters. They were unable to place the species in any existing family or order and ranked it *incertae sedis* in the Copepoda. Franz & Bullock (1990) described both sexes of *Ischnochitonika lasalliana* from the branchial cavity of two polyplacophoran hosts, *Ischnochiton striolatus* (Gray, 1828) and *Stenoplax boogi* (Haddon, 1886), collected in the Caribbean. A comparison was made with the highly modified Herpyllobiidae and a number of enigmatic mollusc-infesting genera such as *Teredoika* Stock,

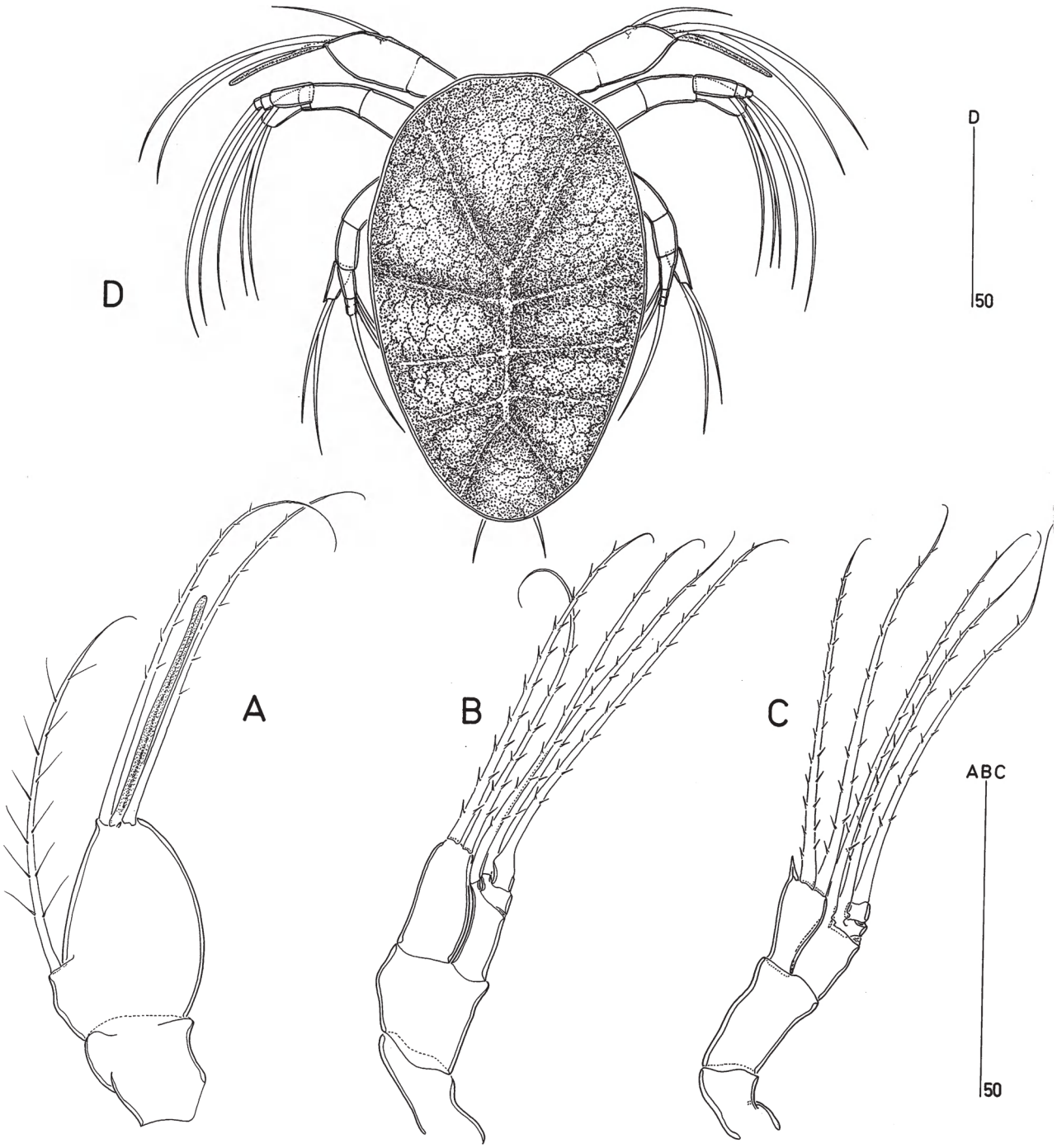

Figure 3. ***Lepetellicola brescianii*** **gen.** et **sp. nov.** Nauplius. A, antennule; B, antenna; C, mandible; D, habitus, dorsal view.

1959, *Axinophilus* Bresciani & Ockelmann, 1966 and *Pectenophilus* Nagasawa, Bresciani & Lützen, 1988, but Franz & Bullock (1990) were unsuccessful in assigning *Ischnochitonika* to any of the parasitic copepod orders. A second species, *I. japonica* was added by Nagasawa *et al.* (1991) from *Ischnochiton* (*Ischnoradsia*) *hakodadensis* (Pilsbry, 1893) in the Sea of Japan but, despite the first description of the nauplius, and SEM observations, their discovery shed no new light on affinities.

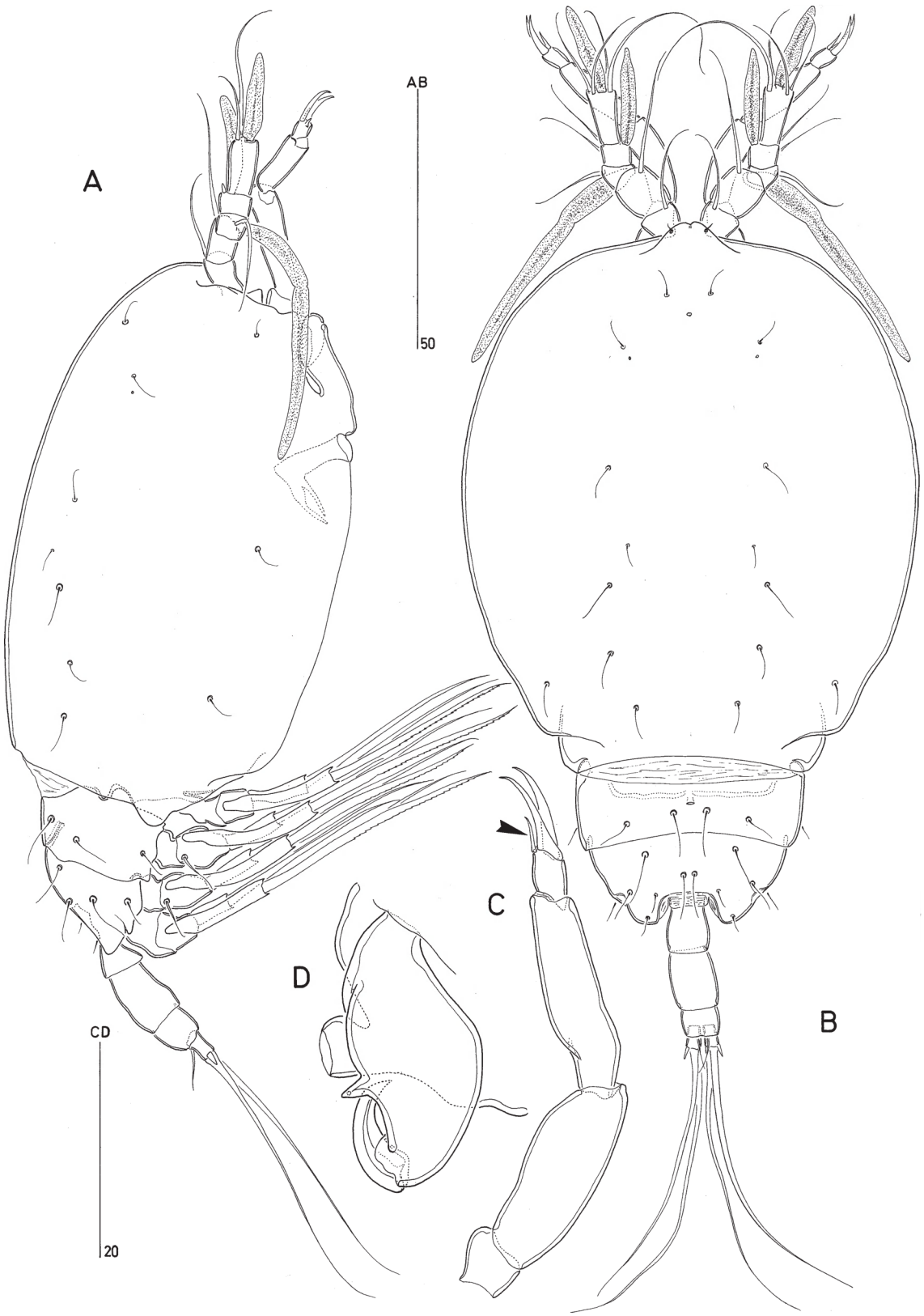

Figure 4. ***Lepetellicola brescianii*** **gen.** et **sp. nov.** Copepodid. A, habitus, lateral view; B, same, dorsal view; C, antenna (minute claw arrowed); D, maxilliped and oral tube, lateral view.

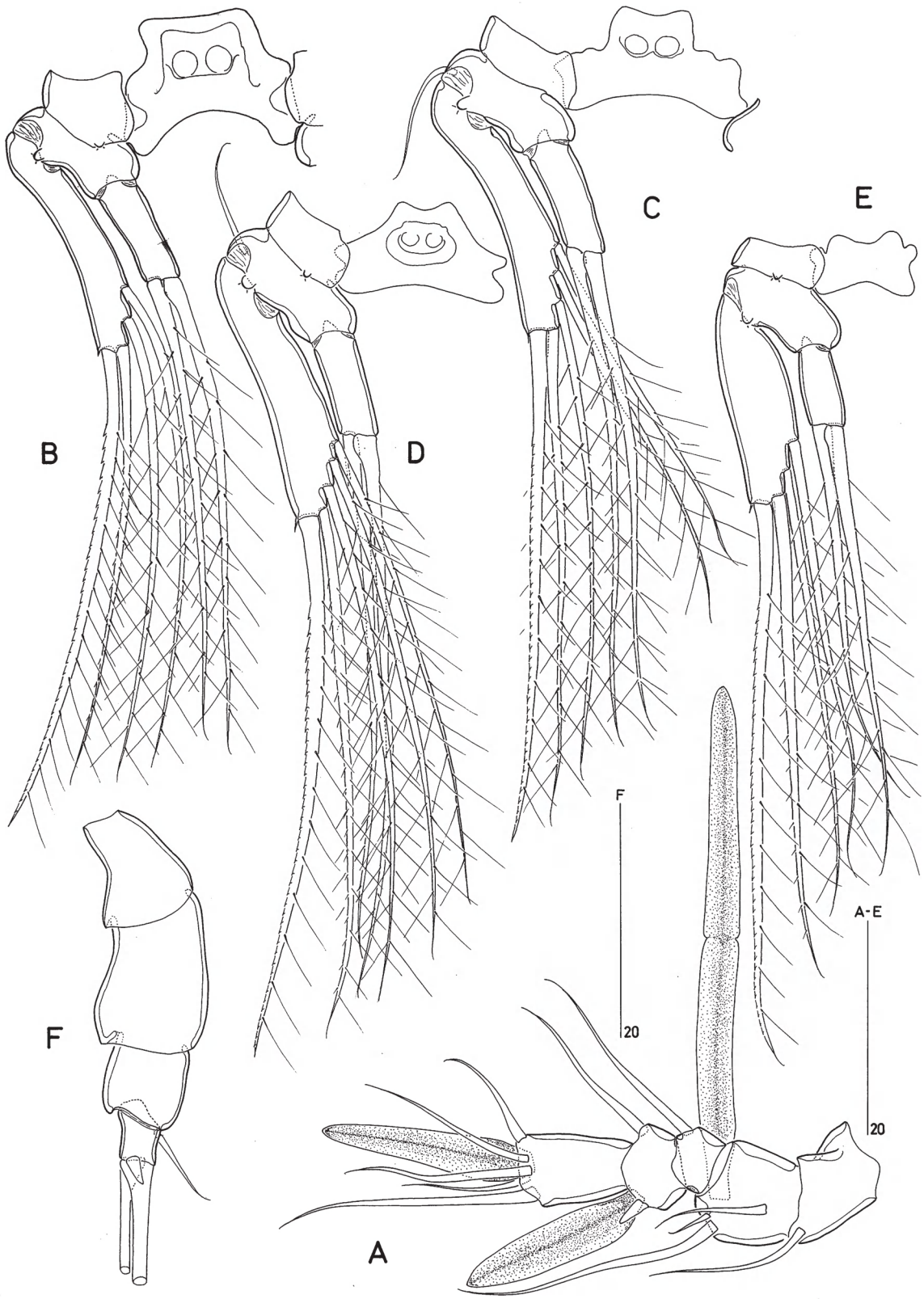

Figure 5. ***Lepetellicola brescianii*** **gen.** et **sp. nov.** Copepodid. A, antennule; B, leg 1 and intercoxal sclerite, anterior; C, leg 2 and intercoxal sclerite, anterior; D, leg 3 and intercoxal sclerite, anterior; E, leg 4 and intercoxal sclerite, anterior; F, urosome, lateral view.

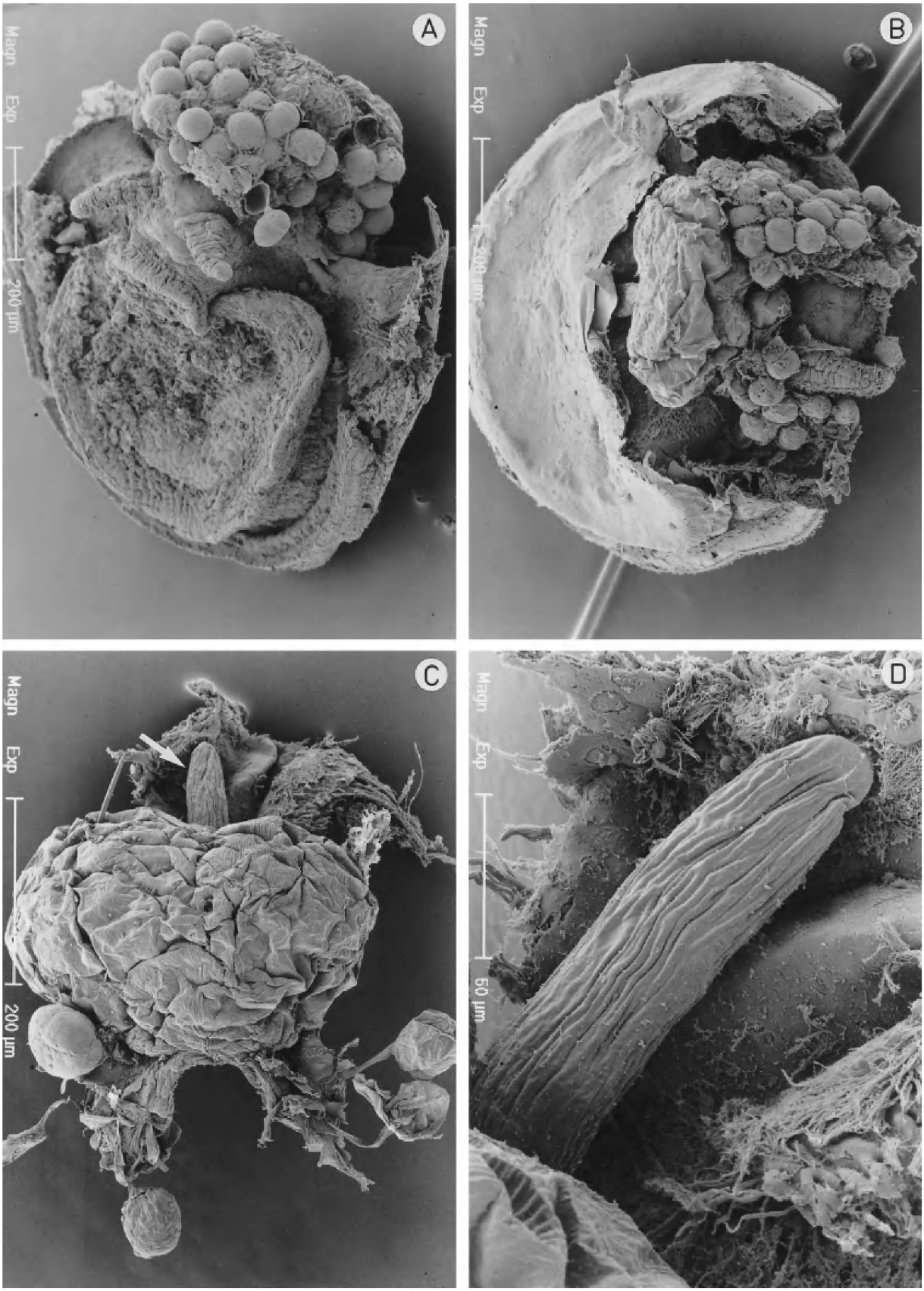

Figure 6. ***Lepetellicola brescianii*** **gen.** et **sp. nov.** (SEM micrographs). A, ovigerous female attached to dorsal side of cephalic region of *Lepetella sierrai* (shell removed); B, same, dorsal view showing paired egg-sacs; C, ovigerous female largely removed from host, dorsal, showing neck (arrowed) and egg-strings of previous batches; D, close-up of neck penetrating host integument.

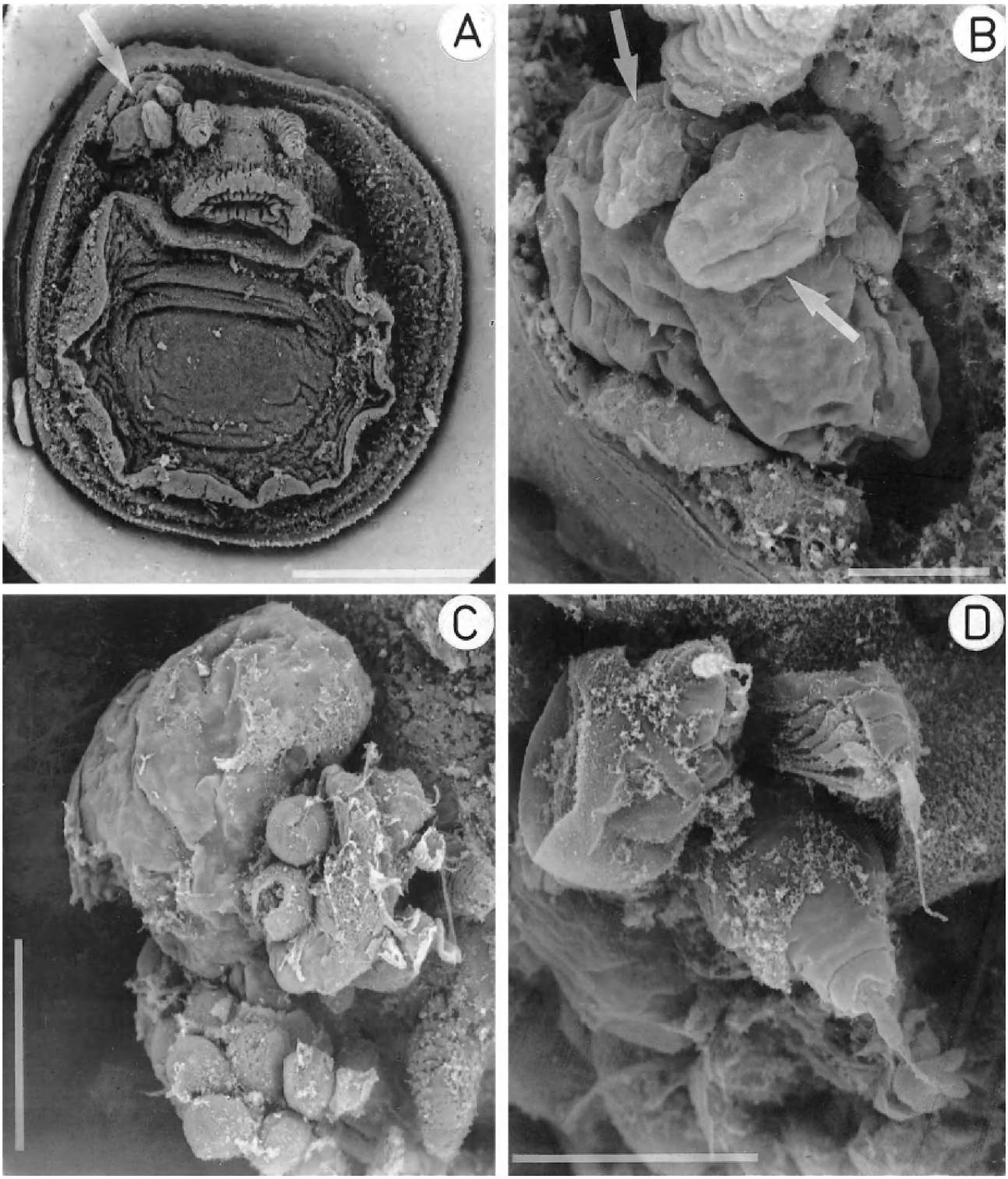

Figure 7. ***Lepetellicola brescianii*** **gen. et sp. nov.** (SEM micrographs). A, female with two attached males in pallial cavity of *Lepetella sierrai* (position arrowed); B, close-up showing males (arrowed); C, ovigerous female with paired egg masses; D, group of three late copepodids found along with nauplii in egg mass. Scale bars: 500 μm (A), 150 μm (B), 250 μm (C), 100 μm (D).

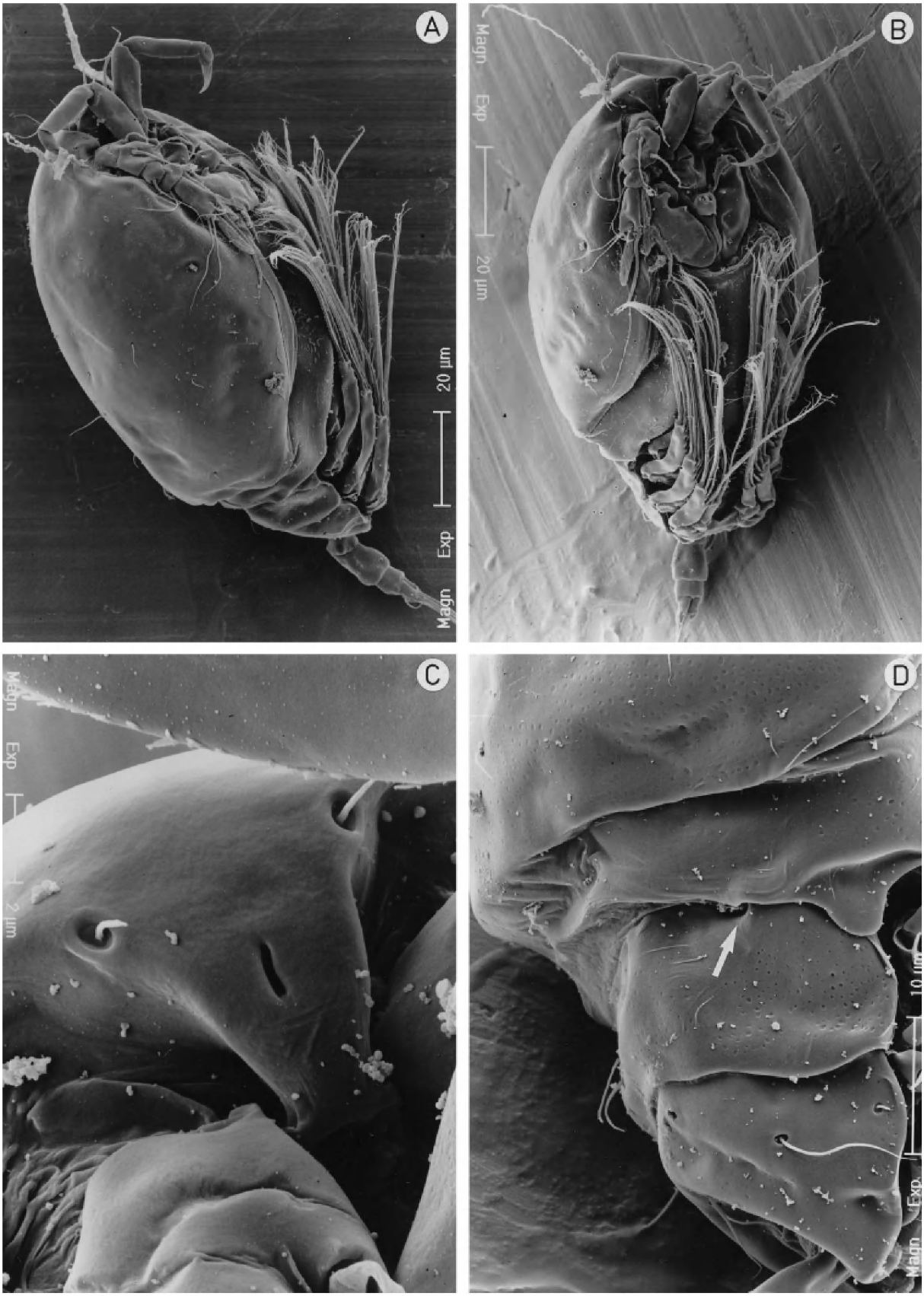

Figure 8. ***Lepetellicola brescianii*** **gen.** et **sp. nov.** (SEM micrographs). Late copepodid. A, body, lateral; B, body, ventral; C, rostrum, frontal; D, cephalothorax (posterior part), P2-bearing somite (condylar articulation arrowed) and pedigerous double-somite, lateral.

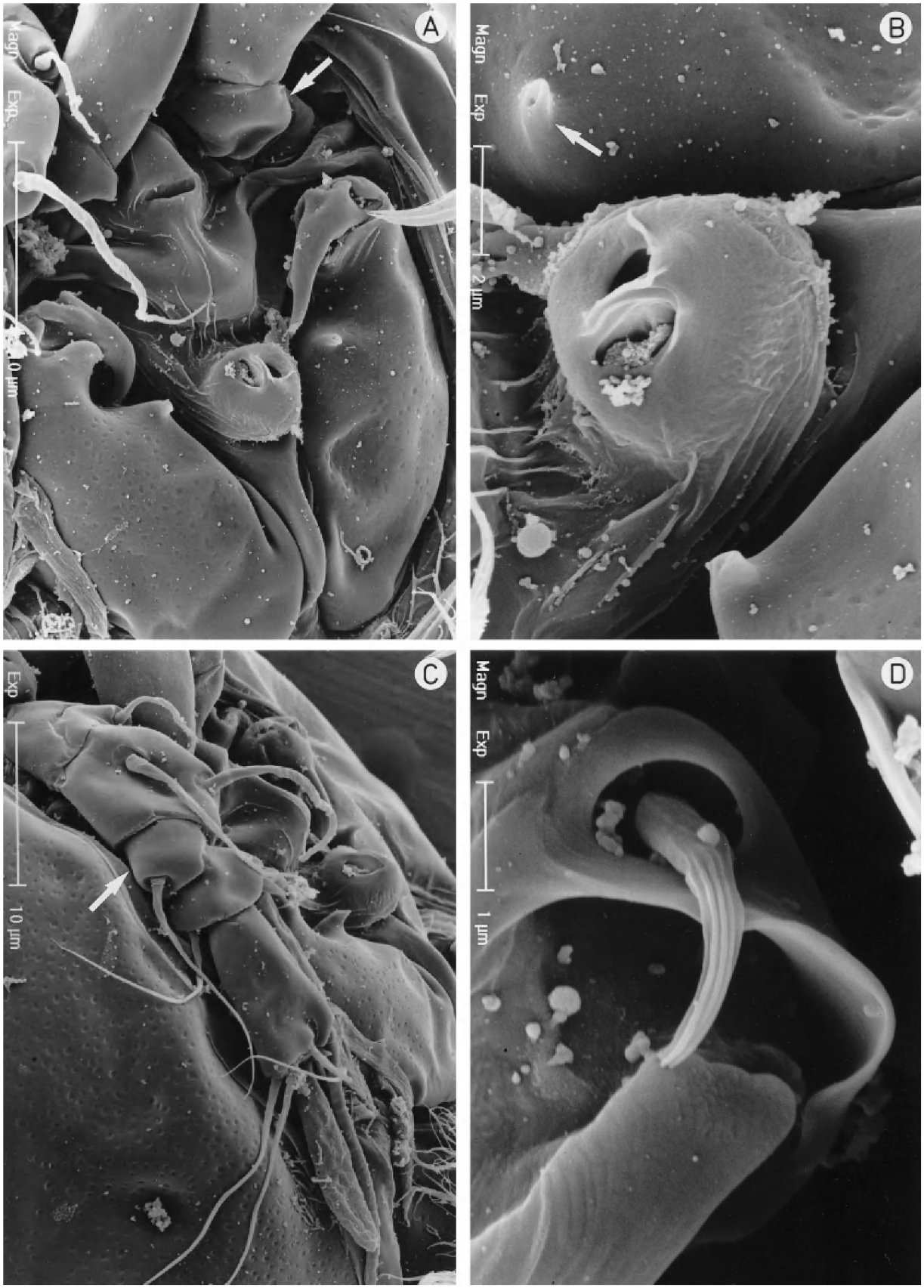

Figure 9. ***Lepetellicola brescianii*** **gen.** et **sp. nov.** (SEM micrographs). Late copepodid. A, cephalothorax, ventral view showing maxillipeds, oral cone and bases of antennae (coxa arrowed); B, oral cone (pore on maxillipedal basis arrowed); C, antennule (segment 3 arrowed); D, P3 outer basal seta.

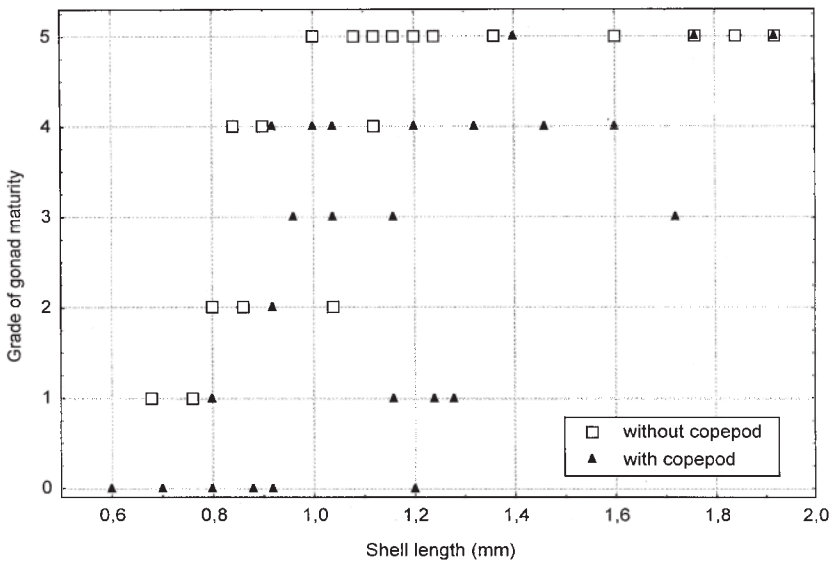

Figure 10. *Lepetella sierrai* Dantart & Luque, 1994. Scatterplot of the level of gonad maturity against shell length (mm). Different levels of gonad maturity: 0, no gonad; 1, spermatids, no ovary; 2, spermatids, previtellogenic oocytes; 3, mature sperm, no ovary; 4, mature sperm, previtellogenic oocytes; 5, mature sperm, vitellogenic oocytes.

These three publications were probably overlooked by Avdeev & Sirenko (1991) when they proposed the family Chitonophilidae for two new genera of highly transformed copepods from shallow water polyplacophorans in the north-western Pacific. *Chitonophilus laminosus* Avdeev & Sirenko, 1991 was described from *Tonicella submarmorea* (von Middendorff, 1848), whereas the host of *Leptochitonicola latus* Avdeev & Sirenko, 1991 was *Leptochiton assimilis* (Thiele, 1909). Little or no justification was provided for the proposal of the new family and no ordinal placement was suggested. In 1994 Avdeev & Sirenko accepted *Cocculinika* and *Ischnochitonika* as valid members of the Chitonophilidae and established two new genera *Tesonesma* and *Cookoides* found in chiton hosts of the genus *Stenosemus* von Middendorff, 1847. Unlike other Chitonophilidae, *Tesonesma reniformis* Avdeev & Sirenko, 1994 was found to be entirely endoparasitic.

Lamb *et al.* (1996) recognized the Chitonophilidae as a poecilostomatoid family but gave no justification for this assignment. They proposed a new family Nucellicolidae for *Nucellicola holmanae* Lamb, Boxshall, Mill & Grahame, 1996, an endoparasite of the dogwhelk *Nucella lapillus* (Linné, 1758) in the British Isles. The same copepod had previously been described as *Nucellicola kilrymontis* in an unpublished Ph.D. thesis (Fitches, 1966). Our results below provide strong evidence for the inclusion of *Nucellicola* in the Chitonophilidae and consequently the Nucellicolidae is relegated here to a junior synonym of the latter.

Diagnosis

Adult female highly transformed, lacking appendages and external traces of segmentation. Body comprising well developed rootlet system, serving as holdfast and absorptive system inside the host, and sac-like trunk, containing reproductive system and digestive tract. Trunk either external (ectosoma) in mesoparasites or entirely internal in endoparasites; in mesoparasites often with lateral lobes anteriorly and/or posteriorly. Genital system paired; position of gonopores variable. Eggs forming grape-like masses in mesoparasitic members, often attached to the genital opening by individual egg-strings; in endoparasites laying free in membranous cyst or tube, communicating with exterior via initial point of entry.

Adult male highly transformed, distinctly smaller than female, lacking segmentation; often with lateral or frontal lobes; with either antennae or maxillipeds as only appendages. Typically attached near the genital apertures of the female. Genital system paired; vasa deferentia each terminating in specialized spermatophore sac; genital apertures either posterior to maxillipeds (when present) or dorsal. Spermatophores elongate-pyriform, attached in clusters to female genital apertures.

Nauplius lecithotrophic; antennule 1- or 2-segmented; antennae and mandibles without gnathobasal elements. Caudal rami represented by one seta each. Nauplii developing in pallial cavity of host in mesoparasitic species or inside egg-tube (or cyst) in endoparasites.

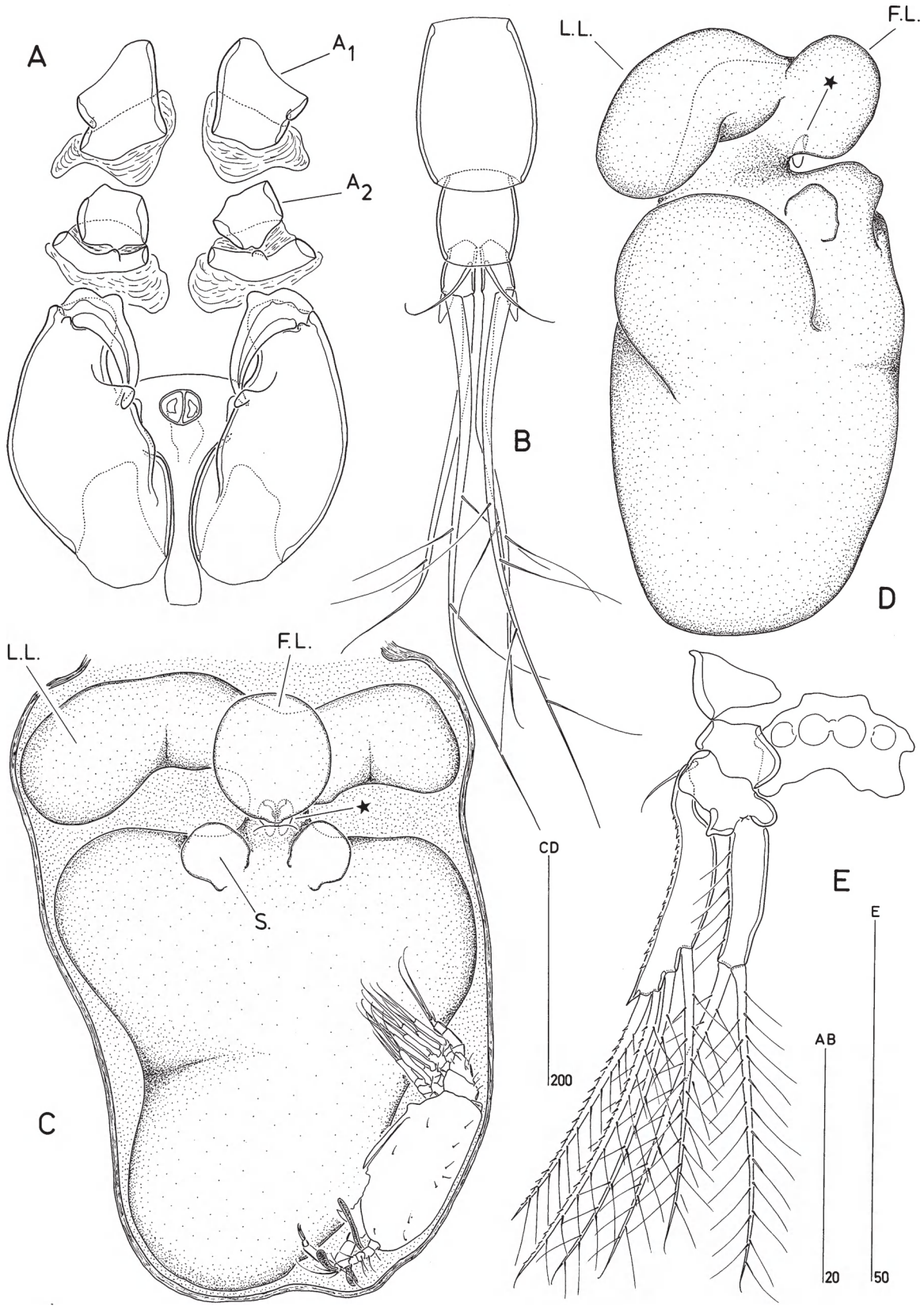

Figure 11. ***Lepetellicola brescianii*** **gen. et sp. nov.** A, copepodid, ventral view of cephalic appendages [A_1, basal segment of antennule; A_2, base of antenna]. *Nucellicola holmanae* Lamb, Boxshall, Mill & Grahame, 1996. B, late male copepodid, last two urosomites and caudal rami, dorsal view; C, adult ♂ enclosed in membranous vesicle together with exuvium of preceding copepodid, ventral view (F.L., frontal lobe; L.L., lateral lobe; S., spermatophore sac); D, adult ♂, lateral view; E, leg 1, anterior view. Asterisk in C–D denotes maxillipeds beneath frontal lobe.

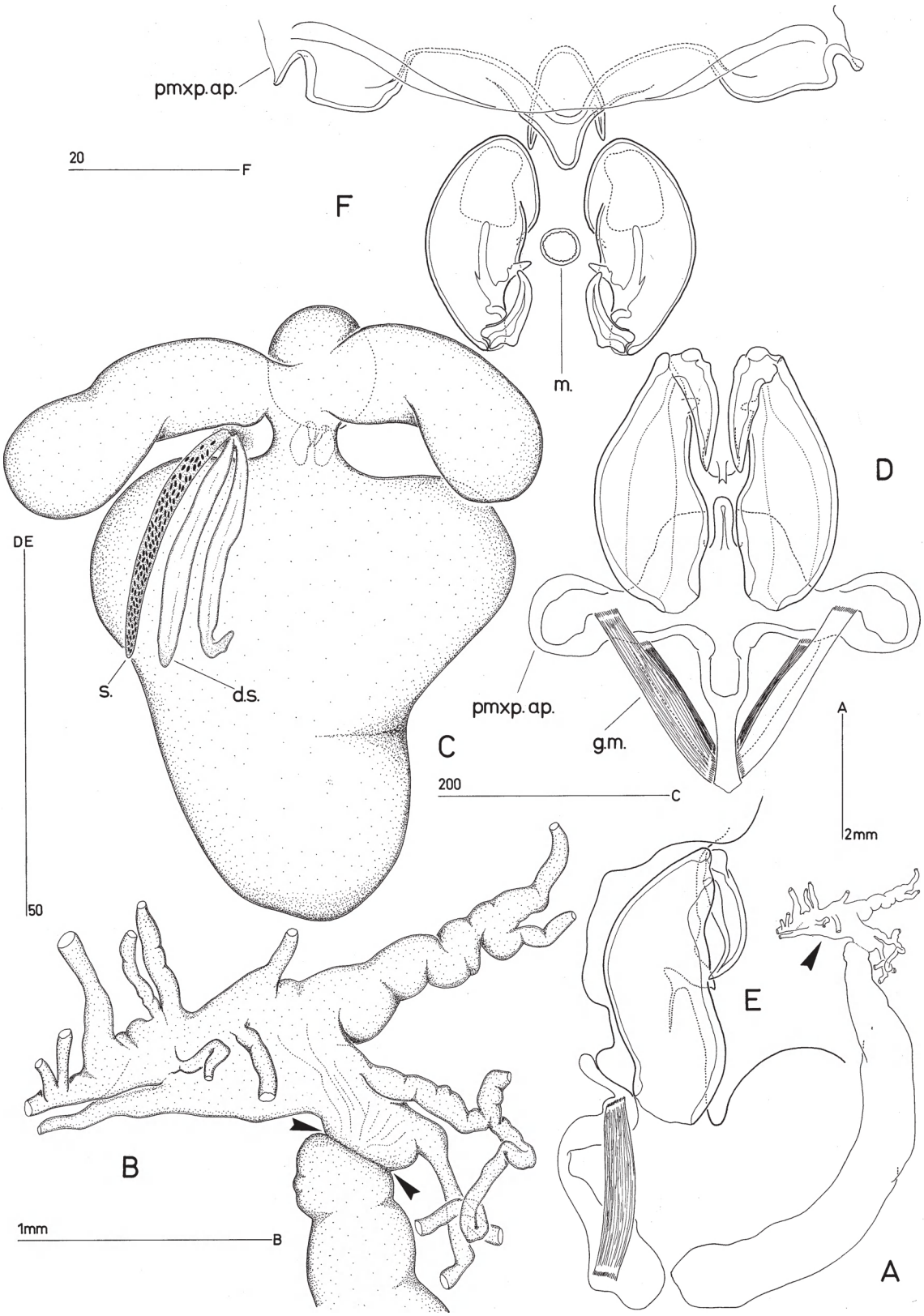

Figure 12. *Nucellicola holmanae* Lamb, Boxshall, Mill & Grahame, 1996. A, adult ♀, habitus (arrow indicates rootlet system); B, rootlet system of adult ♀ shown in (A); arrows marking constriction between cephalic lobe and rootlet system; C, adult ♂, dorsal view (d.s., discharged spermatophore; s., spermatophore; both attached to female); D, adult ♂, maxillipeds, ventral view (g.m., genital muscle; pmxp. ap., postmaxillipedal apodeme; atrophied intrinsic musculature indicated by dotted contours); E, adult ♂, maxilliped, lateral view. ***Lepetellicola brescianii* gen. et sp. nov.** F, adult ♂, maxillipeds, ventral view (m., mouth opening; pmxp. ap., postmaxillipedal apodeme).

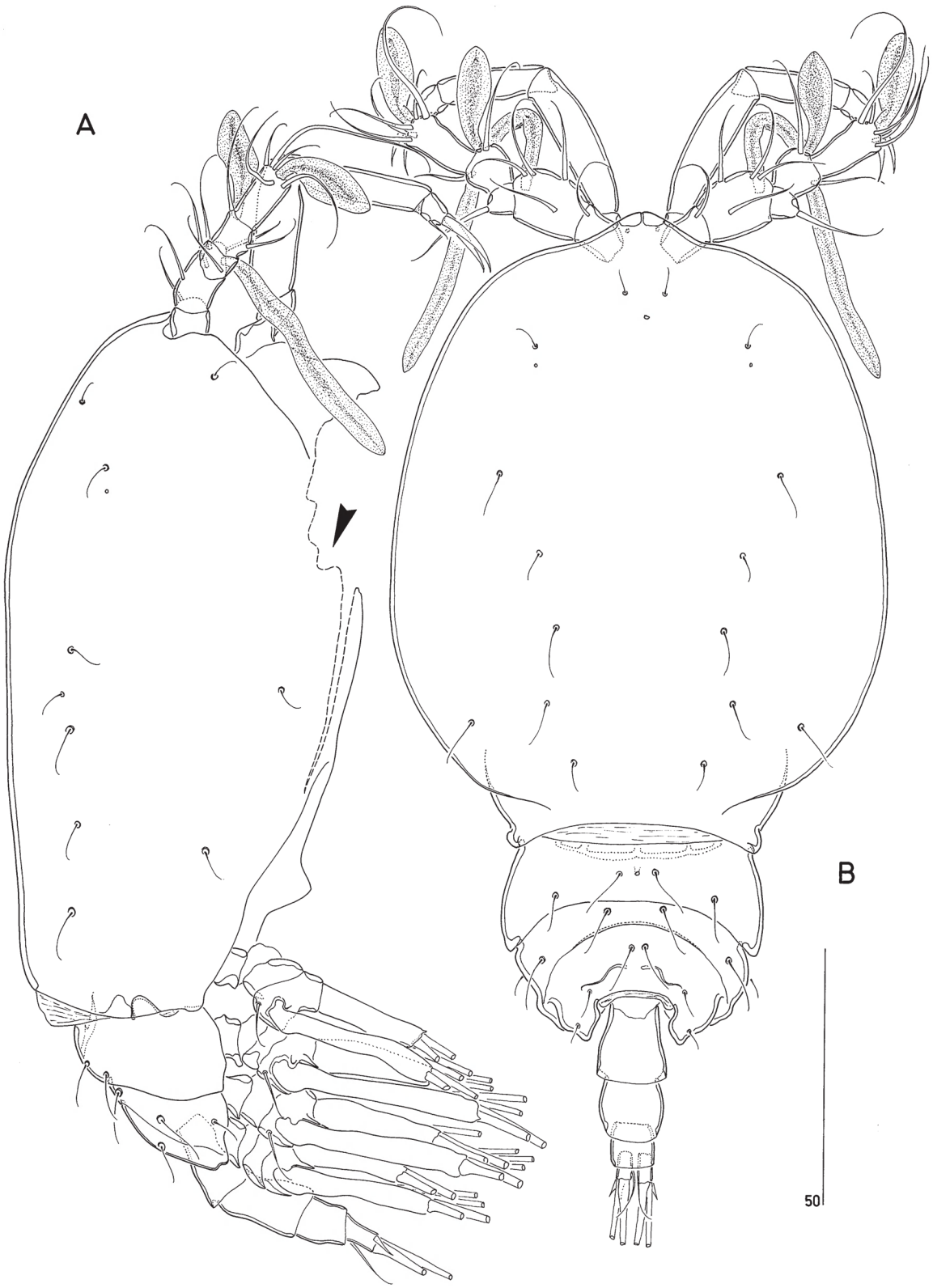

Figure 13. *Nucellicola holmanae* Lamb, Boxshall, Mill & Grahame, 1996. Late male copepodid. A, habitus, lateral view (arrow marking ventral slit in exuvium); B, habitus, dorsal view.

Late copepodid cyclopiform with 3-segmented prosome, comprising cephalothorax, leg 2-bearing somite and pedigerous double-somite bearing legs 3–4; urosome 3-segmented. Cephalothorax with 5-segmented antennules, uniramous antennae bearing three elements at tip, and subchelate maxillipeds; oral cone short, without trace of mandibles. Swimming legs 1–4 biramous; rami 1-segmented with exopodal setae arranged around distal and inner margins. Caudal rami with one dorsal and three terminal elements.

Additional metanaupliar and copepodid stages sometimes present (*Nucellicola*). Infection of host at copepodid stage.

Adults mesoparasitic in pallial groove or endoparasitic in viscera of Polyplacophora, Cocculiniformia and Neogastropoda.

Note: Avdeev & Sirenko (1991, 1994) regarded the fleshy swellings on either side of the mouthcone in the males of *Chitonophilus laminosus* and *Tesonesma reniformis* as highly transformed mandibles. This is highly unlikely as no mandibles are found in the late copepodids of *Nucellicola holmanae* and *Lepetellicola brescianii*.

Type genus
Chitonophilus Avdeev & Sirenko, 1991.

Other genera
Cocculinika Jones & Marshall, 1986; *Ischnochitonika* Franz & Bullock, 1990; *Leptochitonicola* Avdeev & Sirenko, 1991; *Cookoides* Avdeev & Sirenko, 1994; *Tesonesma* Avdeev & Sirenko, 1994; *Nucellicola* Lamb, Boxshall, Mill & Grahame, 1996; *Lepetellicola* gen. nov.

LEPETELLICOLA GEN. NOV.

Diagnosis
Chitonophilidae. Female body comprising ectosoma connected to branching rootlet system (endosoma) inside host via distinct cylindrical neck. Ectosoma typically heart-shaped, with large lateral lobes containing ovaries and elongate paired posterior lobes originating subventrally from genital apertures. Neck arising midventrally from anterior part of ectosoma. Genital system completely paired, comprising ovaries, oviducts and posterodorsal genital apertures located in depressions at base of posterior lobes. Egg masses shaped like bunch of grapes; eggs attached individually by long strings.

Adult male smaller than female, elongate; comprising trilobate cephalic region and sac-like posterior trunk, separated by bilateral constriction; lateral lobes weakly developed. Antennae and antennary projections absent. Subchelate maxillipeds only appendages, with distinct postmaxillipedal apodeme. Oral cone small, without sclerotized mouth-ring. Genital system completely paired, comprising testes, vasa deferentia and spermatophore sacs associated with ventral genital apertures located posterior to maxillipeds. Typically attached midventrally to posterior region of female.

Nauplius with 1-segmented antennules; antennae and mandibles both with 1-segmented bisetose endopod and 3-segmented exopod bearing one seta on each segment.

Late copepodid as for the family; segment 2 of antennule with three setae plus aesthetasc; anterior element on segment 4 spiniform. Leg 1 without outer seta on basis. Caudal rami with smooth terminal setae.

Mesoparasitic in pallial cavity of deepwater cocculiniform limpets.

Type species
***Lepetellicola brescianii* gen. et sp. nov.**

Etymology
The generic name is derived from the generic name of the host, *Lepetella*, and the Latin *colere*, meaning inhabiting. Gender: masculine.

LEPETELLICOLA BRESCIANII GEN. ET SP. NOV.

Type locality
Vizcaya, Bay of Biscay, Fauna Ibérica II Stn 168 A (43°45.13′-43°46.53′N, 8°10.09′-8°9.59′W), 116–120 m depth. Host: *Lepetella sierrai*, paratypes MNCN reg. no. 15.05/5252, coll. 07 June 1991.

Material examined
Following specimens were found on *L. sierrai* from:

(1) Type locality: holotype ovigerous ♀ (MNCN reg. no. 20.04/5259a); paratypes preserved in alcohol are two ovigerous and two juvenile ♀♀ (NHM reg. nos. 2000. 1782–1785), two juvenile ♀♀ and one nauplius (MNCN reg. no. 20.04/5259b), and one ovigerous ♀ (MNCN reg. no. 20.04/5259c); paratypes mounted on slides are four nauplii (on three slides; NHM reg. nos. 2000. 1790–1793), one copepodid (on two slides; NHM reg. no. 2000. 1794), one copepodid and one egg (on one slide; MNCN reg. no. 20.04/5259d), one copepodid (on one slide; MNCN reg. no. 20.04/5259e), one egg mass with developing nauplii (on 1 slide; NHM reg. no. 2000. 1797), and 1 ♀ with attached ♂ (on one slide; NHM reg. no. 2000. 1798); four ovigerous ♀♀ mounted on SEM stubs (not registered);

(2) Off Gijón, Fauna Ibérica II Stn 185 A (43°42.59′-43°43.48′-N, 5°55.40′-5°56.40′W), 116–112 m depth; coll. 4 July 1991; on paratypes of *L. sierrai* (MNCN

reg. no. 15.05/27875): paratype ♂ on slide (MNCN reg. no. 20.04/5260);

(3) Cabo Mayor (N. Cabo Mayor), Fauna Ibérica II Stn 128 A (43°35.92′-43°35.63′-N, 3°47.53′-3°49.21′W), 141–142 m depth; coll. 19 June 1991; on paratypes of *L. sierrai* (MNCN reg. no. 15.05/27877): one ovigerous ♀ with attached ♂, mounted on SEM stub;

(4) BALGIM stn. CP21: Gulf of Cádiz (36°36′-N, 07°24′-W), 478–491 m depth; coll. 31 May 1984; deposited in NRS: paratypes are eight ovigerous females in alcohol (reg. nos MNHN-Cp 1900–1907) and one young ♀ with two ♂♂ mounted on slide (reg. no. MNHN-Cp 1908).

Descriptions based on (1) and (2); SEM observations based on (1) and (3).

Adult female

Body (Figs 1A–E, 6A–C and 7B) highly transformed, consisting of sac-like ectosoma connected via distinct neck to internal rootlet system. Ectosoma with large rounded anterolateral lobes and widely separated elongate posterior lobes extending subventrally; length 400–590 μm (mean: 510 μm, N = 7) including posterior lobes; width 475–630 μm (mean: 570 μm, N= 7. Posterior lobes longer and heart shape of anterior part of ectosoma more pronounced in larger specimens (Fig. 1A). Cylindrical neck arising midventrally from anterior part of ectosoma (arrowed in Fig. 6C), with longitudinal furrows (Fig. 6D); in smaller specimens shorter and originating slightly more anteriorly; integument reinforced around site of penetration. Posterior lobes arising from ventral margin of genital apertures; in bigger specimens often obscured by numerous spermatophores and remains of egg-strings (Figs 1A,D,E and 6C). Genital apertures paired, posterodorsal; concealed in depressions between hind margin of ectosoma and posterior lobes. Genital system clearly visible with paired ovaries, occupying lateral lobes, and long convoluted oviducts (Fig. 1A–B). Radiating branches of digestive system discernible ventrally, connected via neck with rootlet system (Figs 1B,D). Endosoma represented by delicate rootlet system branching inside host; branching pattern irregular and only partly revealed after prolonged preservation in lactic acid.

Egg masses in the shape of a bunch of grapes (Figs 6A–B and 7C). Eggs typically spherical (Fig. 1C, e.) but becoming more ovoid later in development (Fig. 1C, n_1); 110–120 μm in diameter, attached individually by thin egg string to dorsal surface of posterior lobes (Fig. 1A); larger females can have up to 40 eggs attached. Egg strings often remain attached to the female following eclosion of the nauplii (Fig. 6C). Muscles associated with developing naupliar limbs visible through egg membrane.

Infested hosts usually contained a single female but in some cases up to three females were observed (e.g. females illustrated in Figures 1A–B,F–G were found on the same gastropod individual).

Young females

Body (Fig. 1F–G) about 260 μm in length and 390 μm wide. Posterior lobes weakly developed and widely divergent. Reproductive system already fully developed. Young female illustrated in Figure 1F–G with two males attached (not shown) but no spermatophores or traces of egg batches (egg-strings) discernible.

Adult male

Body (Figs 2A–C and 7B) highly transformed, without any traces of segmentation or clear tagmosis; distinctly smaller than ♀; length 250–275 μm (mean: 265 μm), width 105–130 μm (mean: 120 μm, N = 5); comprising trilobate cephalic region and sac-like posterior trunk, separated by bilateral constriction. Frontal and posterolateral expansions of cephalic region lobate, rounded and directed ventrally. Medial mouth cone very small, located just anterior to maxillipeds (Fig. 12F).

Maxillipeds only appendages present; located midventrally on raised area at level of bilateral constriction (Fig. 2B–C); subchelate (Fig. 12F), comprising robust proximal segment (representing basis) and strong endopodal claw; no traces of syncoxa discernible; palmar margin of basis with medially directed spinous process. Maxillipeds internally supported by a symmetrically convoluted postmaxillipedal apodeme running across entire width of cephalic region. No trace of muscles in basis of maxilliped.

Reproductive system completely paired (Fig. 2C). Testes large, extending into frontal lobe and passing dorsally to posterior end of trunk (Fig. 2B); vasa deferentia making characteristic loop with ascending and descending branches in mid-region of trunk; distal parts of vasa deferentia dilated, forming small spermatophore sac opening to exterior via paired gonopores located on either side of mid-ventral protuberance. Spermatophores elongate-pyriform, attached in clusters to female genital apertures (Figs 1A,E).

Adult male attached medially to posteroventral area of female (Fig. 1D); up to six males can be attached to a single female, often with one of them located midventrally between both posterior lobes and any additional males positioned submedially (Fig. 1B; arrowed in Fig. 7B). In one case the site of a dislodged male was revealed by the maxillipeds left behind on the female's ventral surface (arrowed in Fig. 1D).

Nauplius

Lecithotrophic. Body 120–125 µm long and 75–80 µm broad, based on five specimens in lactophenol. Newly released nauplii (Fig. 3D) elongate-ovoid; maximum width measured in anterior half. Exoskeleton extremely thin and fragile. A pigmented nauplius eye appears to be absent. No labrum, functional mouth or anal slit present. Caudal region with pair of naked setae.

Antennule (Fig. 3A) 1-segmented; basal portion corresponding to pedestal, incompletely separated by suture from large paddle-like distal segment. Armature consisting of plumose lateral seta and two pinnate setae plus aethetasc apically.

Antenna (Fig. 3B) biramous; protopod consisting of unarmed coxa and basis, both without gnathobasal projections or setae. Exopod 3-segmented, middle and distal segments minute; each segment with one pinnate seta. Endopod distinctly wider than exopod; 1-segmented; with two apical pinnate setae.

Mandible (Fig. 3C) biramous, more slender than antenna but with similar segmentation and without gnathobasal structures on protopodal segments. Exopod 3-segmented, segments 2–3 minute; each segment with one pinnate seta. Endopod narrower than exopod, dilating distally; inner distal corner produced into pointed process; with two apical pinnate setae.

Several nauplii were observed still attached with their caudal end to the female's body via the proximal part of the egg membrane and its connecting string (Fig. 1C: n_2). Late nauplii with yolk clearly partitioned have been observed, possibly indicating metamery of developing copepodid. All nauplii belonging to same stage; no evidence for metanaupliar instars.

Late copepodid

Body length 160–170 µm (N = 3), greatest width (90 µm) measured mid-way cephalothorax; integument with irregular pattern of surface pits (Fig. 8D). Body cyclopiform (Figs 4A–B, 7D and 8A–B), comprising 3-segmented prosome and 3-segmented urosome. First pedigerous somite incorporated in cephalosome, forming cephalothorax (Fig. 8D). Prosome comprising cephalothorax, P2-bearing somite and pedigerous double-somite bearing P3–P4. Cephalothorax broadly ovoid; posterolateral corners produced into lobate extension; with well developed sensillar pattern and accessory pores as indicated in Figure 4A; separated dorsally from P2-bearing somite by membranous zone (Fig. 8D). Rostrum represented by ventrally directed, tapering protuberance bearing one median slit-like pore and two lateral sensillae (Fig. 8C).

P2-bearing somite with three pairs of sensillae and middorsal pore; anterior margin with lateral rounded protuberances articulating in sockets of posterior margin of cephalothorax, forming condylar articulations (Fig. 4A; arrowed in Fig. 8D); with transverse internal ribs (anterior to middorsal pore; stippled in Fig. 4B) dorsally, representing attachment sites of well developed intersomitic membranes (Fig. 4A); posterior width smaller than that of cephalothorax.

Pedigerous double-somite with six pairs of sensillae; free lateral margin of pleurotergite with short incision marking original segmentation of P3- and P4-bearing somites; posterolateral angles backwardly produced.

Urosome (Fig. 5F) very slender and narrow; anterior margin of first urosomite covered by membranous ring; middle urosomite longest; anal somite shortest. Caudal rami rectangular, longer than wide; each with short dorsal seta, two long terminal setae and one stubby spine at outer distal corner; all elements smooth.

Antennule (Figs 5A and 9C) 5-segmented; armature formula 1-[1], 2-[3 + ae], 3-[1], 4-[1 + 1 spine + ae], 5-[5 + ae]; segment 3 (arrowed in Fig. 9C) much narrower than proximal and distal segments, anterioproximal part often telescoped into distal portion of segment 2. Aesthetasc on segment 2 much longer than others and typically constricted halfway its length.

Antenna (Fig. 4C) 4-segmented, comprising coxa, basis and 2-segmented endopod; exopod absent. Coxa with small sclerite around the base (Fig. 9A, arrowed; Fig. 11A). Coxa, basis and proximal endopod segment unarmed. Proximal endopod segment elongate; distal segment very short, with two equally long claws apically and one minute element (arrowed in Fig. 4C) at outer distal corner.

Mandible, maxillule and maxilla absent. Oral cone short (Figs 4D, 9A–B and 11A), positioned on midventral crest between maxillipeds; with two apical openings separated by medial septum.

Maxilliped (Fig. 4D) subchelate; syncoxa not expressed, basis articulating directly with ventral surface of cephalothorax; 2-segmented, comprising basis and endopod. Basis robust and swollen; palmar margin produced into spinous process bearing apical pore (arrowed in Fig. 9B). Endopod represented by strong curved claw.

Swimming legs 1–4 (Fig. 5B–E) with well developed intercoxal sclerites (distinctly smaller in leg 4), 2-segmented protopods and 1-segmented rami. Coxae unarmed. Bases with outer seta on legs 2 and 3; basal seta with distinct longitudinal ridges (Fig. 9D). Exopods distinctly longer than endopods; without outer spines or setae but outer distal corner produced into minute spinous projection; with two apical and two (legs 1 and 4) or three (legs 2 and 3) inner elements. Endopods with two apical setae. All elements plumose except for outer distal element of exopod being serrate along outer margin and inner distal element tripinnate.

Etymology

The species is named after our colleague Dr José Bresciani, in recognition of his valuable contributions to the biology and anatomy of parasitic copepods.

Affinities

Lepetellicola brescianii is most closely related to *Cocculinika myzorama*, the only other chitonophilid known to be associated with a cocculiniform limpet. Both species occupy the same niche on the host, i.e. above the head region inside the pallial cavity. Adult females are morphologically similar in the possession of lateral expansions but differ in the position of the paired genital apertures. In *Cocculinika* the gonopores are located far anteriorly, near the transition between the ectosoma and the short neck region, whereas in *Lepetellicola* they are concealed in depressions between the hind margin of the ectosoma and the posterior lobes. *L. brescianii* has very well developed posterior lobes that are only incipient in *C. myzorama*. The former is also readily distinguishable by the long cylindrical neck. In view of the uniformity observed in the genus *Ischnochitonika* (Franz & Bullock, 1990; Nagasawa *et al*., 1991) we regard these differences as sufficiently distinct to warrant separate generic status.

Host–parasite relationships

All families of the Cocculiniformia, except for the Choristellidae, have been considered simultaneous hermaphrodites displaying internal fertilization and having distinct vesicles acting as testis and ovary (Haszprunar, 1988b, 1998). Both testis and ovary are mature in fully grown noninfested adults of *Lepetella sierrai*. However, the former always appears, and reaches maturity, before the latter. Mature testes were observed in noninfested individuals as small as 0.8–1.0 mm in shell length (Fig. 10). At this size the ovary consists solely of previtellogenic oocytes, indicating that *L. sierrai* may only function as a male at this size. Testis and ovary both reach maturity in specimens larger than 1.0 mm, which therefore can be considered as simultaneous hermaphrodites. These observations indicate that *L. sierrai* is a functional protandric hermaphrodite like *Addisonia excentrica* Tiberi, 1857, another lepetelloidean belonging to the family Addisoniidae (Roldán & Luque, 1999). In infested individuals the development and maturation of the ovary is strongly affected by the presence of the parasites. In general, the testis matures without development of the ovary, which is a condition (Fig. 10, stage 3) never observed in noninfested specimens where the ovary always contains previtellogenic oocytes at stages 2 and 4. Infection of the host by chitonophilids often results in severe delay in the maturation of the female gonads and, in extreme cases, causes complete cessation of development and probably parasitic castration as reported by Jensen (1987) for splanchnotrophid copepods in shell-less opisthobranchs. Among the infested individuals studied, only three mature simultaneous hermaphroditic specimens were found; these were considerably larger (1.4, 1.8 and 1.9 mm) than fully mature, noninfested specimens (Fig. 10).

Prevalence expressed as the number of host individuals infested vs. the number of host individuals examined was 0.39 (169/425) in station CP21, 0.233 (14/60) in station 185 A, 0.72 (8/11) in station 128 A and 0.48 (36/75) in station 168 A). The intensity (based on the number of female parasites only) was never higher than one parasite per infected host.

New observations on *Nucellicola* Lamb, Boxshall, Mill & Grahame, 1996

Diagnosis (Modified from Lamb et al. (1996))

Chitonophilidae. Female body cylindrical, comprising small cephalic lobe, bearing branching rootlet system, and large vermiform trunk containing highly convoluted gonads. Reproductive system completely paired, comprising ovaries, oviducts and posteriorly located genital apertures. Female enclosed within membranous tube extending through host viscera to point of entry of parasite; tube containing eggs and developing nauplii, not arranged in egg-sacs.

Adult male dwarfed, located in membranous vesicle at posterior end of female; comprising trilobate cephalic region and sac-like posterior trunk, separated by strong bilateral constriction; lateral lobes of cephalic region strongly developed. Antennae and antennary projections absent. Subchelate maxillipeds only appendages, with distinct postmaxillipedal apodeme. Oral cone small, overlain by frontal lobe, without sclerotized mouth-ring. Genital system completely paired, comprising testes, vasa deferentia and spermatophore sacs associated with ventral genital apertures located posterior to maxillipeds.

Nauplius with 1-segmented antennules; antennae and mandibles with 1-segmented bisetose endopod and 3-segmented exopod bearing one seta on each segment. Metanauplius with maxillipeds and two pairs of swimming legs. Early copepodid with 4-segmented prosome and 2-segmented urosome; antennary exopod and mandible vestigial; with four pairs of swimming legs.

Late copepodid as for the family; segment 2 of antennule with four setae plus aesthetasc; anterior element on segment 4 setiform. Leg 1 with outer seta on basis. Caudal rami with sparsely plumose terminal setae.

Endoparasitic in visceral whorl of *Nucella lapillus* (Gastropoda: Muricidae).

Type species
Nucellicola holmanae Lamb, Boxshall, Mill & Grahame, 1996 [by monotypy].

NUCELLICOLA HOLMANAE LAMB, BOXSHALL, MILL & GRAHAME, 1996

Material examined
Holotype ♀ with adult allotype ♂ attached (NHM reg. nos. 1995.664–665); two paratype ♀♀ each with ♂ attached (NHM reg. nos. 1995.666–667); endoparasitic in *Nucella lapillus*; intertidal zone of rocky shore in Robin Hood's Bay, North Yorkshire, England.

Adult female
As described by Lamb *et al.* (1996) except for presence of well developed rootlet system (Fig. 12A–B) separated from the elongated trunk by a distinct constriction (arrowed in Fig. 12B).

Adult male
Enclosed along with exuvium of late male copepodid in membranous vesicle (Fig. 11C); attached in between genital apertures of female. Body highly transformed, 550μm long and 390μm wide; comprising trilobate cephalic region and heart-shaped sac-like trunk, separated by strong bilateral constriction. Cephalic region with very large lateral lobes, extending dorsally; rounded frontal lobe directed ventrally, overlying small oral cone and maxillipeds (Fig. 11C–D). Trunk with lateral lobate extensions in anterior half.

Medial mouth cone very small, located just anterior to maxillipeds. Maxillipeds (indicated by asterisk in Fig. 11C–D) located in deep depression formed by frontal lobe and anterior midventral face of trunk (Fig. 12E) at level of bilateral constriction; subchelate (Fig. 12D), comprising robust proximal segment (representing basis) and strong endopodal claw; no traces of syncoxa discernible; palmar margin of basis with medially directed spinous process. Maxillipeds internally supported by a symmetrically postmaxillipedal apodeme (Fig. 12D–E). Intrinsic muscles in basis atrophied. Two intermaxillipedal processes present (Fig. 12D–E).

Reproductive system completely paired, as described by Lamb *et al.* (1996). Distal part of each vas deferens dilated, forming distinct spermatophore sac (Fig. 11C: S), opening to exterior via paired gonopores located midventrally, posterior to postmaxillipedal apodeme; genital muscles opening gonopores inserting medially on postmaxillipedal apodeme (Fig. 12D–E). Elongate-pyriform spermatophores attached in clusters to genital apertures of ♀ (Fig. 12C); both full and discharged spermatophores present.

Late male copepodid
Description based on exuvium dissected out of membranous sac containing adult male (Fig. 11C). Body length 190μm ($N = 1$), greatest width (100μm) measured at posterior third of cephalothorax. Body cyclopiform (Fig. 13A–B), comprising 3-segmented prosome and 3-segmented urosome. First pedigerous somite incorporated in cephalosome forming cephalothorax. Prosome comprising cephalothorax, P2-bearing somite and pedigerous double-somite bearing P3-P4. Cephalothorax broadly ovoid, abruptly tapering posteriorly; posterolateral corners not produced into lobate extension; with well developed sensillar pattern and accessory pores as indicated in Figure 13A–B; separated dorsally from P2-bearing somite by membranous zone. Rostral area as in *Lepetellicola*. Ventral surface with large slit through which the adult male emerged; corresponding to position of maxillipeds and postmaxillipedal apodeme in copepodid of *Lepetellicola* (compare Fig. 4A).

P2-bearing somite with two pairs of sensillae and middorsal pore; with condylar articulations and transverse internal ribs as in *Lepetellicola*; widening posteriorly so that width at hind margin is greater than posterior width of cephalothorax.

Pedigerous double-somite with nonfunctional articulation middorsally, fading out in transverse surface suture dorsolaterally and laterally (Fig. 13B); free lateral margin of pleurotergite without trace of original segmentation of P3- and P4-bearing somites; with six pairs of sensillae; posterolateral angles backwardly produced; posterior part with sigmoid chitinous ribs dorsally.

Urosome (Figs 11B and 13A–B) less slender than in *Lepetellicola* but somites of similar proportions (foreshortened in Fig. 13B). Caudal rami rectangular, longer than wide; each with short dorsal seta, two long-terminal setae and one stubby spine at outer distal corner; terminal setae sparsely plumose.

Antennule (Fig. 13A–B) 5-segmented; armature formula 1-[1], 2-[4 + ae], 3-[1], 4-[2 + ae], 5-[5 + ae]; segmental proportions and relative lengths of aesthetascs as in *L. brescianii*.

Antenna (Fig. 13A) 4-segmented, comprising coxa, basis and 2-segmented endopod; distal segment very short, with two equally long claws apically and one minute element subapically.

Maxillipeds and mouth area missing.

Swimming legs 1–4 with well developed intercoxal sclerites, 2-segmented protopods and 1-segmented rami. Coxae unarmed, bases with outer seta on legs 1–3. Rami with setal formula as in *L. brescianii*. All elements plumose except for outer distal element of exopod being pinnate along outer margin and inner distal element tripinnate; setae shorter than in *L.*

brescianii. Outer margin of exopod denticulate, that of endopod with long setules (Fig. 11E).

Remarks

Lamb *et al.* (1996) described the prosome of the late male copepodid stage as 5-segmented, comprising a cephalosome and four free pedigerous somites. This is based on a double observational error as the first pedigerous somite is in fact incorporated in the cephalosome forming a cephalothorax (as in the early copepodid; Lamb *et al.*, 1998), and the last two somites are fused, forming a pedigerous double-somite. Excessive squashing of the cephalothorax could have caused the misinterpretation of the narrow posterior part as the first pedigerous somite, whereas the dorsal surface suture on the double-somite was mistakenly drawn as a fully functional articulation separating the last two pedigerous somites. Additional oversights in Lamb *et al.*'s (1996) illustrations include the accessory element on the antennary endopod, the outer basal spine on legs 1–3, the dorsal seta on the caudal ramus, the setal ornamentation on the swimming legs and caudal rami, and the sensillar pattern, which was incompletely illustrated for the pedigerous somites and not shown at all on the cephalothorax. The antennules are clearly 5-segmented instead of 4-segmented and the proximal aesthetasc in the specimen examined is about twice as long as figured by Lamb *et al.* (1996).

Lamb *et al.* (1996) described the late copepodid based on the exuvium enclosed along with the adult male inside the vesicle (Fig. 11C). They emphasized the lack of maxillipeds in this copepodid and postulated that their sudden appearance in the adult male may have been the result of extreme developmental delay as found in some other poecilostomatoids such as the Ergasilidae. In a later report, Lamb *et al.* (1998) claimed that they had overlooked the maxillipeds in the copepodid exuvium because its presence had been demonstrated in preceding stages, including the metanauplius and the early copepodid, as well as the infective copepodid that they obtained in culture. This statement is clearly incorrect as the exuvium invariably shows a longitudinal slit on the ventral surface of the cephalothorax, coinciding with the position of the maxillipeds (arrowed in Fig. 13A). The infective male copepodid attaches to the posterior end of the female, near the genital apertures, using its maxillipeds. The strong muscles in the bases of the maxillipeds (as found in *L. brescianii*) ensure that the copepodid is kept in position during formation of the vesicular wall around it. Although the origin of the vesicle is unknown it is likely to be derived from an extension of the female body wall. Metamorphosis into the adult male presumably initiates once the vesicle has completely enclosed the copepodid, resulting in the shedding of the old cuticle whilst maintaining a grip on the female with the maxillipeds. This explains why no trace of these appendages can be found in the exuvium and why they are of exactly the same size in both the infective copepodid and adult, despite their large disparity in body size (Fig. 11C). As space within the vesicle is limited, the male is almost immobilized and no longer requires attachment devices. Consequently, the maxillipeds become redundant, which is reflected in the atrophy of the basal and extrinsic musculature (Fig. 12D). The genital apertures are located close to the maxillipeds and are intimately associated with the postmaxillipedal apodemes. Two pairs of muscles originate on the common median extension of the apodemes, one inserting directly on the lateral wings of the apodemes, and the other inserting on the body wall posterior to the genital apertures (Fig. 12D–E). These muscles (g.m.) are responsible for opening the genital apertures and are presumably opposed by the elasticity of the thickened cuticle in this area. The maxillipeds and postmaxillipedal apodemes present in the infective copepodid are the only juvenile exoskeletal elements transferred to the adult male during metamorphosis. This developmental mechanism maintains grasping efficiency during the initial stages of attachment of the male and more importantly ensures continuity in the build-up of the genital apparatus. Our observations of the infective copepodid (Fig. 4A) and male (Figs 2B–C and 12F) of *L. brescianii* suggest a similar mechanism of incomplete moulting in all genera that have retained maxillipeds in the adult males (*Nucellicola*, *Lepetellicola*, *Tesonesma* and possibly *Cocculinika*).

The similarity between the copepodids of *N. holmanae* and *L. brescianii* is, to say the least, remarkable. Only a few differences can be observed, all of minor significance: (1) antennule setation pattern (segment 2 with additional seta in *Nucellicola*; anterior element of segment 4 spiniform in *Lepetellicola*) (2) outer basal seta on leg 1 (absent in *Lepetellicola*) (3) degree of fusion of pedigerous somites 3–4 (4) proportional length of urosomites (5) length and ornamentation of swimming leg setae, and (6) terminal setae on caudal rami (plumose in *Nucellicola*).

Familial distinctiveness of Nucellicolidae

The Nucellicolidae and Chitonophilidae are unique in having both sexes highly transformed. Although this similarity was noted by Lamb *et al.* (1996), they distinguished the latter from the former by the presence of a highly developed rootlet system in females, and by the possession of paired antennae and a common median genital aperture in males. These discrepancies, in conjunction with the structure of the swim-

ming legs in the late male copepodid stage, were considered as sufficient justification to attribute separate family status to *Nucellicola*. Our observations have shown that none of these differences is real and that the resemblance between both families extends far beyond the shared transformation of both sexes. A suggested relationship between the Nucellicolidae and Mytilicolidae on the basis of mandibular loss (Lamb *et al.* 1998) could not be corroborated.

A branching rootlet system is clearly developed in *N. holmanae* (Fig. 12A–B) and we suspect that its reported absence in the original description is due to imperfect dissection of the parasite from the host's viscera and the egg-tube enclosing it. Examination of the anterior cephalic lobe in the paratypes of *N. holmanae* showed it to be incomplete, the irregularly shaped fracture plane coinciding with the bilateral constriction between the swollen cephalic region and the much larger rootlet system (arrowed in Fig. 12B).

The diagnostic significance of the presence of paired antennae in male chitonophilids is not to be taken as absolute as this character has thus far only been reported in the males of *Chitonophilus* and *Leptochitonicola* (Avdeev & Sirenko 1991). Antennae are entirely absent in the genera *Cookoides*, *Ischnochitonika*, *Tesonesma* and *Lepetellicola*, all of which resemble *Nucellicola* in this feature. The males of *Cocculinika* are as yet unknown.

Lamb *et al.*'s (1996) claim of a median genital aperture in male Chitonophilidae is based on a misinterpretation of Avdeev & Sirenko's (1991) illustrations of *C. laminosus* and *L. latus*. In both species the male genital system is paired and each vas deferens terminates in a spermatophore sac with associated genital aperture, as in *Nucellicola*. This configuration is also found in *Lepetellicola*, *Ischnochitonika*, *Tesonesma* and possibly also *Cookoides* although Avdeev & Sirenko's (1994) illustrations of the latter are not conclusive in this respect. Lamb *et al.*'s (1996) observational error is in all probability based on a misinterpretation of the conspicuous midventral oral disc illustrated by Avdeev & Sirenko (1991).

The late copepodid stage provides overwhelming evidence for a close relationship between *Nucellicola* and the chitonophilid genera. Lamb *et al.* (1996) had already emphasized the uniqueness of the swimming legs in *N. holmanae*, having elongate 1-segmented rami, and with exopodal setae arranged exclusively along the distal and inner margins. The discovery of the only copepodid stage of *Lepetellicola* revealed a virtually identical swimming leg morphology, differing from *Nucellicola* only in the absence of the outer basal seta on leg 1 and minor deviations in setal length and ornamentation. Copepodids of both genera also share the same tagmosis (including the pedigerous doublesomite), the form and segmentation of the antennules, the presence of two claws plus one accessory element on the antenna and the caudal ramus setation pattern. Concordance is found even in small details such as the integumental sensillar pattern and the specialized condylar articulations between the cephalothorax and the leg 2-bearing somite.

Finally, the adult males of *Lepetellicola* and *Nucellicola*, despite differences in gross body morphology, are remarkably similar in the configuration of the reproductive system, the position of the genital apertures, the possession of a pair of almost identical maxillipeds (with spinous process on the basis but without syncoxa) and the presence of a characteristic postmaxillipedal apodeme. These characters, the presence of a rootlet system in the adult female in conjunction with several lines of evidence based on copepodid morphology decisively reject the claim that *Nucellicola* deserves distinct familial status. Its phylogenetic position within the Chitonophilidae is discussed below.

Phylogeny of Chitonophilidae

The monophyly of the Chitonophilidae is supported by the shared presence of a branching rootlet system embedded in the host's tissues, serving both as an absorptive system and attachment mechanism. The rootlet system transports nutrients to the digestive tract in the ectosoma. Members of the highly transformed family Herpyllobiidae have a similar holdfast, the endosoma, containing the entire digestive system of the copepod (Lützen, 1964, 1966). However, in this family, digestion takes place in the endosoma, indicating that the herpyllobiid holdfast is not homologous to the chitonophilid rootlet system. A close relationship between both families is also ruled out on the basis of copepodid antennule morphology (see below).

Assessing the phylogenetic relationships within the Chitonophilidae is difficult as the adults of both sexes are highly transformed and the more morphologically informative developmental stages are not known for all the genera. Naupliar stages have been described for *Ischnochitonika*, *Cookoides*, *Lepetellicola* and *Nucellicola*, while information on copepodids is only available for the latter two genera. The present analysis is based on nine naupliar and ten adult characters (Table 1) and their character states are summarized in matrix format in Table 2. All characters were set irreversible, which suppresses reversals at the expense of introducing extra convergences and thereby increasing tree length. A 'Branch and Bound search' was run, which guarantees finding all most parsimonious trees, and the 'Acctran' optimization used.

Despite the inclusion of several underdetermined taxa, analysis of the data matrix of 19 characters (Tables 1,2) resulted in one fully resolved, most parsi-

monious tree (MPT) with a length of 21 steps, consistency index 0.905 and retention index 0.931 (Fig. 14). Omission of naupliar characters 1–9 from the data matrix also resulted in one MPT with identical topology but obviously with a shorter tree-length of 12 steps and consistency index 0.833. The topology supports a basal dichotomy dividing the family into a mesoparasitic clade utilizing only polyplacophoran hosts (*Chitonophilus*, *Ischnochitonika*, *Cookoides* and *Leptochitonicola*) and a clade grouping genera associated with cocculiniform limpets (*Cocculinika*, *Lepetellicola*), chitons (*Tesonesma*) and prosobranch gastropods (*Nucellicola*).

The *Chitonophilus* clade is supported by two synapomorphies (17, 19), both relating to the morphology of the adult male. Males of *Chitonophilus*, *Leptochitonicola* and *Ischnochitonika* all have a raised mouth cone characterized by a strongly sclerotized ring circumscribing the mouth opening. Nagasawa *et al.* (1991) showed that this cone has both an anterior and a posterior opening. Avdeev & Sirenko (1994) were unable to observe the mouth opening in the strongly reduced male of *Cookoides cordatus*, possibly resulting from the strong dorsoventral flexure of the anterior part of the body. In males of the *Chitonophilus* clade the spermatophore sacs and associated genital apertures assume a dorsal position. This condition is considered as apomorphic due to secondary displacement as in most copepods the male genital apertures are located ventrally (Huys & Boxshall, 1991). This ancestral position is retained in *Nucellicola*, *Lepetellicola* and *Tesonesma*; it is unknown in *Cocculinika* as the male has yet to be discovered. The genus *Chitonophilus* is identified as the sistergroup of a 3-taxon clade, which is characterized by the possession of paired antennary projections in the male (character 16). These projections are well developed in *Leptochitonicola* and *Ischnochitonika* but are secondarily reduced in *Cookoides* due to the loss of the antennary claws. The loss of these claws (character 15) is shared with *Ischnochitonika* but has yet not affected the size of the antennary projections in this genus (Nagasawa *et al.*, 1991).

The monophyletic group in apposition to the *Chitonophilus* lineage shows a basal split, with a mesoparasitic clade, including *Cocculinika* and *Lep-*

Table 1. Morphological characters used in the phylogenetic analysis. Apomorphic states are referred to in square brackets

1	Antennule nauplius 2-segmented [1-segmented]
2	Antennary endopod nauplius 2-segmented [1-segmented]
3	Antennary endopod nauplius with lateral seta [absent]
4	Antennary exopod nauplius 5-segmented [3-segmented]
5	Antennary exopod nauplius with 4 lateral setae [2 lateral setae]
6	Mandibular endopod nauplius 2-segmented [1-segmented]
7	Mandibular endopod nauplius with lateral seta [absent]
8	Mandibular exopod nauplius 4-segmented [3-segmented]
9	Mandibular exopod nauplius with 3 lateral setae [2 lateral setae]
10	Females mesoparasitic, with egg-sacs or masses [endoparasitic, without egg-sacs]
11	Adult ♀ without lateral lobes [present]
12	Adult ♀ without posterior lobes [present; incipient in *Cocculinika*]
13	Genital apertures of ♀ lateral [dorsal]
14	Cephalothorax ♂ without distinct lobes [trilobate]
15	Antenna present in adult ♂ [absent]
16	Adult ♂ without paired antennary projections [present]
17	Adult ♂ without sclerotized mouth-ring [present]
18	Maxilliped absent in adult ♂ [present]
19	Spermatophore sac and genital apertures of ♂ located ventrally [dorsally displaced]

Table 2. Character data matrix [0 = ancestral (plesiomorphic) state, 1 = derived (apomorphic) state, ? = missing data]

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Chitonophilus	?	?	?	?	?	?	?	?	?	0	0	0	0	0	0	0	1	0	1
Cocculinika	?	?	?	?	?	?	?	?	?	0	1	1	1	?	?	?	?	?	?
Cookoides	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	?	0	?
Ischnochitonika	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	1	0	1
Lepetellicola	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1	0	0	1	0
Leptochitonicola	?	?	?	?	?	?	?	?	?	0	0	0	0	0	0	1	1	0	1
Nucellicola	1	1	1	1	1	1	1	1	1	1	?	0	?	1	1	0	0	1	0
Tesonesma	?	?	?	?	?	?	?	?	?	1	1	0	?	1	1	0	0	1	0

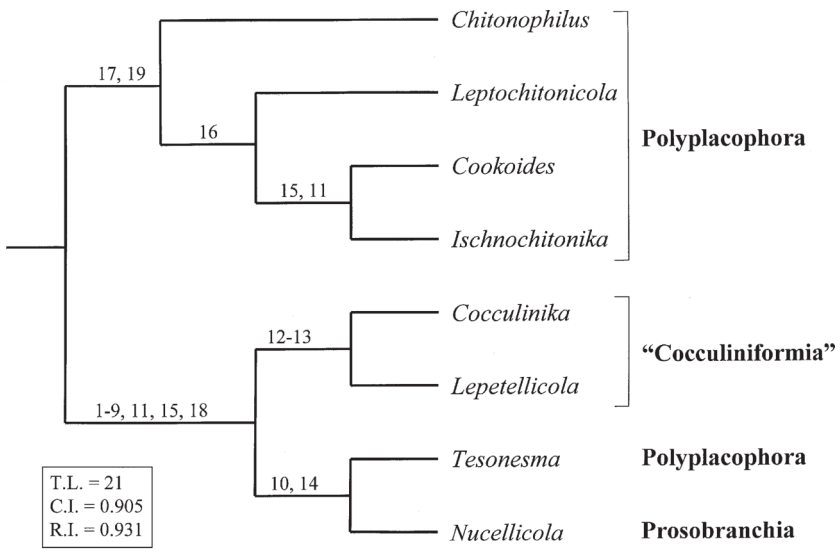

Figure 14. Phylogenetic tree depicting relationships between genera of the Chitonophilidae. Host groups indicated in bold. For explanation of apomorphic states see Tables 1,2 and text.

etellicola, and an endoparasitic clade, encompassing *Tesonesma* and *Nucellicola*. It is supported by a suite of nine naupliar characters, the loss of the antennae in the adult male and the presence of lateral lobes in the adult female. Hypermorphosis along the antero-posterior axis in *Nucellicola* prevented us from scoring the latter character confidently and consequently we have treated it as a missing entry (Table 2: character 11). An additional apomorphy of major importance is the presence of maxillipeds in the males of *Nucellicola*, *Lepetellicola* and *Tesonesma* (the male is unknown in *Cocculinika*). These appendages were misinterpreted as the antennae by Avdeev & Sirenko (1994) in their description of *T. reniformis*, presumably because they had orientated the male incorrectly, the ventral view being the dorsal one, and vice versa. The maxillipeds are not acquired as a result of normal moulting but represent structures retained from the previous developmental stage (see above). It is postulated here that all chitonophilid adults (both sexes) typically acquire their highly transformed body shape through extreme metamorphosis at the final moult, leaving no trace of similarity with the preceding cyclopiform copepodid and resulting in the loss of virtually all appendages. This is a common phenomenon observed in many parasitic copepods that are intimately associated with their hosts. The absence of maxillipeds in males of the *Chitonophilus* lineage is therefore regarded as the plesiomorpic state (Tables 1,2: character 18). Their presence in the other genera is not the result of heterochrony but simply reflects incomplete moulting and is interpreted as the apomorphic state. This explains why the presence of antennae and maxillipeds (or their absences) are mutually exclusive in the two clades. Such a disjunct distribution of major character states (presence/absence) simultaneously expressed in two primary appendages is extremely unusual for a taxon that is highly advanced in all other respects, particularly when they determine the basal dichotomy of the group. This is a typical example demonstrating that what appear to be reversals in copepod evolution should not be taken unquestionably at face value. It is noteworthy that the postmaxillipedal apodeme is only present in those genera that have retained ventral genital apertures, showing once again the close functional correlation between both structures.

The relationship between *Cocculinika* and *Lepetellicola* is supported by two female characters, the dorsal displacement of the genital apertures and the formation of paired posterior lobes. These lobes are incipient in *Cocculinika* (Jones & Marshall, 1986) and strikingly resemble the condition found in young females of *Lepetellicola* (Fig. 1G).

A key innovation in the evolution of the Chitonophilidae is the adoption of a completely endoparasitic life style by the female and the associated loss of egg-sac development. This transition conceivably permitted the major adaptive shift from deepwater hosts to those living in the upper and middle eulittoral zones of rocky shores such as the predatory gastropod *Nucella lapillus*. The completion of the naupliar phase inside the host allows for the offspring to be released at an advanced state of development (metanauplius), possibly in a controlled manner depending on whether tidal conditions are favourable for dispersal. The position of

Nucellicola females inside the dogwhelk is typically near the tip of the visceral whorl, but occasionally they can be found elsewhere in the host nearer to their point of entry in the mantle (Lamb *et al.*, 1996). Each female is contained within a highly convoluted tube that extends through the host viscera to the presumed point of entry. The tube is filled with eggs, nauplii and metanauplii, the latter being closest to the opening through which they are released to the exterior. Avdeev & Sirenko (1994) emphasized that *Tesonesma reniformis*, unlike other chitonophilids, occupies the body cavity of its chiton host. They observed large numbers of eggs at various stages of development as well as nauplii, lying freely in a semitransparent cyst surrounding the female. The cyst wall is of a complex nature, consisting of a ramifying system of membranous partitions that separate the linear, loosely arranged egg-clusters. It is penetrated by the long, simplified rootlet system of the parasite and presumably has a second opening leading to the exterior as in *N. holmanae*. It is highly conceivable that the cyst of *Tesonesma* and the egg-tube of *Nucellicola* are homologous structures. Similarity is also noted in the distinctly coiled paired ovaries and oviducts of *T. reniformis* that are reminiscent of the highly convoluted reproductive system of *N. holmanae* (Lamb *et al.* 1996). Although Avdeev & Sirenko (1994) did not give any information on the exact position of the sexes relative to each other, the resemblance in male morphology provides additional evidence in support of a *Nucellicola-Tesonesma* sistergroup relationship. Males of both genera differ essentially in the degree of development of the lateral lobes and the overall shape of the posterior trunk. Haszprunar (1987a) briefly described (but did not figure) an endoparasite of unknown affinities, living in the haemocoel of the cocculinid *Coccopigya hispida* and maintaining contact with the exterior via a duct opening at the pedal sole, however, it is unclear whether this animal is a chitonophilid.

The outgroup of the Chitonophilidae and hence its host affiliation are as yet unknown. However, as no other copepods have been recorded from chitons thus far, we postulate that the Polyplacophora is the ancestral host group of the Chitonophilidae. This implies that host switching has occurred on two occasions, once in the ancestor of the *Cocculinika-Lepetellicola* clade (cocculiniforms) and once in the *Nucellicola* lineage (prosobranchs) (Fig. 14). It is interesting that both cocculiniform parasites cluster together as the proposed monophyly of the host group (Haszprunar 1988b) has been the subject of recent debate. Ponder & Lindberg's (1996, 1997) phylogenetic analyses indicated that the Cocculiniformia is polyphyletic, comprising two divergent superfamilies of deepwater limpets. The Lepetelloidea (utilized by *Lepetellicola*) may be modified vetigastropods whereas the Cocculinoidea (utilized by *Cocculinika*) are possibly of neritimorph affinities.

DISCUSSION

Brooding in cocculiniform limpets?

Brooding in Lepetellidae was first reported by Warén (1972) in *Lepetella laterocompressa* (De Rayneval & Ponzi, 1854) from the Swedish west coast. Warén observed in the right part of the mantle cavity a heart-shaped egg cluster of about 25 eggs at different stages of development, attached by a narrow string where the anus should be situated. This report, undoubtedly being based on an infection by chitonophilid copepods, has been persistently cited in the literature as evidence for brood protection in lepetelloidean Cocculiniformia (e.g. Haszprunar, 1987a, 1988b, 1998; Haszprunar & McLean, 1996). Subsequent records of eggs in the mantle cavity of other families of the Lepetelloidea fed the conjecture that brooding was widespread in the Cocculiniformia (e.g. Haszprunar 1988b). For example, Moskalev (1978) reported about 100 eggs of irregular triangular shape (about 56 × 140μm), situated under the mantle of *Bathyphytophilus caribaeus* Moskalev, 1978 (Bathyphytophilidae) from the abyssal Caribbean. A similar case of presumed brooding was recently reported in the congener *B. diegensis* Haszprunar & McLean, 1996 from the San Diego Trough. Haszprunar & McLean (1996) photograph of a brooding limpet shows eggs at the left side of the mantle roof and subpallial cavity, near the head region. Haszprunar (1988a) reported the presence of many ripe eggs in the pallial and subpallial cavities of two specimens of *Notocrater ponderi* Marshall, 1986 that he regarded as clear evidence for brood protection in the family Pseudococculinidae. In the second superfamily of the Cocculiniformia, the Cocculinoidea, supposed brooding has been observed by Haszprunar (1987a) who found ripe eggs in the pallial cavity of a single specimen of *Coccopigya hispida*.

Dantart & Luque (1994) pointed out that many specimens of *L. sierrai*, collected in the Bay of Biscay, had the pallial cavity filled with a mass of up to 70 white spherical eggs, measuring about 100μm in diameter. In accordance with previous reports of brooding in cocculiniform limpets they regarded these as developing embryos of the gastropod. However, during a recent study of the Lepetellidae from the Iberian-Moroccan Gulf and North Spain it was concluded that these 'embryos', typically found as a cluster dorsal of the cephalic tentacles (Dantart & Luque, 1994: fig. 52; fig. 6A herein), are in reality the egg-masses of mesoparasitic copepods. Warén (pers. comm.) con-

firmed the presence of similar ovigerous parasites in the pallial cavity of *Caymanabyssia vandoverae* McLean, 1991, collected from sunken driftwood at a depth of 2750 m off the coast of Oregon (44°45′47′-N, 125°31′14′-W).

The published records for both superfamilies of the Cocculiniformia made Haszprunar (1988b, 1998) postulate that brood protection was a common phenomenon and possibly typical for the whole group, but all these records should now be re-interpreted in the light of the present results. The position of *L. brescianii* on *L. sierrai* (Figs 6A and 7A) is similar to that described for *Cocculinika myzorama* by Jones & Marshall (1986) who found a single ovigerous female attached above and behind the head, near the anus of each infested specimen of the cocculinid host *Coccopigya hispida*. The latter is the only known host of *C. myzorama* and it is therefore highly conceivable that Haszprunar's (1987a) report of brooding in this species is based on an observational error. The similar position of the 'ripe eggs' described in *Notocrater ponderi* by Haszprunar (1988a) and in *Lepetella laterocompressa* by Warén (1972) leave little doubt that they were dealing with gastropods infested by chitonophilid copepods. Although Haszprunar & McLean's (1996) illustration of *Bathyphytophilus diegensis* lacks sufficient focus to be conclusive, the position and size of the egg-masses raise the strong suspicion that they also belong to a chitonophilid.

Haszprunar (1988b, 1998) assumed that the reduced ciliated gill leaflets displayed by many families of the Lepetelloidea are no longer primarily respiratory in function but are instead involved in creating water currents in the pallial cavity, supplying the brooded eggs with fresh, oxygenated water. A similar inference of brood protection based on anatomical features was made specifically for the Lepetellidae. Haszprunar (1988b) stated that this family is exceptional in possessing a deeper mantle cavity that serves as a 'brood pouch' for the lecithotrophic eggs, and he considered this a synapomorphy for the Lepetellidae. However, detailed examination of more than 500 specimens of *L. sierrai* and other *Lepetella* species from different localities and depths in the Atlantic failed to reveal any evidence of brooding. These observations have demonstrated unequivocally that at least in the Lepetellidae the reported phenomenon of brooding is fallacious. Conversely, members of the lepetelloidean family Addisoniidae possess a well developed gill, and the development of the nonrespiratory epithelial zones of the gill, for which Haszprunar (1987b) suggested a possible function in protecting eggs or embryos, is significantly related to the grade of gonad maturity (Roldán & Luque, 1999). Nevertheless, no specimens of any species of *Addisonia* Dall, 1882 have been found to brood eggs thus far even though more than 500 individuals of the Atlantic-Mediterranean species *A. excentrica* have been examined until now. The scanty data available for the Pseudococculinidae and Bathyphytophilidae are based on very few specimens and leave considerable scope for re-interpretation, whereas the single specimen record for the Cocculinidae (Haszprunar, 1987a) is almost certainly based on an ovigerous female of *C. myzorama*. Hence, the function of the supposed 'brood pouch' formed by the hypobranchial gland in the Cocculinidae (Haszprunar, 1998) requires reconsideration. In conclusion, there is at present no sound evidence for brooding in cocculiniform limpets.

Heterochrony in Chitonophilidae

The development in *Nucellicola holmanae* and other chitonophilids is a remarkable example of dissociated heterochrony, whereby different appendages or growth fields can undergo different heterochronic changes. In the male developmental sequence for example (Fig. 15) predisplacement and postdisplacement are combined in a single stage. Lamb *et al.* (1998) identified the early copepodid of *N. holmanae* as the equivalent of copepodid II observed in copepods passing through the complete series of six postnaupliar stages. This identification was based on body segmentation, comprising a cephalothorax and five free somites, which serves as a useful reference background against which the timing of appearance of the various appendages can be assessed. The mandible and antennary exopod were shown by Lamb *et al.* (1998) as shrivelled appendages. The illustration of the former is not convincing, however, the rudimentary antennary exopod is clearly reminiscent of the condition found in copepodid I of many Cyclopoida (e.g. Dudley, 1966) and Poecilostomatoida (e.g. Izawa, 1986). The persistence of this vestige in copepodid II of *N. holmanae* is the result of postdisplacement, i.e. the late initiation of the reduction of the antennary exopod. On the contrary, the presence of the fourth pair of swimming legs in this stage contradicts the generalized pattern of leg development proposed by Ferrari (1988). As this condition is typical for copepodid III, the early onset of the development of this character is due to predisplacement. Lamb *et al.* (1998) attributed the mosaic morphology of the copepodid II to the combination of retarded (antenna, mandible) and accelerated development (leg 4). This is somewhat misleading as these heterochronic changes are rate concepts, commonly known as neoteny (slower rate) and acceleration (increased rate), and describe perturbations in the rate of developmental events. Predisplacement and postdisplacement are time concepts describing the relative timing of onset (initiation) and offset (cessation) of ontogenetic processes as expressed, for example, in

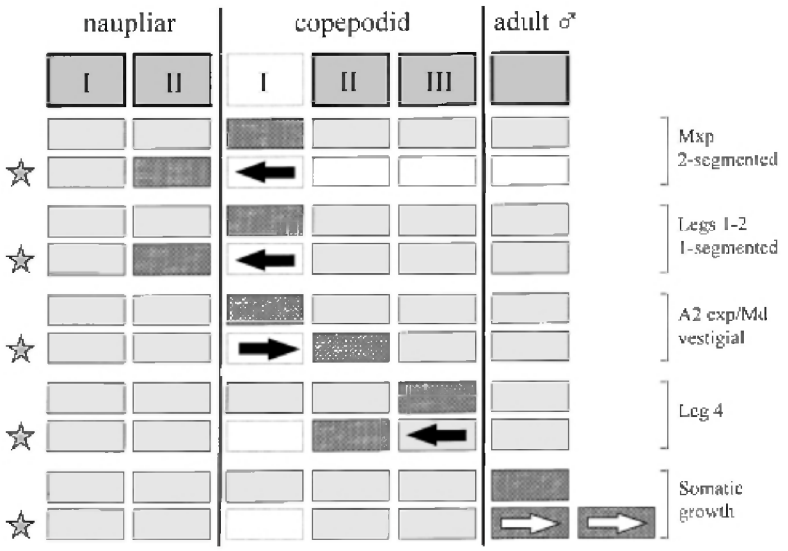

Figure 15. Heterochronic events in development of male *Nucellicola holmanae* (indicated by asterisk) compared with normal development. Arrows indicate predisplacement, postdisplacement or hypermorphosis.

the early copepodid of *N. holmanae*. The metanauplius of the latter is a unique case of predisplacement, demonstrated by the presence of a 2-segmented maxilliped and two pairs of 1-segmented swimming legs, both of which are characters normally expressed at copepodid I (Fig. 15). Lamb *et al.* (1998) regarded this as an adaptation to enhance the locomotory capability of the metanauplius.

The abbreviation of the life cycle in *Nucellicola* is extreme, comprising only two naupliar and two copepodid stages plus the adult. There is virtually no size increase between the nauplius and the late male copepodid, suggesting a rapid moulting sequence. The simultaneous presence of eggs, first nauplii and late copepodids still maintaining close contact to the adult female in *L. brescianii* (Fig. 1C) suggests that rapid moulting may also be common in other Chitonophilidae. The adults of both sexes of *N. holmanae* not only undergo extreme transformation at the final moult but also gross size increase as a result of hypermorphosis. The extent of hypermorphosis expressed in the adult male is demonstrated by comparison with the exuvium size of the late copepodid (III) (Fig. 11C). In the adult female the effect is an order of magnitude larger and is correlated with extreme elongation along the antero-posterior axis as a result of allometric growth.

Ordinal position of Chitonophilidae

Ordinal placement of the Chitonophilidae was not discussed by Avdeev & Sirenko (1991, 1994) but Lamb *et al.* (1996) ranked the family among the Poecilostomatoida without giving any explicit reason. They also placed the Nucellicolidae in this order on the basis of the structure of the antenna in the copepodid stage, and the presence of maxillipeds in males combined with their apparent absence in females. One of the antennary characters used by Lamb *et al.* was the alleged presence of a coxo-basis, which is a diagnostic autapomorphy of the Poecilostomatoida (Huys & Boxshall, 1991). This claim is however, contradicted by their own illustration (Lamb *et al.*, 1996: Fig. 2C and our observations (Fig. 13A–B), showing that there is no evidence for such compound segment and that the coxa is discrete from the basis as in *Lepetellicola* (Figs 4C, 9A and 11A). This antennary segmentation pattern precludes placement of the Chitonophilidae in the Poecilostomatoida.

The segmentation pattern of the male copepodid antennules is identical in *Nucellicola* and *Lepetellicola* (Figs 5A, 9C and 13B). Lamb *et al.* (1996) erroneously described the antennule as 4-segmented, discounting the small third segment as a true segment. However, this segment is crucial as it permits unequivocal homologization of the four segment boundaries (Fig. 16). The presence of the posterior seta on this segment unambiguously identifies it as ancestral segment XXIV as no other posterior setae are found on more proximally located segments. The fourth segment, carrying a posterior seta and an anterodistal seta with associated aesthetasc, is identified as segment XXV. The distal compound segment corresponds to fused ancestral segments XXVI-XXVIII. Proximal to segment XXIV is another compound segment bearing a large aesthetasc and three (*Lepetellicola*) or four setae (*Nucellicola*). The aesthetasc

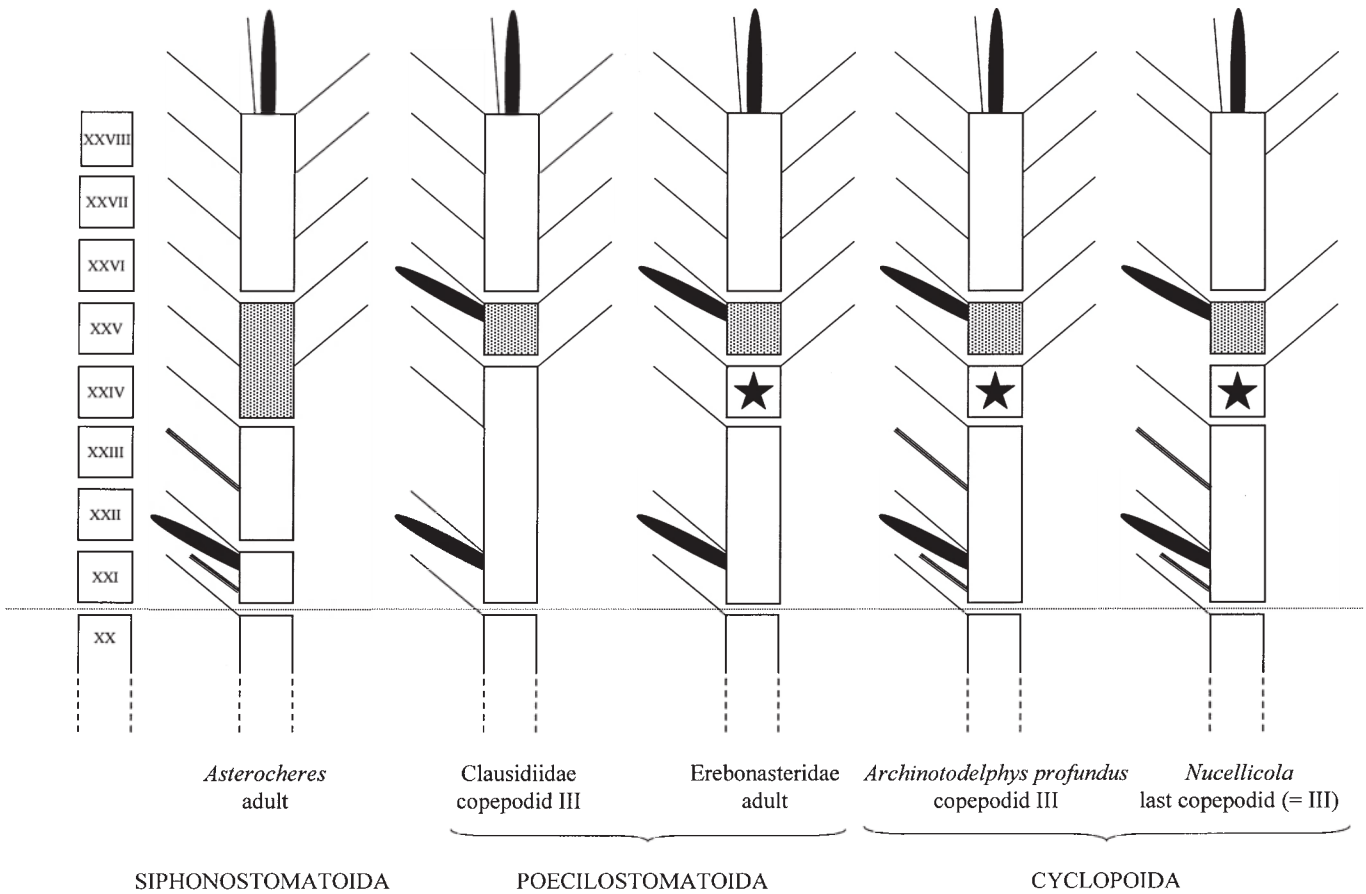

Figure 16. Segmentation and setation pattern of antennule part distal to XX–XXI articulation (equivalent to neocopepodan geniculation) for various taxa. Based on personal observations (*Archinotodelphys profundus*, *Nucellicola*) or data derived from Boxshall & Huys (1998) (*Asterocheres*), Kim & Ho (1992) (*Hemicyclops*) and Huys & Boxshall (1990) (Erebonasteridae). See text for explanation.

originates from ancestral segment XXI, inferring that the second segment represents the fused ancestral segments XXI–XXIII. The boundary between the first two segments is homologous to the articulation separating ancestral segments XX and XXI, indicating that no separation has occurred proximal to this boundary (i.e. segments I–XX). This articulation is highly conserved, both in ontogeny and phylogeny, and is already expressed at copepodid I in most copepods. Examination of a range of calanoid developmental stages showed that it is the first articulation to be expressed in ontogeny, often from nauplius I onwards (Huys & Kršinic, unpublished data).

The male copepodid antennule in Chitonophilidae reveals three characters of particular interest: segmentation, aesthetasc pattern and armature on the compound segment XXI–XXIII.

Boxshall & Huys (1998) pointed out that the penultimate segment (XXV) of virtually every copepodid stage of almost every described member of the Poecilostomatoida is expressed as a distinct segment, carrying two setae and an aesthetasc. Although this signature can be regarded as an ordinal characteristic, it is by no means exclusively diagnostic as many cyclopoids, both free-living (e.g. Gurney, 1933) and associated (e.g. Dudley, 1966), show a similar pattern (Fig. 16). Consequently, it does not provide conclusive evidence justifying placement of the Chitonophilidae in the Poecilostomatoida, however, it unequivocally excludes the family from the Siphonostomatoida in which the double segment XXIV–XXV remains undivided (Fig. 16) or is part of a larger compound segment incorporating additional proximal and/or distal segments (Boxshall & Huys, 1998). Particularly relevant is segment XXIV, which is distinct in the Chitonophilidae (arrowed in Fig. 9C). Within the Poecilostomatoida, this segment is only expressed in the Erebonasteridae (e.g. Martínez Arbizu, 1996/97; Huys & Boxshall, 1990), that is generally accepted as the most primitive lineage in the order (Huys & Boxshall,

1991). The presence of this feature in the highly modified Chitonophilidae would imply a basal position in the poecilostomatoid phylogenetic tree for this family, a scenario that is not, however, reinforced by additional morphological evidence.

Copepodid stages of poecilostomatoids typically have three aesthetascs on the distal part of the antennule. These aesthetascs originate from ancestral segments XXI, XXV and XXVIII (Boxshall & Huys, 1998) and are found on the last three segments in the majority of poecilostomatoids. In the Erebonasteridae and Chitonophilidae, four segments are expressed distal to the XX–XXI articulation but the origin and number of aesthetascs on them is similar. The poecilostomatoid aesthetasc pattern is highly conservative during postnaupliar development, being expressed from copepodid I onwards, and differs from the siphonostomatoid arrangement by the presence of an aesthetasc on segment XXV. This dissimilarity demonstrates that the current placement in the Siphonostomatoida (Bowman & Abele, 1982; Huys & Boxshall, 1991) of some highly transformed families such as the Herpyllobiidae is unjustified as the infective copepodid stage exhibits the poecilostomatoid aesthetasc pattern. However, it does not unequivocally substantiate assignment of the Herpyllobiidae and Chitonophilidae to the Poecilostomatoida as many cyclopoids, such as members of the Fratiidae (Ho *et al.*, 1998) and Notodelphyidae (Dudley, 1966), display the same pattern.

In poecilostomatoids, the anterior seta on segment XXIV appears at copepodid II but those on segments XXI and XXII never appear due to the early offset of setal development on these segments (local progenesis). This results in the presence of only three anterior and one posterior setae on the compound segment XXI`–XXIV in most families and only two anterior setae on the compound segment XXI–XXIII in the Erebonasteridae (Fig. 16). The report of six setae on antennulary segment 5 (= XXI–XXIV) in *Clausidium vancouverense* (Haddon, 1912) by Huys & Boxshall (1991) is based on an observational error, the tiny seta being a reduced aesthetasc (originating from XXI) and the long seta arising from a socle near the proximal articulation being the anterodistal seta of segment XX. In *Nucellicola* (Fig. 13B) the second compound segment possesses the full complement of four setae and one aesthetasc, representing the ancestral setation of segments XXI (two anterior setae + aesthetasc), XXII and XXIII (each with one anterior seta). Either the anteroproximal seta on segment XXI or the anterior seta on segment XXII failed to develop in the copepodid of *Lepetellicola* (Fig. 5A) as only three elements plus an aesthetasc are present on segment 2. The retention of the full setation on these segments in *N. holmanae* indicates that the Chitonophilidae cannot possibly be accommodated in the Poecilostomatoida, an ordinal position already refuted on the basis of antennary segmentation. Placement in the Cyclopoida is supported by antennulary segmentation and aesthetasc pattern, both of which preclude assignment to the Siphonostomatoida. This position is, however, admittedly based on the principle of elimination rather than on common ancestry. The plesiomorphic condition of the three antennulary characters provides further evidence for the paraphyly of the order Cyclopoida, a status already indicated by the recent discovery of the Fratiidae. Ho *et al.* (1998) placed this family in the Cyclopoida but its mosaic morphology, combining gnathostome and poecilostome features, clearly reflects the phylogenetic continuum between both orders.

The Chitonophilidae is the third family in the Cyclopoida utilizing molluscan hosts. The three genera of the Mantridae are all associated with bivalves of the families Chamidae and Mytilidae (Huys, 1990; Ohtsuka *et al.*, 2000). The only known representative of the Ozmanidae inhabits the haemocoel of the freshwater mesogastropod snail *Pomacea maculata* Perry, 1810 in the Brazilian Amazon (Ho & Thatcher, 1989).

ACKNOWLEDGEMENTS

We thank Dr Anders Warén (Naturhistoriska Riksmuseet Stockholm) for arranging the loan of the BALGIM material and for additional information on *Lepetella*. Dr Bruce A. Marshall (National Museum of New Zealand) is acknowledged for the initial suggestion about the copepod. This work was supported by a research grant of the Dirección General de Investigación Científica y Técnica within the framework of the Fauna Ibérica II project (PB-89-0081), and a F.P.I. Fellowship from the Comunidad Autónoma de Madrid to E. Roldán. PJL-G acknowledges financial support for a visit to the Natural History Museum (London) under the EC-funded TMR Bioresource Large-Scale Facility Programme. We also acknowledge the assistance of Esperanza Salvador, Luis Ángel Garrido and José Luis Poderoso (SIDI, U.A.M) in preparing the SEM micrographs, and Zara Frenkiel (NHM) for help in translating Russian literature.

REFERENCES

Abdelhalim AI, Lewis JW, Boxshall GA. 1993. The external morphology of adult female ergasilid copepods (Copepoda: Poecilostomatoida): a comparison between *Ergasilus* and *Neoergasilus*. *Systematic Parasitology* **24:** 45–52.

Amado MA, Pinto da Motta, Ho J-S, da Rocha CEF. 1995. Phylogeny and biogeography of the Ergasilidae (Copepoda, Poecilostomatoida), with reconsideration of the taxonomic

status of the Vaigamidae. *Contibutions to Zoology* **65:** 233–243.

Avdeev GV, Sirenko BI. 1991. Chitonophilidae fam. n., a new family of parasitic copepods from the chitons of the North-Western Pacific. *Parazitologiya* **25:** 370–374.

Avdeev GV, Sirenko BI. 1994. New species of copepod endoparasites of chitons. *Parazitologiya* **28:** 110–118.

Bowman TE, Abele LG. 1982. Classification of the recent Crustacea. In: Abele, LG, ed. *The biology of crustacea, vol. 1. Systematics, the fossil record, and biogeography.* New York: Academic Press. pp. 1–27.

Boxshall GA, Huys R. 1998. The ontogeny and phylogeny of copepod antennules. *Philosophical Transactions of the Royal Society of London B* **353:** 765–786.

Dantart L, Luque ÁA. 1994. Cocculiniformia and Lepetidae (Gastropoda: Archaeogastropoda) from Iberian waters. *Journal of Molluscan Studies* **60:** 277–313.

Dudley PL. 1966. Development and systematics of some Pacific marine symbiotic copepods. A study of the biology of the Notodelphyidae, associates of ascidians. *University of Washington Publications in Biology.* **21:** 1–282.

Ferrari FD. 1988. Developmental patterns in numbers of ramal segments of copepod post-maxillipodal legs. *Crustaceana* **54:** 256–293.

Fitches CE. 1966. *Parasitological studies on the structure, biology and taxonomy of Nucellicola kilrymontis.* PhD Thesis, University of St Andrews, Scotland.

Franz JC, Bullock RC. 1990. *Ischnochitonika lasalliana*, new genus, new species (Copepoda), a parasite of tropical western Atlantic chitons (Polyplacophora: Ischnochitonidae). *Journal of Crustacean Biology* **10:** 544–549.

Garstang W. 1890. Report on the nudibranchiate Mollusca of Plymouth Sound. *Journal of the Marine Biological Association of the United Kingdom* **1:** 173–198.

Gurney R. 1933. *British fresh-water Copepoda*, Volume 3. London: The Ray Society.

Haszprunar G. 1987a. Anatomy and affinities of cocculinid limpets (Mollusca, Archaeogastropoda). *Zoologica Scripta* **16:** 305–324.

Haszprunar G. 1987b. The anatomy of *Addisonia* (Mollusca, Gastropoda). *Zoomorphology* **106:** 269–278.

Haszprunar G. 1988a. Anatomy and affinities of pseudococculinid limpets (Mollusca, Archaeogastropoda). *Zoologica Scripta* **17:** 161–179.

Haszprunar G. 1988b. Comparative anatomy of cocculiniform gastropods and its bearing on archaeogastropod systematics. In: Ponder WF, ed. *Prosobranch phylogeny. Malacological reviews, supplement 4.* Ann Arbor, Museum of Zoology, University of Michigan, 64–84.

Haszprunar G. 1998. Superorder Cocculiniformia. In: Beesley BPL, Ross GJB, Wells A, eds. *Mollusca: the southern synthesis. Fauna of Australia, Vol. 5, Part B.* Melbourne, Australia: CSIRO Publishing, pp. 653–664.

Haszprunar G, McLean JH. 1996. Anatomy and systematics of bathyphytophilid limpets (Mollusca, Archaeogastropoda) from the northeastern Pacific. *Zoologica Scripta* **25:** 35–49.

Ho J-S, Conradi M, López-González PJ. 1998. A new family of cyclopoid copepods (Fratiidae) symbiotic in the ascidian (*Clavellina dellavallei*) from Cádiz, Spain. *Journal of Zoology, London* **246:** 39–48.

Ho J-S, Thatcher VE. 1989. A new family of cyclopoid copepods (Ozmanidae) parasitic in the hemocoel of a snail from the Brazilian Amazon. *Journal of Natural History* **23:** 903–911.

Huys R. 1990. Allocation of the Mantridae Leigh-Sharpe to the Cyclopoida (Crustacea: Copepoda) with notes on *Nearchinotodelphys* Ummerkutty. *Bijdragen tot de Dierkunde* **60:** 283–291.

Huys R. 2001. Splanchnotrophid systematics: a case of polyphyly and taxonomic myopia. *Journal of Crustacean Biology* **21:** 106–156.

Huys R, Boxshall GA. 1990. Discovery of *Centobnaster humesi*, new genus, new species (Erebonasteridae), the most primitive poecilostomatoid copepod known, in New Caledonian deep waters. *Journal of Crustacean Biology* **10:** 504–519.

Huys R, Boxshall GA. 1991. *Copepod evolution.* London: The Ray Society.

Izawa K. 1986. On the development of parasitic Copepoda. IV. Ten species of poecilostome cyclopoids, belonging to Taeniacanthidae, Tegobomologidae, Lichomolgidae, Philoblennidae, Myicolidae, and Chondracanthidae. *Publications of the Seto Marine Biological Laboratory* **31:** 81–162.

Jensen KR. 1987. *Ismaila monstrosa* Bergh (Copepoda: Splanchnotrophidae) found parasitizing in *Ercolania funerea* (Costa) (Gastropoda: Ascoglossa). *Ophelia* **28:** 75–84.

Jones JB, Marshall BA. 1986. *Cocculinika myzorama*, new genus, new species, a parasitic copepod from a deep-sea wood-ingesting limpet. *Journal of Crustacean Biology* **6:** 166–169.

Kim I-H, Ho J-S. 1992. Copepodid stages of *Hemicyclops ctenidis* Ho and Kim, 1990 (Clausidiidae), a poecilostomatoid copepod associated with a polychaete. *Journal of Crustacean Biology* **12:** 631–646.

Lamb EJ, Boxshall GA, Mill PJ, Grahame J. 1996. Nucellicolidae: a new family of endoparasitic copepods (Poecilostomatoida) from the dog whelk *Nucella lapillus* (Gastropoda). *Journal of Crustacean Biology* **16:** 142–148.

Lamb EJ, Boxshall GA, Mill PJ, Grahame J. 1998. Postembryonic stages of *Nucellicola holmanae* (Copepoda, Poecilostomatoida), an endoparasite of the dog whelk *Nucella lapillus* (Gastropoda). *Journal of Marine Systems* **15:** 261–267.

Lützen J. 1964. A revision of the family Herpyllobiidae, (parasitic copepods) with notes on hosts and distribution. *Ophelia* **1:** 241–274.

Lützen J. 1966. The anatomy of the family Herpyllobiidae (parasitic copepods). *Ophelia* **3:** 45–64.

Martínez Arbizu P. 1996/97. The monophyly of Erebonasteridae, with the description of *Centobnaster severnicus* sp. n. (Copepoda: Poecilostomatoida) from the Laptev Sea (Arctic Ocean). *Zoologischer Anzeiger* **235:** 263–270.

Moskalev LI. 1978. The Lepetellidae (Gastropoda, Prosobranchia) and forms similar to them. *Works of the P.P. Shirshov Institute of Oceanology, Academy of Sciences of the USSR* **113:** 132–146.

Muñoz MA, Valdés A, Ortea J. 1996. The genus *Okenia* Menke, 1830 (Nudibranchia: Goniodorididae) in Chile. *Haliotis* **25:** 101–106.

Nagasawa K, Bresciani J, Lützen J. 1991. *Ischnochitonika japonica*, new species (Copepoda), a parasite on *Ischnochiton* (*Ischnoradsia*) *hakodadensis* (Pilsbry) (Polyplacophora: Ischnochitonidae) from the Sea of Japan. *Journal of Crustacean Biology* **11:** 315–321.

Ohtsuka S, Boxshall GA, Torigoe K. 2000. A new genus and species of the family Mantridae (Copepoda: Cyclopoida) infesting the bivalve *Pseudochama retroversa* from the Seto Inland Sea, western Japan. *Journal of Natural History* **34:** 1967–1976.

Ponder WF, Lindberg DR. 1996. Gastropod phylogeny– challenges for the 90s. In: Taylor JD, ed. *Origin and evolutionary radiation of the Mollusca*. Oxford: Oxford University Press, pp. 135–154.

Ponder WF, Lindberg DR. 1997. Towards a phylogeny of gastropod molluscs—an analysis using morphological characters. *Zoological Journal of the Linnean Society* **119:** 83–265.

Roldán E, Luque ÁA. 1999. Notes on the reproduction and larval shell of *Addisonia excentrica* (Tiberi, 1855) (Gastropoda, Cocculiniformia, Lepetelloidea, Addisoniidae). *Journal of Molluscan Studies* **65:** 510–514.

Scott T, Scott A. 1895. On some new and rare British Copepoda. *Annals and Magazine of Natural History* **6:** 353–362.

Swofford DL. 1993. *PAUP (Phylogenetic Analysis Using Parsimony), Version 3.1.1.* Washington, DC: Laboratory of Molecular Systematics, Smithsonian Institution.

Thatcher VE. 1986. The parasitic crustaceans of fishes from the Brazilian Amazon. 16. *Amazonicopeus elongatus* gen. et sp. nov. (Copepoda: Poecilostomatoida) with the proposal of Amazonicopeidae fam. nov. and remarks on its pathogenicity. *Amazoniana* **10:** 49–56.

Thatcher VE, Robertson BA. 1984. The parasitic crustaceans of fishes from the Brasilian Amazon. 11. Vaigamidae fam. nov. (Copepoda: Poecilostomatoida) with males and females of *Vaigamus retrobarbatus* gen. et sp. nov. and *V. spinicephalus* sp. nov. from plankton. *Canadian Journal of Zoology* **62:** 716–729.

Warén A. 1972. On the systematic position of *Fissurisepta granulosa* Jeffreys, 1882 and *Patella laterocompressa* de Rayneval & Ponzi, 1854 (Gastropoda Prosobranchia). *Sarsia* **51:** 17–24.