

**First record of a mesoparasite (Crustacea, Copepoda)
infesting a polyplacophoran (Mollusca, Polyplacophora)
in Chilean waters,
with an overview of the family Chitonophilidae**

(Crustacea & Mollusca)

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A detailed re-description of the mesoparasitic chitonophilid copepod *Ischnochitonika lasalliana* Franz & Bullock, 1990 is given, based on light- and scanning electron microscopy and microscopic X-ray computed tomography observations. The species was found in the mantle cavity of *Chaetopleura benaventei* Plate, a polyplacophoran constituting a new host record for this parasite. The new discovery considerably extends the distribution of *I. lasalliana* to the southeastern Pacific and is the first record of a polyplacophoran-copepod association from Chilean waters. New morphological and anatomical observations, e.g. the lack of a digestive track in the adult female, the presence of antennary claws in males, the 7-segmented exopod of the nauplius antenna, contradict earlier interpretations of these characters. For the first time features of the nauplii, complex spermatophores and fine surface structures of *I. lasalliana* are presented while a summary of the known members of the family Chitonophilidae is also provided.

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Introduction

In the course of an inventory of Chilean polyplacophorans, an exclusively marine group of molluscs with worldwide distribution, a single specimen of Chaetopleuridae containing an egg cluster in its mantle cavity was found. Close examination of this cluster however revealed that it is not of chiton origin but indicated the presence of a mesoparasitic copepod of the family Chitonophilidae, which is known

to infest gastropod and polyplacophoran hosts (e.g. Huys et al. 2002, Avdeev & Sirenko 2005).

Chitonophilids are highly adapted copepods with extreme sexual dimorphism. Females are hypermorphosed without external segmentation. They are reduced to a well-developed rootlet system and a variable shaped trunk. In mesoparasitic forms the trunk is found in the host's pallial cavity, but in endoparasitic forms it lies entirely within the viscera of its host. Males are distinctly smaller than

females, also not segmented, but retaining either antennae or maxillipeds as the only appendages. In mesoparasitic females, eggs are usually connected to the genital openings by individual egg strings, while endoparasitic forms have eggs free lying in cysts or tubes (see Huys et al. 2002).

The genus *Ischnochitonika* Franz & Bullock, 1990 comprises four described and two as yet undescribed species and is the most speciose genus within the Chitonophilidae. Representatives of *Ischnochitonika* utilize a broad range of polyplacophoran hosts. While all other chitonophilids are restricted to a single host family, species of this genus infest 10 host species, belonging to six genera spread over three families. Most species are known from the Northern Pacific (Avdeev & Sirenko 2005). According to Avdeev & Sirenko (2005) there is only a single record of the genus from the southern hemisphere, in the western Atlantic Ocean off Brazil. Despite the numerous *Ischnochitonika* records our knowledge of this genus is limited and de facto restricted to the description of the gross-morphology of both sexes. Only a single description of a nauplius stage of *I. japonica* is known (Nagasawa et al. 1991) and copepodid stages are unknown.

The aim of this study is not only to describe a new host record for *Ischnochitonika*, but also to provide data about the female anatomy, scanning electron micrographs for previously unknown male characteristics as well as information on the second record of a naupliar stage within this genus. Finally, the current knowledge of the family Chitonophilidae is summarized, making the information about this group available to a broader audience, as several earlier descriptions are only available in Russian.

Material and methods

Sampling of chitons along the Valdivian coast was conducted by scuba diving up to a depth of 20 m, using an underwater georeferencing method coupled to image analyses to assess species distributions with an error range of less than 10 m (Schories & Niedzwiedz 2012, Niedzwiedz & Schories 2013).

Most individuals collected were photographed in situ, scraped from their substrate and placed into individually labelled plastic bags containing 100–200 ml of seawater. Additionally, images for habitat description were taken. Collected material was fixed in 96 % alcohol within 6 h after sampling. All collected material was deposited at the Bavarian State Collection of Zoology, Munich, Germany (ZSM Mol).

Light microscopy photographs were taken with a Jenoptik ProgRes C12P^{plus} digital camera mounted on

an Olympus SZX12 stereo microscope. Image acquisition was controlled by ProgRes Capture Pro 1.0.0-control software, and afterwards z-stacks were processed with Helicon Focus Pro, Version 5.3. Prior to the scanning electron microscopy (SEM) the examined males and nauplii were placed in a dish with hexamethyldisilazane (Sigma-Aldrich) and permitted to evaporate for at least 24 h. The chemical dried objects were gold sputter coated for 120 s (POLARON Equipment Ltd., Watford, United Kingdom) and afterwards examined with a LEO 1430VP SEM (Electron Microscopy Ltd., Cambridge, United Kingdom).

Prior to microscopic X-ray computed tomography (microCT) scanning, the female was contrasted with saturated 25 % Lugol iodine (Degenhardt et al. 2010) for two days. Subsequently the treated female was placed centrally in a minute vial filled with agarose gel. The agarose gel was first heated to 80 °C, dropped partly in the vial and cooled down. At room temperature the object was placed in the gel. Once oriented, it was slowly covered with additional heated gel, ensuring a bubble-free coverage of the object. The vial was distally fixed on a glass stick which was mounted in a microCT scanner. Scanning was performed with a Nanotom m (GE Sensing & Inspection Technologies GmbH, Wunstorf, Germany) at 50 kV and 440 mA for 2 h (1440 projections at 360°, 1.03 µm voxel size).

The microCT dataset was visualized by volume rendering with DRISHTI 2.3 software (Limaye 2012). In DRISHTI we applied transfer functions in the 2D histogram. Individual colour and transparency settings for multiple transfer functions permitted discerning tissues with different density attributes (following Handschuh et al. 2013).

Terminology for the female's orientation in the host follows Lützen (1966) while systematics of the copepod follow Boxshall (2012) and the host nomenclature refers to Sirenko (2006).

Abbreviations

LACM	Los Angeles County Museum of Natural History, United States
MNCN	Museo Nacional de Ciencias Naturales, Madrid, Spain
MZUSP	Museu de Zoologia da Universidade de Sao Paulo, Brazil
NHM	National History Museum, London, England
NSMT	National Science Museum (Natural History), Tokyo, Japan
NMNZ	National Museum of New Zealand, Te Papa Tongarewa, Wellington, New Zealand
USNM	United States National Museum of Natural History, Washington, United States
ZISP	Zoological Institute of Russian Academy of Sciences, St. Petersburg, Russia
ZSM	Bavarian State Collection of Zoology (Departments Mollusca and Arthropoda), Munich, Germany

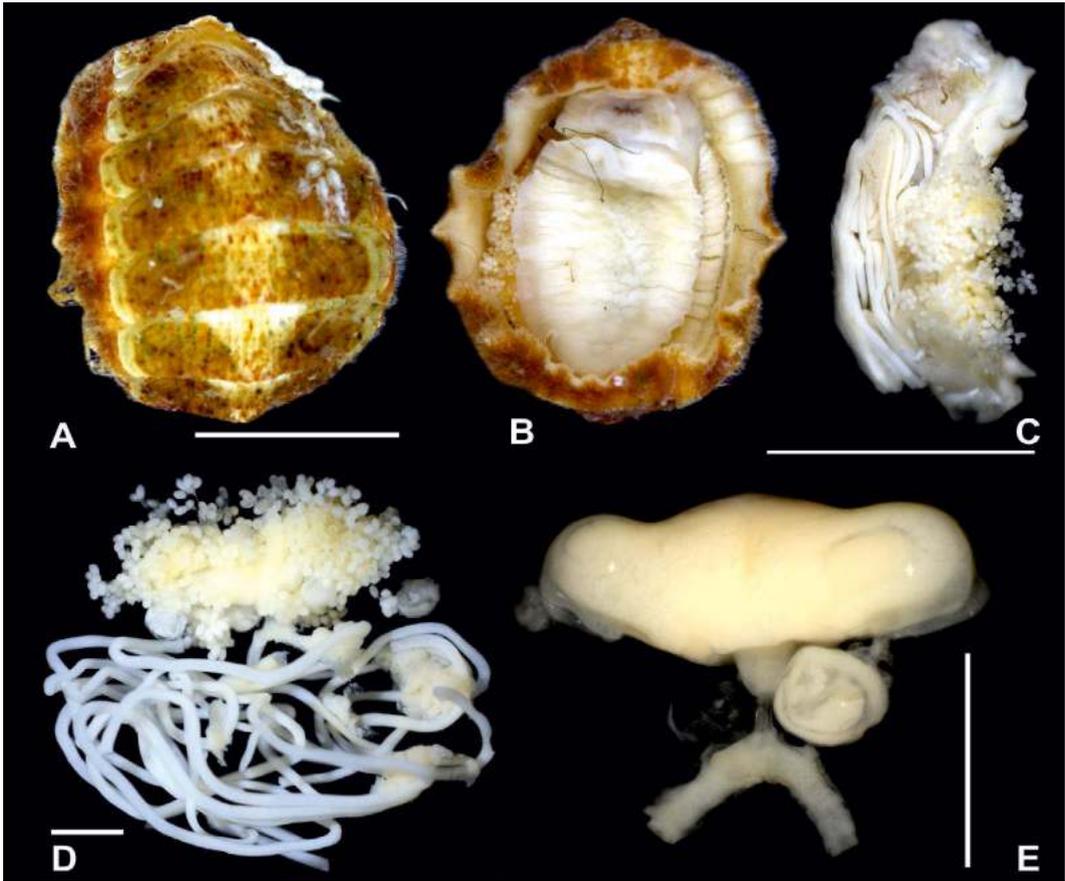


Fig. 1. *Ischnochitonika lasalliana* Franz & Bullock, 1990 and its host species *Chaetopleura benaventei* Plate, 1899 (ZSM Mol 20130036). **A.** Dorsal view of *C. benaventei*; **B.** ventral view of *C. benaventei*, showing the ovigerous female of *I. lasalliana* in the left mantle cavity; **C.** *C. benaventei* with valves and mantle removed to show full extension of *I. lasalliana* in relation to the host's soft parts, right dorso-lateral view of host species, to show the parasite rootlet system among the digestive tract; **D.** dorsal view of dissected ovigerous female of *I. lasalliana*; **E.** same as D, egg masses removed and rootlet system shortened, showing the larger attached male with its dorsal side directed towards female trunk. Scale bars: A–C=5 mm, D=2 mm, E=1 mm. Please note, in figures A–B the girdle elements were partly removed to smoothen the edge of the perinotum.

Systematics

Order Cyclopoida Burmeister

Family Chitonophilidae Avdeev & Sirenko, 1991 (p. 370) = Nucellicolidae Lamb, Boxshall, Mill & Grahame, 1996 (p. 142) fide Huys et al. (2002: 190)

Type genus: *Chitonophilus* Avdeev & Sirenko, 1991, by original designation.

Genus *Ischnochitonika* Franz & Bullock, 1990 (p. 544)

Type species: *Ischnochitonika lasalliana* Franz & Bullock, 1990, by original designation.

Genus distribution: Caribbean Sea, SW Atlantic Ocean, NW Pacific Ocean, NE Pacific Ocean, SE Pacific Ocean. Recent.

Ischnochitonika lasalliana Franz & Bullock, 1990

Figs 1–5

Ischnochitonika lasalliana Franz & Bullock 1990: 545, figs 2–7; Nagasawa et al. 1991: 318; Avdeev & Sirenko 1994: 110; 2005: 527, fig. 4.1–8; Huys et al. 2002: 190.

Material examined. Single ovigerous female (Fig. 1D), eight associated males and three nauplii from one of three specimens of *Chaetopleura benaventei* Plate (ZSM Mol 20130036).

Locality. Chile, Región de los Ríos, 20 km North of Corral, Chaihuin, 39°57'26" S, 73°36'08" W; water depth 4.37 m, temperature 11 °C. Leg. DS & JH, 24 March 2011.

The locality lies in the southern intermediate zone of Chile. Salinity is fully marine and does not vary significantly between seasons. The area is exposed to the open sea, but is partly protected against wind and waves from the southwest, which is the prevailing wind direction during the year. Mean tide range is about 1.5 m. Kelp algae such as *Durvillaea antarctica* and *Macrocystis pyrifera* are present in the lower intertidal. Only *M. pyrifera* grows in protected parts of the location in patches down to 6 m, whereas the kelp algae *Lessonia trabeculata* starts growing at this depth down to 15 m.

Position in host. Mesoparasitic (in the mantle cavity of a specimen of *Chaetopleura benaventei* measuring 9.7 × 7.2 mm (Figs 1A–B). Ectosoma situated between ctenidia and foot approximately halfway along the left gill row (seen from ventral, Figs 1B–C). Penetrating neck divides immediately into two long branches (rootlets) (Fig. 1E), which follow the digestive track of the host, one branch from the anterior side, the other from posterior, meeting in the body cavity beneath the midgut gland, without detectable damage to the host's internal organs.

Habitat. The host species was found under encrusted stones among barnacles and hydrozoans. Accompanying macro-fauna comprised the chitons *Tonicia chilensis* (Frembly), *T. smithii* Leloup, *T. elegans* (Frembly), the nudibranch *Thecacera darwini* Pruvot-Fol and the brachiopod *Discinisca lamellosa* (Broderip).

Description

Female. Ectosoma (Figs 1E, 2) slightly glossy, beige, dorsoventrally flattened, length 1.11 mm (including the 0.12 mm long and 0.08 mm wide tissue piercing neck), height 1.00 mm and width 2.13 mm. Appendages and segmentation absent. Trunk (Figs 1E, 2A–B, F) obtusely cone-shaped, slightly covered by anterior margins of dorsum, and to a lesser degree, of ventrum, length about 0.29 mm, diameter at base as wide as total length, tapering to a distal diameter of 0.15 mm where the neck arises centrally. Endosoma (Figs 1D) (rootlet system) splits into two branches with a diameter of 0.34 mm and an approximate length of 80 mm. Ectosoma (Fig. 2) anteriorly more or less straight with two indistinct rounded thickenings; curved laterally, forming two more or less globular genital lobes, posteriorly convex; with dorsolaterally situated eggs on individual strings, partly clustering. Eggs (Figs 1D, 5A) oval, measuring about 0.17 × 0.10 mm. Associated males (Fig. 1E) attached to ectosoma mainly anteriorly or laterally adjacent to genital openings. Genital lobes (Figs 2C, F, 3B) dorsally flattened forming a ridge-

like protuberance, less distinct ventrally, tending to be roundish, genital apertures about 0.31 mm in length.

Besides the brief observations of the internal anatomy of adult females of *Nucellicola holmanae* Lamb, Boxshall, Mill & Grahame, 1996 and *Lepetellicola brescianii* Huys, López-González, Roldán & Luque, 2002 in their respective original descriptions, information on the anatomy of chitonophilid females remains scarce, especially for the polyplacophoran-infesting taxa that are restricted to a few vague sketches of what is visible through the female's body (e.g. Nagasawa et al. 1991, Avdeev & Sirenko 2005). Principally the organisation of the reproductive system is quite similar to that described by Lützen (1966) for the herpyllobiid species *Herpyllobius poly-noes* (Krøyer). The germinal portion of the ovary (go) is centrally situated and cross-shaped (Figs 3A–B). Laterally it leads into a bipartite coiled system of lobes (ov) which occupies nearly the whole body (Figs 3A–C). The lobes each terminate in a short genital duct (gd) at the base of the genital slits (Fig. 3B). Ventrally with a gland-like structure (cg) (Figs 3B, C) interpreted as a cement gland. Secretion does not occur via the genital duct, as we found independent pores (p) in the walls of the aperture (Fig. 3B). No digestive tract was observed in the present species (stated by Huys et al. 2002: 198, as being characteristic for female chitonophilids). There are a high number of cross running branches (mt) (Fig. 3C) referred to as mesenchymatous tissue (sic Lützen 1966), suggesting nutrition is osmotic.

Males. The males (Fig. 4A) of *I. lasalliana* were described by earlier authors (Franz & Bullock 1990, Avdeev & Sirenko 2005). The largest specimen we examined measures 0.98 mm in total length, 0.73 mm in width and has a total height of 0.77 mm. In this specimen the lateral lobes are more distinct than in smaller specimens. Additional features include a sclerotized mouth-ring (Fig. 4C) (cf. Nagasawa et al. 1991, for *I. japonica*) on top of a slightly elevated mouth cone, ventrally at the base of the bifurcated antennary processes, with a diameter of ca. 41 µm and a deep posterior notch. The bifurcated antennary processes (Fig. 4A) have a squarish distal segment bearing a single blackish tridentate claw (Fig. 4D). Each antennary process carries at mid-length a ventrally directed secondary process, showing a dense surface ornamentation of minute burr-like structures (Fig. 4B). The same surface ornamentation is distributed more or less over the dorsum of the male, with the highest density around the genital openings (Fig. 4E) situated dorso-laterally of the lateral lobes. A bulb (Fig. 4B) ("cone-shaped projection" sensu Franz & Bullock 1990: 547; "rectangular

lappet” sensu Nagasawa et al. 1991: 318, figs 3, 4; “genital tubercle” sensu Avdeev & Sirenko 2005: 528, fig. 4.5), overlaying the spermatophore sacs is located dorsally at the base of the antennary processes. We were unable to see a connection between the paired spermatophore sacs and this single structure. One individual (Fig. 1E) is orientated with the bulb (and not the mouth) to the female’s body, while being attached by the antennary projections.

A specimen (Fig. 4A) about 0.43 mm in height shows a complex spermatophore (Fig. 4F) attached to the base of its antennary projections. This spermatophore terminates in a divided finely lamellar coupling plate. Whether the spermatophore was released by this specimen or an artefact remains unclear.

Nauplius. With the egg cluster, we found three nauplii (Figs 5B–F) and additionally a hatching one (Fig. 5A). The body (Fig. 5B) is oval in outline, slightly narrowing posteriorly, caudal rami (Fig. 5F) represented by single seta each. A pigmented nauplius eye, a labrum, an anal opening and a mouth opening were not detected. The specimen’s size of $210 \times 133 \mu\text{m}$ is comparable to the measurements of Nagasawa et al. (1991) for the nauplius of *I. japonica* ($205 \times 150 \mu\text{m}$). Antennule (Fig. 5B) 2-segmented, basal segment rectangular, with a rim of minute denticles midway, a small spine in the distal third and a short seta at the suture, distal segment distinctly slender, but of equal length, apically with two long setae, inner distal corner with pointed process. Antenna (Fig. 5C) biramous, protopod short, rectangular without ornamentation. Exopod 7-segmented, segments 3–7 with a single seta (Fig. 5E), segments 1–2 without ornamentation. Endopod 2-segmented, distinctly shorter than exopod, first segment without ornamentation, distal segment with two apical setae and a short one laterally below the inner distal corner. Mandible (Fig. 5D) biramous, protopod 2-segmented, coxa armed with basal segment of endopod, exopod 4-segmented, each segment with one seta, terminal one situated apically. Second segment with short inner seta, other segments with small pointed process at inner distal corner. Endopod 2-segmented, second segment with short inner seta and two apical setae. All setae fully agree with the conditions found in *I. japonica* (Nagasawa et al. 1991) in being “flexible and flat, suggesting elongated prickly leaves of a holly shrub”.

Copepodid stages. Unknown.

Remarks. Until now *Ischnochitonika lasalliana* was known from a total of 19 ischnochitonid polyplacophoran host specimens of the species *Ischnochiton striolatus* (Gray), *Stenoplax boogi* (Haddon) and

S. fallax (Carpenter in Pilsbry). From these chitons, 20 copepod females (indicating at least one double infestation) are reported. Despite the large number of collected specimens, some details are not described, e.g. the internal anatomy of the female and a more precise description of the rootlet arrangement, the fine ornamentation and position of the genital pores and mouth opening of the males, and the structure of the spermatophores.

Although our material differs slightly from the size range given by Avdeev & Sirenko (2005: 535) and the female’s shape does not exactly fit the holotype (Franz & Bullock 1990: fig. 3), we interpret these variations as being intraspecific. It has already been demonstrated that the shape is very variable (Franz & Bullock 1990: fig. 5), and is likely to depend upon several factors, e.g. maturity stage of the parasite, number of attached males, exact position in host, size of host, and host species.

In Franz & Bullock (1990: fig. 4) the endosoma lies irregularly in the visceral cavity, a condition we did not find. Unfortunately Avdeev & Sirenko (2005) did not mention the arrangement of the rootlets in their records and consequently nothing is known about the parasites’ impact on their hosts. No detectable damage to the host’s internal organs was observed in the examined specimens. The copepod’s rootlets follow exactly along the chiton’s digestive tract and do not impact the gonads. However, the arrangement of the rootlets of *I. lasalliana* in *Ischnochiton striolatus* as illustrated by Franz & Bullock (1990: fig. 4) resembles the condition Nagasawa et al. (1991: 318) stated for the endosoma of *I. japonica* which lays “spirally wound up within the host’s periintestinal blood sinus”.

Huys et al. (2002: 209) postulated that the antennary claws in *Ischnochitonika* males are lost. However, the presence of antennary claws was reported by Avdeev & Sirenko (2005) and we confirm their observations. In their phylogenetic analysis of chitonophilids Huys et al. (2002) simplified the only available nauplius characters described by Nagasawa et al. (1991). Nagasawa et al. (1991) clearly described the antennary exopod of the nauplius of *I. japonica* with 7 segments – an observation we confirmed for *I. lasalliana* – but Huys et al. (2002: 209) reduced the number of segments to 5. In addition, the genus *Cookoides* Avdeev & Sirenko, 1994 was interpreted as mesoparasitic, while it was found entirely embedded in the host’s body cavity (Avdeev & Sirenko 1994). The reason for this interpretation was probably the presence of eggs attached to the female’s trunk, which Huys et al. (2002) interpreted as characteristic for the mesoparasitic forms. Until now it remains unclear, whether these eggs are permanently attached to the female or if they can also be present free-lying

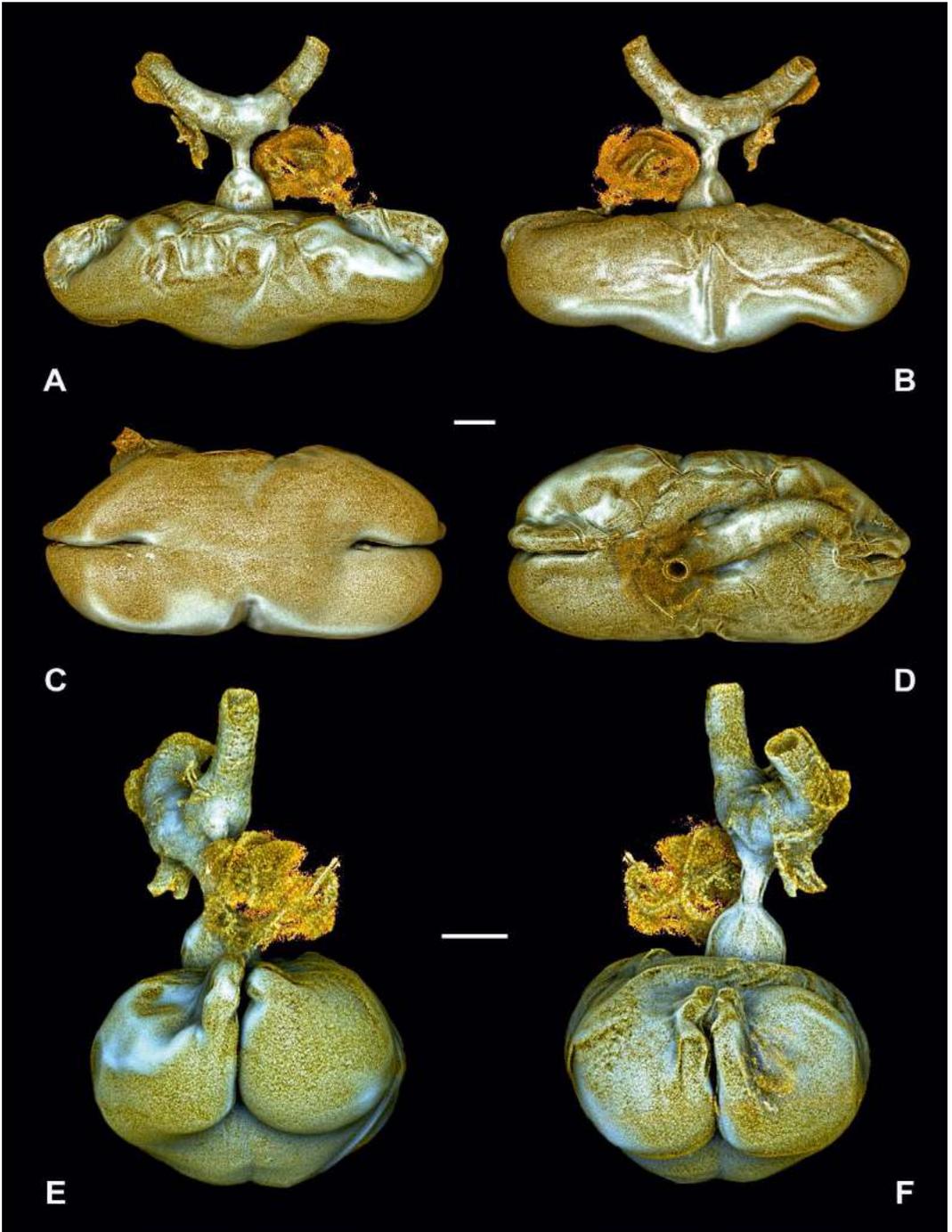


Fig. 2. MicroCT data (volume rendering) and DRISHTI 2.3. software visualization. Different views of female of *Ischnochitonika lasalliana* Franz & Bullock, 1990 with eggs removed and shortened rootlet system. Larger males from former images still attached but with low resolution probably due to inadequate staining. **A-B, E-F.** Rootlet system orientated to top. **A.** Dorsal view, **B.** ventral view, **C.** posterior view, **D.** anterior view, **E.** right lateral view, **F.** left lateral view. All scale bars = 100 μm .

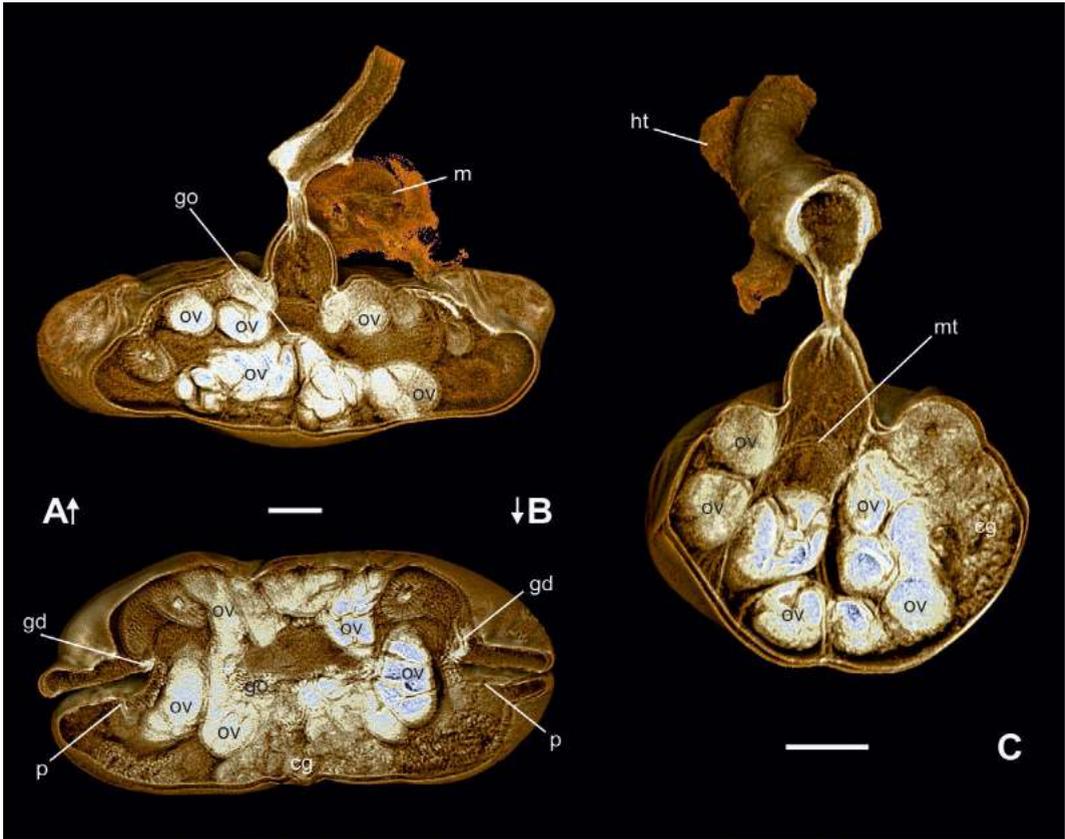


Fig. 3. MicroCT data (volume rendering) and DRISHTI 2.3. software visualization. Different views of female of *Ischnochitonika lasalliana* Franz & Bullock, 1990 using clipping functions to highlight internal organs. **A.** Horizontal section at level of genital slits, dorsal view; **B.** vertical section at mid body, seen from posterior; **C.** cross section, right lateral view. All scale bars = 100 μ m. Abbreviations: **cg**, cement gland; **gd**, genital duct; **go**, germinal position of ovary; **ht**, host tissue; **m**, male; **mt**, branches of mesenchymatous tissue; **p**, pores (potentially for secretion of cement gland); **ov**, lobes of ovary).

in the body cavity of the host (as is known for the sipunculan endoparasite *Coelotrophus nudus* Ho, Katsumi & Honma). Considering the current findings and additional morphological features described, we conclude that the phylogeny as presented by Huys et al. (2002) requires further testing.

Chitonophilidae and the included taxa

Huys et al. (2002) made an attempt to unify highly transformed symbiotic copepods parasitic on gastropods and polyplacophorans under the family Chitonophilidae. These authors pointed out that several “brood”-records of limpets (if not all) most probably reflect chitonophilid-gastropod associations and could thus contribute much to our knowl-

edge of this group. We summarize the present stage of knowledge of the Chitonophilidae as Avdeev & Sirenko (2005) subsequently introduced new taxa and records of previously known species. Huys et al. (2002) provide a history of the recognized genera and the systematic placement of the family under Cyclopoida versus Poecilostomatoida, based on the antennary segmentation of copepodid stages, or the number of swimming legs (Huys et al. 2006). However, copepodid characters are only known for three of the nine recognized genera currently grouped in Chitonophilidae, and the phylogenetic relationships between the taxa are also unclear (see above).

The family Chitonophilidae comprises the following taxa according to the revision of Huys et al. (2002), the additions of Avdeev & Sirenko (2005) and later records.

Genus *Chitonophilus* Avdeev & Sirenko, 1991 (p. 370) mesoparasitic

Type species: *Chitonophilus laminosus* Avdeev & Sirenko, 1991, by original designation.

Species included:

laminosus Avdeev & Sirenko, 1991 (p. 373, fig. 1)

Primary type: Female holotype (ZISP).

Type locality: Lesser Kurile Ridge, Kurile Islands.

Host: Originally described from the pallial groove of *Tonicella submarmorea* (von Middendorff). Subsequently Avdeev & Sirenko (2005) also found it in *Boreochiton granulata* (Jakovleva) and *Tonicella zotini* Jakovleva.

Distribution: Beside the type locality also known from the Sea of Japan (Avdeev & Sirenko 2005).

Depth range: Intertidal–17 m.

Remarks: All host species belong to the family Tonicellidae. Avdeev & Sirenko (1994: 110) restricted the type locality to Iturup and Shikotan, but only the latter belongs to the Lesser Kurile Ridge.

Genus *Cocculinika* Jones & Marshall, 1986 (p. 166) mesoparasitic

Type species: *Cocculinika myzorama* Jones & Marshall, 1986, by original designation.

Species included:

myzorama Jones & Marshall, 1986 (p. 1166, fig. 1)

Primary type: Female holotype (NMNZ 3355).

Type locality: Pacific Ocean, New Zealand, North Island, off Castlepoint, 41°09.7' S 176°31.3' E.

Host: In the mantle cavity of *Coccapigya hispida* Marshall.

Distribution: East of New Zealand.

Depth range: 1198–1514 m.

Remarks: The host species belongs to the family Cocculinidae and is wood-associated. Marshall (1986: 514) also mentioned an endoparasite within one of the specimens of *Coccapigya hispida*, which could potentially also be a crustacean, being briefly described by Haszprunar (1987: 316). However, the systematic position of this parasite remains unclear (see Huys et al. 2002: 211). Haszprunar (1987: 307) investigated four paratypes of *C. hispida* from the type locality and found ripe eggs ventrally of the pallial roof of one specimen, which led Huys et al. (2002: 212) to doubt this brooding record in favour of a chitonophilid infestation.

Genus *Cookoides* Avdeev & Sirenko, 1994 (p. 114) mesoparasitic (according to Huys et al. 2002, but see above)

Type species: *Cookoides cordatus* Avdeev & Sirenko, 1994, by original designation.

Species included:

cordatus Avdeev & Sirenko, 1994 (p. 111, figs 1–2)

Primary type: Female holotype (ZISP).

Type locality: Near South Georgia Islands, 53°45' S 39° W.

Host: In the body cavity of *Stenosemus exarata* (G. O. Sars).

Distribution: Only known from type locality.

Depth range: 267 m.

Remarks: The host species belongs to the family Ischnochitonidae.

Genus *Ischnochitonika* Franz & Bullock, 1990 (p. 544) mesoparasitic

Type species: *Ischnochitonika lasalliana* Franz & Bullock, 1990, by original designation.

Species included:

aleutica Avdeev & Sirenko, 2005 (p. 527, figs 3.9–3.10)

Primary type: Female holotype (ZISP).

Type locality: Not defined.

Host: In the pallial groove of *Leptochiton* cf. *belknapi* Dall.

Distribution: NW part of the Pacific Ocean and Bering Sea.

Depth range: 150–700 m.

Remarks: The host species belongs to the family Leptochitonidae.

japonica Nagasawa, Bresciani & Lützen, 1991 (p. 315, figs 1–5)

Primary type: Female holotype (NSMT-Cr 10320).

Type locality: Sea of Japan, Japan, W-Hokkaido, at Oshoro.

Host: In the pallial groove of *Ischnochiton hakodadensis* Carpenter in Pilsbry.

Distribution: Sea of Japan, W Sakhalin Island and Lesser Kurile Ridge (Avdeev & Sirenko 2005: 529).

Depth range: 0.5–4 m.

Remarks: The host species belongs to the family Ischnochitonidae.

kurochkini Avdeev & Sirenko, 2005 (p. 526, figs 3.4–3.8)

Primary type: Female holotype (ZISP 18069).

Type locality: Northern part of the Sea of Okhotsk, Russia.

Host: Originally described from the pallial groove of *Tripoplax kobjakovae kobjakovae* (Jakovleva). Additionally found in *Lepidozona multigranosa* Sirenko and *Tripoplax albrechtii* (von Schrenck).

Distribution: Only known from the Sea of Okhotsk and the Sea of Japan.

Depth range: 2–150 m.

Remarks: The host species belongs to the family Ischnochitonidae.

lasalliana Franz & Bullock, 1990

Primary type: Female holotype (USNM 241681).

Type locality: Venezuela, Isla de Margarita, Guaya-cancito, 10°56.1'N 64°12.6'W.

Host: Originally described from the pallial groove of *Ischnochiton striolatus* (Gray), but also from *Stenoplax boogi* (Haddon). Avdeev & Sirenko (2005: 527, figs 4.1–4.8) also found the species in *S. fallax* (Carpenter in Pilsbry). Herein we report the species from *Chaetopleura benaventei* Plate.

Distribution: Caribbean Sea (Isla de Margarita, Venezuela and SE coast of Pensacola, Florida, United States). Avdeev & Sirenko (2005) reported the species from the NE Pacific Ocean (Monterey Peninsula, California). Herein it is also recorded from the SE Pacific.

Depth range: 0–39 m.

Remarks: The host species belong to the families Ischnochitonidae and Chaetopleuridae.

sp. 1 Avdeev & Sirenko, 2005 (p. 533, figs 5.4–5.5)

Material: 2 females (LACM).

Locality: United States, Californian peninsula, 22° 57'N 109°47'W.

Host: In the pallial groove of *Callistochiton elenensis* (Sowerby in Broderip & Sowerby).

Distribution: NE Pacific Ocean.

Depth range: Intertidal.

Remarks: The host species belongs to the family Callistoplacidae.

sp. 2 Avdeev & Sirenko, 2005 (p. 541)

Material: 1 female and one male (MZUSP 36095).

Locality: Atlantic Ocean, Brazil, 8°07.03'S 34°48.01'W.

Host: In *Stenoplax marcusii* (Righi).

Distribution: SW Atlantic Ocean.

Depth range: 21.5 m.

Remarks: The host species belongs to the family Ischnochitonidae.

Genus *Lepetellicola* Huys, López-González, Roldán & Luque, 2002 (p. 202) mesoparasitic

Type species: *Lepetellicola brescianii* Huys, López-González, Roldán & Luque, 2002, by original designation.

Species included:

brescianii Huys, López-González, Roldán & Luque, 2002 (p. 202)

Primary type: Female holotype (MNCN 20.04/5259a).

Type locality: Spain, Galicia, Vizcaya, Bay of Biscay, 43°45.13'–43°46.53'N, 8°10.09'–8°9.59'W.

Host: In the pallial cavity of *Lepetella sierrai* Dantart & Luque.

Distribution: Atlantic side of the northern and southern Iberian Peninsula.

Depth range: 116–491 m.

Remarks: The host species belongs to the family Lepetellidae. Dantart & Luque (1994: 285, fig. 52) first illustrated the host species with “eggs” in the mantle cavity.

Genus *Leptochitonicola* Avdeev & Sirenko, 1991 (p. 373) mesoparasitic

Type species: *Leptochitonicola latus* Avdeev & Sirenko, 1991, by original designation.

Species included:

attenuata Avdeev & Sirenko, 2005 (p. 522, figs 2.7–2.12, 3.1)

Primary type: Female holotype (ZISP).

Type locality: NW Pacific Ocean, Bering Sea, Russia, Commander Islands, Bering Island.

Host: In the pallial groove of *Leptochiton* cf. *rugatus* (Carpenter in Pilsbry).

Distribution: Only known from type locality.

Depth range: 105–300 m.

Remarks: The host species belongs to the family Leptochitonidae.

hanleyellai Avdeev & Sirenko, 2005 (p. 520, figs 2.1–2.6)

Primary type: Female holotype (ZISP).

Type locality: NW Pacific Ocean, Bering Sea, Russia, Commander Islands.

Host: In the pallial groove of *Hanleyella asiatica* Sirenko.

Distribution: Only known from type locality.

Depth range: 100 m.

Remarks: The host species belongs to the family Leptochitonidae.

intermedia Avdeev & Sirenko, 2005 (p. 519, figs 1.6–1.12)

Primary type: Female holotype (ZISP).

Type locality: NW Pacific Ocean, Russia, E Kamchatka 53°26'N 160°21'E.

Host: In the pallial groove of *Leptochiton* sp.

Distribution: Only known from type locality.

Depth range: 1814–1920 m.

Remarks: The host species belongs to the family Leptochitonidae.

lata Avdeev & Sirenko, 1991 (p. 373, fig. 2) emendation in Avdeev & Sirenko (2005: 519)

Primary type: Female holotype (ZISP).

Type locality: Not defined.

Host: In the pallial groove of *Leptochiton assimilis* (Thiele).

Distribution: In the original description mentioned from the Lesser Kurile Ridge and Strait of Tartar.

Depth range: 30–100 m.

Remarks: The host species belongs to the family Leptochitonidae.

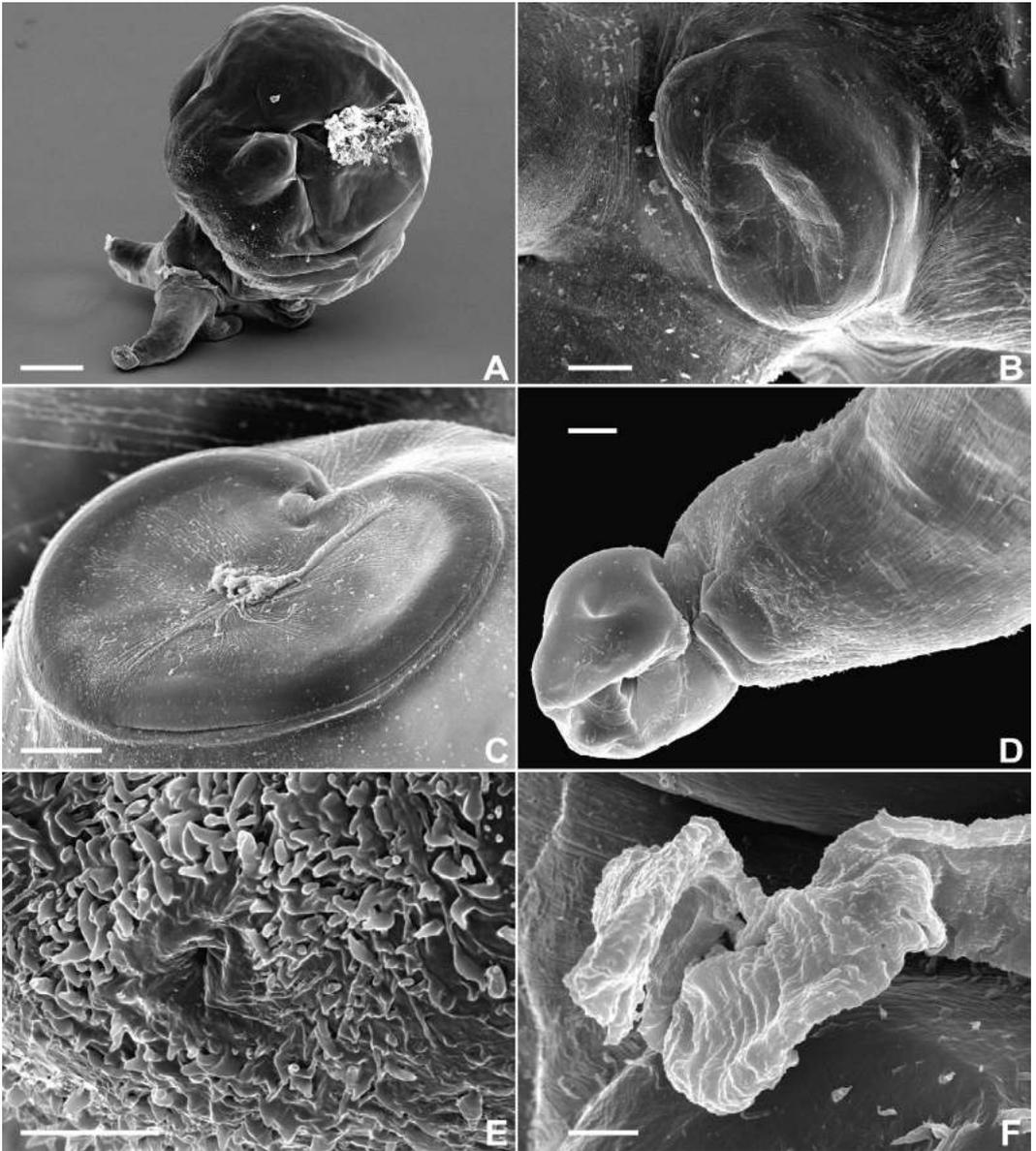


Fig. 4. SEM images of a male of *Ischmochitonika lasalliana* Franz & Bullock, 1990. **A.** Left dorso-lateral view of complete specimen; **B.** dorsal bulbous lobe at the base of the antennary processes; **C.** sclerotized mouth-ring; **D.** distal element of antennary process; **E.** genital pore, ventral view of male, **F.** complex spermatophore located by the antennary process. Scale bars: A=100 μ m; B=500 μ m, C-F=10 μ m.

sphaerica Avdeev & Sirenko, 2005 (p. 517, figs 1.1-1.5)

Primary type: Female holotype (ZISP 18064).

Type locality: Sea of Japan, Russia, Posyet Bay [42°30' N 130°55' E].

Host: In the pallial groove of *Leptochiton rugatus* (Carpenter in Pilsbry).

Distribution: Western part of the Sea of Japan.

Depth range: 4-12 m.

Remarks: The host species belongs to the family Leptochitonidae.

? sp. Avdeev & Sirenko, 2005 (p. 541)

Material: One female specimen (LACM 77-133).

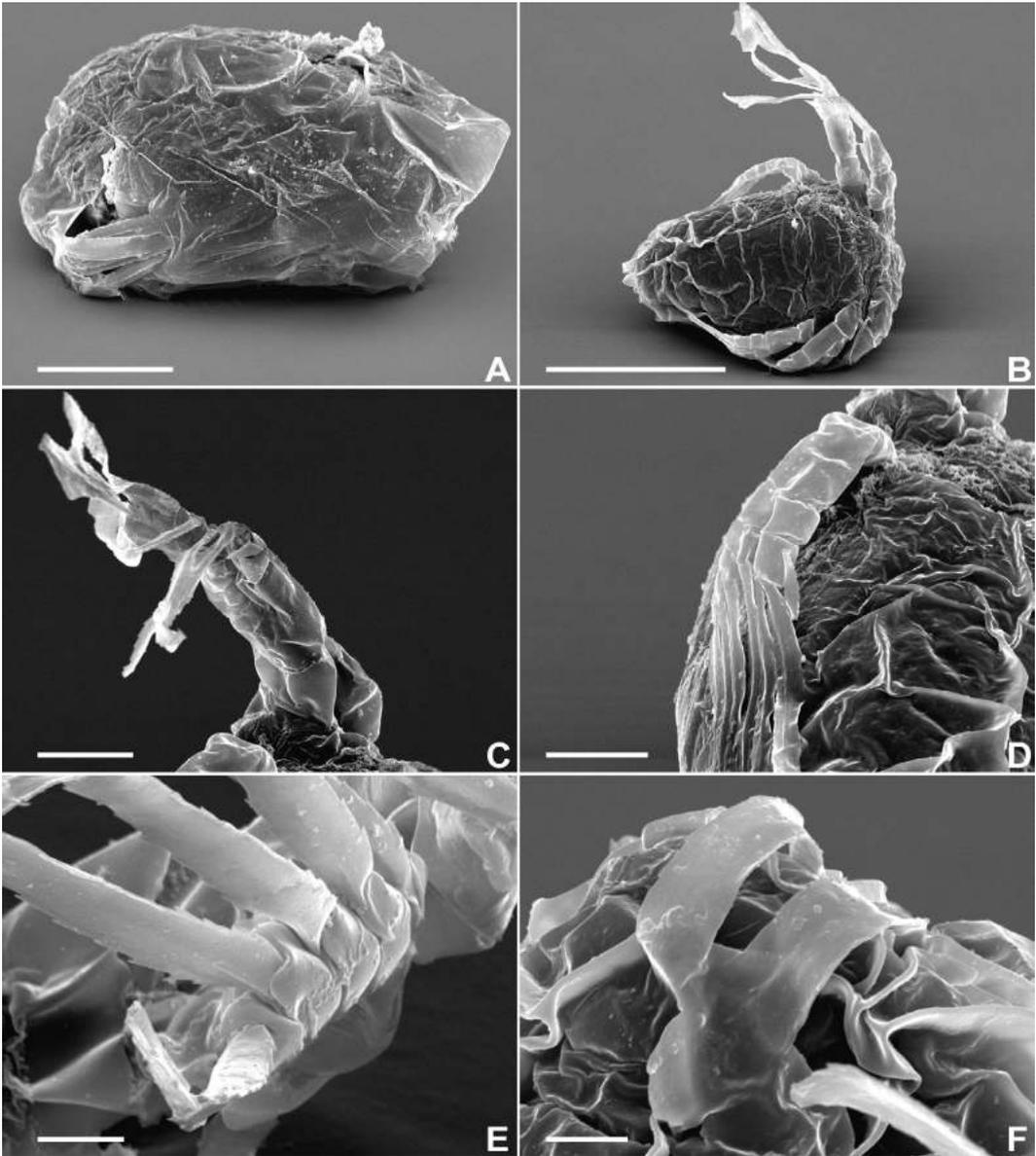


Fig. 5. SEM images of nauplii of *Ischnochitonika lasalliana* Franz & Bullock, 1990. B–D. Nauplius specimen 1; E–F. nauplius specimen 2. A. Freshly hatching nauplius; B. ventral view; C. antenna; D. mandible; E. last setation of the five distal segments of exopod of antenna; F. naked caudal rami. Scale bars: A=50 μ m; B=100 μ m; C–D=20 μ m; E–F=5 μ m.

Locality: NE Pacific Ocean, United States, California, San Nicolas Island, 32°59' N 119°32.8' W.

Host: In *Hanleyella oldroydi* (Bartsch MS, Dall).

Distribution: NE Pacific.

Depth range: 374–384 m.

Remarks: The host species belongs to the family Leptochitonidae.

sp. Sirenko pers. comm.(e-mail 31.07.2013)

Material: At least one female specimen (ZISP).

Locality: NW Atlantic Ocean, Canada, Newfoundland Bank, 46°40' N 50° W.

Host: In *Leptochiton alveolus* (Sars MS, Lovén).

Distribution: NW Atlantic.

Depth range: 1190 m.

Remarks: The host species belongs to the family Leptochitonidae.

Genus *Leptochitonoides* Avdeev & Sirenko, 2005 (p. 536) mesoparasitic

Type species: *Leptochitonoides vitiazi* Avdeev & Sirenko, 2005, by original designation.

Species included:

vitiazi Avdeev & Sirenko, 2005 (p. 537, figs 5.6–5.8, 6.1)

Primary type: Female holotype (ZISP).

Type locality: NE Pacific Ocean, United States, Alaska, off Alexander Archipelago, off Prince Wales Island, 55°23' N 134°46' W.

Host: In the pallial groove of *Leptochiton* cf. *belknapi* Dall.

Distribution: Only known from type locality.

Depth range: 300 m.

Remarks: The host species belongs to the family Leptochitonidae.

Genus *Nucellicola* Lamb, Boxshall, Mill & Grahame, 1996 (p. 143) endoparasitic

Type species: *Nucellicola holmanae* Lamb, Boxshall, Mill & Grahame, 1996, by monotypy.

Species included:

holmanae Lamb, Boxshall, Mill & Grahame, 1996 (p. 143, figs 1–2)

Primary type: Female holotype (NHM 1995.664).

Type locality: North Sea, England, North Yorkshire, Robin Hood's Bay.

Host: In the viscera of *Nucella lapillus* (Linnaeus).

Distribution: NE Atlantic Ocean.

Depth range: Intertidal.

Remarks: The host species belongs to the family Muricidae.

Genus *Tesonesma* Avdeev & Sirenko, 1994 (p. 110) endoparasitic

Type species: *Tesonesma reniformis* Avdeev & Sirenko, 1994, by original designation.

Table 1. Summary of taxon composition of the family Chitonophilidae, with special references to their geographic and bathymetric distribution, host preference and microhabitat in the host (“type”: M, mesoparasitic; E, endoparasitic). Host classes are defined as: P, Polyplacophora; G, Gastropoda. Asterisk indicates a doubtful description; the genus was originally described being endoparasitic, while Huys et al. (2002) re-classified it. Species numbers in brackets refer to “records” it remains unclear whether all records reflect true species.

Taxon	Type	Species number	Geography	Bathymetry (m)	Host classes	Host families
<i>Chitonophilus</i>	M	1	Sea of Japan and adjacent NW Pacific Ocean	0–17	P	Tonicellidae
<i>Cocculinika</i>	M	1	E New Zealand waters	1198–1514	G	Cocculinidae
<i>Cookoides</i>	M*	1	S Atlantic, subantarctic	267	P	Ischnochitonidae
<i>Ischnochitonika</i>	M	6	Sea of Japan, Okhotsk Sea, Bering Sea, NE Pacific, SE Pacific, Caribbean Sea, SW Atlantic	0–700	P	Leptochitonidae, Ischnochitonidae, Callistoplacidae, Chaetopleuridae
<i>Lepetellicola</i>	M	1	NE Atlantic	116–491	G	Lepetellidae
<i>Leptochitonicola</i>	M	7	N Pacific; NW Atlantic	4–1920	P	Leptochitonidae
<i>Leptochitonoides</i>	M	1	NE Pacific	300	P	Leptochitonidae
<i>Nucellicola</i>	E	1	NE Atlantic	0	G	Muricidae
<i>Tesonesma</i>	E	1	Sea of Japan, Sea of Okhotsk, Bering Sea	37–194	P	Ischnochitonidae
Chitonophilidae gen. sp.	M	(6)	Sea of Japan, NE Pacific, SW Pacific, NE Atlantic, Mediterranean Sea	3–2420	G	Mopaliidae, Lepetodrilidae, Lepetellidae, Pseudococculinidae
?Chitonophilidae gen. sp.	M	(4)	NE Pacific, NW Atlantic	1224–2750	G	Pseudococculinidae, Bathysciadiidae, Bathyphytophilidae

Species included:

reniformis Avdeev & Sirenko, 1994 (p. 111, figs 1-2)

Primary type: Female holotype (ZISP).

Type locality: Not defined.

Host: In the body cavity of *Stenosemus albus* (Linnaeus).

Distribution: The original description mentioned Shantar Island 55°33' N 136°23' E, Strait of Tartar (at Mys Zolotoy [47°18' N, 139°01' E]), and Bering Strait 66°02' N 169°29' W. Avdeev & Sirenko (2005) show a distributional range from the Sea of Japan, the Sea of Okhotsk to the Bering Sea.

Depth range: 37-194 m.

Remarks: The host species belongs to the family Ischnochitonidae.

Incertae sedis

Chitonophilidae gen. sp.

– Avdeev & Sirenko, 2005 (p. 541)

Material: One female specimen (ZISP).

Locality: Sea of Japan, Vostok Bay, Russia.

Host: In *Mopalia schrencki* Thiele.

Distribution: Sea of Japan.

Depth range: 3-4 m.

Remarks: The host species belongs to the family Mopaliidae.

– Tunncliffe et al. (2008) (p. 1283) mesoparasitic
Material: Several stages and both sexes.

Locality: NE Pacific Ocean, from Explorer to Juan de Fuca Ridges.

Host: In the afferent branchial vein of *Lepetodrilus fucensis* McLean.

Distribution: NE Pacific.

Depth range: 1520-2420 m.

Remarks: The host species belongs to the family Lepetodrilidae and is a hot-vent species.

– Warén (1972) (: 21) mesoparasitic

Material: At least one ovigerous female.

Locality: NE Atlantic Ocean, west coast of Sweden, between Lille Sotra and Store Sotra, 60°19'50" N, 5°8'30" E.

Host: In the mantle cavity of *Lepetella laterocompressa* (de Rayneval & Ponzi).

Distribution: NE Atlantic.

Depth range: 30-60 m.

Remarks: The host species belongs to the family Lepetellidae. The identification of this as a chitonophilid copepod is based on Huys et al. (2002: 211).

– Haszprunar (1988) (p. 165) mesoparasitic

Material: At least two ovigerous females.

Locality: Australia, New South Wales, east of Brush Island, 35°33' S 150°44' E.

Host: In the pallial and subpallial cavity of *Notocrater ponderi* Marshall.

Distribution: SW Pacific.

Depth range: 384 m.

Remarks: The host species belongs to the family Pseudococculinidae and is wood associated. The identification of this as a chitonophilid copepod is based on Huys et al. (2002: 211).

– Michalak (2001) (p. 21, Figs 9A-F; 11C) mesoparasitic

Material: At least one ovigerous female (ZSM), but see under remarks.

Locality: France, Banyuls-sur-Mer.

Host: In the mantle cavity of "*Lepetella* aff. *sierrai*".

Distribution: Western Mediterranean Sea.

Depth range: 60-90 m.

Remarks: The host species belongs to the family Lepetellidae. The host species is currently under study by Haszprunar et al. (pers. comm.), but it is not *Lepetella sierrai*. Michalak mentioned he examined 12 animals of the *Lepetella* species and later stated that in all treated samples (it remains unclear, if he speaks of the specimens he used for histology only) the "endoparasite" occurred. However, his critical point dried animal does not show an infestation. He

Host genera	Remarks on host
<i>Tonicella</i> , <i>Boreochiton</i>	
<i>Coccapigyra</i>	Wood associated
<i>Stenosemus</i>	
<i>Leptochiton</i> , <i>Ischnochiton</i> , <i>Tripoplax</i> , <i>Lepidozona</i> , <i>Stenoplax</i> , <i>Callistochiton</i> , <i>Chaetopleura</i>	
<i>Lepetella</i>	
<i>Leptochiton</i> , <i>Hanleyella</i>	
<i>Leptochiton</i>	
<i>Nucella</i>	
<i>Stenosemus</i>	
<i>Mopalia</i>	
<i>Lepetodrilus</i> , <i>Lepetella</i> , <i>Lepeta</i> , <i>Notocrater</i>	<i>Lepetodrilus</i> is a hot-vent species, <i>Notocrater</i> is wood associated.
<i>Caymanabyssia</i> , <i>Bathysciadium</i> , <i>Bathyphtyphilas</i>	<i>Caymanabyssia</i> and <i>Bathyphtyphilas</i> are wood or plant remains associated.

wrongly used the term “endoparasite” but clearly shows the ectosoma situated in the host’s neck in the mantle cavity. The endosoma is widely distributed in the body cavity of the host.

– Schwabe (2013) (p. 88, text fig.) mesoparasitic
Material: One ovigerous female (ZSM) with an attached male.

Locality: Sea of Japan, 43°13.5215' N 135°04.3071' E
– 43°13.6778' N 135°04.4447' E.

Host: In the mantle cavity of *Lepeta caeca* (Müller).

Distribution: Sea of Japan.

Depth range: 532 m.

Remarks: The host species belongs to the family Lepetellidae.

? Chitonophilidae gen. sp.

– Huys et al. (2002) (p. 212) mesoparasitic

Material: At least one ovigerous female.

Locality: NE Pacific Ocean, off Oregon, 44°45'47" N
125°31'14" W.

Host: In the pallial cavity of *Caymanabyssia vando-
verae* McLean.

Distribution: NE Pacific.

Depth range: 2750 m.

Remarks: The host species belongs to the family Pseudococculinidae and is wood associated. Huys et al. (2002) do not explicitly speak of a chitonophilid, but an ovigerous mesoparasite, that is why it is tentatively placed here in the Chitonophilidae.

– Hartmann et al. (2011) (p. 274) mesoparasitic

Material: Not specified.

Locality: Azores, 38°33'57" N 28°19'15" W.

Host: In the mantle cavity of *Bathysciadium costu-
latum* (Locard).

Distribution: N Atlantic.

Depth range: 1266 m.

Remarks: The host species belongs to the family Bathysciadiidae and feeds on squid beaks. Hartmann et al. (2011) do not explicitly speak of a chitonophilid, but refer to a parasite similar to those mentioned by Tunnicliffe et al. (2008) and Huys et al. (2002).

– Moskalev (1978) (p. 141) mesoparasitic

Material: At least one ovigerous female.

Locality: NW Atlantic Ocean, from the southeastern
slope of the Grand Bahama Bank, 22°24' N 75°26' W.

Host: In the mantle cavity of *Bathyphtophilas cari-
baeus* Moskalev.

Distribution: NW Atlantic.

Depth range: 2450 m.

Remarks: The host species belongs to the family Bathyphtophilidae and is associated with plant remains. While Moskalev (1978) mentions eggs in the mantle cavity Huys et al. (2002: 212) doubted the

brooding behaviour in this family and commented that the occurrence of eggs in the mantle cavity of this deep-sea limpet could be reinterpreted in the light of our new understanding of chitonophilids, that is why the record is tentatively placed here in Chitonophilidae.

– Haszprunar & McLean (1996) (p. 37, fig. 1) meso-
parasitic

Material: At least one ovigerous female (LACM 2398
holotype of host species).

Locality: NE Pacific Ocean, Mexico, Baja California,
off Tijuana, San Diego Trough at the base of Cor-
onado Canyon, 32°18.2' N 117°29.8' W.

Host: In the mantle cavity of *Bathyphtophilas dieg-
ensis* Haszprunar & McLean.

Distribution: NE Pacific.

Depth range: 1224 m.

Remarks: The host species belongs to the family Bathyphtophilidae and is associated with plant remains. While Haszprunar & McLean (1996) speaks about eggs in the mantle cavity Huys et al. (2002: 212) doubted the brooding behaviour in this family and commented that the occurrence of eggs in the mantle cavity of this deep-sea limpet could be reinterpreted in the light of our new understanding of chitonophilids, that is why the record is tentatively placed here in Chitonophilidae.

These records (Table 1) show that chitonophilids have a worldwide distribution (Fig. 6) from the splash zone down to abyssal depths (Fig. 7). The lack of any records from the Indian Ocean is remarkable with the main occurrence in the north western Pacific Ocean. The majority of chitonophilid taxa were described from polyplacophoran hosts, within which the order Lepidopleurida is most often infested. Lepidopleurida are the earliest basally-diverging group of extant polyplacophorans (Sigwart et al. 2011) with a potential deep sea origin (Sirenko 2004). This is interesting given that all gastropod infesting chitonophilids are known from deep-sea limpets with the exception of *N. holmanae*. Several forms of these limpets colonize chemosynthetic or biogenic substrata and these environments may serve as so-called stepping stones in deep-sea limpet diversification. Such ideas have been tested (Kano et al. 2013) and this scenario was also hypothesized by Sirenko (2004) for deep-sea lepidopleurid chitons. Thus, it seems likely that an ancient form of chitonophilids evolved in the deep sea and host switching from Polyplacophora to Gastropoda (or vice versa) occurred already in the deep (see also Sobecka 2012 for evolutionary association).

Huys et al. (2002) doubted the brooding behav-
iour of deep-sea limpets (see above) and speculated

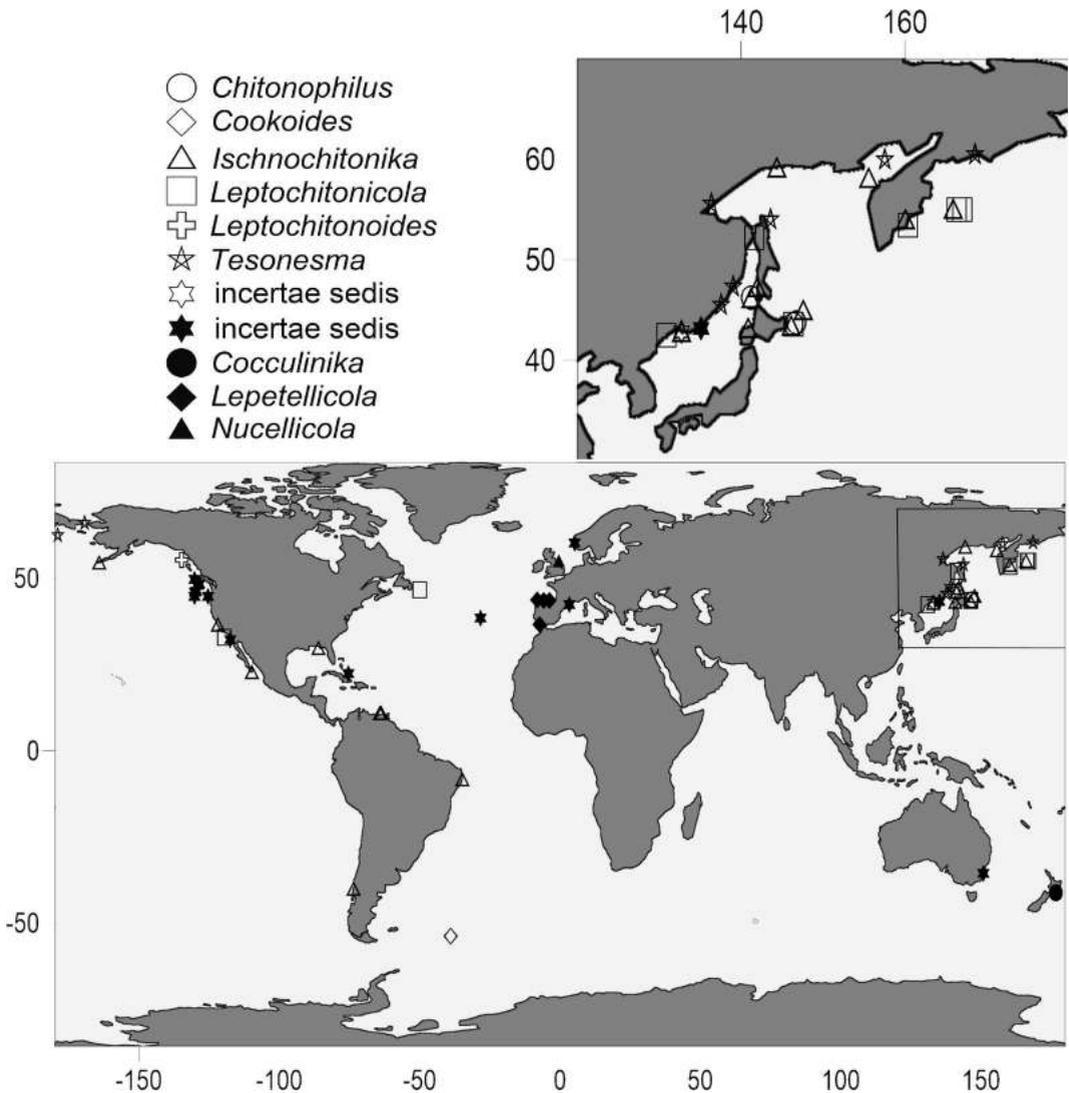


Fig. 6. Distribution pattern of Chitonophilidae. Valid genera refer to species records, “incertae sedis” to unconfirmed records, open symbols to polyplacophoran-copepod associations, filled symbols to gastropod-copepod associations.

that the egg masses instead refer often (if not always) to ovigerous females of a chitonophilid association. We checked the available information on brooding chitons to test the circumstances in polyplacophorans and found a single record of a brooding female (Turner 1978: 46). *Paricoplax crocinus* was described with “eggs ... are partially encased in a damaged, membranous sac” and “the brood is situated in the mantle cavity at the anterior end of the right side of the foot” (sic Turner 1978). This would be rather unusual for chitons and thus we tried to examine this specimen (Natural Museum Victoria, Australia

F30097), but Dr. Julian Finn informed us (email: 08/09/2013) that whatever Turner observed there, is lost.

In total, the family Chitonophilidae comprises nine recognized genera and several unconfirmed records. Focusing on the described genera, six are strictly host specific infesting only polyplacophoran hosts, while the remaining three are gastropod associated. Following Huys et al. (2002) interpretation of the copepod’s microhabitat, there are only two endoparasitic genera, a single one for each host class, whereas all others are mesoparasitic. Of the 20

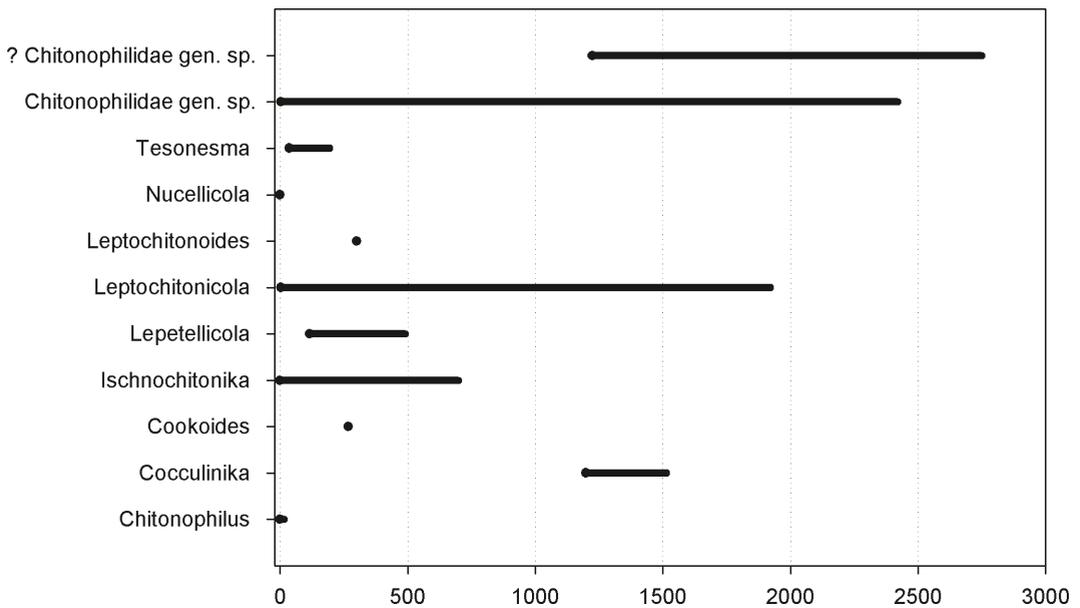


Fig. 7. Bathymetric distribution of the family Chitonophilidae. While the valid genera refer to attributed species, the "(?) Chitonophilidae gen. sp." refer to unconfirmed records.

species, including undescribed species, attributed to particular genera, copepodid stages are known for only three genera, and naupliar stages for only four while males are only known for eight genera.

The genus *Ischnochitonika* with six recognized species is the only genus known from more than one host family. Representatives were found in four families, comprising seven genera and 11 host species, indicating an ongoing diversification (Anton & Schrödl 2013).

While infestations of individual host specimens by more than one female are not uncommon in chitonophilids (e.g. Nagasawa et al. 1991, Avdeev & Sirenko 1994, 2005, Tunnicliffe et al. 2008, ES pers. obs.), "host sharing" is rare. If we ignore the unconfirmed "chitonophilid sp." records, two *Leptochitonids* (Polyplacophora) are seemingly the only species, to be infested by more than one chitonophilid species. *Leptochiton* (cf.) *belknapi* Dall serves as a host species for *I. aleutica* Avdeev & Sirenko, 2005 and *Leptochitonoides vitiazii* Avdeev & Sirenko, 2005, while *Leptochiton* (cf.) *rugatus* (Carpenter in Pilsbry) is infested by *Leptochitonicola attenuata* Avdeev & Sirenko, 2005 and *L. sphaerica* Avdeev & Sirenko, 2005.

The intensity of infestation has not been estimated in most chitonophilids. Infestation rates have been reported for a handful species (e.g. Huys et al. 2002, Avdeev & Sirenko 2005, Tunnicliffe et al. 2008). Tunnicliffe et al. (2008) reported an infestation rate

of 5 % (198 specimens with a single parasite plus 20 specimens with more than one parasite per limpet) in 3211 examined specimens of *Lepetodrilus fucensis*. However, the analysed specimens originated from different areas and the authors simultaneously reported that the limpet may locally reach densities of up to 390 000 individuals per m². Although more than 3000 examined specimens is an unusually high number, the infestation may be patchy and could distort the real number.

Summary

The discovery of *I. lasalliana* on the Chilean coast allowed new observations on this mesoparasitic copepod belonging to the family Chitonophilidae. This is the first record from the southern hemisphere and thus a considerable range extension, and also demonstrates the low host specificity of this species. The species belongs to a genus of chitonophilids with members being mostly generalists in terms of host selection. While the remaining eight genera currently placed in the Chitonophilidae are each restricted to a single host family, *Ischnochitonika* members utilize four families of polyplacophorans with a total of 11 host species in seven genera. It is inferred that chitonophilids originated in a deep-sea habitat, as the host taxa mainly belong to species of deep-sea limpets (gastropods) and lepidopleurids

(polyplacophorans) which are generally seen as the most primitive chitons and have a deep-water origin.

Despite several detailed examinations of chitonophilid species, our knowledge is still too limited to make precise predictions about aspects of the lifestyle, phylogeny and even anatomy of these highly modified parasitic copepods. First attempts were made by Huys et al. (2002), but due to additional information the estimation of phylogenetic relationships should probably be redone. Additional information to consider includes the absence of a digestive tract in the examined females (the presence of which was considered a family character of the chitonophilids) and the antennary exopod segmentation of the first naupliar stage comprising of seven instead of five segments (also see Nagasawa et al. 1991).

To better our knowledge of chitonophilids, future records need to provide information on infestation intensities linked with more precise specification about investigated material, e.g. used sample gear, the total number of examined host species versus infested ones, accompanying biotic and abiotic factors such as temperature and salinity (e.g. Sobecka 2012), number of females and maturity stage of males, and date of collection. Dates might be of interest as our own data on polyplacophoran-infesting chitonophilids reveal that ovigerous females occurred mainly in the first half of the year, irrespective of their host taxon, depth and region.

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