

Two new polychaete species living in the mantle cavity of *Calypptogena gallardoi* (Bivalvia: Vesicomidae) at a methane seep site off central Chile (~36°S)

EDUARDO QUIROGA¹ and JAVIER SELLANES^{2,3}

¹ Centro de Investigación en Ecosistemas de la Patagonia (CIEP), Bilbao 449, Coyhaique, Chile.
E-mail: eduardo.quiroga@ciep.cl

² Universidad Católica del Norte, Departamento de Biología Marina, Facultad de Ciencias del Mar, Larrondo 1281, Coquimbo, Chile.

³ Centro de Investigación Oceanográfica en el Pacífico Sur-Oriental (COPAS), Universidad de Concepción, Casilla 160-C, Concepción, Chile.

SUMMARY: Two new polychaete species belonging to Nautiliniellidae and Antonbruunidae were found in the mantle cavity of the vesicomid bivalve *Calypptogena gallardoi* Sellanes and Krylova, 2005, at a methane seep site off central Chile. *Shinkai robusta* n. sp. is characterized by having modified parapodia with robust notopodia and nine simple hooks per parapodium on the middle setigers, and an anteriorly truncated sub-triangular prostomium, with a pair of small cirriform antennae. The new species closely resembles *Shinkai longipedata* Miura and Ohta, 1991, and *Shinkai semilonga* Miura and Hashimoto, 1996. *Antonbruunia gerdesi* n. sp. is characterized by having a trapezoidal prostomium, with five sub-equal occipital antennae and a conspicuous pygidium with two short, well-developed digitiform anal cirri. These two new species constitute the first report of polychaetes living in symbiosis with chemosymbiotic bivalves in the south-eastern Pacific.

Keywords: methane seep, Nautiliniellidae, Antonbruunidae, symbiont polychaetes, Chile.

RESUMEN: DOS NUEVAS ESPECIES DE POLIQUETOS QUE HABITAN LA CAVIDAD DEL MANTO DE *CALYPTOGENA GALLARDOI* (BIVALVIA: VESICOMYIDAE) EN UN AFLORAMIENTO DE METANO FRENTE A CHILE CENTRAL (36°S). – Dos nuevas especies de poliquetos simbiotes pertenecientes a las familias Nautiliniellidae y Antonbruunidae se encontraron en la cavidad del manto del bivalvo vesicomíido *Calypptogena gallardoi* Sellanes y Krylova, 2005, proveniente de una zona de afloramiento de metano frente a Chile central. *Shinkai robusta* n. sp. se caracteriza por presentar parapodios modificados con notopodios bien desarrollados y robustos, nueve setas simples por parapodio en la región media del cuerpo y un prostomio sub-triangular, truncado anteriormente, con un par de pequeñas antenas cirriformes. La nueva especie es similar a *Shinkai longipedata* Miura y Ohta, 1991, y *Shinkai semilonga* Miura y Hashimoto, 1996. *Antonbruunia gerdesi* n. sp. se caracteriza por presentar un prostomio trapezoidal, con cinco antenas occipitales sub-iguales, incluyendo una antena central bien desarrollada, insertada en la superficie dorsal posterior del prostomio, y un pigidio conspicuo con dos cirros anales cortos digitiformes. Estas dos nuevas especies constituyen la primera cita de poliquetos simbiotes de bivalvos quimiosimbióticos en el Pacífico sur-oriental.

Palabras clave: afloramiento de metano, Nautiliniellidae, Antonbruunidae, poliquetos simbiotes, Chile.

INTRODUCTION

Benthic communities thriving in reducing habitats such as methane seeps and hydrothermal vents are constituted by rich invertebrate assemblages

that host chemoautotrophic symbionts (e.g. Sibuet and Olu, 1998; Sibuet and Olu-Le Roy 2002; Sahl-ing *et al.*, 2002; Van Dover *et al.*, 2003), as well as many heterotrophic species that also benefit from the chemosynthesis and methane-derived carbon (Bis-

coito *et al.*, 2002; Van Dover *et al.*, 2003; Levin, 2005; Cordes *et al.*, 2007). Communities of benthic animals associated with methane seeps (also known as cold seeps) have been found in several locations on active and passive continental margins of the Pacific and Atlantic Ocean (Olu *et al.*, 1996; Olu-Le Roy *et al.*, 2004; Ravara *et al.*, 2007; Cordes *et al.*, 2007). There have been some recent studies on the occurrence of cold seepage and gas hydrates on the central Chile margin (Morales, 2003; Sellanes *et al.*, 2004, 2008). Clams dominate the chemosymbiotic fauna at a cold seep site located off central Chile (36°S) called the Concepción Methane Seep Area (CMSA) (Sellanes *et al.*, 2004; Sellanes and Krylova, 2005). The chemosymbiotic assemblages at the CMSA include vesicomid, solemyid, lucinid and thyasirid bivalves (Sellanes and Krylova 2005; Holmes *et al.*, 2005; Oliver and Sellanes, 2005), as well as a siboglinid tubeworm of the genus *Lamelibrachia* (Sellanes *et al.*, 2008).

Vesicomid bivalves are typical organisms of marine chemosynthesis-based communities (e.g. Sibut and Olu-Le Roy, 2002; Van Dover *et al.* 2003; Sellanes *et al.*, 2008). In the CMSA, the most frequently collected species is *Calypptogena gallardoii*, and two polychaete species belonging to the families Nautiliniellidae (Miura and Laubier, 1989) and Antonbruuniidae Fauchald, 1977 were found within its mantle cavity. Nautiliniellids are a small group of polychaetes and all the species reported so far live as symbionts within deep-sea bivalve molluscs from hydrothermal vents and cold seeps (e.g. Miura and Laubier, 1989,

1990; Blake, 1993; Miura and Hashimoto, 1996; Dreyer *et al.*, 2004; Ravara *et al.*, 2007). This family has eleven genera, which are characterized by having a muscularized foregut, paired antennae, simple neuropodial hooks, and a rounded pygidium without anal cirri (Blake, 1993; Dreyer *et al.*, 2004). However, the taxonomic status and ecology of this family are still not fully understood (Blake, 1993, 1997). The family Antonbruuniidae was recognized by Fauchald (1977), and is closely related to the family Nautiliniellidae (Martin and Britayev, 1998). *Antonbruunia viridis* Hartmann and Boss, 1965, the only species described so far, is associated with the shallow water (62 to 82 m depth) bivalve mollusc *Lucina fosteri* Hartmann and Boss, 1965.

In fact, since both families are morphologically very close, it has been suggested that their taxonomic position should be redefined (see Martin and Britayev, 1998). The two new species described in this paper constitute the first report of symbiont polychaetes of chemosymbiotic bivalves in the south-eastern Pacific.

MATERIALS AND METHODS

Study site

The site is located 72 km NW off Concepción Bay, Chile, at the slope zone (750 to 900 m water depth) and near a mound separated by a shallow depression from another mound (Fig. 1). The pres-

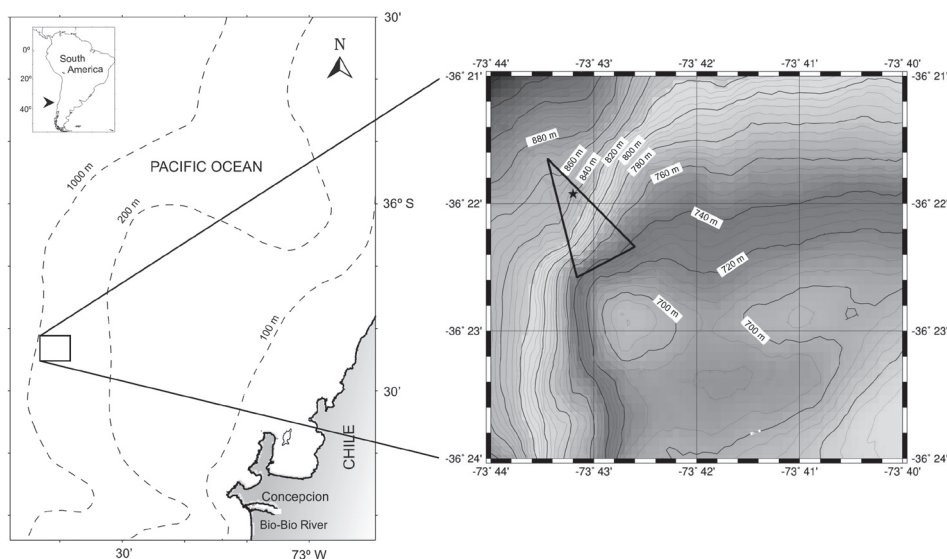


FIG. 1. – Study site, located on the slope zone off central Chile (36°S). The triangle indicates the area in which trawls successfully collected evidence of active methane seepage (carbonate blocks, live chemosymbiotic clams and shell fragments). The star indicates the locations where shallow sub-surface gas hydrates have been observed.

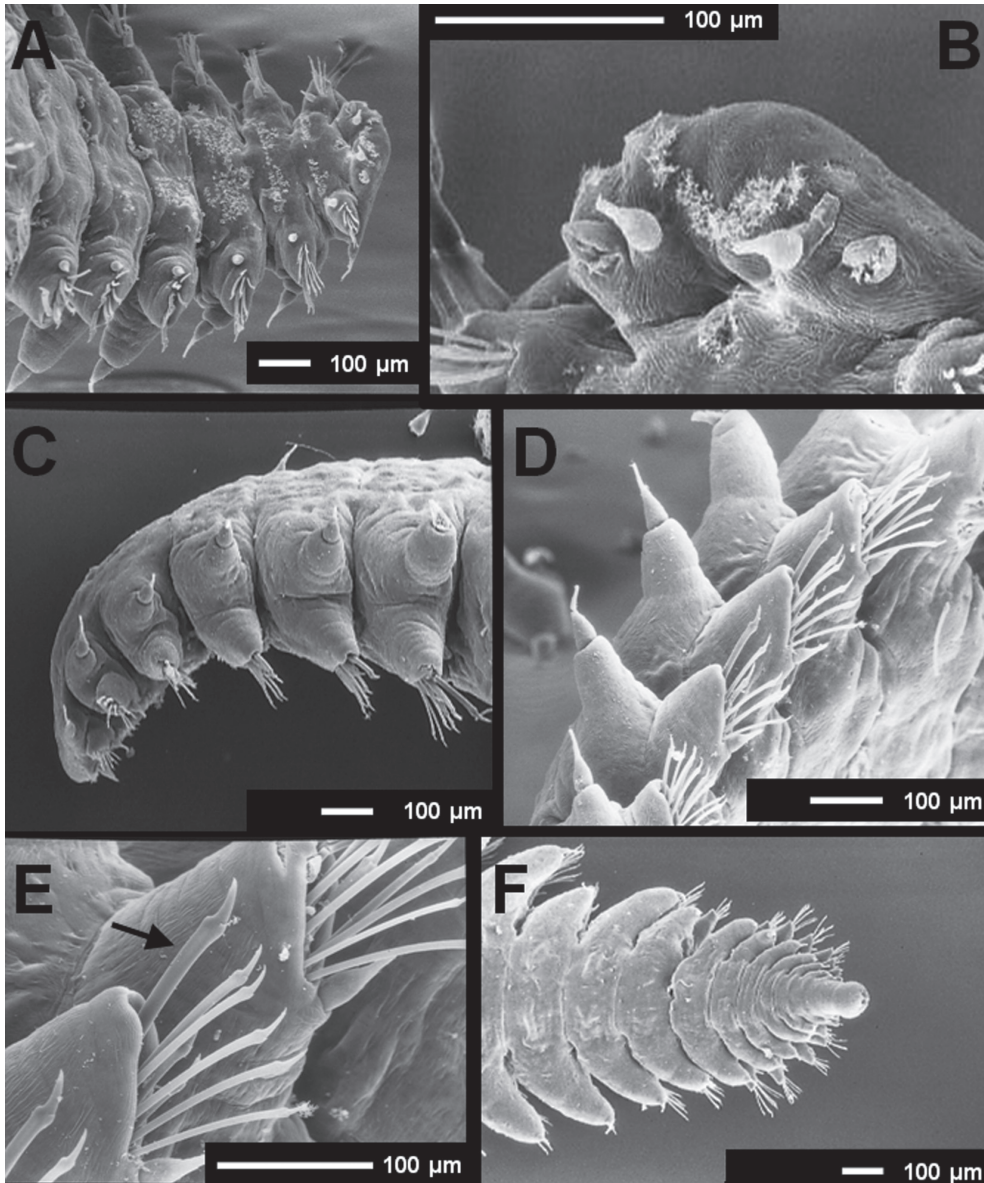


FIG. 2. – *Shinkai robusta* n. sp. (AGT10/VG-07): A, anterior region, ventral view; B, prostomium, ventral view; C, anterior region, lateral view; D, parapodia of the middle region; E, hooks of anterior parapodia (arrow showing a stout hook); F, pygidium, dorsal view.

ence of mounds and pockmarks has been previously documented for areas rich in subsurface gas hydrate deposits (Dando *et al.*, 1991; Sassen *et al.*, 2001, 2003). The sediment surface is characterized by the presence of abundant carbonate-cemented mud blocks (Sellanes *et al.*, 2004, 2008), while high concentrations of methane and sulfide, as well as gas hydrates, have been documented in sediment cores (Coffin *et al.*, 2006). Chemosymbiotic bivalves include vesicomysids, the solemyid *Acharax* sp., the lucinid *Lucinoma anemiophila*, and the thyasirids *Thyasira methanophila* and *Conchocele* sp. (Holmes *et al.*, 2005; Oliver and Sellanes, 2005; Sellanes *et al.*, 2008).

Collection of samples

Polychaete specimens were collected during oceanographic cruises conducted onboard RV “Vidal Gormáz” of the Chilean Navy during October 2004 (VG-04 cruise), September 2006 (SeepOx cruise), and September 2007 (VG-07 cruise). The biological material was obtained with an Agassiz trawl (mouth opening 1.5 x 0.4 m, mesh size 10 x 10 mm at the cod-end) in 20 minute hauls.

Specimens of *Calyptogena gallardo* were dissected onboard immediately after collection and polychaetes were extracted and fixed in 10% formalin and later preserved in 70% ethanol. Details of

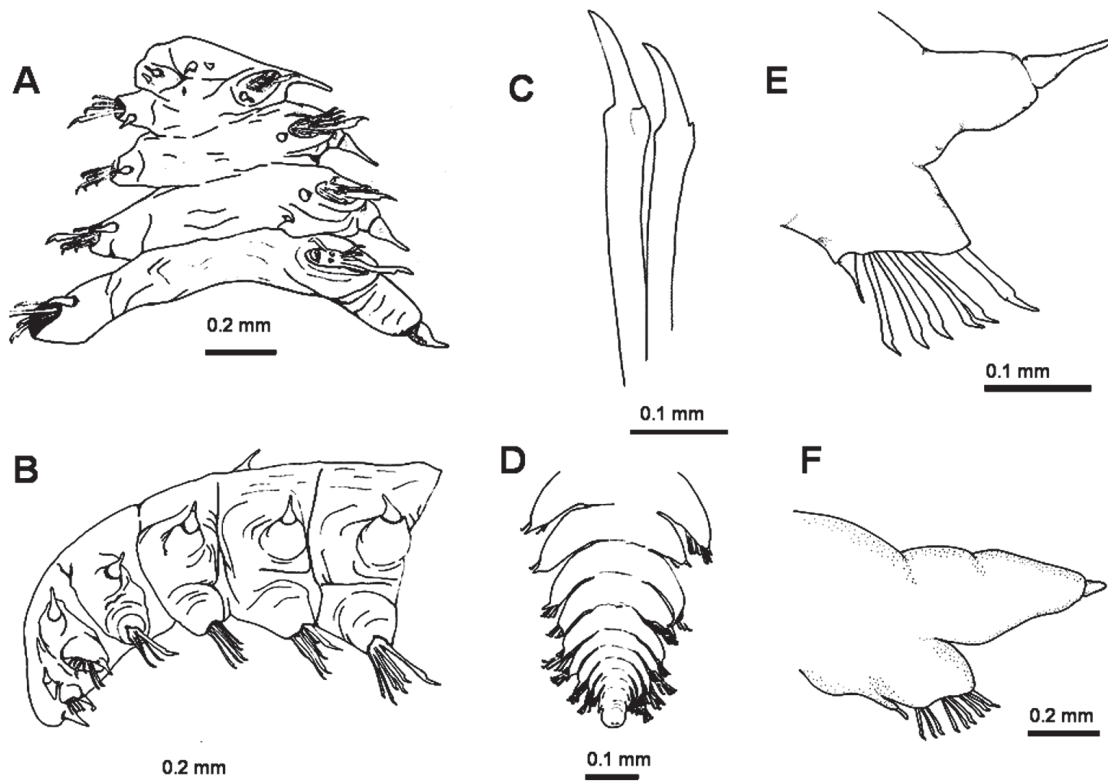


FIG. 3. – *Shinkai robusta* n. sp. (AGT10/VG-07): A, anterior region, ventral view; B, anterior region, lateral view; C, neuropodial hooks from parapodium 6; D, pygidium, dorsal view; E, parapodium 6 of the holotype; F, middle parapodium.

the setae were examined under immersion oil with a compound microscope. The terminology used follows Miura and Laubier (1989, 1990), Miura and Ohta (1991), Miura and Hashimoto (1993, 1996) and Blake (1990, 1993). Scanning electronic microscope (SEM) photographs were obtained (JEOL JSM-T300 microscope) of critical-point dried, gold coated specimens (JEOL JFC-1100 fine coat ion sputter; Rouse and Pleijel, 2001).

Type specimens are deposited in the “Museo Nacional de Historia Natural”, Santiago, Chile (MNH-NCL) and the reference collection of the Centre for Advance Studies in Patagonian Ecosystems (CIEP), Coyhaique, Chile.

SYSTEMATIC ACCOUNT

Family NAUTILINIELLIDAE (Miura and Laubier, 1989)

Genus *Shinkai* Miura and Laubier, 1990

Shinkai robusta n. sp.

(Figs. 2 and 3)

Type material. Holotype (MNHNCL-AN2043), complete specimen, collected from the mantle cavity of *Calyptogena gallardoii*. Type locality: off Concepción, Chile (36°21'64"S,

73°43'57"W), 865-926 m deep. October, 11, 2004 (AGT7/VG-04). Paratype (1 specimen, used for SEM photographs), complete specimen, collected from the mantle cavity of *Calyptogena gallardoii*. Type locality: off Concepción, Chile (36°22'01"S, 73°43'10"W), 764-843 m deep. October 02, 2007 (AGT10/VG07).

Description. (Holotype); complete specimen with 78 segments, measuring 13.86 mm long, 2.13 mm wide, including parapodia. Paratype; complete specimen with 82 setigers, measuring 19.20 mm long, 2.32 mm wide, including parapodia. Body elongated, vermiform, flattened ventrally, strongly arched dorsally and with longitudinal ventral groove. Colourless in alcohol. Prostomium short, sub-triangular with pair of small cirriform antennae, without eyes or other appendages. Mouth opening ventrally between prostomium and first setiger (Fig. 2A-C, 3A-B). Foregut with well-developed muscular region without jaws and paragnaths. First segment partially fused with prostomium. Achaetous peristomial ring absent (Fig. 2C). Parapodia subbiramous with well-developed dorsal cirri and reduced ventral cirri (Fig. 2D-E and 3A-B, E-F). Neuropodia well developed, supported by single, stout neuroacicula; ventral cirri shorter than dorsal cirri (Fig. 3E, F). Anterior neuropodia with simple hooks (Fig. 3C), six on parapodia

1-10, one of the hooks is stouter than the others (see Fig. 2C, E and 3C, E, F). Posterior neuropodia with up to nine simple hooks per neuropodium, all of them similar (Fig. 3F). Pygidium rounded, without anal cirri (Fig. 2F, 3D).

Remarks. The new species closely resembles the other congeneric species. In general, the parapodia and the head region of *Shinkai robusta* n. sp. are similar to those of the *Shinkai semilonga* and *Shinkai longipedata* specimens; however, the main diagnostic characters are the shape of the neuropodial hooks, their number on each parapodium and the sub-triangular prostomium with a pair of small cirriform antennae. In fact, *S. robusta* n. sp. differs from all other species of the genus as it has anterior neuropodia with six hooks on parapodia 1-10, and one of the hooks is stouter than the others. In *S. semilonga*, the head and first eight setigers are depressed antero-posteriorly. The neuropodial hooks are simple and slightly curved at the distal end. The number of hooks per parapodium is about 15 on parapodia 1-3, and more than 25 on parapodia 4-6, then decreases to about ten on parapodium 10, and then five to eight on parapodia 50 to 200 (Miura and Hashimoto, 1996; Fig. 4A-E). In *S. longipedata* the middle and posterior parapodia are armed by a single neuropodial hook with a strongly curved distal fang (Fig. 4F-I; Miura and Hashimoto, 1996; Dreyer *et al.*, 2004). *Shinkai sagamensis* differs from the above three species as it has very short notopodia and a different number of hooks on each parapodium (Fig. 4J-L). In fact, several hooks projected from each neuropodium on the anterior parapodia, e.g. 3-4 on parapodium 1, 5-8 on parapodia 2-6, 1-3 on parapodia 7-20 and 1 on the posterior parapodia (Miura and Laubier, 1990).

Habitat. All previously known nautiliniellid species are symbionts associated with bivalve molluscs from cold seeps or hydrothermal vents (Table 1). The genus *Shinkai* is close to *Nautiliniella*, but differs in having up to eight hooks per neuropodium instead of one, and a single pair of antennae instead of two. In contrast, *Natsushima* differs from the other two genera as it has two types of neuropodial spines instead of only one (Blake, 1993). The genus *Shinkai* comprises, at present, three described species: *Shinkai longipedata* Miura and Otha, 1991, *Shinkai semilonga* Miura and Hashimoto, 1996 and *Shinkai sagamensis* Miura and Laubier, 1990, all from Japan. The occurrence rate in bivalves of *S.*

TABLE 1. – Families Nautiliniellidae and Antonbruunidae: List of species, host bivalve species, collection depth, location and author references.

Species	Host bivalve	Depth (m)	Location	References
Family Nautiliniellidae				
<i>Flascarpia albinae</i> Blake, 1993	Unknown	3303	Florida Escarpment	Blake, 1993
<i>Iheomytilicola tridentatus</i> Miura and Hashimoto, 1996	<i>Bathymodiolus adulooides</i> Hashimoto and Okutani, 1994 (Mytilidae)	1395	Okinawa Trough	Miura and Hashimoto, 1996
<i>Laubierius mucronatus</i> Blake, 1993	Mytilidae	3243	Florida Escarpment	Blake, 1993
<i>Miura spinosa</i> Blake, 1993	Unknown	565	Santa Maria Basin	Blake, 1993
<i>Mytilidiphila enseisensis</i> Miura and Hashimoto, 1993	<i>Bathymodiolus</i> sp. (Mytilidae)	625	Okinawa Trough	Miura and Hashimoto, 1993
<i>Mytilidiphila okinawaensis</i> Miura and Hashimoto, 1993	<i>Bathymodiolus adulooides</i> Hashimoto and Okutani, 1994 (Mytilidae)	701	Okinawa Trough	Miura and Hashimoto, 1993
<i>Natsushima bifurcata</i> Miura and Laubier, 1990	<i>Solemya</i> sp. (Solemyidae)	1114	Sagami Bay	Miura and Laubier, 1990
<i>Natsushima graciliceps</i> Miura and Hashimoto, 1996	<i>Solemya</i> sp. (Solemyidae)	98	Kagoshima Bay	Miura and Hashimoto, 1996
<i>Nautiliniella calyptogenicola</i> (Miura and Laubier, 1989)	<i>Calyptogenia phasecoliformis</i> Mériuier <i>et al.</i> , 1986 (Vesicomomyidae)	5650	Japan Trench	Miura and Laubier, 1989
<i>Petrecca thyasira</i> Blake, 1990	<i>Thyasira insignis</i> (Verrill and Bush, 1898) (Thyasiridae)	3700	Laurentian Fan	Blake, 1990
<i>Thyasiridicola branchiatus</i> Miura and Hashimoto, 1996	<i>Conchocele disjuncta</i> Gabb, 1866 (Thyasiridae)	1160	Sagami Bay	Miura and Hashimoto, 1996
<i>Vesicomynicola trifurcatus</i> Dreyer <i>et al.</i> , 2004	<i>Calyptogenia</i> sp. (Vesicomomyidae)	2155	Blake Ridge Diapir	Dreyer <i>et al.</i> , 2004
<i>Shinkai longipedata</i> Miura and Otha, 1991	<i>Calyptogenia</i> sp. (Vesicomomyidae)	1400	Okinawa Trough	Miura and Otha, 1991
<i>Shinkai sagamensis</i> Miura and Laubier, 1990	<i>Calyptogenia soyaae</i> Okutani, 1957 (Vesicomomyidae)	1170	Sagami Bay	Miura and Laubier, 1990
<i>Shinkai semilonga</i> Miura and Hashimoto, 1996	<i>Calyptogenia solidissima</i> Okutani <i>et al.</i> 1992 (Vesicomomyidae)	625	Okinawa Trough	Miura and Hashimoto, 1996
<i>Shinkai robusta</i> n. sp.	<i>Calyptogenia gallardoi</i> Sellanes and Krylova, 2005 (Vesicomomyidae)	764-926	Central Chile, CMSA	Present study
Family Antonbruunidae				
<i>Antonbruunia viridis</i> Hartman and Boss, 1965	<i>Lucina fosteri</i> Hartman and Boss, 1965 (Lucinidae)	68-82	Mozambique Channel	Hartmann and Boss, 1965
<i>Antonbruunia gerdesi</i> n. sp.	<i>Calyptogenia gallardoi</i> Sellanes and Krylova, 2005 (Vesicomomyidae)	795-843	Central Chile, CMSA	Present study

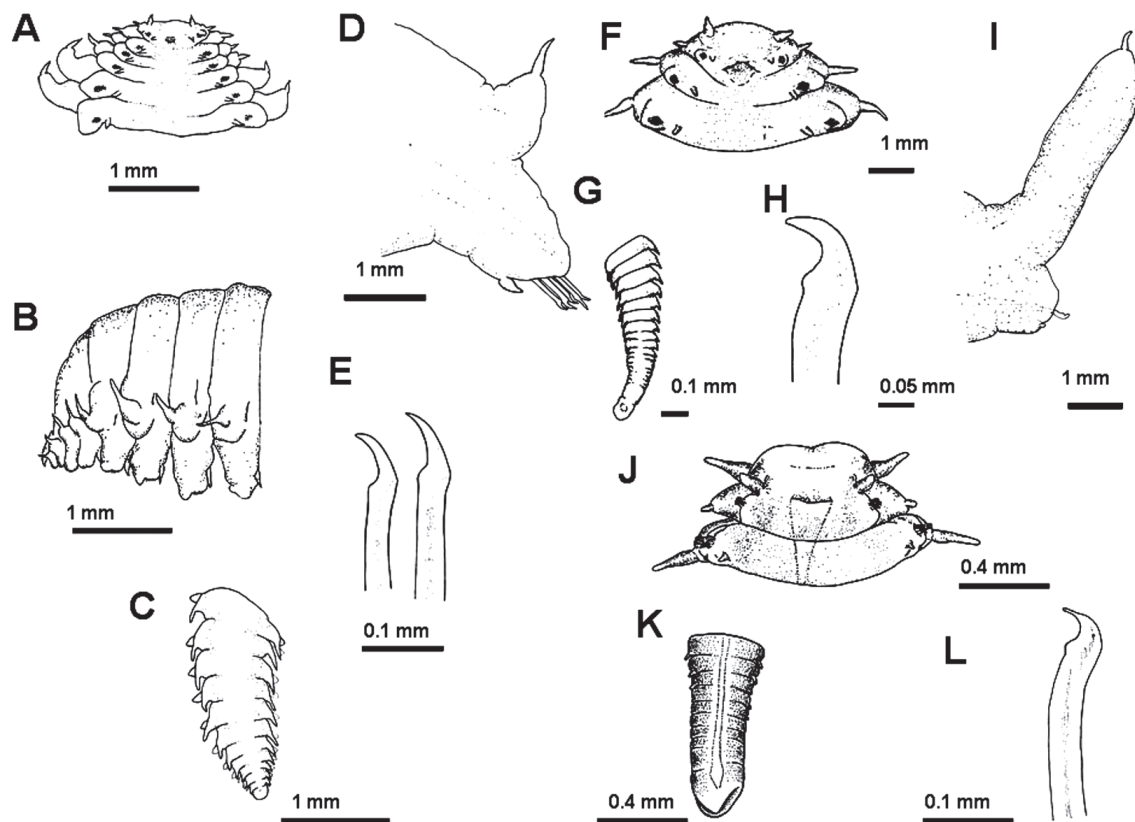


FIG. 4. – *Shinkai semilonga* (after Miura and Hashimoto, 1996): A, anterior region, dorsal view; B, anterior region, lateral view; C, pygidium, dorsal view; D, parapodium 100; E, neuropodial hooks. *Shinkai longipedata* (after Miura and Otha, 1991): F, anterior region, dorsal view; G, pygidium, dorsal view; H, neuropodial hook; I, parapodium 100. *Shinkai sagemensis* (after Miura and Laubier, 1990): J, anterior region, dorsal view; K, pygidium, dorsal view; L, neuropodial hook.

robusta n. sp. is very low, and only two of the 35 specimens of *C. gallardoi* analyzed hosted a single individual of this polychaete species.

Distribution. Only known from the type locality off Concepción, at a depth of 764 to 926 m. *Shinkai robusta* n. sp. is the first nautiliniellid species reported at a cold seep site in the south-eastern Pacific area. To date, its host bivalve *C. gallardoi* is also only known from the type locality.

Etymology. The species' name is derived from their characteristically elongated and robust notopodia.

Family ANTONBRUUNIDAE Fauchald, 1977
Genus *Antonbruunia* Hartman and Boss, 1965
Antonbruunia gerdesi n. sp.
(Figs. 5 and 6)

Type material. Holotype (MHNCL-AN2044), complete specimen, collected from the mantle cavity of *Calyptogena gallardoi*. Type locality; off Concepción, Chile (36°22'19"S, 73°43'36"W),

843-846 m deep. 04 September, 2004 (AGT6-9/SeepOx). Paratype (1 specimen, used for SEM photographs), complete specimen, collected from the mantle cavity of *Calyptogena gallardoi*. Type locality; off Concepción, Chile (36°21'93"S, 73°42'84"W), 795-843 m deep. 02 September, 2006 (VG-06/SeepOx).

Description. (Holotype); complete specimen with 33 segments, measuring 5.86 mm long, 1.10 mm wide, including parapodia. Paratype; complete specimen with 53 segments, measuring 16.40 mm long, 2.04 mm wide, including parapodia. Body vermiform, flattened ventrally, slightly arched dorsally, without longitudinal ventral groove (Fig. 5A-C, and Fig. 6A). Colour green in life, white after preservation. Prostomium short and trapezoidal with five sub-equal cirriform antennae, including an unpaired median antenna, without eyes or other appendages. Frontal antennae shorter, inserted ventrally on prostomium (Fig. 5A, B and 6B). Median antenna inserted posteriorly on the dorsal surface of the prostomium. Pharynx without jaws, paragnaths. Peristomial achaetous ring with two pairs of long, well-developed cirri (Fig. 5A, B and 6B). Parapodia increasing gradually to the post-anterior and mid-body region (Fig. 5A). All parapodia

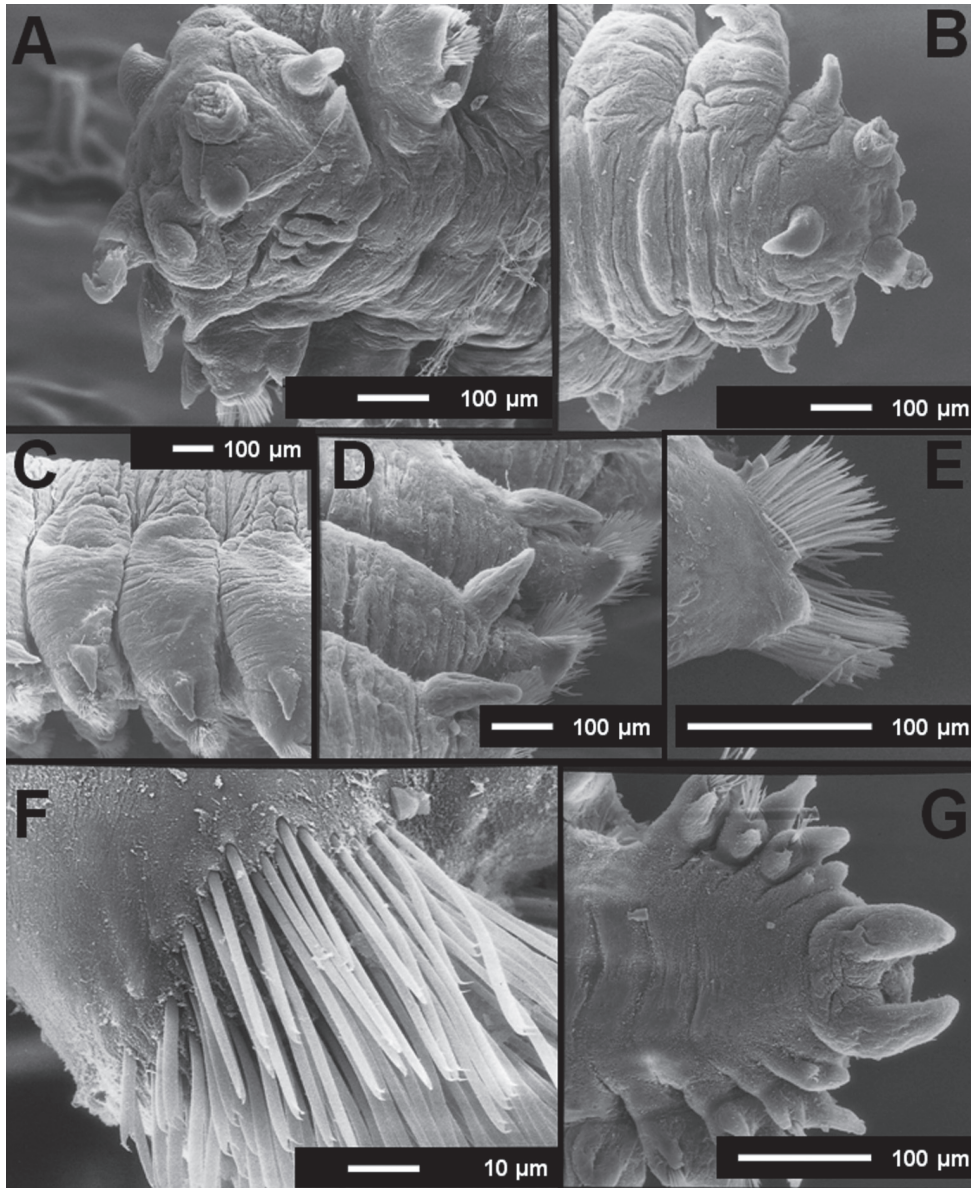


FIG. 5. – *Antonbruunia gerdesi* n. sp. (AGT6-9/SeepOx): A, anterior region, ventral view; B, anterior region, dorsal view; C, parapodia of the middle region lateral view; D, parapodia of the middle region, ventral view; E, hooks of the middle region; F, hooks; G, pygidium, ventral view.

dia subbiramous (Fig 5C-E and 6C). Notopodia with dorsal cirrus and a slender embedded notoaciculum, straight and pointed. All setae simple with long straight shaft, bifid, with two straight teeth of similar length (Fig. 5E, F and 6B, D). Pygidium conspicuous, with two well-developed digitiform cylindrical anal cirri (Fig. 5G, 6C).

Remarks. *Antonbruunia gerdesi* n. sp. differs from *A. viridis* in the shape of its notopodia (elongated and wide), and the presence of five sub-equal occipital antennae on the prostomium (Fig. 6A-D, 6F-H). The hooks of *A. gerdesi* n. sp are simple, with

a long, straight, distally bifid shaft (Fig. 6E). In contrast, those of *A. viridis* are curved distally (Miura and Laubier, 1990; Fig. 6H). The only known species that represents the family Antonbruunidae so far, *Antonbruunia viridis* inhabits the mantle cavity of its host bivalve *Lucina fosteri* (Table 1), which occurs in hypoxic sediments of black-brown oozy mud and detritus, off the coast of Mozambique, Indian Ocean (Hartman and Boss, 1965).

Habitat. The host bivalve of *A. gerdesi* n. sp. (*C. gallardoi*) is only known from the type locality in the south-eastern Pacific (Sellanes and

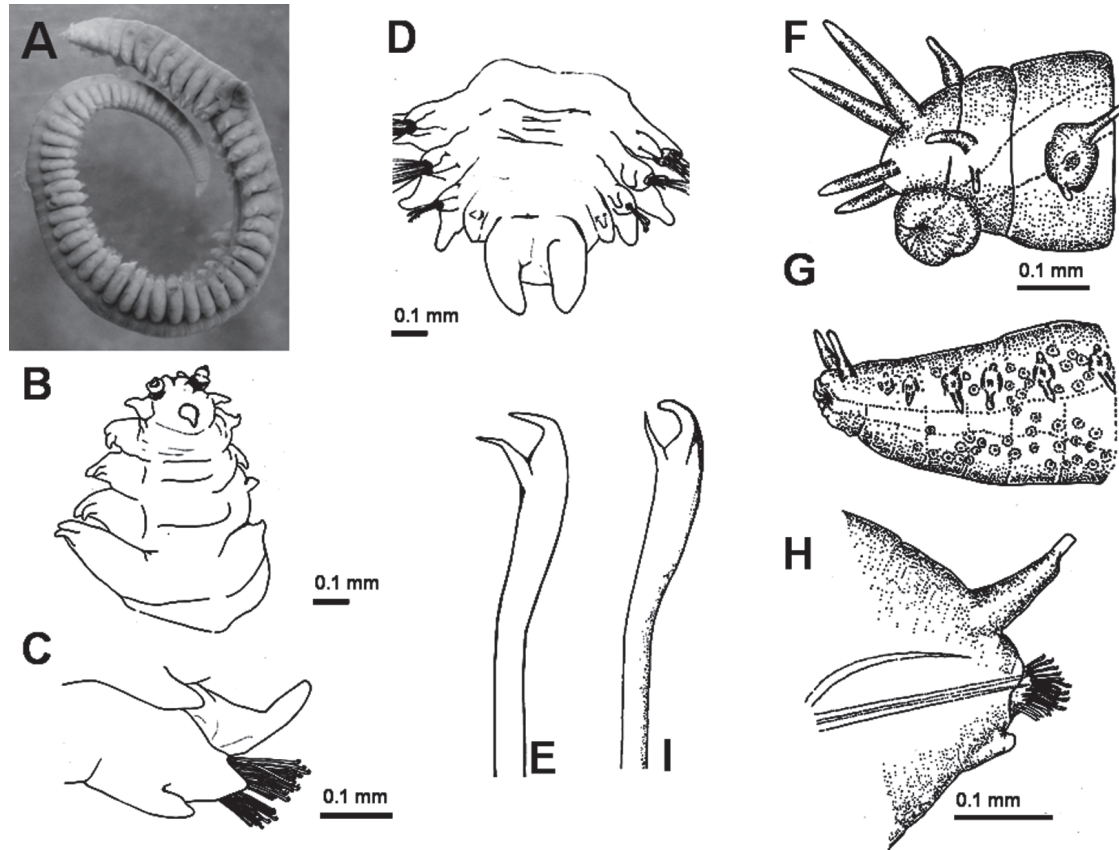


FIG. 6. – *Antonbruunia gerdesi* n. sp. (AGT6-9/SeepOx): A, photograph of whole body, lateral view; B, anterior region, dorsal view; C, middle parapodium; D, pygidium, ventral view; E, hook. *Antonbruunia viridis* Hartman and Boss, 1965 (after Miura and Laubier, 1990): F, anterior region, lateral view; G, pygidium, lateral view; H, posterior parapodium; I, hook (after Hartman and Boss, 1965).

Krylova, 2005), where it inhabits sticky dark grey sediments smelling of sulphide. Carbonate crusts also occur in the area, and gas hydrates were retrieved from subsurface sediments nearby. Like the other species described in the present study, the occurrence rate of *A. gerdesi* n. sp. is low: four of the 35 specimens of *C. gallardoi* analyzed hosted an individual of this polychaete. Although in general each bivalve hosted a single polychaete, three specimens were found in one host bivalve that measured 9 mm long. It is also worth noting that no co-occurrence of *A. gerdesi* n. sp. and *S. robusta* n. sp. has been observed within a single specimen of *C. gallardoi*, but to our knowledge this is the first time that two different polychaete species have been found associated with the same vesicomymid host species.

Distribution. Only known from the type locality off Concepción (Chile), 795–846 m depth. *Antonbruunia gerdesi* n. sp. is the second known species of the family, the first antonbruunid found in the Pacific Ocean, and the first report of the family at a cold seep site.

Etymology. The species is named in honour of Dr. Dieter Gerdes (Alfred Wegener Institute for Polar and Marine Research, Germany) who has studied marine benthic communities of the South American and Antarctic margins extensively.

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