Reproductive characteristics of *Calyptogena gallardoi* (Bivalvia: Vesicomyidae) from a methane seep area off Concepción, Chile

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Calyptogena gallardoi is a vesicomyid bivalve inhabiting a methane seep area located at a depth of 740–870 m off the Bay of Concepción, Chile. Vesicomyids host chemoautotrophic sulphide-oxidizing endosymbiont bacteria and are always found associated to reducing environments. In this study, the gonadal structure and the gametes produced by C. gallardoi *are described. Light microscopy is used to examine serial histological sections of the gonads, and scanning electron microscopy is used to visualize the external morphology of gametes. The gonads of both males and females are organized in ramified tubular acini. In males, mature sperm are stored near genital openings in acini lined with a secretor epithelium that resembles a seminal receptacle. Spermatozoids have bullet-like heads with an average length of 30.3 ± 2.6 μm (mean ± 1 SD). In females, the mature oocytes are driven toward the genital opening through evacuator conduits lined by 'paddle' cilia. The average diameter of oogonias is 11.6 ± 2.5 μm and that of mature oocytes is 273.8 ± 23.1 μm, making the size of the mature oocyte among the largest reported for bivalves. In addition,* C. gallardoi *is shown to have external sexual dimorphism. Shells of males are significantly smaller and more elongated with sloping postero-dorsal margin compared with shells of females. The data are discussed in the context of available information on reproductive biology of vesicomyids.*

INTRODUCTION

Chemosynthesis-based communities supported by reducing environments are distributed widely throughout the World Ocean. Despite their broad occurrence, these communities are scattered across the 'regular' deep-sea floor, interspersed among areas that are not suitable for hydrothermal vent and seep animals. In this aspect, reproductive biology of the organisms inhabiting chemosynthesis-based communities is particularly important, as it may improve our understanding of the mechanisms involved in their dispersal among remote habitats and recruitment into reducing communities.

Although a considerable body of data has been collected on the life histories of the main groups of chemosynthesis-based communities, the information on reproductive biology remains fragmented (see Tyler & Young, 1999 for review). Among bivalves, some reproduction data has been collected for *Acharax alinae* (Solemyidae) (Beninger & Le Pennec, 1997), *Bathypecten vulcani* (Pectinidae) (Le Pennec *et al.*, 2002), some species of *Bathymodiolus* (Mytilidae) (Le Pennec & Beninger, 1997; Eckelbarger & Young, 1999; Healy *et al.*, 2001; Colaço *et al.*, 2006; Dixon *et al.*, 2006; Tyler *et al.*, 2007), and some representatives of vesicomyids

(Berg, 1985; Endow & Ohta, 1990; Cary & Giovanni, 1993; Lisin *et al.*, 1997; Fujiwara *et al.*, 1998; Heyl *et al.*, 2007). It has been shown that bivalves from chemosynthesis-based communities do not have a common reproductive strategy (Tyler & Young, 1999), show different types of development (planktotrophic or lecithotrophic) and can have either the presence or absence of reproductive seasonality. Furthermore, reproduction patterns are conservative and considerably phylogenetically-constrained (Eckelbarger & Watling, 1995).

Vesicomyid bivalves are consistently one of the dominant components of reducing communities. This family is morphologically disparate and comprises about a hundred described living species. These highly specialized molluscs live in symbiosis with sulphide-oxidizing bacteria allocated in their gills.

Information regarding the reproductive patterns of vesicomyids is rather limited. Further study of these patterns would help in the understanding of the mechanisms of establishment and maintenance of the broad, though disconnected, areas characteristically inhabited by some vesicomyids (Kojima *et al.*, 2004; Krylova & Janssen, 2006; Krylova & Sahling, 2006). Moreover, comparing the reproductive patterns of vesicomyids with other chemosymbiotic bivalves found in reducing communities could help differentiate adaptive strategies specific to the family. Additionally, the morphology of the reproductive system, particularly the structure of spermatozoids, is helpful for the study of phylogenetic relationships and taxonomy (Popham, 1979; Healy, 1996).

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Chemosynthetic-based communities associated with methane seepage have been recently discovered in the bathyal zone off central Chile (Concepción Methane Seep area (CMSA)) (Sellanes *et al.*, 2004). A diverse assemblage of chemosymbiotic bivalves (including some species of vesicomyids, a lucinid, two thyasirids and a solemyid) has been reported for this region (Holmes *et al.*, 2005; Oliver & Sellanes, 2005; Sellanes & Krylova, 2005), with the most abundant species being the vesicomyid *Calyptogena gallardoi* (Sellanes & Krylova, 2005). The genus *Calyptogena* is widely distributed throughout the Pacific Ocean, occurring exceptionally along continental and island margins. However, in the south-eastern Pacific, *C. gallardoi* remains the only species of the genus described so far.

This paper aims to present data on the reproduction patterns of *C. gallardoi*. The gonadal structure and morphology of gametes were studied using a qualitative analysis made through the observation of serial histological sections and scanning electronic microscopy (SEM). We also report on the external morphological aspects of the shell which give evidence for the presence of sexual dimorphism, a trait uncommon among bivalves, though characteristic for the genus *Calyptogena* (Coan *et al.*, 2000; Krylova & Sahling, 2006).

MATERIALS AND METHODS

The study site is located 72 km north-west off Concepción Bay, Chile (36° 27.87 S 73° 43.25 W), in the upper slope zone at a water depth of 740 m to 870 m (Figure 1). General sampling was conducted onboard AGOR 'Vidal Gormáz' of the Chilean Navy during October 2004 (VG-04 cruise) and September 2006 (SeepOx cruise). The biological samples were collected by an Agassiz trawl (AGT; mouth opening 1.5 × 0.5 m, mesh size 10 × 10 mm at the cod end), in 20 minute hauls. Animals were sorted from the non-biological material and preserved onboard. A total of 21 specimens of *C. gallardoi* were allocated for this study. Seven specimens (5 females and 2 males) were collected during the VG-04 cruise and 14 (12 females and 2 males) during the SeepOx cruise.

Samples for SEM were fixed with 3% glutaraldehyde in a 0.025 M solution of cacodylate in micro-filtered seawater (pH: 7.2–7.4). The samples were then rinsed in cacodylate buffer, dehydrated in an ascending ethanol gradient from 20% to absolute ethanol, critical-point dried, mounted on tape, coated under vacuum with gold and examined with a JEOL T-300 scanning electron microscope.

Samples for histological analysis were preserved in Davidson's fixative for 24 hours, then rinsed in micro-filtered seawater, dehydrated from 30% to absolute ethanol and included in paraffin blocks. The 5 μm slices were dyed using haematoxylin–eosin according to standard technique and mounted for observation under light microscopy. The gonad morphology was studied using serial cuts from one specimen of each gender. The diameter of at least 100 oocytes was measured in three females in order to construct size–frequency histograms. Measurements were limited to oocytes with visible nucleoli.

A morphometric analysis of 14 specimens (only those with unbroken valves) was performed using digital images, which were analysed using the software Image-Pro Plus. Different measurements, including the total area of the valves, were obtained for each specimen. As an indicator of sexual dimorphism, the relation of total area over the length to height ratio was used to compare the valves of males and females. This relation should be higher for rounded shells and smaller for more elongated shells, which have a larger length to height ratio. The Student's statistical test (two way *t*-test; Zar, 1998) was used to asses morphological differences between sexes.

RESULTS

Calyptogena gallardoi is a gonochoric species. In both sexes the large gonads are embedded in the posterior–dorsal part of the visceral mass, behind the digestive gland, and
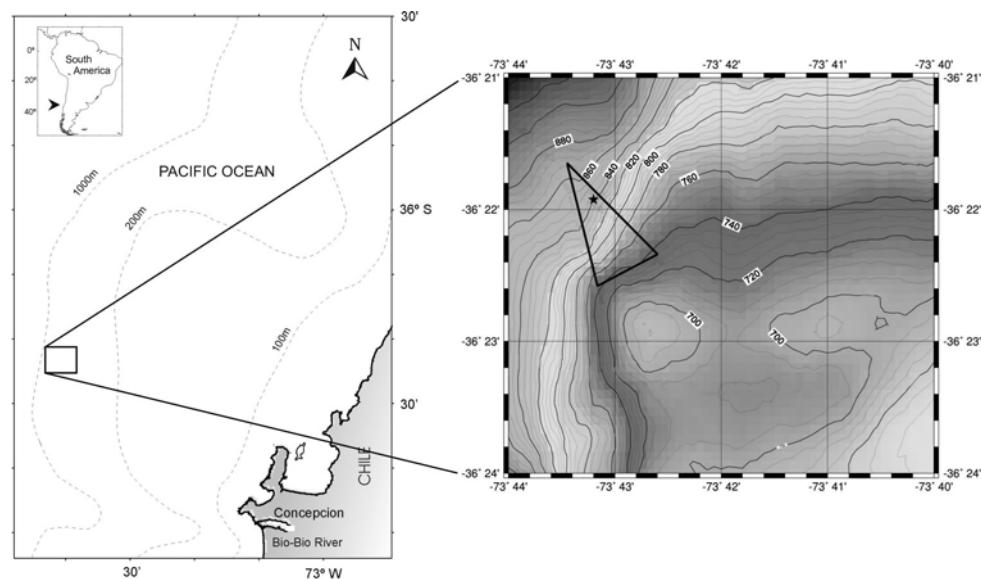

Fig. 1. Study area off Concepción Bay, central Chile. The triangle indicates the area where most of the trawlings that retrieved living specimens of *Calyptogena gallardoi* were performed. The star indicates the position in which the presence of shallow sub-surface gas hydrates has been observed.
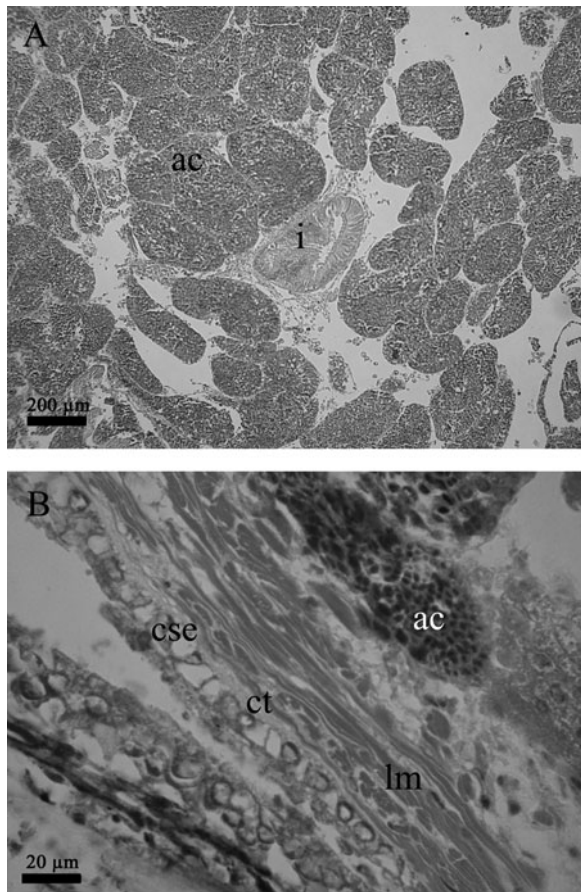
Fig. 2. Male gonad and lining epithelium. (A) General view of the gonad; (B) lining tissue. ac, acini with spermatozoids; cse, cubic simple epithelium; ct, connective tissue; i, intestine; lm, longitudinal musculature.
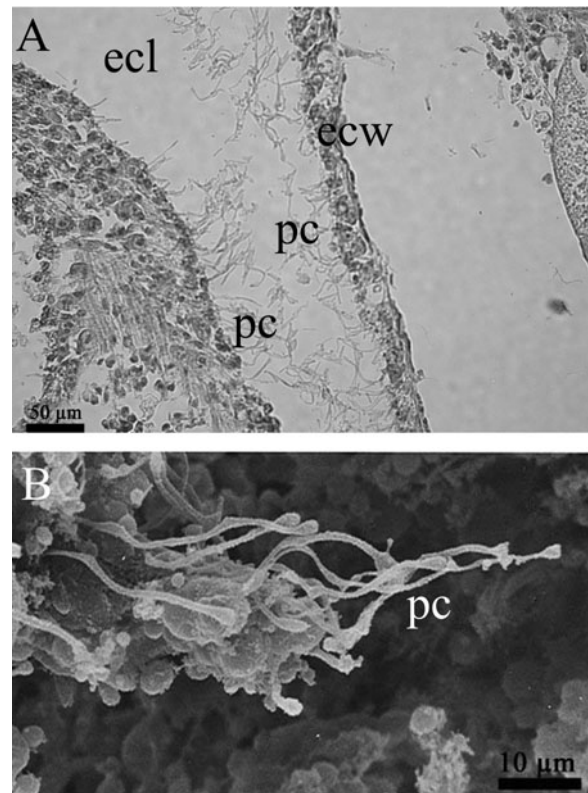

Fig. 3. Section of the ovary. (A) Light microscopy view of the evacuator conduit; (B) scanning electronic microscopy view of the evacuator conduit with the ciliae. ecl, evacuator conduit lumen; ecw, evacuator conduit wall; pc, paddle cilia.

surrounding the reduced digestive tube (Figure 2A). Slit-like genital apertures are located near the base of the posterior pedal retractor. The gonad has a lobular structure with many divisions that contain central tubes branching into smaller, tubular acini.

Female gonad

The internal surfaces of the evacuator conduits found in the acini are lined by cilia that form a bulky, or 'paddle', structure in the distal portion (Figure 3A, B); the length of the cilia is $31.6 \pm 4.5 \, \mu m$ (mean $\pm$ 1 SD).

Acini walls are lined by a thin, germinal epithelium where it is possible to distinguish oogonia with an average diameter of $11.6 \pm 2.5 \, \mu m$ (Figure 4A). The oogonia originate oocytes I of an average diameter of $17.7 \pm 2.4 \, \mu m$ (Figure 4A). Vitellogenesis appears to start once oocyte diameters reach $93.1 \pm 23.2 \, \mu m$ (Figure 4B, C) expanding to $190.1 \pm 27.0 \, \mu m$ (Figure 4B). The previtellogenic oocytes attach to the acini walls through strong pedicels that get longer as the development advances (Figure 4B). The mature oocytes measured $273.8 \pm 23.1 \, \mu m$ in diameter, and generally they are localized in the lumen of the acini. They group in large-sized platelets and are characterized by a great quantity of yolk. The germinal vesicle (Figure 4D) represents ~32% of the diameter of the mature oocytes and ~50% of the diameter of the previtellogenic oocytes. Within each gonad, and even within individual acini, sexual cells of all different developmental stages can be found (Figure 4D). Oocyte size–frequency analysis of 3 representative mature females demonstrated that, in spite of the predominance in number of previtellogenic oocytes, there is also a significant share of mature oocytes (Figure 5).

Male gonad

Tubular acini come together toward bigger acini. The parts of the wall of some larger acini located close to the genital aperture contain only mature sperm and are constituted of cubical ciliate epithelium composed of relatively big cells with a nucleus of great size. These regions of ciliate epithelium are limited by simple epithelium with flattened non-ciliated cells (Figure 2B).

The germinal epithelium of tubular acini is surrounded by a thin layer of connective tissue (Figure 2B). Spermatogonia, spherical in shape and granular in appearance, line the inside of the acini. The diameter of spermatogonia is $5.6 \pm 0.5 \, \mu m$. Toward the lumen of acini, it is possible to distinguish sexual cells in sequentially mature stages— spermatocytes, spermatides and spermatozoids. The mature spermatozoid has an elongated, bullet-shaped head measuring $3.7 \pm 0.2 \, \mu m$ in length from the tip of the acrosome to the base of middle piece constituted by a complex of 4 spherical mitochondria (Figure 6). The length of the flagellum is $26.5 \pm 2.5 \, \mu m$.
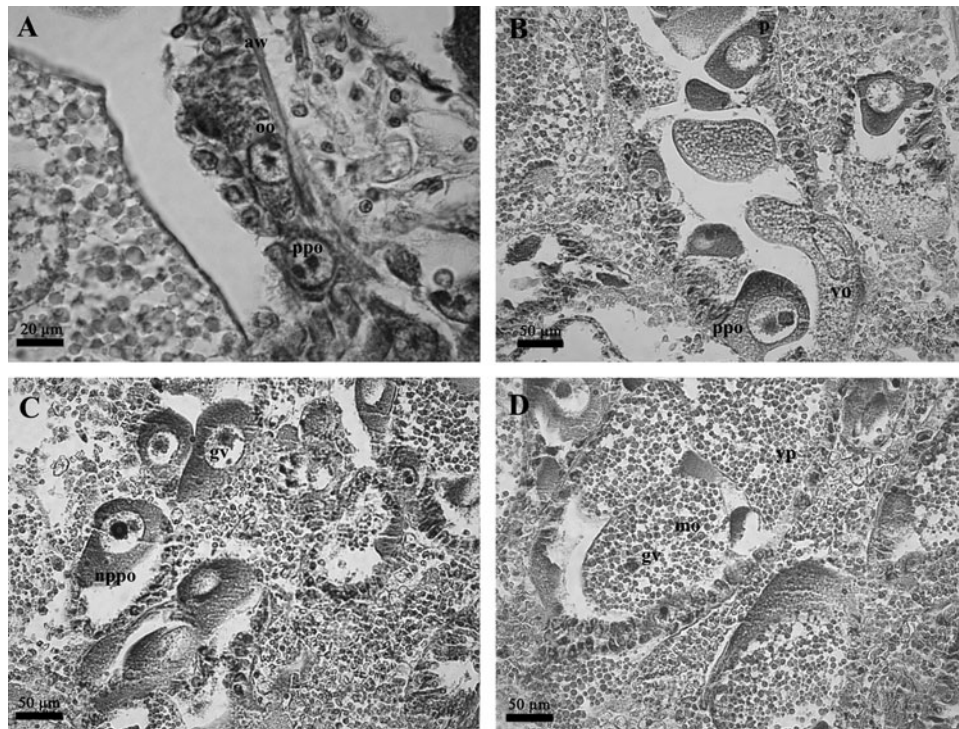
Fig. 4. Oocyte development stages within the ovary of *Calyptogena gallardoi*. (A) Early stages; (B) advanced stages; (C) previtellogenic oocytes; (D) mature stage. aw, acini wall; gv, germinal vesicle; mo, mature oocyte; nppo, non-pedunculated previtellogenic oocyte; oo, oogonia; p, pedicel; ppo, pedunculated previtellogenic oocyte; vo, vitellogenic oocyte; yp, yolk platelets.

Morphometric analysis of the valves

Calyptogena gallardoi has a stout shell up to 45 mm in length. The valves are elongate–elliptical, with a slightly convex, ventral margin. Significant differences in shell shape between males and females were found by comparing the relation of valve area to the ratio between the length and height. This relation, which is significantly higher in females (443.8) than in males (133.8) (*t*-test, $P < 0.01$), reflects that the shells of the females tend to be more rounded, while those of the males are more elongated (Figure 7). Males also have a more angulated posterior margin, sloping in its dorsal region. Furthermore, on average, the size of the female (31.4 ± 7.3 mm) is significantly larger than the male (21.5 ± 0.7; *t*-test, $P = 0.017$).

DISCUSSION

The new reproductive data gathered on *C. gallardoi* has been summarized in Table 1, along with the respective data for the seven other vesicomyid species that have been studied to date. These species have separate sexes, and the reported sex ratio for '*Calyptogena*' *magnifica* is 1:1 (Berg, 1985). All studied vesicomyids have large gonads embedded in the posterior–dorsal part of visceral mass adjacent to the digestive gland
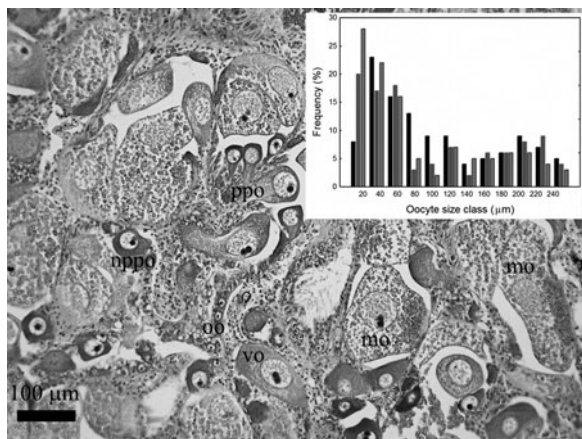

Fig. 5. Oocyte size–frequency distribution for three representative mature specimens of *Calyptogena gallardoi*, and image that exemplifies the presence of all oocyte development stages within the same gonad. mo, mature oocyte; nppo, non-pedunculated previtellogenic oocyte; oo, oogonia; ppo, pedunculated previtellogenic oocyte; vo, vitellogenic oocyte.
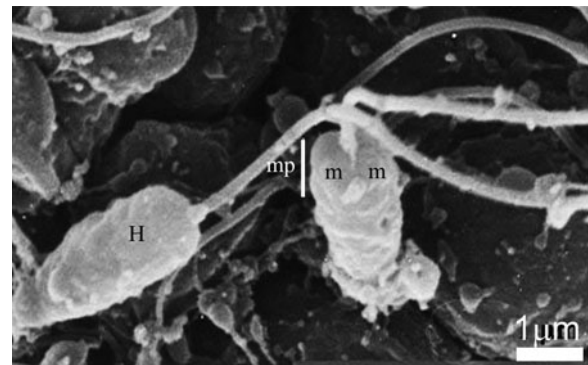

Fig. 6. Lateral and posterior views of the head of spermatozoids of *Calyptogena gallardoi*. H, head of the spermatozoid; m, mitochondria; mp, middle plate.
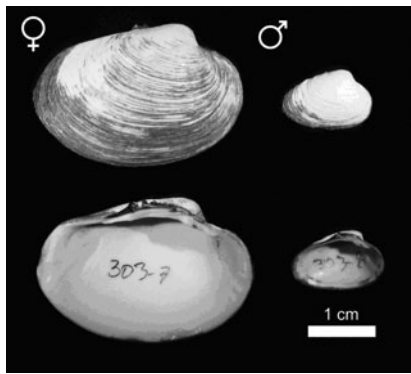
Fig. 7. Sexual dimorphism in *Calyptogena gallardoi*. External and internal views of the valves of a female (left) and of a male (right). Shells of females are larger, more rounded while males have a more angular posterior edge.

and surround the reduced gut. Genital apertures are slit-like and located in excurrent chambers near the base of posterior pedal retractor. The gonad of *C. gallardoi*, having tubular acini with branched divisions, follows this common pattern of gonadal organization.

Female reproductive system

In *C. pacifica*, '*C.*' *kilmeri* and a vesicomyid from Blake Ridge, the developing gametes are arranged peripherally around a central lumen within reproductive acini, which simultaneously contain gametes in all stages of development (Lisin *et al.*, 1997; Heyl *et al.*, 2007). The succession of the stages of oogenesis is typical for bivalves: oogonia, previtellogenic oocytes, pedunculated oocytes, vitellogenic oocytes and mature oocytes. In general, the gonadal organization in *C. gallardoi* is similar. The simultaneous presence of all developing stages of oogenesis would suggest that *C. gallardoi* displays continuous partial spawning, as it was proposed for other species of vesicomyids (Berg, 1985; Heyl *et al.*, 2007).

The average diameter of the female gametes in *C. gallardoi* (ranging from $11.6 \pm 2.5\,\mu m$ in oogonia to a maximum of $273.8 \pm 23.1\,\mu m$ in mature oocytes) is larger than the average size of the oocytes of most bivalves, and corresponds to, approximately, the dimensions of oocytes of most vesicomyids (Table 1). Among vesicomyids, the largest oocyte diameter is found in '*C.*' *magnifica* (Berg, 1985) which reaches $482\,\mu m$. In this species, 1–2 eggs are enclosed in gelatinous capsules (Berg & Alatalo, 1982).

In *C. pacifica*, a species of comparable size, the variation of the average diameter during the development of the oocytes is similar, and goes from $68.0–74.4\,\mu m$ in previtellogenic oocytes, to $80–100\,\mu m$ in vitellogenic oocytes, to $180–220\,\mu m$ in mature oocytes (Lisin *et al.*, 1997). The size of mature oocyte is related to the quantity of yolk and the type of feeding strategy of the larvae (Bruce, 1991; Lisin *et al.*, 1997; Tyler & Young, 1999). The large dimensions of the oocytes of vesicomyids indicate a lecithotrophic type of development. Beninger & Le Pennec (1997), in discussing the large oocytes of *Acharax alinae*, pointed out that the traditional interpretation of lecithotrophic development as short-term planktonic stage in conditions found in reducing habitats could be not true. Possibly, large amounts of yolk enable a prolonged larval stage to disperse through deep-sea oligotrophic areas which surround the scattered reducing environments. This

could also be true for vesicomyids, and would explain the wide but disconnected areas inhabited by some species.

A particular type of ciliature was observed in the evacuator conducts of *C. gallardoi*. This ciliature has been described as modified normal cilia, and named by several authors as 'paddle' cilia (Ehlers & Ehlers, 1978; Campos & Mann, 1988; Deiner & Tamm, 1991; Deiner *et al.*, 1993). The paddle-shaped cilia were previously seen in the veliger larval stage of some molluscs. Campos & Mann (1988) reported the presence of disc-like cilia and palette cilia in larvae of the bivalves *Mulinia lateralis* and *Spisula solidissima*, and suggested a possible locomotive and sensorial function for them. There are also indications that the palette cilia (as well as other modifications in the ciliature of molluscs) are probably artefacts generated by the formaldehyde, sodium phosphate and sodium cacodylate solutions used during the SEM treatment (Ehlers & Ehlers, 1978) or by the exposure of the samples to hypotonic media with variations in salinity (Deiner & Tamm, 1991; Deiner *et al.*, 1993). However, the results of this study suggest that the paddle-shaped cilia found in the evacuator conducts of *C. gallardoi* are not artefacts. Besides being observed through SEM, they were also found in gonadal tissue preserved in 70% ethanol and subjected to histological treatment. There are no reports of the occurrence of this type of artefact due to ethanol preservation. Paddle cilia in *C. gallardoi* are thought to have a transportation function inside the ovary, helping the movement of mature oocytes during their transit through the conduct. Further study of the nature of this ciliature is needed to confirm its function.

Male reproductive system

Spermatozoid morphology is a useful tool for taxonomic investigations, especially in such a taxonomically complicated group as vesicomyids. Data on spermatozoid morphology are available for '*Calyptogena*' *soyoae* (Fujiwara *et al.*, 1998); for '*C.*' *magnifica* and *C. pacifica* only some data on dimensions has been published (Le Pennec unpublished, in Beninger & Le Pennec, 1997). The species, '*C.*' *soyoae* and *C. gallardoi*, have 4 spherical mitochondria in the middle piece. This number of mitochondria is typical for most bivalves and for all studied veneroids (Popham, 1979). The shape of the spermatozoid head of *C. gallardoi* and '*C.*' *soyoae* is slightly different: both species have a bullet-shaped head, but in *C. gallardoi* it is more elongated than in '*C*' *soyoae* (Y. Fujiwara, personal communication).

The spermatozoids of *C. gallardoi* and those of its closely related species *C. pacifica* differ considerably in their flagellum length, with *C. gallardoi* having nearly twice the length of that found in *C. pacifica* (Le Pennec unpublished, in Beninger & Le Pennec, 1997).

There is an aspect of the male gonadal structure of *C. gallardoi* that has not been reported in other vesicomyids. In *C. gallardoi*, the walls of some large acini, located close to the genital opening consist of two types of specialized (probably secretor) epithelia: (i) cubical ciliate epithelium, with relatively large cells, each containing a nucleus of great size; and (ii) simple epithelium with flattened, non-ciliated cells that limit these acini. In this zone, only mature sperm that is ready for spawning has been found. This suggests the zone is a place for sperm storage, which is a characteristic that has not been reported for other members of this family. A similar receptacle for storing sperm is reported for the

Table 1. Reproductive characteristics of some representatives of the family Vesicomyidae.

Species	Size of mature oocyte, μm	Head shape of spermatozoid, number of mitochondria	Maximum length of head with middle piece; length of flagellum (μm)	Synchrony of gametogenesis within specimen	Temporal variations in gametogenesis	Seasonality in spawning	Synchrony of spawning	Site	Reference
'Calyptogena' magnifica	482		3; 8.5	Non-synchronous				East Pacific Rise, Galapagos Rift, 2450–2700 m and 21°N, 2000–2620 m	Berg & Alatalo, 1982; Berg, 1985; Le Pennec unpublished, in Beninger & Le Pennec, 1997
'Calyptogena' phaseoliformis	200							Japan Trench, 5640–5695 m	Fiala-Medoni & Le Pennec, 1989
'Calyptogena' soyae		Cylindrical-shaped, 4 mitochondria	4.5			Absent, 11 events in 1.5 years	Present	Sagami Bay, 1175 m	Fujiwara *et al.*, 1998
'Calyptogena' lauberi	200							Tenryu Canyon, 3787–3835 m	Fiala-Medoni & Le Pennec, 1989
'Calyptogena' kilmeri	180–237			Non-synchronous	Present			Monterey, 900 m	Lisin *et al.*, 1997
'Vesicomya' sp.	200			Non-synchronous				Blake Ridge, 2155 m	Heyl *et al.*, 2007
Calyptogena pacifica	180–220		3.5; 12	Non-synchronous				Monterey, 600 m	Lisin *et al.*, 1997
Calyptogena gallardoi	273.78 ± 23.12	Bullet-shaped, 4 mitochondria	3.92; 29	Non-synchronous				North-west off the Bay of Concepción, 760–800 m	This paper

hermaphroditic bivalves *Mysella bidentata*, *Barrimysia siphonosomae* (Jespersen & Lützen, 2001) and *Pseudopythina ochetostomae* (Jespersen *et al.*, 2002). According to Jespersen & Lützen (2001), it is not usual for a seminal receptacle to occur in gonochoric bivalves. The presence of a seminal receptacle in *C. gallardoi* would contribute to the continuous production of sperm. Once reaching maturity in the acini, sperm would pass into the seminal sac which would allow the acini to continue sperm producing. Further histological investigation would probably indicate such structure for other vesicomyids.

Reproductive cycle of vesicomyids

So far, information concerning temporal variations and seasonal-relative reproductive patterns of vesicomyids is unclear. A temporal investigation of gametogenesis was conducted only for 'C.' kilmeri and *C. pacifica* (Lisin *et al.*, 1997). While in 'C.' kilmeri, analysis of gonadal development through the year suggest a peak in reproductive output during winter, in *C. pacifica* analyses of variations in reproductive tissues were insufficient to resolve seasonal changes. In both species however, there were oocytes of spawning size found during all sampling periods (Lisin *et al.*, 1997).

In a vesicomyid from Blake Ridge, five reproductive condition stages were distinguished for the female gametes, all of them were simultaneously present and most females were in the developing and ripe reproductive stages, while all males were in ripe stage (Heyl *et al.*, 2007). A continuous reproductive pattern was also suggested for 'C.' magnifica given the simultaneous presence of all stages of gametes in the gonads (Berg, 1985).

For 'C.' soyoae, over the course of 1.5 years, eleven *in situ* spawning events have been recorded at the same site (Fujiwara *et al.*, 1998). These data, together with the TEM observations of gonads having similar maturity in specimens collected in summer and autumn, suggest a continuous spawning. The absence of seasonal peaks does not mean that there is no synchrony in reproduction efforts, at least among part of the population. Spawning of 'C.' soyoae was shown to be induced by a 0.1–0.2°C temperature rise. Males spawn first usually followed 7–11 minutes later by females releasing eggs. However, not every male spawning event was followed by the spawning of females (Fujiwara *et al.*, 1998). This observation supports the view that the presence of a sperm sac could be a generalized feature in vesicomyids. Such a structure would allow for the storage of large volumes of spermatozoids ready to be spawned once unpredictable appropriate environmental conditions occur.

Sexual dimorphism

Although most bivalves are gonochoric, external sexual dimorphism is unusual. Saleuddin (1964) describes the sexual dimorphism for *Astarte elliptica*, *A. sulcata* and *A. borealis*, reporting that the shell of the female is more elongated than that of the male. Except for these and a few other cases, the sex of bivalves can only be determined by direct examination of the gonads (generally under microscope) or by the observation of the spawning process (Sastry, 1979).

For *C. pacifica*, variation in the shell shape was described by Dall (1981) and this was later suggested to demonstrate sexual dimorphism impressed in the shell shape: the females have larger and more expanded posterodorsal shells (J.P. Barry, personal communication, in Coan *et al.*, 2000). Later the dimorphism was proposed for the whole genus *Calyptogena* (Krylova & Sahling, 2006), but it was not confirmed.

Variations in the shape of the shell of *C. gallardoi* were reported by Sellanes & Krylova (2005), who indicated the presence of more or less elongated specimens. They also stated that the outline of posterior margin varied from rounded to tapering. However, these observations were based on the valves of dead specimens. Currently, the parallel investigation of the reproductive system of *C. gallardoi* has allowed us to confirm that these morphs actually exhibit sexual dimorphism. Among vesicomyids described to date, sexual dimorphism is reported only for the genus *Calyptogena s.s.*

Concluding remarks

Many reproductive aspects of *C. gallardoi* such as the presence of external sexual dimorphism and the presence of large eggs, suggesting a lecithotrophic development of the larvae, confirm previous observations (often poorly documented) in other vesicomyids. Other aspects, like the presence of a sperm sac in males, as well as the evacuator conduits of the gonads lined by paddle cilia, though reported for different bivalves, are novel for this family. Future studies of the recently discovered seep habitats in the south-eastern Pacific will certainly shed light on further interesting biological aspects of this species, as well as on other representatives of the rich local fauna.

REFERENCES

Beninger P.G. and Le Pennec M. (1997) Reproductive characteristics of a primitive bivalve from a deep-sea reducing environment: giant gametes and their significance in *Acharax alinae* (Cryptodonta: Solemyidae). *Marine Ecology Progress Series* 157, 195–206.

Berg C.J. (1985) Reproductive strategies of mollusks from abyssal hydrothermal vent communities. *Bulletin of the Biological Society of Washington* 6, 185–197.

Berg C.J. and Alatalo P. (1982) Reproductive strategies of bivalves from deep-sea hydrothermal vents and interidal sulfide-rich environments. *Biological Bulletin. Marine Biological Laboratory, Woods Hole* 163, 397.

Bruce D. (1991) *Atlas of invertebrate reproduction and development.* 1st edition. New York: Wiley-Liss. 252 pp.

Campos B. and Mann R. (1988) Discocilia and paddle cilia in the larvae of *Mulinia lateralis* and *Spisula solidissima* (Mollusca: Bivalvia). *Biological Bulletin. Marine Biological Laboratory, Woods Hole* 175, 343–348.

Cary S.C. and Giovannoni S.J. (1993) Transovarial inheritance of endosymbiotic bacteria in clams inhabiting deep-sea hydrothermal vents and cold seeps. *Proceedings of the National Academy of Sciences of the United States of America* 90, 5695–5699.

Coan E.V., Scott P.V. and Bernard F.R. (2000) *Bivalve seashells of western North America. Marine bivalve molluscs from Arctic Alaska to BajaCalifornia.* Santa Barbara, CA: Santa Barbara Museum of Natural History.

Colaço A., Martins I., Laranjo M., Pires L., Prieto C., Costa V., Lopes H., Rosa D., Dando P.R. and Serrão-Santos R. (2006) Annual spawning of the hydrothermal vent mussel, *Bathymodiolus azoricus*, under controlled aquarium, conditions at atmospheric pressure. *Journal of Experimental Marine Biology and Ecology* 333, 166–171.

Dall H.W. (1981) On some new or interesting West American shells obtained from the dredgings of the U.S. Fish Commission steamer "Albatross" in 1888, and other sources. *Proceedings of the United States National Museum* 17, 675–733.

Deiner M. and Tamm S.L. (1991) Mechanism of paddle cilia formation in molluscan veligers. *Biological Bulletin. Marine Biological Laboratory, Woods Hole* 181, 335–336.

Deiner M., Tamm S.L. and Tamm S. (1993) Mechanical properties of ciliary axonemes and membranes as shown by paddle cilia. *Journal of Cell Science* 104, 1251–1262.

Dixon D.R., Lowe D.M., Miller P.I., Villemin G.R., Colaco A., Serrao-Santos R. and Dixon L.R.J. (2006) Evidence of seasonal reproduction in the Atlantic vent mussel *Bathymodiolus azoricus*, and an apparent link with the timing of photosynthetic primary production. *Journal of the Marine Biological Association of the United Kingdom* 86, 1363–1371.

Eckelbarger K.J. and Watling L. (1995) Role of phylogenetic constraints in determining reproductive patterns in deep-sea invertebrates. *Invertebrate Biology* 114, 256–269.

Eckelbarger K.J. and Young C.M. (1999) Ultrastructure of gametogenesis in a chemosynthetic mytilid bivalve (*Bathymodiolus childressi*) from a bathyal, methane seep environment (northern Gulf of Mexico). *Marine Biology* 135, 635–646.

Ehlers U. and Ehlers B. (1978) Paddle cilia and discocilia, genuine structures? *Cell Tissue Research* 192, 489–501.

Endow K. and Ohta S. (1990) Occurrence of bacteria in the primary oocytes of vesicomyid clam *Calyptogena soyoae*. *Marine Ecology Progress Series* 64, 309–311.

Fiala-Medoni A. and Le Pennec M. (1989) Adaptive features of the bivalve molluscs associated with fluid venting in the subduction zones off Japan. *Palaeogeography, Palaeoelimatology, Palaeoecology* 71, 161–167.

Fujiwara Y., Tsukahara J., Hashimoto J. and Fuikura K. (1998) *In situ* spawning of a deeep-sea vesicomyid clam: evidence for an environmental cue. *Deep-Sea Research* 45, 1881–1889.

Heyl T.P., Gilhooly W.P., Chambers R.M., Gilchrist G.W., Macko S.A., Ruppel C.D. and Van Dover C.L. (2007) Characteristics of vesicomyid clams and their environment at the Blake Ridge cold seep, South Carolina, USA. *Marine Ecology Progress Series* 339, 169–184.

Healy J.M. (1996) Molluscan sperm ultrastructure: correlation with taxonomic units within the Gastropoda, Cephalopoda and Bivalvia. In Taylor J. (ed.) *Origin and evolutionary radiation of the Mollusca.* Oxford: Oxford University Press, pp. 99–113.

Healy J., Buckland-Nicks J. and Barrie G.M. (2001) Spermatozoal ultrastucture of spiny oysters (Spondylidae, Bivalvia) including a comparison with other bivalves. *Invertebrate Reproduction and Development* 41, 27–37.

Holmes A., Oliver P.G. and Sellanes J. (2005) A new species of *Lucinoma* (Bivalvia: Lucinoidea) from a methane gas seep off the southwest coast of Chile. *Journal of Conchology* 38, 673–682.

Jespersen A. and Lützen J. (2001) Ultrastructure of the seminal receptacle and the dimorphic sperm in the commensal bivalve *Mysella bidentata* (Veneroida; Galeommatoidea; Montacutidae). *Acta Zoologica (Stockholm)* 82, 107–111.

Jespersen A., Lützen J. and Morton B. (2002) Ultrastructure of dimorphic sperm and seminal receptacle in the hermaphrodites *Barrimysia siphonosomae* and *Pseudopythina ochetostomae* (Bivalvia, Galeommatoidea). *Zoomorphology* 121, 159–172.

Kojima S., Fujikura K. and Okutani T. (2004) Multiple trans-Pacific migrations of deep-sea vent/seep-endemic bivalves in the family Vesicomyidae. *Molecular Phylogenetics and Evolution* 32, 396–406.

Krylova E.M. and Janssen R. (2006) Vesicomyidae from Edison Seamount (South Western Pacific: Papua New Guinea: New Ireland fore-arc basin) (Bivalvia: Glossoidea). *Archiv für Molluskenkunde* 135, 233–263.

Krylova E. and Sahling H. (2006) Recent bivalve molluscs of the genus *Calyptogena* (Vesicomyidae). *Journal of Molluscan Studies* 72, 359–395.

Le Pennec M. and Beninger P.G. (1997) Ultrastructural characteristics of spermatogenesis in three species of deep-sea hydrothermal vent mytilids. *Canadian Journal of Zoology* 75, 308–316.

Le Pennec G., Le Pennec M., Beninger P. and Dufour S. (2002) Spermatogenesis in the archaic hydrothermal vent bivalve, *Bathypecten vulcani*, comparison of spermatozoon ultrastructure with littoral pectinids. *Reproduction and Development* 41, 13–19.

Lisin E.S., Hannan E.E., Kochevar R.E., Harrold C. and Barry J.P. (1997) Temporal variation in gametogenic cycles of vesicomyid clams. *Invertebrate Reproduction and Development* 31, 307–318.

Oliver P.G. and Sellanes J. (2005) Thyasiridae from a methane seepage area off Concepción, Chile. *Zootaxa* 1092, 1–20.

Popham J.D. (1979) Comparative spermatozoon morphology and bivalve phylogeny. *Malacological Review* 12, 1–20.

Sastry A.N. (1979) Pelecypoda (excluding Ostreidae). In Giese A. and Pearse J.S. (eds) *Reproduction of marine invertebrates. Volume V, Molluscs: pelecypods and lesser classes.* New York: Academic Press, pp. 113–292.

Saleuddin A. (1964) The gonads and reproductive cycle of *Astarte sulcata* (Da Costa) and sexuality in *A. elliptica* (Brown). *Proceedings of the Malacological Society of London* 36, 141–148.

Sellanes J. and Krylova E. (2005) A new species of *Calyptogena* (Bivalvia: Vesicomyidae) from a recently discovered methane seepage area off Concepción Bay, Chile (∼36° S). *Journal of the Marine Biological Association of the United Kingdom* 85, 969–976.

Sellanes J., Quiroga E. and Gallardo V.A. (2004) First direct evidences of methane seepage and associated chemosynthetic communities in the

bathyal zone off Chile. *Journal of the Marine Biological Association of the United Kingdom* 84, 1065–1066.

Tyler P.A. and Young C.M. (1999) Reproduction and dispersal at vents and cold seeps. *Journal of the Marine Biological Association of the United Kingdom* 79, 193–208.

Tyler P.A., Young C.M., Dolan E., Arellano S.M., Brooke S.D. and Baker M. (2007) Gametogenic periodicity in the chemosynthetic cold-seep mussel '*Bathymodiolus*' *childressi*. *Marine Biology* 150, 829–840.

and

Zar H. (1998) *Biostatistical analysis.* 4th edition. Upper Saddle River, NJ: Prentice-Hall.

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