

## A swimming medusoid gonophore in the life cycle of *Ventromma halecioides* (Alder, 1859) (Hydrozoa: Leptothecata: Kirchenpaueriidae)

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**Abstract:** The swimming gonophore of *Ventromma halecioides* (Alder, 1859) is described for the first time. It is a short-lived cryptomedusoid, provided with a well-formed, elongated-ovoid umbrella with thick mesoglea which, in turn, lacks a gastrovascular system, tentacles and tentacle bulbs, as well as sense organs. Its solid spadix is attached laterally to the blastostyle, and is surrounded by a compact mass of gametes, filling completely the subumbrellar cavity. The bell margin is provided with a raised collar comprising numerous refringent granules, and a velum is present. Scattered pseudostenoteles occur on exumbrella, while transversely-set muscle cells line the subumbrella.

**Keywords:** Life cycle - medusoid - cryptomedusoid.

### INTRODUCTION

The production of swimming medusoid gonophores [for details, see Boero & Bouillon (1989)] among many “unusual” families of thecate hydroids is poorly documented and relatively misunderstood, as their occurrence is still unpredictable and often unexpected when it is discovered. To date, the presence of such a dispersive stage was already described in the lovenellid genus *Hydranthea* (Hincks, 1868) (Boero & Sarà, 1987), the hebellid genus *Anthohebella* Boero *et al.*, 1997 (original account), the haleciid genus *Nemalecium* Bouillon, 1986 (Gravier-Bonnet & Migotto, 2000; Galea *et al.*, 2012), the sertulariid genera *Amphisbetia* L. Agassiz, 1862 (Motz-Kossowska, 1907), *Sertularia* Linnaeus, 1758 (Migotto, 1998) and *Sertularella* Gray, 1848 (Gravier-Bonnet & Lebon, 2002), the plumulariid genera *Dentitheca* Stechow, 1919 (Migotto & Marques, 1999; Galea *et al.*, 2012) and *Monothea* Nutting, 1900 (Motz-Kossowska, 1907), the halopteridid genus *Antennella* Allman, 1877 (Bourmaud & Gravier-Bonnet, 2005), and the aglaopheniid genus *Macrorhynchia* Kirchenpauer, 1872 (Bourmaud & Gravier-Bonnet, 2004). Additional indications of occurrence of medusoids in other genera and/or species are scattered in the literature, and need to be confirmed through accurate observations.

The main obstacle to the discovery of swimming gonophores is, by far, the study of fixed material, followed by the disregard of histological studies. Except for the gonophores of *Hydranthea* and *Anthohebella*,

all those described until now in the families mentioned above belong to the cryptomedusoid type [for definition and classification, see Bouillon *et al.* (2006)], characterized by the presence of a well-defined umbrella which, in turn, is devoid of tentacles and tentacle bulbs, gastro-vascular system, and sense organs. The spadix is eccentric, solid and, consequently, does not open distally into a mouth. The subumbrella is provided with muscle cells, and a velum is present in the majority of cases, allowing the gonophores to swim, although these appear to be likely absent in rare instances (Bourmaud & Gravier-Bonnet, 2005; Galea *et al.*, 2012). In all but one family (*viz.* Sertulariidae), the bell margin bears a raised collar comprising a belt of refringent corpuscles whose function is not as yet totally understood, though it is possibly involved in the medusoid buoyancy. These bodies are labile in many fixatives, except perhaps the alcohol. Indeed, Kirchenpauer (1872: 16), noted – for the first time – their presence in nearly all species of *Macrorhynchia* he studied, but erroneously interpreted them as being oocytes. Additional observations by Bale (1894: 106; 1914: 30) and Allman (1883: 34) were likely done on alcohol-preserved specimens. The occurrence of refringent corpuscles in the gonophores is, most probably, a good indication of the production of swimming medusoids. However, their absence in the Sertulariidae could be an impediment even when working with living material, unless the spawning is monitored.

Leclère *et al.* (2009) have shown that swimming medusoid gonophores “are probably not secondary

simplified medusae but simple pelagic forms re-acquired independently from polyp-only ancestors”, provided with a set of morphological characters (e.g. umbrella with muscle cells, presence of a velum) gained subsequently after a previous lost.

*Ventromma halecioides* (Alder, 1859) is a well-known, nearly cosmopolitan species (Gravili *et al.*, 2015). Although its gonothecae were originally described and illustrated accurately by Alder (1859, pl. 12 fig. 4), the gonophore received lesser attention (“Pressed between plates of glass, the capsules showed the ova in a very early stage”). Some subsequent authors (e.g. Calder, 1997), reported the presence of fixed sporosacs, although Gravier-Bonnet & Migotto (2000) cite unpublished data of Migotto & Marques, according to which medusoids occur in this species.

Abundant, fertile colonies of this species were collected recently from the French coast of the Mediterranean Sea, allowing its reproduction to be documented.

## MATERIAL AND METHODS

Colonies of *V. halecioides* growing on floating docks in the port of La Ciotat were collected by scraping. The material was brought to the laboratory in a cooler, within an hour. The colonies were placed in crystallizing dishes covered by a fine mesh, and immersed in two 5 L tanks filled with fresh seawater (from the collecting site) and supplied with aeration, either in the dark or exposed at the daylight, at *ca.* 25°C. The dishes were inspected every 15 minutes for the medusoid release under a Huvitz HSZ-TR30 trinocular stereomicroscope equipped with a Canon EOS 60D camera. Male and female individuals were immediately placed in separate dishes, and kept in the same conditions as stated above. Photomicrographs were taken using a Tucsen IS1000 CMOS (10 MPixel) camera mounted on a Optika B-500 trinocular compound microscope, with either transmitted light or darkfield illumination. The experiment was done four times. All studied material was subsequently fixed in Bouin’s liquid, and part of it was transferred to 70% ethanol for long-term storage, while another part was stained in Grenacher’s borax carmine according to the protocol given by Gibbons & Ryland (1989), and mounted on microslides. To test the tolerance of the refringent granules to the pH, 20 µL of a 1 M HCl or 1 M NaOH solutions were pipetted into the seawater containing a few medusoids placed on a depression slide covered with a coverslip, and their behavior was monitored under the microscope. Part of the material studied here was deposited in the collection of the *Muséum d’histoire naturelle* of Geneva, Switzerland (registration is indicated by MHNG-INVE- followed by a 5-digit number), while another part is housed in the private collection of the author (registered as HRG- followed by a 4-digit number).

## RESULTS

Family Kirchenpaueriidae Stechow, 1921

Genus *Ventromma* Stechow, 1923

*Ventromma halecioides* (Alder, 1859)

Figs 1-2

*Plumularia halecioides* Alder, 1859: 353, pl. 12.

**Material examined:** MHNG-INVE-99622; France, La Ciotat, old harbor, 43.172512° 5.610192°, 0-0.3 m; 05, 07 and 11 Sep. 2017; fertile colonies, detached stolon gonothecae, and medusoids in alcohol. – HRG-1342; France, La Ciotat, old harbor, 43.172512° 5.610192°, 0-0.3 m; 01 Sep. 2017; fertile colonies, detached stolon gonothecae, and medusoids mounted on slides.

**Systematics:** Evidence from molecular work (Leclère *et al.*, 2007, 2009; Moura *et al.*, 2008; Peña Cantero *et al.*, 2010; Maronna *et al.*, 2016) indicate that this species should be assigned to the genus *Ventromma*, which forms a monophyletic group, different from *Kirchenpaueria* Jickeli, 1883, within the family Kirchenpaueriidae. Unlike the latter, with which it has been synonymized by a number of authors (e.g. Bouillon, 1985; Ansín Agís *et al.*, 2001), *Ventromma* is morphologically characterized by the presence of nematophores protected by nematothecae (Fig. 1A). A comprehensive synonymy of *V. halecioides* is provided by Ansín Agís *et al.* (2001).

**Description:** For a description of this species, refer to Calder (1997). Billard (1903) documented the morphological variations of its trophosome. Migotto (1996) noted the presence of two distinct regions, delimited by a transverse constriction, in the hydranth, an observation made earlier by Goette (1907: 322, pl. 12 fig. 258), and confirmed by the present study (Fig. 1B); however, their respective functions could not be ascertained at this stage.

The present material is fully fertile, with both cauline and stolon gonothecae containing gonophores at various stages of development. Since the specimens could not be kept alive for more than 12 hours, the development of their gonophores could not be studied sequentially. However, their life history could be traced based on the present observations, and by homology with other species whose similar life cycles are well-characterized. Only gonothecae of one sex were borne on a given stem but, due to the high density of plumes and the likely occurrence of multiple colonies on the same substrate, it is impossible to state whether *V. halecioides* is a mono- or dioecious species. Male and female gonothecae are indistinguishable morphologically, although their respective gonophores are immediately identifiable under the stereomicroscope.

One large and, often, a second, basal and comparatively smaller gonophore occur per gonotheca (Fig. 1C). This is especially obvious when female gonothecae are



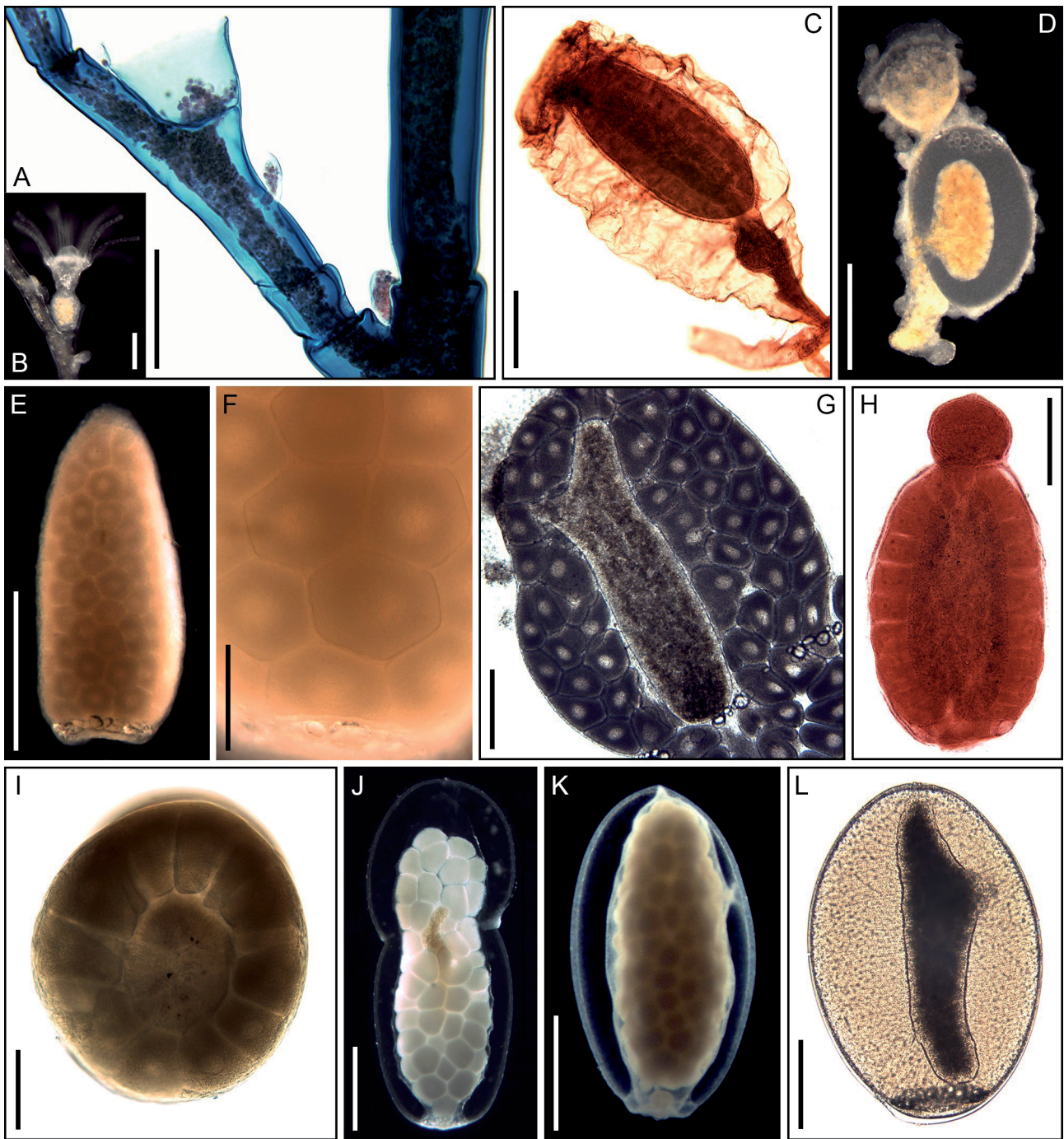


Fig. 1. (A) Portion of colony showing the cauline apophysis and its associated nematotheca, and proximal part of a cladium with first hydrothecate internode and its thecae. (B) Expanded hydranth, showing colors in life. (C) Gonotheca with female gonophore. (D) Whole young male gonophore extracted from its gonotheca. (E-F) Newly-liberated female medusoid (E) and close-up showing polygonal oocytes (F). (G) Squashed female medusoid showing Y-shaped spadix. (H-I) Stained (H) and cross-section (I) through female medusoid showing the spadix encircled by single layer of oocytes. (J-K) Female medusoid escaping from its protective membrane (J) and newly-liberated individual (K). (L) Male medusoid enveloped in membrane. Scale bars: 50  $\mu$ m (B), 100  $\mu$ m (A, F), 200  $\mu$ m (C, G-I, L), 400  $\mu$ m (D, E, J, K).

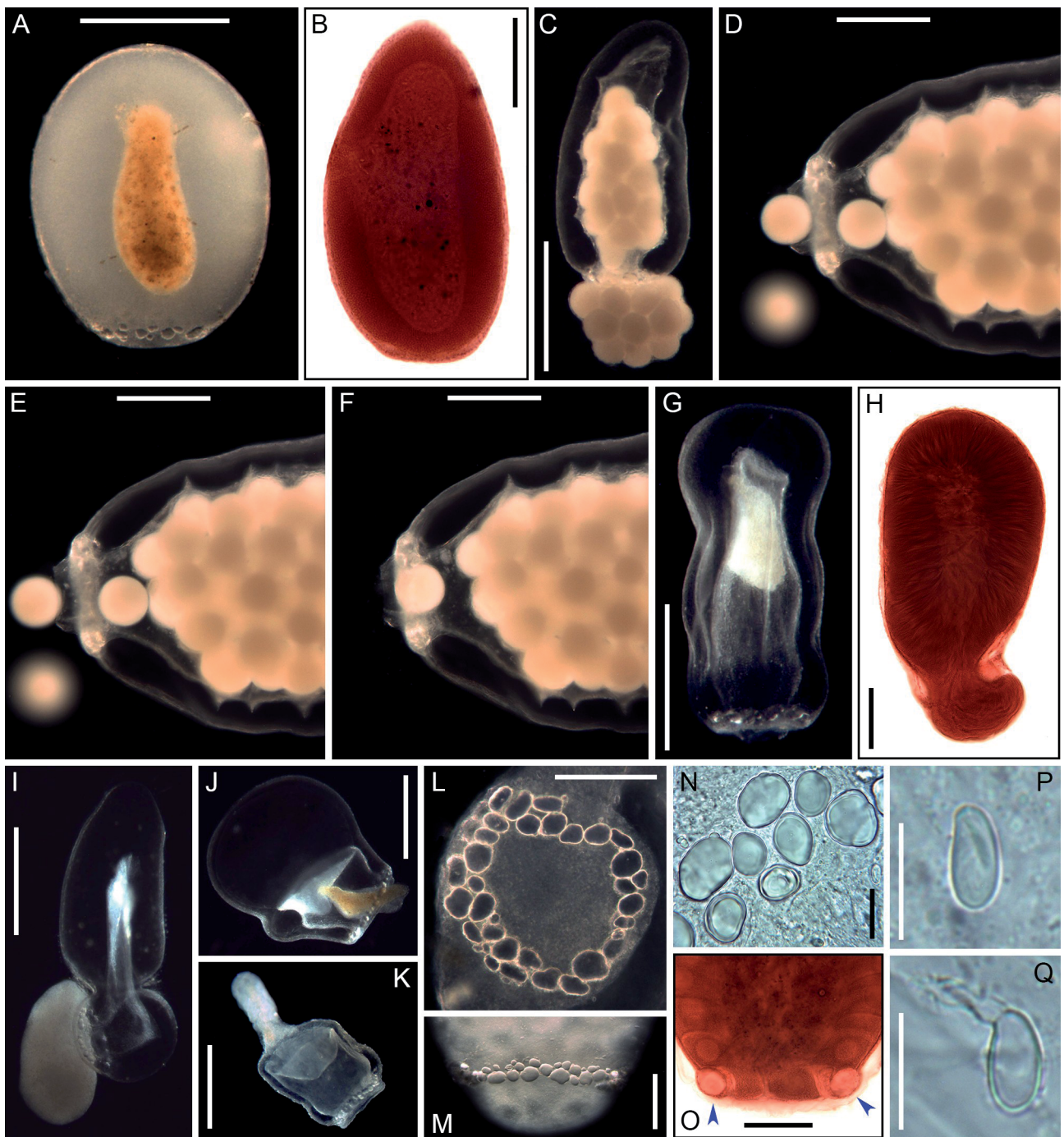


Fig. 2. (A-B) Male medusoid either showing colors in life (A), or stained (B) and displaying an Y-shaped spadix. (C) Partially spawned female medusoid with aggregate of oocytes. (D-F) Three steps of spawning (note presence of velum). (G) Emptied female medusoid. (H) Stained male medusoid partly liberating its gametes, showing directional arrangement of sperm cells. (I) Spawned male medusoid partly liberated from its membrane. (J, K) Spawned medusoids of unknown sex(es), the latter with the bell inside-out. (L-M) Belt of refringent corpuscles seen apically in a male medusoid (L) and laterally in a female (M). (N) Close-up of the refringent corpuscles. (O) Bell margin of a female medusoid showing large, vacuolated cells after the dissolution of the concretions (blue arrowheads). (P-Q) Pseudostenoteles from exumbrella either undischarged (P) or discharged (Q). Scale bars: 10  $\mu$ m (P, Q), 20  $\mu$ m (N), 100  $\mu$ m (L, M, O), 200  $\mu$ m (B, D-F, H), 400  $\mu$ m (A, C, G, I-K).



examined, as the large oocytes are easily distinguishable from other cell types occurring in the gonophore. The main gonophore is budded off laterally from the lower half of a slender, tubular blastostyle running longitudinally from the proximal to the distal end of the gonotheca. In young thecae, the blastostyle ends up distally in a broad, globular, hollow apical plate (Fig. 1D), most likely involved in the building of the distal, and hence youngest, portion of the perisarcal sheet of the gonotheca. The more the gonotheca becomes morphologically complete and, simultaneously, its gonophore undergoes maturation, the more the apical plate becomes flattened (Fig. 1C), until it eventually disappears. Tall, columnar cells are easily noted on the side of the apical plate in contact with the inner gonothecal wall.

The very young gonophore is nearly spherical in shape and contains a globular spadix in its center (especially visible in males) linked laterally to the blastostyle. The latter assumes at this stage a sickle-shaped appearance, as it partly encircles the gonophore from one end to the other. A few conspicuous, refringent bodies form a belt towards the distal end of the gonophore. In males, the mass of spermatids is translucent while, in females, ovoid oocytes of various sizes encircle the spadix. The blastostyle, gonophore, and apical plate are enveloped together by a monostriated mantle. Numerous claw-like holdfasts emerge from the latter, anchoring – on one hand – the gonophore to the inner gonothecal wall, and – on the other hand – the border of the apical plate all the way around the insertion points of the future gonothecal operculum.

As the gonophore develops, it becomes gradually elongated, and its central spadix follows the same process. At these more advanced stages, one can easily note that the brownish spadix is linked to the blastostyle through a lateral “arm” (Fig. 1D). In the female gonophores, the oocytes aggregate around the spadix into a single layer (Fig. 1H, I); they assume a polygonal (penta- or hexagonal) shape, their cytoplasm is translucent, allowing large nuclei with visible nucleoli to be noted (Fig. 1F, G). The number of refringent bodies increases gradually until a belt of up to 3 superimposed whorls is formed (Fig. 2L, M).

In more advanced male and female gonophores, a thin, transparent, unicellular membrane surrounding them, and distinct from the mantle, could be noted. It is likely that it is formed simultaneously with the gonophore, but its presence is indiscernible at earlier developmental stages, unless demonstrated by histological sections. It is composed of elongated cells, with large nuclei, arranged in parallel, intercalating rows. Among them, a few scattered pseudostenoteles occur [undischarged capsules  $(8.0\text{--}9.1) \times (3.6\text{--}4.2) \mu\text{m}$ ].

As the gonophores grow up in their already fully formed gonothecae, the apical plate regresses completely, leaving only the complex blastostyle-gonophore-mantle within the gonotheca. When fully formed, the gonophores escape

from the gonotheca through a narrow, arc-shaped passage created by the partial cleave of the apical perisarc. All gonophores obtained during the four experiments carried out were liberated from their gonothecae still enclosed in their thin, transparent membrane (Fig. 1L). On the other hand, the jagged mantle and remains of the blastostyle remain trapped within the gonotheca. There was no apparent correlation between the medusoid liberation and the exposure of the colonies to light or dark, as a similar number of individuals were obtained in both cases.

The male gonophores subsequently undergo a change in the appearance of their content: from translucent, they become opaque, most probably upon the maturation of spermatids into sperm cells. Similarly so, the nuclei of the oocytes are no more visible, and the cells change in shape from polygonal to spherical (Fig. 1J). In a few instances, medusoids of both sexes were seen getting free (Fig. 1J), or already free (Fig. 1K), from their envelope. At this point, their mesoglea, previously thin and unnoticeable, expanded significantly and the gonophores adopted the characteristic shape of medusoids.

Structurally, they are true cryptomedusoids, as no radial or ring canals, no tentacles or tentacular bulbs, and no sense organs could be noted. A few pseudostenoteles (Fig. 2P, Q) are scattered on the exumbrella. The female medusoids are up to  $1165 \mu\text{m}$  long and  $655 \mu\text{m}$  wide, and produce up to 90 oocytes, while their male counterparts are up to  $820 \mu\text{m}$  long and  $585 \mu\text{m}$  wide. No natatory behavior has been ever observed after the liberation of the medusoids from their protecting membrane.

Spawning has been documented in a couple of female medusoids. An oocyte, whose diameter is *ca.*  $110 \mu\text{m}$ , was released approximately every 2 minutes through a progressive contraction of the subumbrellar cavity, but not of the medusoid as a whole. Upon the passage of the oocytes through the bell aperture, the presence of a velum could be noted (Fig. 2D–F). The oocytes either fell individually to the bottom of the crystallizing dishes (Fig. 2D–F), or formed aggregates at the bell aperture (Fig. 2C). Male medusoids were not observed during the spawning, although one spent specimen (partially extracted from its envelope) was found (Fig. 2I). Similarly so, one spent female medusoid (Fig. 2G) was found in the crystallizing dish with female individuals. In addition, a couple of spent medusoids of unidentifiable sex(es) (Fig. 2J, K) were found among the hydroid colonies. One of them had its bell inside out (Fig. 2K), which allowed not only the presence of transverse muscle cells in the subumbrella to be noted, but also the absence of a mouth. The lack of the latter was also confirmed in the second spent medusoid, in which the spadix was partially extruded from the bell cavity (Fig. 2J). In addition, these two spent medusoids displayed jerking contractions of the bell, not noted in unspent individuals, probably due to the crowded condition of their gametes, completely filling the subumbrellar cavity. No correlation between the exposure to light or dark of the medusoids and their spawning was noted.

Table 1. List of species in which swimming medusoid gonophores presumably exist, or were described thoroughly.

Taxa	Reference(s)	Brief description and remarks
<b>LOVENELLIDAE</b>		
<i>Hydranthea margarica</i> (Hinks, 1863)	Motz-Kossowska (1911, as <i>Halecium billardi</i> sp. nov.); Boero & Sarà (1987: 134, fig. 5)	Spherical, 4 radial canals, ring canal, gonad surrounding the spadix, 8 statocysts, nematocysts scattered on exumbrella; no tentacles.
<b>HEBELLIDAE</b>		
<i>Anthoebella brevitheca</i> (Leloup, 1938)	Boero <i>et al.</i> (1997: 27, fig. 14)	Ovoid, 4 radial canals, 4 large atentaculate bulbs, velum present, spadix with 4 distinct interrational gonads, mouth present, striated muscles in subumbrella.
<i>Anthoebella communis</i> (Calder, 1991)	Galea (2013: 13, fig. 3R)	Male medusoids with 4 radial canals; no other details discernible in fixed gonothecae.
<i>Anthoebella darwiniensis</i> Watson, 2000	Watson (2000: 8, fig. 5D)	4 vestigial radial canals; 4 long rudimentary tentacles contracted into a wavy pattern; no velum or manubrium.
<i>Anthoebella najimaensis</i> (Hirohito, 1995)	Hirohito (1995: 131, as <i>Scandia najimaensis</i> ); Boero <i>et al.</i> (1997: 28, fig. 16C-D)	Spadix surrounded by gonad, without radial canals, ring canal or marginal tentacles.
<i>Anthoebella parasitica</i> (Ciamician, 1880)	Boero (1980: 136, fig. 5, as <i>Hebella parasitica</i> ); Boero <i>et al.</i> (1997: 25)	Ovoid, 4 radial canals, ring canal, 4 perradial bulbs with tentacle rudiments, 4 smaller interrational atentaculate bulbs, gonad encircling spadix, <i>ca.</i> 200 oocytes/medusoid. Presence of mouth invalidated subsequently (Boero & Bouillon, 1989; Boero, <i>pers. comm.</i> ) Swim vigorously. Life span: 3-4 days under laboratory conditions.
<i>Anthoebella tubithec</i> (Millard & Bouillon, 1973)	Boero <i>et al.</i> (1997: 27, fig. 15)	Ovoid, gonads around spadix, 4 radial canals.
<b>HALECHIDAE</b>		
<i>Nemalium lighti</i> (Hargitt, 1924)	Bouillon (1986: 77, pl. 2 figs 2 & 4, pl. 3); Gravier-Bonnet & Migotto (2000: 208, figs 1-2)	Histological study done by Bouillon (1986), who demonstrates the occurrence of medusoids within the gonothecae, and noted the presence of a velum, most probably allowing the gonophore to become free and lead a short free life. Observational data on this species artificially mixed up with those of a putative undescribed species (see below) by Gravier-Bonnet & Migotto (2000). Colonies dioecious. Gonotheca opens apically. Medusoid elongated-ovoid, (1050-1400) × (420-550) µm, female producing 40-62 oocytes (120-135 µm wide). Mesoglea thick; exumbrella with scattered microbasic mastigophores; subumbrella with muscle cells. Bell margin provided with ring of large, ciliated cells enclosing one to more refringent corpuscles within large vacuoles. Velum present; active swimmers.
<i>Nemalium</i> sp.	Gravier-Bonnet & Migotto (2000: 208, figs 1-2)	Observational data on this species and <i>N. lighti</i> artificially mixed up. Colonies either mono- or dioecious. Release at dawn. Medusoids similar to those of <i>N. lighti</i> .
<i>Nemalium gracile</i> Galea <i>et al.</i> (2012)	Galea <i>et al.</i> (2012: 48, fig. 3)	Colonies and individual stems either mono- or dioecious. Gonophores liberated at sunset through rupture of apical perisarc of the gonotheca; elongated-ovoid, male (730-1085) × (255-340) µm, female (1025-1305) × (355-400) µm and carrying 30-35 oocytes. Mesoglea thick. A belt of refringent corpuscles in 1-3 whorls. Exumbrella with scattered pseudostenoteles. Subumbrella with muscle cells. Velum present.
<b>SERTULARIIDAE</b>		
<i>Amphisbetia operculata</i> (Linnaeus, 1758)	Teissier (1922: 358, figs 1-4, as <i>Sertularia operculata</i> ), Motz-Kossowska (1907: cxvi, fig. 3, as <i>S. operculata</i> )	Liberated at night. Elongated-ovoid, spadix eccentric, females carrying <i>ca.</i> 50 oocytes 150-180 µm wide. Muscle cells on subumbrella; velum present, provided with muscle cells; active swimmers. Life span 10 minutes.
<i>Sertularella diaphana</i> (Allman, 1885)	Gravier-Bonnet & Lebon (2002: 105, fig. 1)	Observational data on this and following species reportedly similar and, consequently, pooled together. Colonies dioecious. Release at dawn through opening of gonothecal operculum. Medusoids bell-shaped, male (2157-2431) × 1200 µm, female (1875-2513) × (863-1313) µm and containing <i>ca.</i> 77 oocytes ( <i>ca.</i> 184 µm wide). Mesoglea relatively thin, subumbrella with muscle cells. No nematocysts on exumbrella, no refringent corpuscles around bell aperture. Velum present; active swimmers. Spawning within 12 minutes after release.



Taxa	Reference(s)	Brief description and remarks
<i>Sertularella</i> sp.	Gravier-Bonnet & Lebon (2002: 105, fig. 1)	Putative undescribed species. Medusoids similar to those of <i>S. diaphana</i> ; male (1765-2078) × (980-1375) µm, female (1875-2250) × (1188-1450) µm and containing 34-51 oocytes (ca. 235 µm wide).
<i>Sertularia loculosa</i> Busk, 1852	Unpublished data by Migotto cited by Gravier-Bonnet & Lebon (2002: 107)	Unpublished results.
<i>Sertularia marginata</i> Kirchenpauer, 1864	Migotto (1998: 5, fig. 2)	Colonies dioecious. Medusoid release after dusk, through narrow opening of gonothecal operculum; elongated-ovoid; male (852-1080) × (540-768) µm; female (960-1200) × (588-960) µm and producing 21-37 eggs. Mesoglea relatively thin; exumbrella with scattered microbasic mastigophores. No refringent corpuscles. Velum present; active swimmers.
<i>Sertularia turbinata</i> (Lamouroux, 1816)	Unpublished data by Migotto cited by Gravier-Bonnet & Lebon (2002: 107)	Unpublished results.
<b>PLUMULARIIDAE</b>		
<i>Dentitheca asymmetrica</i> (Bale, 1914)	Bale (1914: 30, as <i>Plumularia asymmetrica</i> )	“A cluster of rounded highly refractive granules near the aperture”.
<i>Dentitheca bidentata</i> (Jäderholm, 1920)	Migotto (1997: 170), Migotto & Marques (1999: 956, fig. 4)	Colonies either mono- or dioecious. Gonophore ovoid, (720-940) × (500-720) µm. Mesoglea thick; exumbrella with scattered microbasic mastigophores; refringent corpuscles in 2-3 irregularly-concentric rings. Velum present; active swimmers. Spawning 15 min after release; life span ca. 2 hours. Females producing 10-12 oocytes (deduced from fig. 4C), 130-145 µm wide.
<i>Dentitheca dendritica</i> (Nutting, 1900)	Galea <i>et al.</i> (2012: 44, fig. 1)	Colonies dioecious. Medusoids liberated at sunset; ovoid to spherical, female ca. 550 × 240 µm and producing up to 20 oocytes (diameter 110-150 µm), male ca. 415 × 250 µm. Mesoglea thin; belt of refringent corpuscles. Presence of velum and/or muscle cells in the subumbrella could not be confirmed. Spawning within gonotheca. No contractions of the bell have been noted.
<i>Monothecha obliqua</i> (Johnston, 1847)	Motz-Kossowska (1907: cxv, figs 1-2, as <i>Plumularia obliqua</i> )	Only males documented. Elongated-ovoid. Muscle cells on subumbrella. A well-developed velum, though reportedly devoid of muscle cells, although spawning accompanied by contractions of the bell.
<i>Monothecha margaretta</i> Nutting, 1900	Unpublished data by Migotto & Marques, cited by Gravier-Bonnet & Migotto (2000: 212)	Unpublished results.
<b>KIRCHENPAUERIIDAE</b>		
<i>Ventromma halecioides</i> (Alder, 1859)	Unpublished data by Migotto & Marques, cited by Gravier-Bonnet & Migotto (2000: 212); present study	Individual stems dioecious. The gonothecal operculum opens only partially to allow the escape of the medusoid; the latter is elongated-ovoid, ca. 820 × 585 µm in males, and ca. 1165 × 655 µm in females, the latter producing up to 90 oocytes, ca. 110 µm in diameter. Exumbrella with scattered pseudostenoteles; subumbrella with muscle cells; velum present. Marked contractions of the bell observed after spawning.
<b>HALOPTERIDIDAE</b>		
<i>Antennella</i> sp.	Bourmaud & Gravier-Bonnet (2005: 56, figs 1-2)	Colonies monoecious. Medusoid not released from gonotheca; ovoid to nearly spherical; mesoglea thin; female producing ca. 5 oocytes. No muscle cells on subumbrella, no velum, no nematocysts on exumbrella. Spawning during the night.
<b>AGLAOPHENIIDAE</b>		
<i>Aglaophenia latecarinata</i> Allman, 1877	Unpublished data by Migotto & Marques, cited by Gravier-Bonnet & Migotto (2000: 212)	Unpublished results.
<i>Aglaophenia</i> sp.	Unpublished data by Boero & Bouillon, cited by Boero & Bouillon (1989: 37)	Putative undescribed species from Papua New Guinea. Spadix “displaced towards bell margin”. No additional data available.
<i>Gymnangium ascidioides</i> (Bale, 1882)	Bale (1894: 106, pl. 5 fig. 1, as <i>Halicornaria ascidioides</i> )	“[...] a very distinct circle of highly refractive granules just below the aperture”.
<i>Gymnangium bryani</i> (Nutting, 1906)	Ronowicz <i>et al.</i> (2017: 30)	“Gonophores with medusoid inside (release not observed)”.
<i>Gymnangium ferlusi</i> (Billard, 1901)	Jarvis (1922: 355, fig. 5, as <i>Halicornaria ferlusi</i> var. <i>brevis</i> ); unpublished data by Gravier-Bonnet, cited by Gravier-Bonnet & Migotto (2000: 212)	“The mouth is circular, surrounded by a double ring of highly refractive discs” (Jarvis, 1922).

Taxa	Reference(s)	Brief description and remarks
<i>Gymnangium hians</i> (Busk, 1852)	Unpublished data by Gravier-Bonnet, cited by Gravier-Bonnet & Migotto (2000: 212); Ronowicz <i>et al.</i> (2017: 30)	“Gonophores with free medusoids” (Ronowicz <i>et al.</i> , 2017).
<i>Lytocarpia angulosa</i> (De Lamarck, 1816)	Allman (1883: 34, pl. 20 fig. 3, as <i>Ac-anthocladium huxleyi</i> )	“gonophore [...] encircled just below its summit by a wreath of refringent spherules”.
<i>Macrorhynchia filamentosa</i> (De Lamarck, 1816)	Kirchenpauer (1872: 43, pl. 2 fig. 21, as <i>Aglaophenia ligulata</i> ); Kirchenpauer (1872: 44, pl. 2 fig. 23, as <i>Aglaophenia patula</i> )	Medusoids undoubtedly depicted in pl. 2 fig. 21 (as <i>A. ligulata</i> ) and pl. 2 fig. 23 (as <i>A. patula</i> ), with their characteristic belt of refringent corpuscles.
<i>Macrorhynchia philippina</i> Kirchenpauer, 1872	Kirchenpauer (1872: 17, Fig.; pl. 2 fig. 26, as <i>A. philippina</i> ); Kirchenpauer (1872: 46, pl. 2 fig. 27, as <i>A. urens</i> ); Bale (1888: 787, pl. 21 figs 6-7, as <i>Lytocarpus philippinus</i> ); Gravier (1970: 255, fig. 1B, C); Watson (2000: 68, fig. 53D); Bourmaud & Gravier-Bonnet (2004: 368, figs 1-2)	Colonies monoecious. Gonotheca opens wide into two valves; release at dusk. Gonophore ovoid, <i>ca.</i> 1315 × 950 µm [deduced from fig. 1C in Gravier (1970)]; female producing <i>ca.</i> 40 oocytes. Mesoglea relatively thin; exumbrella with pigment cells and scattered microbasic mastigophores; subumbrella with muscle cells. Marginal belt composed of 2-3 whorls of refringent corpuscles, soluble in acid. Velum present; active swimmers. Spawning within 1-2 minutes; life span of the medusoid <i>ca.</i> 2 hours.
<i>Macrorhynchia racemifera</i> (Allman, 1883)	Allman (1883: 41, pl. 13 fig. 5, as <i>Lytocarpus racemiferus</i> )	“The gonophore occupies the centre of the gonangium, from whose walls it is separated by a clear space, across which may be seen a wreath of highly refringent spherules, by which the summit of the gonophore is encircled”.
<i>Macrorhynchia</i> sp.	Unpublished data by Gravier-Bonnet, cited by Gravier-Bonnet & Migotto (2000: 212)	Any data available on this species from Madagascar.
<i>Taxella eximia</i> Allman, 1874	Allman (1876: 278, pl. 22 fig. 2, as <i>Halicornaria saccaria</i> )	“[...] sporosac encircled near its summit by a band of refringent roundish corpuscles”.

As noted above, there is a belt of refringent bodies around the bell aperture in the medusoids of both sexes. Their shape ranges from nearly spherical to irregularly elongated, and their size varies from 10 to 33 µm long (Fig. 2N). They are likely of a mineral nature, and dissolve within less than 30 seconds in an acidic medium (though without obvious effervescence), but remain unaffected in an alkaline solution. Stained and mounted medusoids, in which the marginal bodies have dissolved, show an accumulation of quite large, polygonal cells around the bell margin that seem to delimit large “compartments” for housing these structures, possibly vacuolated cells. Two such diametrically opposed compartments may be mistakenly taken for a ring canal (Fig. 2O, arrowheads). Whether these compartments are represented by large, vacuolated cells could not be established at this stage, and a histological study is expected to clarify the case.

**Remarks:** The so-called “sketch of ring canal” noted by Bouillon (1986) in sections of medusoids of *N. lighti* likely correspond to the “ring of large cells enclosing [the] refringent corpuscles” observed by Gravier-Bonnet & Migotto (2000) in the same species. A similar histological structure may be also met with in the medusoid of *V. halecioides*, with the large “compartments” mentioned above corresponding to these vacuolated cells.

Watson (2011) created a new species, *V. bellarensis*, from southern Australia, and distinguished it from *V. halecioides* through the constant absence of ahydrothecate

internodes on cladia. Given that her description was based on a single colony only and, taking into account that many authors (*e.g.* Naumov, 1969; Migotto, 1996; Ansin Agís *et al.*, 2001) described – besides the otherwise “distinctive” heteromorous segmentation of cladia – the occurrence of only hydrothecate internodes, or a combination of both, in *V. halecioides*, it is assumed that *V. bellarensis* is no more than a junior synonym of Alder’s (1859) species.

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