SUMMARY: Two new polychaete species belonging to Nautiliellidae and Antonbruunidae were found in the mantle cavity of Calyptogena gallardoi (Bivalvia: Vesicomyidae) at a methane seep site off central Chile (~36°S). 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, Nautiliellidae, 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). Las nuevas especies de poliquetos simbiontes pertenecientes a las familias Nautiliellidae y Antonbruunidae se encontraron en la cavidad del manto del bivalvo vesicómiido Calyptogena 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 simbiontes de bivalvos quimiosimbóticos en el Pacífico sur-oriental.

Palabras clave: afloramiento de metano, Nautiliellidae, Antonbruunidae, poliquetos simbiontes, 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-
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 vesicomyid, 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 Lamellibrachia (Sellanes et al., 2008).

Vesicomyid bivalves are typical organisms of marine chemosynthesis-based communities (e.g. Sibuet and Olu-Le Roy, 2002; Van Dover et al., 2003; Sellanes et al., 2008). In the CMSA, the most frequently collected species is Calyptothena gallardoi, 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-
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 vesicomyids, 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 gallardoi were dissected onboard immediately after collection and polychaetes were extracted and fixed in 10% formalin and later preserved in 70% ethanol. Details of
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 (MNHNC1) and the reference collection of the Centre for Advance Studies in Patagonian Ecosystems (CIEP), Coyhaique, Chile.

SYSTEMATIC ACCOUNT

Family Nautiliellidae (Miura and Laubier, 1989)
Genus Shinkai Miura and Laubier, 1990

Shinkai robusta n. sp.
(Figs. 2 and 3)

Type material. Holotype (MHNCL-AN2043), complete specimen, collected from the mantle cavity of Calyptogena gallardoi. 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 gallardoi. 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, vermiciform, flattened ventrally, strongly arched dorsally and with longitudinal ventral groove. Colourless in alcohol. Prostomium short, sub-triangulair 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 parapodium 1-3, and more than 25 on parapodium 4-6, then decreases to about ten on parapodium 10, and then five to eight on parapodium 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, e.g. 3-4 on parapodium 1, 5-8 on parapodia 2-6, 1-3 on parapodium 7-20 and 1 on the posterior parapodium (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 *Nautiliellia*, 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.

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

<table>
<thead>
<tr>
<th>Species</th>
<th>Host Bivalve</th>
<th>Depth (m)</th>
<th>Location</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Flascarpia alvinae</em></td>
<td><em>Laubierus mucronatus</em></td>
<td>398-450</td>
<td>Sagami Bay</td>
<td>Miura and Laubier, 1990</td>
</tr>
<tr>
<td><em>Iheyomytilidicola tridentatus</em></td>
<td><em>Bathymodiolus aduloides</em></td>
<td>625-701</td>
<td>Sagami Bay</td>
<td>Miura and Hashimoto, 1993</td>
</tr>
<tr>
<td><em>Miura spinosa</em></td>
<td><em>Bathymodiolus sp.</em> (Mytilidae)</td>
<td>358</td>
<td>Sagami Bay</td>
<td>Miura and Hashimoto, 1993</td>
</tr>
<tr>
<td><em>Mytilidiphila okinawaensis</em></td>
<td><em>Conchocele disjuncta</em></td>
<td>98-1160</td>
<td>Sagami Bay</td>
<td>Miura and Hashimoto, 1996</td>
</tr>
<tr>
<td><em>Nautiliniella calyptogenicola</em></td>
<td><em>Thyasiridicola branchiatus</em></td>
<td>1114-1170</td>
<td>Sagami Bay</td>
<td>Miura and Laubier, 1990</td>
</tr>
<tr>
<td><em>Phascolosoma phaseoliformis</em></td>
<td><em>Calyptogena sp.</em> (vesicomyidae)</td>
<td>1400</td>
<td>Sagami Bay</td>
<td>Miura and Otha, 1991</td>
</tr>
<tr>
<td><em>Shinkai longipedata</em></td>
<td><em>Calyptogena gallardoi</em></td>
<td>795-843</td>
<td>Central Chile</td>
<td>Present study</td>
</tr>
<tr>
<td><em>Shinkai robusta</em></td>
<td><em>Calyptogena soyoae</em></td>
<td>625-701</td>
<td>Sagami Bay</td>
<td>Miura and Hashimoto, 1996</td>
</tr>
<tr>
<td><em>Shinkai semilonga</em></td>
<td><em>Calyptogena solidissima</em></td>
<td>625-701</td>
<td>Sagami Bay</td>
<td>Miura and Hashimoto, 1996</td>
</tr>
<tr>
<td><em>Shinkai sagamensis</em></td>
<td><em>Calyptogena gallardoi</em></td>
<td>98-1160</td>
<td>Sagami Bay</td>
<td>Miura and Hashimoto, 1996</td>
</tr>
<tr>
<td><em>Shinkai robusta</em></td>
<td><em>Calyptogena gallardoi</em></td>
<td>98-1160</td>
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<td>Miura and Hashimoto, 1996</td>
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<td>Sagami Bay</td>
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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 (MNHCL-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 vermiciform, 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 parapo-
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
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 vesicomysid 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 *antonbruunia* 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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