



## New records for the solenogaster *Proneomenia sluiteri* (Mollusca) from Icelandic waters and description of *Proneomenia custodiens* sp. n.

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**Abstract:** During August–September 2011, scientists aboard the R/V *Meteor* sampled marine animals around Iceland for the IceAGE project (Icelandic marine Animals: Genetics and Ecology). The last sample was taken at a site known as “The Rose Garden” off northeastern Iceland and yielded a large number of two species of *Proneomenia* (Mollusca, Aplacophora, Solenogastres, Cavibelonia, Proneomeniidae). We examined isolated sclerites, radulae, and histological section series for both species. The first, *Proneomenia sluiteri* Hubrecht, 1880, was originally described from the Barents Sea. This is the first record of this species in Icelandic waters. However, examination of aplacophoran lots collected during the earlier BIOICE campaign revealed additional Icelandic localities from which this species was collected previously. The second represents a new species of *Proneomenia*, which, unlike other known representatives of the genus, broods juveniles in the mantle cavity. We provide a formal description, proposing the name *Proneomenia custodiens* sp. n. Interestingly, the sclerites of brooded juveniles are scales like those found in the putatively plesiomorphic order Pholidoskepia rather than hollow needles like those of the adults of this species. Cytochrome c oxidase subunit I (COI) DNA barcode sequences are provided for both species of *Proneomenia*.

Key words: Icelandic waters, Aplacophora, Neomeniomorpha, brooding, taxonomy.

### Introduction

Solenogastres (= Neomeniomorpha) is a small but understudied lineage of shell-less, worm-like molluscs. Solenogastres can most easily be distinguished from the other group of aplacophoran molluscs, Caudofoveata (= Chaetodermomorpha), by the complete lack of a foot in the latter. Interestingly, most of the de-

scribed species of Solenogastres are known from polar regions, especially the Antarctic (García-Álvarez and Salvini-Plawen 2007), although this may be due to sampling bias. During the 2011 IceAGE (Icelandic Animals: Genetics and Ecology) research cruise (IceAGE I) aplacophorans received special attention, as two aspiring aplacophoran taxonomists were onboard. Consequently, a large number of specimens were observed alive and preserved for a variety of morphological and molecular studies. Although most aplacophoran specimens were relatively small (most <1 cm in length) and were predominantly collected via epibenthic sled (EBS), larger species were retained by the coarser mesh net of the Agassiz trawl (AGT). The vast majority of these larger specimens belonged to two species of the genus *Proneomenia* (Proneomeniidae).

Proneomeniidae is a relatively homogenous family including large-bodied species (up to 16 cm, but mostly 2–5 cm in length) with a thick cuticle, epidermal sclerites in the shape of hollow needles arranged in multiple layers, a polystichous radula, and long, tube-shaped type C (Salvini-Plawen 1978) or *Epimenia*-type (Handl and Todt 2005) ventrolateral foregut glands. The family includes two genera: *Proneomenia* and *Dorymenia*, which differ in the presence or absence of copulatory stylets (summarized by García-Álvarez and Salvini-Plawen 2007; García-Álvarez *et al.* 2009). Of the eleven described species of *Proneomenia*, six are known from polar waters. However, five of these species are from the Southern Ocean while only *Proneomenia sluiteri* Hubrecht, 1880 is known from polar waters in the Northern Hemisphere. Previous collection localities of *P. sluiteri* include the Norwegian Barents Sea and the Russian Kara and Laptev Seas (Hubrecht 1880, 1881; Heuscher 1892; Thiele 1911). Here, we report collection of *Proneomenia sluiteri* during the IceAGE project in the Norwegian Sea off northeastern Iceland, extending the biogeographic range of this species. Additional specimens of this species were identified from other localities in aplacophoran lots from the BIOICE campaign. The inter-Nordic BIOICE project was a survey of the benthic invertebrate fauna in the Icelandic exclusive economic zone. During seventeen cruises in 1992 to 2002 with the Icelandic R/V *Bjarni Sæmundsson* and the Norwegian R/V *Håkon Mosby*, sampling was conducted all around Iceland. This resulted in more than 1300 samples from 530 localities (Tendal 1998; Guðmundsson 2000; Dauvin *et al.* 2012).

We provide a diagnosis of *P. sluiteri* to facilitate identification of this, in some habitats, very abundant species and illustrate important morphological characters. Hubrecht (1881) and Heuscher (1892) give detailed descriptions of the internal anatomy and histology. Additionally, Thiele (1911) presents a concise description (in German) with very useful illustrations. Furthermore, we report the collection of a second, undescribed species of *Proneomenia* from one IceAGE locality. These relatively small-bodied animals (body length about 1 cm) have the habit of brooding their young in the mantle cavity. We provide a formal species description based on extraction of hard parts and histological sectioning and propose the name *Proneomenia custodiens* sp. n.

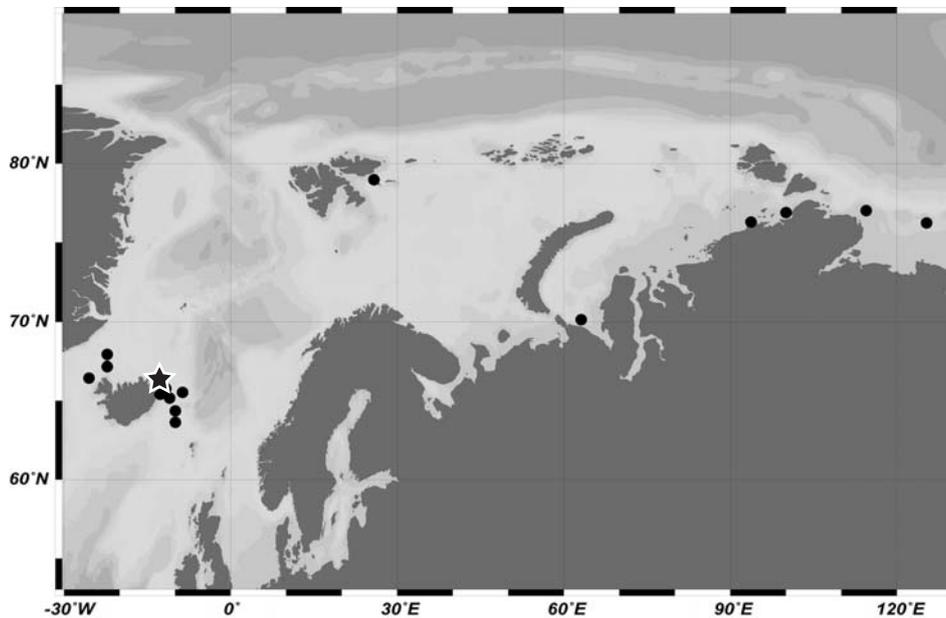


Fig. 1. Schematic map indicating collection localities of *Proneomenia sluiteri*. IceAGE station 1223, the type locality of *Proneomenia custodiens* sp. n., is indicated by a star.

## Materials and methods

During the IceAGE cruise, specimens of two distinct species of *Proneomenia* were collected during a single cast of the Agassiz trawl. The trawl sampled the benthos at IceAGE station 1223 (Fig. 1, indicated by a star) between 66.29533°N–12.36267°W and 66.30166°N–12.37716°W at a depth of approximately 284 meters. This site is colloquially known as “The Rose Garden.” The trawl was filled with soft mud that was sieved on deck through a series of sieves as fine as 1 mm. Aplacophorans were rinsed with sea water and placed into plastic dishes with clean, cold filtered seawater as they were collected.

Specimens for histological sectioning and examination of hard parts were relaxed in a 1:1 mixture of 7.5% MgCl<sub>2</sub> and filtered seawater and fixed in buffered 4% formalin for approximately 24 hours followed by transfer to 70% ethanol. Specimens for molecular work were fixed in RNAlater (Ambion) or 100% ethanol buffered with sodium borate (saturated solution) and stored at -20°C.

For histological sectioning, the anterior and posterior body ends of three individuals of the undescribed brooding *Proneomenia* and one individual of *P. sluiteri* were decalcified in a dilute solution of HCl in ethanol overnight (initially about 1 drop of HCl in 5 ml of 70% ethanol). Notably, the specimens were highly resistant to decalcification and several more drops of HCl needed to be added. After no remnants of the sclerites could be detected, the specimens were dehydrated stepwise

and embedded in paraffin. The paraffin blocks were trimmed and transverse section series (10  $\mu\text{m}$  thickness of cross-sections) were made using a Leica 2255 rotation microtome. Sections were stained with Gomori's trichrome stain and mounted in Araldite epoxy resin (Huntsman Advanced Materials). Histological sections were imaged on a Leica DM 6000B microscope with a Leica DFC 420 digital camera using differential interference contrast (DIC) or brightfield.

For the undescribed *Proneomenia* species, reconstructions of internal anatomy were made by hand based on the histological cross section series of the holotype. Distances in the x-axis were calculated by counting sections and multiplying the number with the section thickness (10  $\mu\text{m}$ ). Distances in the y-axis were measured with an ocular micrometer on a Leitz Wetzlar Dialux 20 compound microscope.

Sclerites were isolated by dissolving pieces of mantle tissue in dilute sodium hypochlorite (household bleach) on a microscope slide until they could be easily separated away from the cuticle with a pair of fine needles. The bleach was then rinsed away from the extracted sclerites with multiple rinses of deionized water and the slide was allowed to air dry. Sclerites were then embedded in Araldite epoxy resin (Huntsman Advanced Materials) and polymerized overnight at 70°C. Additional sclerite preparations were made by mounting sclerites in Euparal (Bioquip).

Cytochrome c oxidase subunit I (COI) barcode sequences were obtained from transcriptome data. For *Proneomenia sluiteri*, mantle tissue from one animal was used. Special care was taken to avoid contamination with gut contents. For the undescribed *Proneomenia* species, eight unhatched juveniles were removed from the brooding chamber of one adult. RNA extraction was performed using the Ambion RNAqueous micro kit. Complementary DNA libraries were synthesized using the SMART cDNA Library Construction Kit (Clontech) and Advantage 2 PCR reagents (Clontech). Full-length cDNA was sent to Hudson Alpha (Huntsville, AL) for Illumina sequencing library preparation and 2 X 100 bp paired-end sequencing on an Illumina HiSeq 2000 instrument using approximately 1/6 lane per taxon. Transcriptome data were assembled using the October 2012 version of Trinity (Grabherr *et al.* 2011). COI sequences were identified by comparing the assembled contigs to the *Katharina tunicata* COI sequence (NCBI NC001636.1 / NP008173.1; positions 1-1548 of the mitochondrial genome) using blastn (Altschul *et al.* 1990) with an e-value cutoff of 0.0001.

## Systematics

Following García-Álvarez and Salvini-Plawen (2007).

Order Cavibelonia Salvini-Plawen, 1978

Hollow acicular sclerites in one or more layers within a thick cuticle, or, if with solid sclerites (rare), with a biserial radula and ventrolateral foregut glands not of type A. Radula of different types or absent. Ventrolateral foregut glands of different types.

## Family Proneomeniidae Simroth, 1893

Cuticle thick. Hollow acicular sclerites arranged in multiple layers. Radula polystichous. Ventrolateral foregut glands with epithelial gland cells (type C, *Epimения* type). With at least one pair of seminal receptacles.

*Proneomenia* Hubrecht, 1880

With epidermal papillae. Mouth opening within atrium (vestibulum). Midgut with regular constrictions. Secondary genital opening unpaired. Without copulatory stylets. With or without abdominal sclerites. With dorsoterminal sense organ. Without respiratory organs.

*Proneomenia sluiteri* Hubrecht, 1880

**Collection localities** (Fig. 1, Table 1). — During the IceAGE cruise 2011, the species was recorded from *The Rose Garden*, IceAGE station 1223, between 66.29533°N–12.36267°W and 66.30166°N–12.37716°W, depth 284 m, 22 September, 2011.

For locality and cruise data from the BIOICE material (Icelandic Museum of Natural History) (Fig. 1, Table 1).

**Material.** — A total of 49 specimens of *Proneomenia sluiteri* (Fig. 2A) were collected from IceAGE station 1223 off Northeastern Iceland (Fig. 1). All IceAGE specimens of *P. sluiteri* were observed alive by K.M.K. The anterior and posterior ends of one IceAGE specimen were histologically sectioned, Ap 122.1F, University Museum of Bergen, accession number 94113. Additional material from IceAGE location 1223: permanent mounts of sclerites and abdominal sclerites of one speci-

Table 1  
*Proneomenia sluiteri* collected from Iceland, location and cruise data.

| Cruise | Station | Longitude (N) | Latitude (W) | Depth (m) | Date       |
|--------|---------|---------------|--------------|-----------|------------|
| IceAge | 1223    | 66.2953       | -12.3627     | 284       | 22.09.2011 |
| BIOICE | 2009    | 65.2992       | -11.2316     | 611       | 20.07.1991 |
| BIOICE | 2047    | 65.4209       | -12.5260     | 272       | 25.07.1991 |
| BIOICE | 2321    | 63.5600       | -10.0000     | 833       | 03.05.1993 |
| BIOICE | 2322    | 63.5500       | -10.0400     | 627       | 03.05.1993 |
| BIOICE | 2323    | 63.5500       | -10.0500     | 623       | 03.05.1993 |
| BIOICE | 2363    | 64.2900       | -10.2600     | 493       | 07.05.1993 |
| BIOICE | 2364    | 64.3500       | -10.0300     | 605       | 08.05.1993 |
| BIOICE | 2518    | 66.3694       | -25.3359     | 749       | 13.07.1993 |
| BIOICE | 2583    | 67.3590       | -22.2323     | 605       | 16.07.1993 |
| BIOICE | 3140    | 67.8650       | -22.2482     | 768       | 25.08.1999 |
| BIOICE | 3198    | 65.5083       | -8.5217      | 935       | 08.07.2001 |
| BIOICE | 3250    | 65.8398       | -12.0117     | 196       | 14.07.2001 |

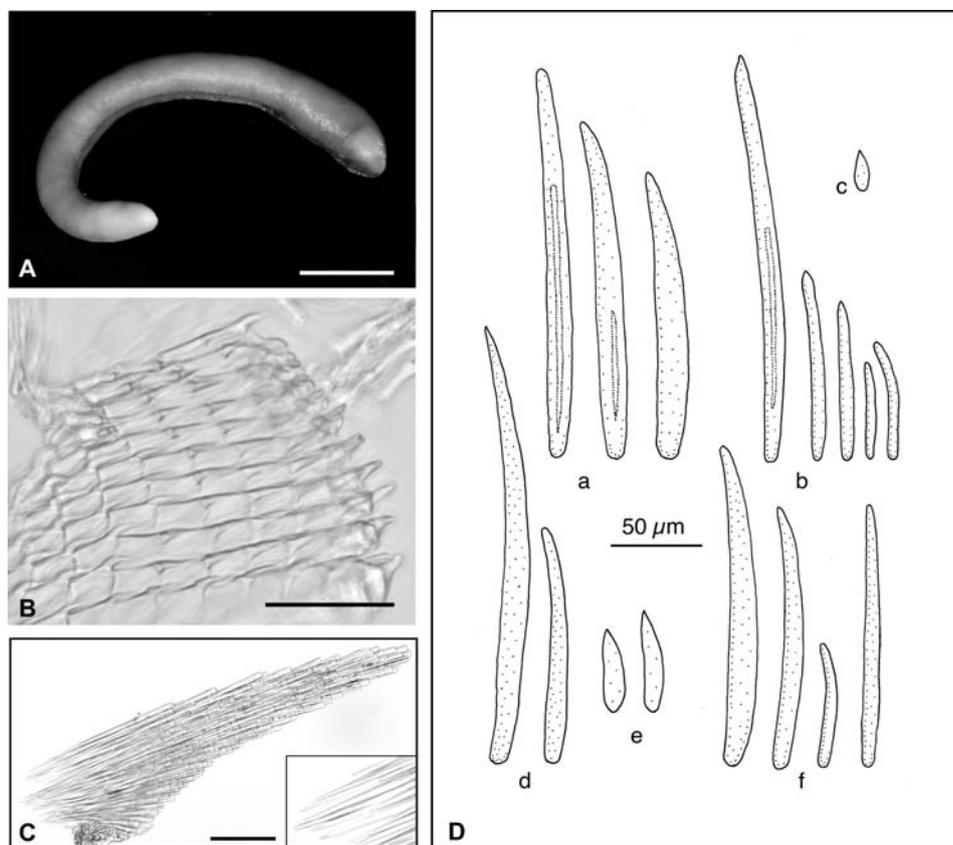


Fig. 2. *Proneomenia sluiteri*. **A.** Habitus (96% ethanol-fixed specimen), anterior body end to the left; scale bar 1 cm. **B.** Radula, light micrograph of whole mount preparation; scale bar 50  $\mu\text{m}$ . **C.** Abdominal spicules, light micrograph of whole mount preparation, the insert shows the slender tips of the spicules in higher magnification; scale bar 200  $\mu\text{m}$ . **D.** Illustration of sclerite types according to body region: **a**, main body, dorsal and lateral body wall; **b**, anterior body end; **c**, surrounding vestibular opening; **d**, ventral side, along foot groove; **e**, directly flanking foot groove; **f**, posterior body end, sclerites surrounding pallial cavity.

men, University Museum of Bergen, accession number 94114; permanent mounts of sclerites, abdominal sclerites, and radula from another specimen, University Museum of Bergen, accession number 94115; two partial specimens fixed in 96% ethanol, University Museum of Bergen, accession number 94116; and four intact specimens fixed in 4% formaldehyde and stored in 70% ethanol, Smithsonian Institution of Natural History, accession number 1231341. Sclerites and abdominal sclerites of one specimen from BIOICE station 2323 were extracted and permanently mounted (all BIOICE material deposited at the Icelandic Museum of Natural History).

**Diagnosis.** — Large-bodied (up to 15 cm long) with rounded anterior and pointed posterior body end; body cylindrical in cross section, no dorsal keel; body of

both living and fixed specimens stiff due to thick cuticle and skeletal sclerites; body surface nearly smooth, color whitish to light tan in life but occasionally dark tan in fixed specimens; epidermal sclerites at the body ends mostly hollow spines with small cavity, but predominantly flattened and solid spines at midbody, characteristic spines with flattened tips around vestibular opening; openings of paired abdominal spicule pouch may be visible as small pits just anterior to the pallial cavity opening; dorsoterminal sense organ visible as small dorsal pit above the anterior part of the pallial cavity opening; pallial cavity epithelium ciliated; numerous slender vestibular papillae; polystichous radula with 15–20 teeth per row; genital tract with a somewhat convoluted tubular seminal receptacle at the fusion site of pericardioduct and spawning duct; pericardioduct with few seminal vesicles close to the opening of the seminal receptacle; pericardioduct smaller in diameter than paired spawning duct.

**Remarks.** — The IceAGE specimens of *Proneomenia sluiteri* range in size from around 20 mm to 120 mm total length. Examination of aplacophoran lots collected during the BIOICE research cruises revealed twelve additional Icelandic localities for *Proneomenia sluiteri*. Previously, *Proneomenia sluiteri* was known from its type locality in the Barents Sea close to Svalbard (201 to 293 m depth; Hubrecht 1881), the Kara Sea (105 m, 45 m) and the Laptev Sea (28 m, 50 m, 60 m; Thiele 1913), and east of Spitsbergen (160 m, 140 m; Heuscher 1892). Comparing depth regimes where the species has been observed, it is obvious that the Icelandic sites are deeper than the northeastern sites (especially those in the Russian Laptev Sea). Thiele (1913) remarks that some of the Russian specimens he studied were smaller and he found some differences in anatomy, namely “the receptaculum seminis is a straight, posteriorly-pointing, narrow tube” (translated from German) and in histology, including the mantle cavity epithelium containing “clusters of lightly-colored cells with round nuclei” (translated from German). This could be due to differences in ontogenetic stage, but it could also point to the presence of another species of *Proneomenia* in the area, maybe even the new species described herein.

Comparison of the radula (Figs 2B and 3) and histological sections (Fig. 4A–D) of the IceAGE and BIOICE specimens agreed with previous detailed descriptions of

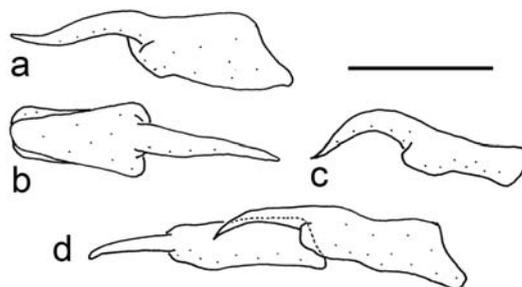


Fig. 3. *Proneomenia sluiteri*, schematic drawings of isolated teeth of voucher specimen Ap122.3F, University Museum of Bergen, accession number 94115, in obliquely lateral (a), dorsal (b); and lateral (c) views; two articulated teeth in obliquely lateral view (d). Scale bar 20  $\mu$ m.

*P. sluiteri* (Hubrecht 1881; Heuscher 1892; Thiele 1911). These publications, however, did not describe or depict epidermal sclerites in detail. Therefore, we here show the range of sclerite types on different body parts of a specimen with a body length of about 4 cm (Fig. 2D, a–f). The main body sclerites (Fig. 2D, a) are solid or hollow spines with a very small cavity, which are up to 300  $\mu\text{m}$  long, but mostly measure about 200  $\mu\text{m}$ . On the anterior body end (Fig. 2D, b), there are also spines with a peculiar short flattened tip (150–250  $\mu\text{m}$  long), which are either hollow or solid and short and slender solid spines, 50–100  $\mu\text{m}$  long, with a blunt tip. Minute drop-shaped scales (Fig. 2D, c), measuring 25  $\mu\text{m}$  in length, surround the vestibulum. The pedal groove is lined by leaf-shaped scales (Fig. 2D, e), which are 50–60  $\mu\text{m}$  long, flanked by slender, pointy solid spines, up to 220  $\mu\text{m}$  long (Fig. 2D, d). Similar pointy solid spines (50–120  $\mu\text{m}$  long) also surround the mantle cavity opening (Fig. 2D, f).

Hubrecht (1881) draws the epidermal sclerites as solid, such as found in the midbody and close to the pedal groove and mantle cavity opening of our specimens. He does not mention hollow sclerites in the text. In contrast, Heuscher (1892) and Thiele (1911) show them to be hollow, as common in the anterior and posterior body ends of our specimens. This varying distribution of sclerite types has to be taken into account for identification. In addition, it is possible that there is some intraspecific variation concerning the abundance of solid *versus* hollow sclerites.

In our dissected specimen, the thick bundle of abdominal spines is about 1.1 mm long (Fig. 2C). It is difficult to determine the maximum length of individual spines because they break easily when one is trying to tease the bundle apart. The spines are very slender and pointy at the distal end (Fig. 2C, insert) and have a wide cavity that is open at the proximal end. Hubrecht (1881) interpreted the abdominal sclerites as reminiscent of byssus glands, an interpretation that was rejected by Heuscher (1892) and Thiele (1911), who recognized them to represent specialized calcareous spines.

The radula (Figs 2B, 3, 4A) is similar to earlier descriptions. Heuscher (1892) detected crustacean body parts in the midgut and thus mentioned small crustaceans (“Entomostraca”) as possible food organisms for the animals. We cannot confirm this observation, but want to remark that there were no cnidocysts found in histological sections of the midgut. However, in one large specimen that was dissected for radula and sclerite preparations, there was a large amount of sclerites from an unidentified soft coral (Alcyonaria) in the hindgut and mantle cavity, indicating that soft corals make up at least part of the diet of *P. sluiteri*. Notably, a large number of colonies of a small, white, unidentified soft coral (Alcyonaria, Alcyoniidae?) were collected from IceAGE station 1223 along with the *P. sluiteri*.

*Proneomenia custodiens* sp. n.

**Collection locality.** — *The Rose Garden*, IceAGE station 1223, between 66.29533°N–12.36267°W and 66.30166°N–12.37716°W, depth 284 m, 22 September, 2011.

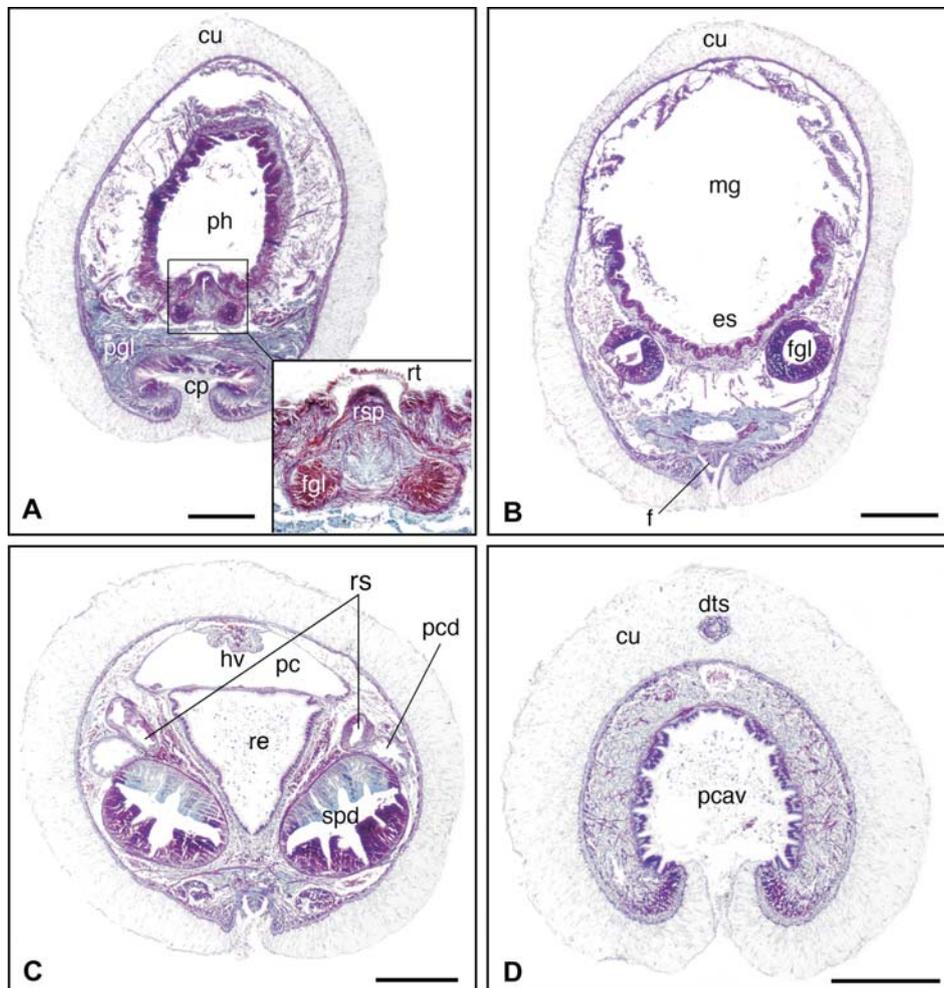


Fig. 4. *Proneomenia sluiteri*, histological cross-sections. **A.** Anterior body with pharynx (ph), ciliary pedal pit (cp), and pedal gland (pgl); note the thick cuticle (cu). The insert shows a detail of the radula apparatus with muscular support (rsp), and the radula membrane carrying teeth (rt), as well as the ducts of the ventrolateral foregut glands (fgl). **B.** Anterior body with the esophagus (es) opening into the midgut (mg), and the paired ventrolateral foregut glands (fgl); (f) foot, (cu) cuticle. **C.** Posterior body with pericardium (pc), heart ventricle (hv), rectum (re), paired portion of spawning duct (spd), pericardioduct (pcd), and seminal receptacle (rs). **D.** Posterior body with pallial cavity (pcav) and dorsoterminal sense organ (dts) embedded in the thick cuticle (cu). All scale bars 500 µm.

**Material.** — A total of eleven specimens of *Proneomenia custodiens* sp. n. (Fig. 4A) were collected from *The Rose Garden* site (IceAGE station 1223) off Northeastern Iceland. All specimens were observed alive by K.M.K. The anterior and posterior ends of three specimens were embedded in paraffin and histologically sectioned and examined. The sclerites and radulae of two additional specimens were mounted and examined.

Holotype, Ap125.1F, mounted histological sections, 10 glass slides, University Museum of Bergen, accession number 94109. Paratype 1, Ap126.1F, whole specimen fixed in 4% formalin and preserved in ethanol, University Museum of Bergen, accession number 94110. Paratype 2, Ap124.1F, mounted histological sections, 14 glass slides, University Museum of Bergen, accession number 94111. Paratype 3, Ap126.2E.1, extracted radula and sclerites mounted on four glass slides, University Museum of Bergen, accession number 94112. Paratype 4, whole specimen fixed in 4% formalin and preserved in ethanol, Icelandic Museum of Natural History, accession number IINH 28834. Paratype 5, Ap126.1F, mounted histological sections, 16 glass slides, Icelandic Museum of Natural History, accession number IINH 28835. Paratype 6, Ap126.2E.2, extracted radula and sclerites mounted on three glass slides, Icelandic Museum of Natural History, accession number IINH 28836.

**Diagnosis.** — Up to 10 mm long (maybe slightly larger) with rounded posterior body end; in brooding specimens the posterior end is slightly keeled and swollen and thus thicker than the anterior end; body stiff due to thick cuticle and skeletal sclerites; body surface rough, color tan (in living specimens with a pinkish hue); main epidermal sclerites hollow spines with long flattened tips, close to the vestibular and mantle cavity openings very characteristic spatula-shaped sclerites (mostly solid); pallial cavity serves as a brood pouch, lined by glandular epithelium and lacking cilia; dorsoterminal sense organ above the posterior part of the mantle cavity (subterminal); numerous slender vestibular papillae; polystichous radula with 18–22 teeth per row; genital tract with two globular seminal receptacles and one shortly-tubular receptacle at the fusion site of pericardioduct and spawning duct; pericardioduct very wide, lined by seminal vesicles throughout (only portion closest to pericardium ciliated and free of vesicles); pericardioduct larger in diameter than paired spawning duct.

**Etymology.** — *custodiens* from lat. *custodire* – to guard, to take care of, referring to the parental care the animals are providing to their young.

**Description.** — All observed specimens are approximately 10 mm in length by 3.5 mm in diameter at their midsection. The body has a rough surface and the posterior end is slightly dorsally keeled. The anterior end can be pointed while the posterior end is always rounded. In some specimens (carrying young in the brood pouch) the posterior end is distinctly thicker than the anterior end. All specimens were curled up in a characteristic crescent-moon shape to C-shape at the time of collection. Living specimens were light tan with a slight pink hue. Specimens fixed in 4% formalin and preserved in 70% ethanol appear rather tan than pink. Vestibular-buccal opening and pallial cavity opening are visible as short longitudinal slits, the ventral groove is distinct. Most specimens were somewhat dirty upon collection with detritus and sand adhering to their sclerites. Notably, apparently living rotaliid foraminiferans were attached to some specimens.

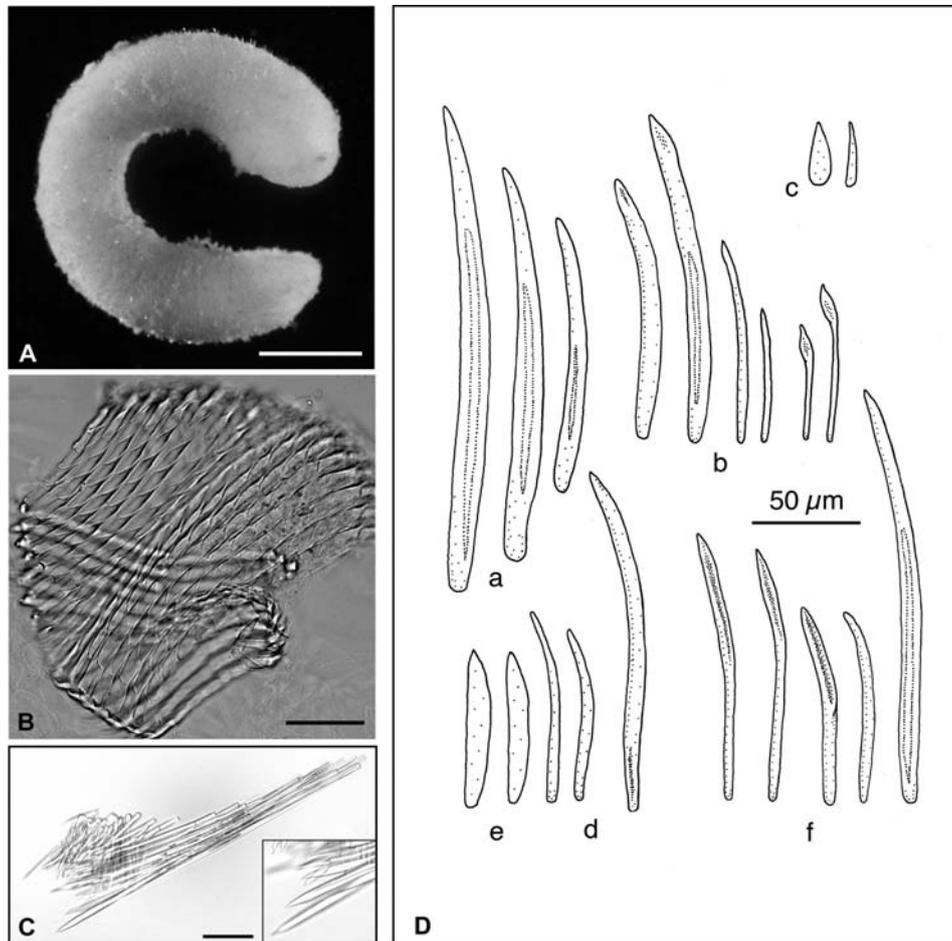


Fig. 5. *Proneomenia custodiens* sp. n. **A.** Habitus (living specimen, photo by D. Fiege), anterior body end in the upper right corner; scale bar 0.8 mm. **B.** Radula, light micrograph of whole mount preparation; scale bar 50 µm. **C.** Abdominal sclerites, light micrograph of whole mount preparation; the insert shows the broadened tips of the spicules in higher magnification; scale bar 100 µm. **D.** Illustration of sclerite types according to body region; **a**, main body, dorsal and lateral body wall; **b**, anterior body end; **c**, surrounding vestibular opening; **d**, ventral side, along foot groove; **e**, directly flanking foot groove; **f**, posterior body end, sclerites surrounding pallial cavity.

The following anatomical and histological descriptions are based on evidence from the five paratypes. Measurements of anatomical features were taken from the holotype, which also was used for reconstruction of the internal organization (see below). We focus here mostly on features that are important for species identification.

Sclerites according to body regions are shown in Fig 5D. The main epidermal sclerites in dorsal and lateral position (sclerites a) are acicular (needles) and arranged in multiple layers. These needles are up to 350 µm but mostly about 220 µm long,

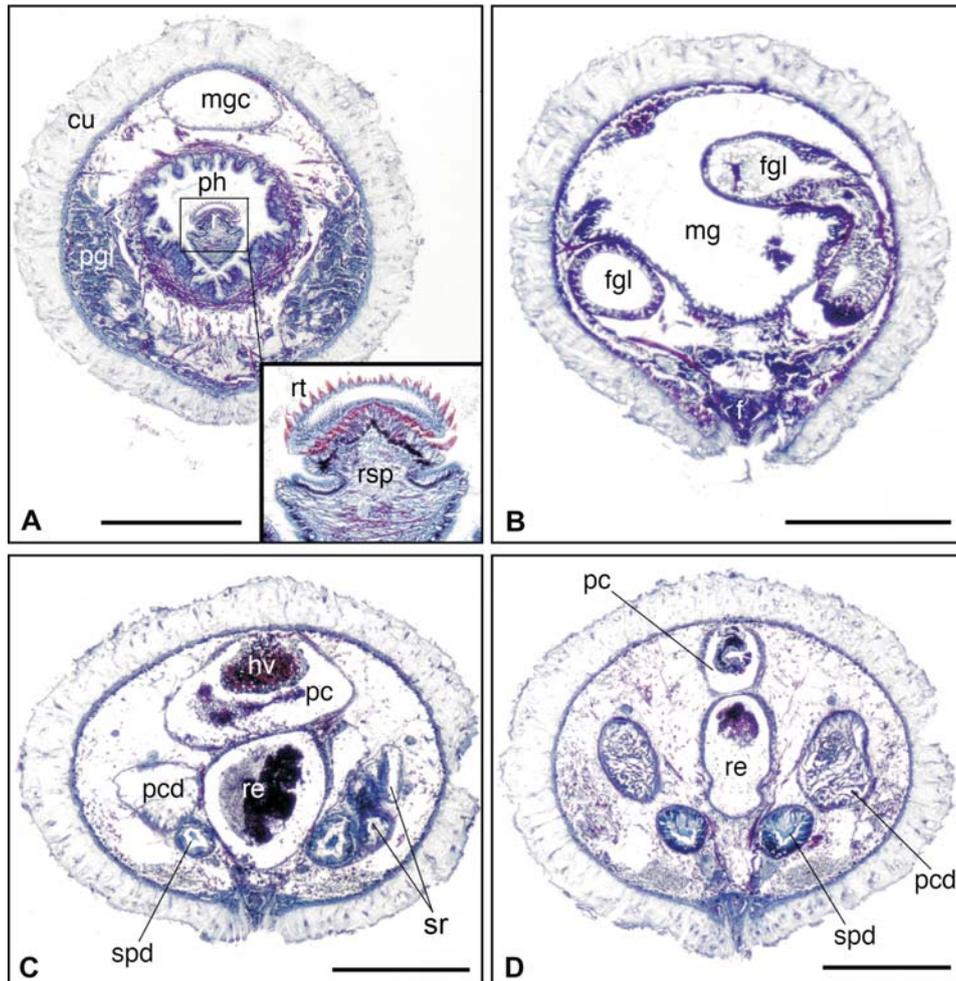


Fig. 6. *Proneomenia custodiens* sp. n., histological cross-sections of paratype 1. **A.** Anterior body with radula (r), pharynx (ph), midgut caecum (mgc), pedal gland (pgl), and cuticle (cu). The insert shows a detail of the radula apparatus with muscular support (rsp), and radula membrane carrying teeth (rt). **B.** Anterior body with midgut (mg), and ventrolateral foregut glands (fgl); note that one of the foregut glands (to the right in this micrograph) is curving upwards instead of pointing backwards in this specimen; f: foot. **C.** Slightly oblique section through posterior body with pericardium (pc), heart ventricle (hv), rectum (re), paired portion of spawning duct (spd), pericardioduct (pcd), and seminal receptacles (sr). **D.** Posterior body with pericardium (pc), rectum (re), paired portion of spawning duct (spd), and the conspicuously wide pericardioducts (pcd). Scale bars 500  $\mu$ m.

bluntly rounded proximally and tapering to a rounded point distally. Most have a hollow internal cavity but it usually extends only about two-thirds of the length of the sclerite in the distal direction, leaving the distal flattened third of the sclerite solid. In some cases, usually in smaller sclerites of the same general shape, no hollow internal cavity is present. On the anterior body end (Fig. 5D, b), there are also

hollow sclerites (up to 200  $\mu\text{m}$  long) with a broadened tip that bears a shallow groove and short, slender solid spines, 40–60  $\mu\text{m}$  long, with a blunt tip, and very conspicuous solid spatula-shaped sclerites, 50–80  $\mu\text{m}$  long. Small drop-shaped scales measuring 40  $\mu\text{m}$  in length (Fig. 5D, c) surround the vestibulum. Ventral sclerites (Fig. 5D, d) include slender, pointy solid spines (60–80  $\mu\text{m}$  long), and solid sclerites with flattened tips with a groove, 100–120  $\mu\text{m}$  long. The pedal groove is lined by leaf-shaped scales, 60–80  $\mu\text{m}$  long, (Fig. 5D, e). Posterior sclerites (Fig. 5D, f) include scalpel-shaped solid elements, 80–110  $\mu\text{m}$  long, short acicular solid spines close to the mantle cavity opening, and long, pointy hollow needles (240–260  $\mu\text{m}$  long) dorso-laterally on the posterior body end.

A pair of bundles of abdominal spines is present in the posterior region of the body. The spines are up to 750  $\mu\text{m}$  long and have a broadened but pointy distal end (Fig. 5C). The longest ones have a narrow cavity that is open on the proximal end and runs along less than half the length of the spine.

The cuticle is about 200  $\mu\text{m}$  thick and pierced by slender papillae with bulbous apices. In fixed specimens with the foot retracted, the pedal groove is about 200–300  $\mu\text{m}$  deep. The pedal pit is large with associated voluminous pedal glands. The foot has one main ciliated fold, on each side flanked by a very small ciliated fold and a large lateral fold lacking cilia.

The vestibulum contains numerous slender sensory papillae. The mouth is situated at the posterior end of the vestibulum. The pharynx is muscular and lined by a low, somewhat glandular epithelium (Fig. 6A). Extraepithelial (subepithelial) foregut glands are altogether lacking. No dorsal pharyngeal gland was observed but there is a dorsal tubular extension of the anterior pharynx. The function of this pharyngeal pouch, which was observed in all three specimens studied, is unknown and there are no distinct glandular cells associated with it. The radula is polystichous (Fig. 5B) with the central four of the in total 18–22 teeth (Fig. 7) in each row being the smallest (Fig. 6A). The radula sheath is short and the radula is almost as wide as long (300  $\mu\text{m}$   $\times$  400  $\mu\text{m}$  in the holotype). Anteriorly, the radula is supported by a highly muscularized “tongue” (Fig. 6A, insert). The ventrolateral foregut glands are type C or *Epimения*-type (Fig. 6B).

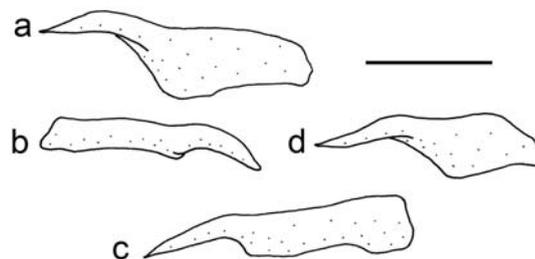


Fig. 7. *Proneomenia custodiens* sp. n., schematic drawings of isolated teeth of specimen paratype 3, **a**, obliquely lateral view; **b**, nearly dorsal view; **c**, lateral view; **d**, tooth of paratype 6 in an obliquely lateral view. Scale bar 20  $\mu\text{m}$ .

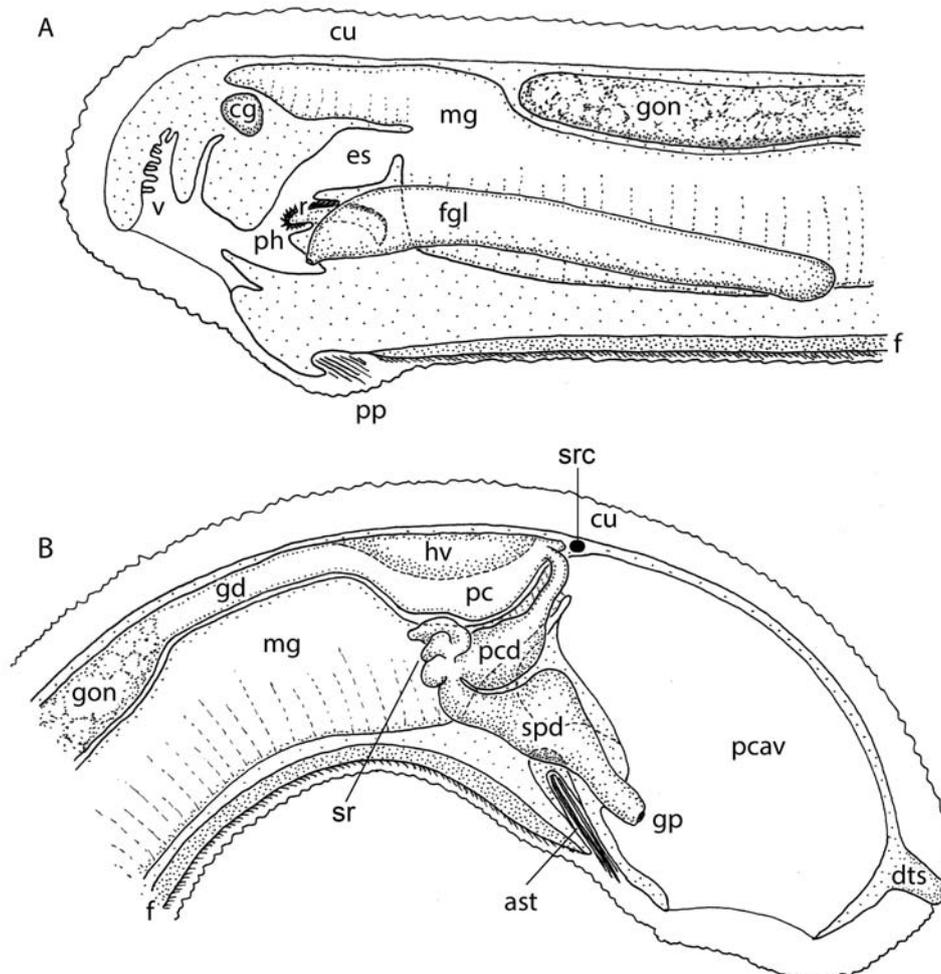


Fig. 8. *Proneomenia custodiens* sp. n., lateral reconstruction of internal anatomy, schematic drawings. **A.** Anterior body end. **B.** Posterior body end. Abbreviations: (ast) abdominal stylets (= abdominal spicules; not to be confused with copulatory stylets), (cg) cerebral ganglion, (cu) cuticle, (dts) dorsoterminal sense organ, (es) esophagus, (f) foot, (fgl) ventrolateral foregut gland, (gd) gonopericardioduct, (gon) gonad, (gp) gonopore, (hv) heart ventricle, (mg) midgut, (pc) pericardium, (pcd) pericardioduct, (pcav) pallial cavity, (ph) pharynx, (pp) pedal ciliary pit, (spd) spawning duct, (sr) seminal receptacles, (src) supra-rectal commissure, (v) vestibulum.

They are long and tubular, about ten times longer than wide. They open with narrow pores into a short subradular pouch (Fig. 8A). Notably, in the holotype, one of the ventrolateral foregut glands curves sharply upwards rather than projecting posteriorly (Fig. 6B). The esophagus is short, broadening dorsally prior to expanding into the midgut. The midgut has regular constrictions and the rectum is quite short and ciliated (Fig. 6C, D). The anus is located in the anterodorsal portion of the mantle cavity.

The nervous system is similar to that of other members of the family. Briefly, the cerebral ganglion is dumbbell-shaped, approximately  $500 \times 100 \times 100 \mu\text{m}$  (width  $\times$  maximum height  $\times$  maximum length). The lateral nerve cords are inconspicuous whereas the ventral ones are distinct. The suprarectal commissure is located dorsal to the anal region (Fig. 8B). A single dorsoterminal sensory organ is present at the body end. In brooding specimens, the mantle cavity opening is almost completely closed and at least in this case the dorsoterminal sense organ comes to lie in a terminal position (Fig. 8B).

The large and muscular heart ventricle is located within a spacious pericardium (Fig. 6C). Two types of erythrocytes were observed. One type is elongate and smooth in appearance with a fusiform to teardrop shape. These cells stained light pink and measure up to about  $3 \mu\text{m}$  in length and  $0.75 \mu\text{m}$  in diameter. No nuclei or longitudinal grooves were observed. The other type is generally spherical and covered with surface granulations. These cells stained a more deep red and are up to  $1 \mu\text{m}$  in diameter. Accumulations of erythrocytes can be found in the ventral and dorsal sinus, but interestingly also in lip-like lateral extensions in the mouth cavity.

All three of the histologically sectioned specimens were sexually mature. The hermaphroditic gonad reaches anteriorly almost to the foregut region. The gonopericardioducts are wide and paired throughout. From the posteriormost tip of the pericardium the paired pericardioduct extends ventrally, then widens and curves slightly anteriorly (Fig. 8B). The widening is due to the fact that the pericardioduct wall is differentiated into a multitude of small compartments that most likely represent seminal vesicles (Fig. 6D). In addition, there might be some glandular activity of the pericardioduct cells. Shortly before fusing with the spawning duct, the pericardioduct narrows again and there are three seminal receptacles connected to it (Figs 8B and 6C). Two of these receptacles are laterally flattened pouches and one is a short tube. The paired portion of the spawning duct (Fig. 6C, D) is quite slender, smaller in cross section than the pericardioduct, and about as long as the unpaired portion. The single genital pore is located on a short papilla extending into the mantle cavity (Fig. 8B).

In adult specimens, the mantle cavity is enlarged to a brooding chamber that may be filled with developing embryos or juveniles (Fig. 9A), each enclosed in a very thin egg hull (Fig. 9C). There are short, paired frontal pallial cavity pouches, but the majority of juveniles are kept in the pallial cavity proper, filling out all available space (Fig. 9C). In one case, a juvenile was even found in the rectum. Three specimens were observed to be brooding their young, some of which were clearly visible in the mantle cavity opening (Fig. 9A). In all three cases, the egg hulls contained well-developed juveniles bearing scale-like sclerites (Fig. 9B). No younger larval stages were observed. Thus we cannot state if the animals are direct developers or if they have intracapsular pericalymma larvae. We sectioned one specimen that was not brooding and two specimens that were. Histology revealed that about 90 juvenile specimens were carried by one of the brooding specimens

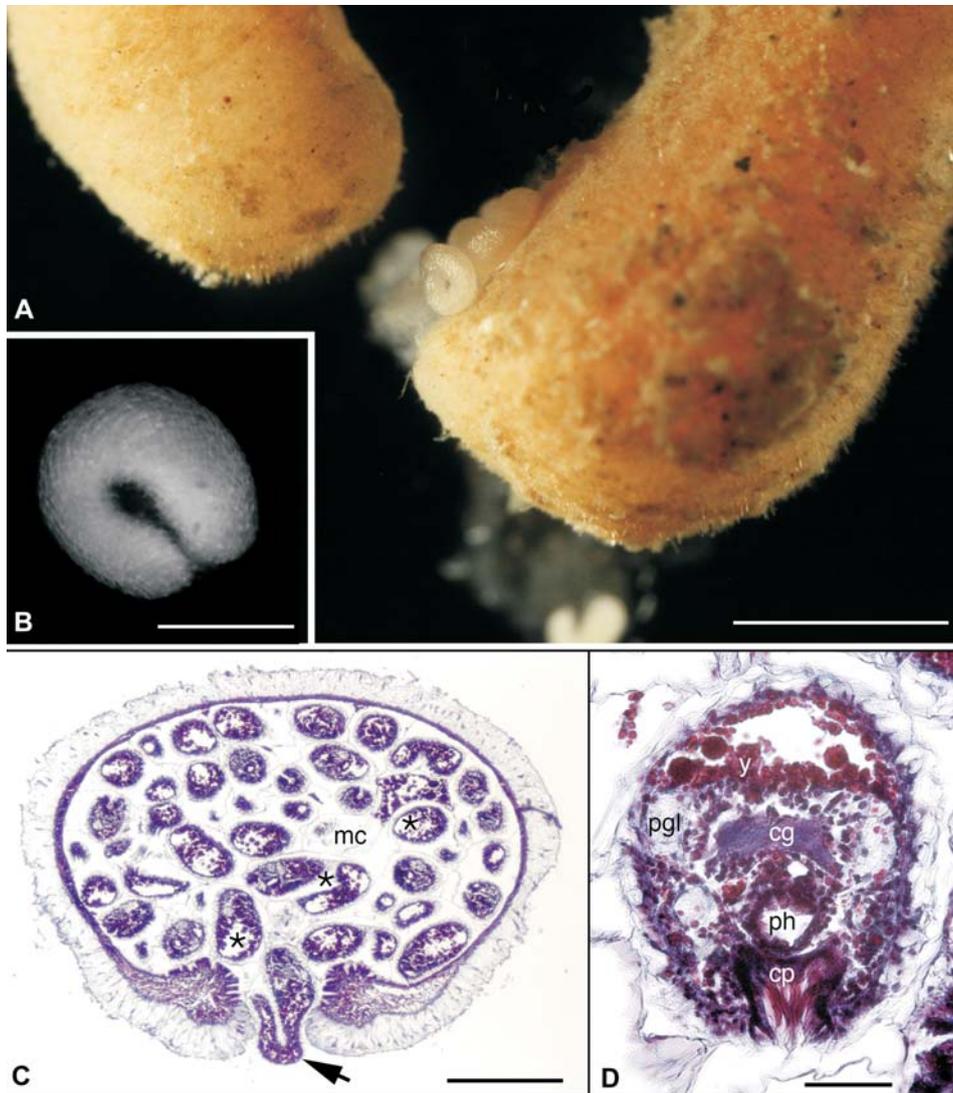


Fig. 9. *Proneomenia custodiens* sp. n. **A.** Photograph of a brooding specimen in the process of expelling its offspring, presumably a stress response (photo by D. Fiege); scale bar 0.8 mm. **B.** Microphotography of one juvenile (photo by M. Błażewicz-Paszkowycz); scale bar 100  $\mu$ m. **C.** Histological cross-section through the mantle cavity (mc) of the holotype = oding juveniles (asterisks); one juvenile is situated in the mantle cavity opening (arrow); scale bar 500  $\mu$ m. **D.** Cross-section of a juvenile from within the mantle cavity of the holotype, showing the cerebral ganglion (cg), pharynx (ph), pedal ciliary pit (cp), and pedal gland (pgl) of one of the juveniles. The specimen still contains yolk (y) and it is covered in cuticle and surrounded by the folded egg hull; scale bar 50  $\mu$ m.

(holotype). After collection and being placed in a dish, animals in the laboratory were observed to release their young over time, possibly as a stress response. Therefore, it seems likely that some of the developing juveniles were lost during

the sieving process and that this number could have been larger before the animals were disturbed. Likewise it is possible that the specimen with an empty brood pouch, which we sectioned, had released its young during the stress of collection. In histological section, the juveniles appear almost as miniature versions of their parents (Fig. 9D), each with a distinct cerebral ganglion, pharynx, foot and radula, except for a relatively thinner cuticle and an undeveloped genital tract.

**Taxonomic remarks.** — Of the eleven described species of *Proneomenia*, only *P. sluiteri* was previously known from polar waters in the Northern Hemisphere. Here we report the finding of a second species of *Proneomenia* from Icelandic waters in the Norwegian Sea. The new species, *P. custodiens* sp. n., is mature at a body length of 1 cm and thus of much smaller size than *P. sluiteri* with a maximum documented body length of 14.8 cm (Hubrecht 1881). Externally, *Proneomenia custodiens* sp. n. is easily distinguished from *P. sluiteri* by body shape (both body ends rounded whereas *P. sluiteri* has a pointed posterior end), surface characteristics of the integument (rougher surface than *P. sluiteri*) and – in living specimens – coloration (pinkish tan *versus* whitish). Additional differences are: the shape and size of sclerites, especially those close to the vestibular opening; higher number of radula teeth per row in specimens approximately 1 cm long (about 20 in *P. custodiens* sp. n. *versus* about 16 in *P. sluiteri*) and larger size of radula teeth; three short seminal receptacles at the junction of spawning duct and pericardioduct (as compared to one long tubular receptacle in *P. sluiteri*); mantle cavity in mature specimens developed into a brood pouch, mantle cavity epithelium lacks ciliation (densely ciliated in *P. sluiteri*). Thiele (1911) described another species of *Proneomenia*, *P. thulensis*, from similar collecting sites as *P. sluiteri* in Norwegian and Russian waters. This species was later transferred to the Genus *Thieleherpia* (Salvini-Plawen 2004) due to the presence of lateroventral foregut glands of type A (*Pararrhopalia*-type).

## Discussion

A single cast of the Agassiz trawl at IceAGE station 1223 yielded a large number of specimens of two species of *Proneomenia*. In addition to the proneomeniids, a single specimen of a third relatively large species of Solenogastres, tentatively identified as a member of the family Amphimeniidae, was also collected at this site. This species is easily distinguished from the proneomeniids by having a shorter and stouter body (*ca.* 1.5 cm long), having sclerites arranged in one layer, and being light pink in coloration.

Our collection of *Proneomenia sluiteri* from Icelandic waters in the Norwegian Sea during the IceAGE1 cruise and identification of this species in several BIOICE aplacophoran lots greatly expands the known biogeographic range for this species, making it one of the most widely distributed species of Solenogastres.

It has to be remarked here, however, that species records for Solenogastres in general are very patchy and thus only little is known about the biogeography of most taxa. Although we detected no differences between the specimens we examined and the detailed descriptions by Hubrecht (1881) and Heuscher (1892), it is possible that cryptic species may exist. Future studies employing molecular tools could help address this question. Sequences for the mitochondrial gene cytochrome c oxidase subunit I (COI) for both *P. sluiteri* and *P. custodiens* sp. n. are available on GenBank (*P. sluiteri*, accession number KJ568517; *P. custodiens*, accession number KJ568518). The sequence for *P. sluiteri* is for the entire coding region of the gene while the sequence of *P. custodiens* is incomplete at the 3' end.

The most notable characteristic of *Proneomenia custodiens* sp. n. is that it retains its young in a brood pouch. Brooding of juveniles in the mantle cavity was previously reported in other solenogasters including *Epimenia* (Baba 1938, 1951; Scheltema and Jebb 1994), *Halomenia gravida* (Heath, 1918), and some other pruvotinids (Thiele 1913; Salvini-Plawen 1978; C. Todt unpublished). All these cases are representatives of the order Cavibelonia and it is the pallial cavity that is adapted to form a brood pouch. In general, respiratory organs (folds or papillae) are small or reduced in the brooding taxa (own observation). An interesting biological aspect is that, at least in *P. custodiens* sp. n., there is no compartmentalization within the mantle cavity, which means that there is not only respiratory surface lost for the mother, but also that either all waste products get discharged onto the young or that the mother does not feed when breeding.

Heath (1918) describes larval development in *Halomenia gravida* based on histological sections and found that essentially all stages known from free-living larvae are found in the brood pouch and enclosed in egg-hulls (intracapsular larval development). The young in *P. custodiens* are intracapsular, as well, but we could only detect small juveniles and no younger embryos or larvae. One important difference between the juveniles and their mother is the type and distribution of sclerites on the body. Adult solenogasters ascribed to the order Cavibelonia (such as *Proneomenia*) generally have hollow, needle-like sclerites covering most of their body with solid scale-like or blade-like sclerites associated with the pedal groove. However, Okusu (2002) observed that around the time of metamorphosis, juveniles of *Epimenia babai* have flat, solid, blade-like sclerites evenly distributed over the body surface along with the hollow, needle-like adult type spicules and a second type of solid and flat sclerite associated with the pedal groove. Similarly, we observed flat, apparently solid, blade-like sclerites covering the body of juveniles of *Proneomenia custodiens* sp. n. Scale-like sclerites are typical of the putatively plesiomorphic solenogaster order Pholidoskepia. Similar sclerites in juvenile cavibelonians may represent a relict of the plesiomorphic condition of Solenogastres. Comparative mineralogical and developmental gene expression studies would be of particular interest towards addressing such questions about the evolution of the aplacophoran scleritome.

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