

## Gonangium development and medusoid of *Nemalecium lighti* (Hargitt, 1924) (Cnidaria: Hydrozoa, Haleciidae)\*

NICOLE GRAVIER-BONNET<sup>1</sup> and ALVARO E. MIGOTTO<sup>2</sup>

<sup>1</sup>Université de la Réunion, Faculté des Sciences, 15 Avenue René Cassin, BP n° 7151, 97715 Saint-Denis Messag Cedex 9, France. E-mail: Nicole.Gravier-Bonnet@univ-reunion.fr

<sup>2</sup>Centro de Biologia Marinha, Universidade de São Paulo, Caixa Postal 83, 11600-970, São Sebastião, SP, Brazil. E-mail: aemigott@usp.br

**SUMMARY:** Based on live specimens of *Nemalecium lighti* collected in the coasts of La Réunion (Indian Ocean) and São Sebastião (Southeastern coast of Brazil) and kept in the laboratory, we observed the release of short-lived medusoids. The gonangia pass through six developmental phases: *growing*, *ripening*, *migrating*, *stripping*, *liberating* and *spawning*. The medusoids are tall, lack tentacles, bulbs, circular and radial canals, and the sexual products are packed around the eccentric manubrium; they are provided with a velum and with a subumbrellar ectoderm rich in transverse striated muscle fibers. There are refringent and isotropic corpuscles within vacuolated and ciliated large cells located around the aperture of the medusoid, which possibly function as statoliths. The corpuscles are similar to those already described for the families Plumulariidae and Aglaopheniidae. The gametes are liberated shortly after the release of the medusoid from the gonotheca. The female medusoid spawned 40-62 ova; spermatozoa exhibited a semicircular nucleus, and planulae were formed *c.* twelve hours after fertilization. Colonies with medusoids of only one sex or with both male and female medusoids.

**Key words:** Cnidaria, Leptomedusae, hydroids, reproduction, life cycle, medusoid, gametes, hermaphroditism

### INTRODUCTION

*Nemalecium* is a haleciid genus with a relatively wide distribution on the tropical regions of the world, being remarkable by having a pair of nematodactyls: short and thick fingerlike tentacles armed with large, pseudostenotele nematocysts (Bouillon, 1986; Bouillon *et al.*, 1986; Calder, 1991; Migotto, 1996).

Bouillon (1986) thoroughly redescribed *Nemalecium lighti* (Hargitt, 1924) from Papua-New Guinea,

including an account of the female gonophore. By histological sections he established the presence of a velum and of remains of a circular canal, and hypothesized that these gonophores could be released into the water and have a short free life. Calder (1991: 27) described the male gonotheca of specimens from Bermuda. Sexual reproduction by medusoids during the austral summer (November to April), together with data on feeding and on ecological preferences, has been described previously from Indian Ocean material (Gravier-Bonnet and Mioche, 1996).

Up to now the genus *Nemalecium* is monotypic. But some characters (see Discussion) differ slightly

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among specimens from São Sebastião, La Réunion and those described by Bouillon (1986) and Calder (1991), what could indicate that there are more than one species of *Nemalecium*. And in Papua New-Guinea, Boero suspected the presence of two species (pers. comm.). However, as the specific name *Nemalecium lighti* were already cited from São Sebastião (Migotto, 1996) and La Réunion (Gravier-Bonnet and Mioche, 1996), we decided not to name new species until thoroughly morphological comparisons are not undertaken.

Based on live specimens of *N. lighti* collected in the coasts of La Réunion island (SW Indian Ocean) and São Sebastião (southeastern coast of Brazil) that were kept in the laboratory, the development of male and female gonangia was followed until the release of mature, short-lived medusoids. The successive developmental phases of the gonangia and the released medusoids are herein described.

The similarities of the medusoid of *N. lighti* with those few already known from Plumulariida (for references and discussion on medusoids and swimming gonophores see Migotto, 1998) stress the importance of these structures for phylogenetic inferences for higher taxa.

## MATERIALS AND METHODS

Colonies of *N. lighti* were collected on experimental plates and rocks of the shallow infralittoral of the São Sebastião Channel, São Paulo State, Brazil (see Migotto, 1996 for a brief description of the collecting area) and from crevices on the littoral reef platform of Cap Homard in La Réunion (see Gravier-Bonnet and Mioche, 1996 for a description of the collecting area).

Stems with gonothecae, from São Sebastião, were transferred to glass beakers (600 and 1000 ml) with filtered seawater, and kept in a constant temperature chamber at 24°C, until the release of medusoids. Poorly ramified colonies from La Réunion, collected with fragments of the hard substratum, were kept at 23-25°C in an aquarium with natural seawater (closed system, 6000 ml) and aeration, and the formation and growth of the gonangia were observed from the outset. The development of the gonangia was seen in material from La Réunion (phases 1-4) and the liberation of the medusoid in the material from São Sebastião (phases 5-6), but newly released medusoids and gametes were seen in materials from both locations.

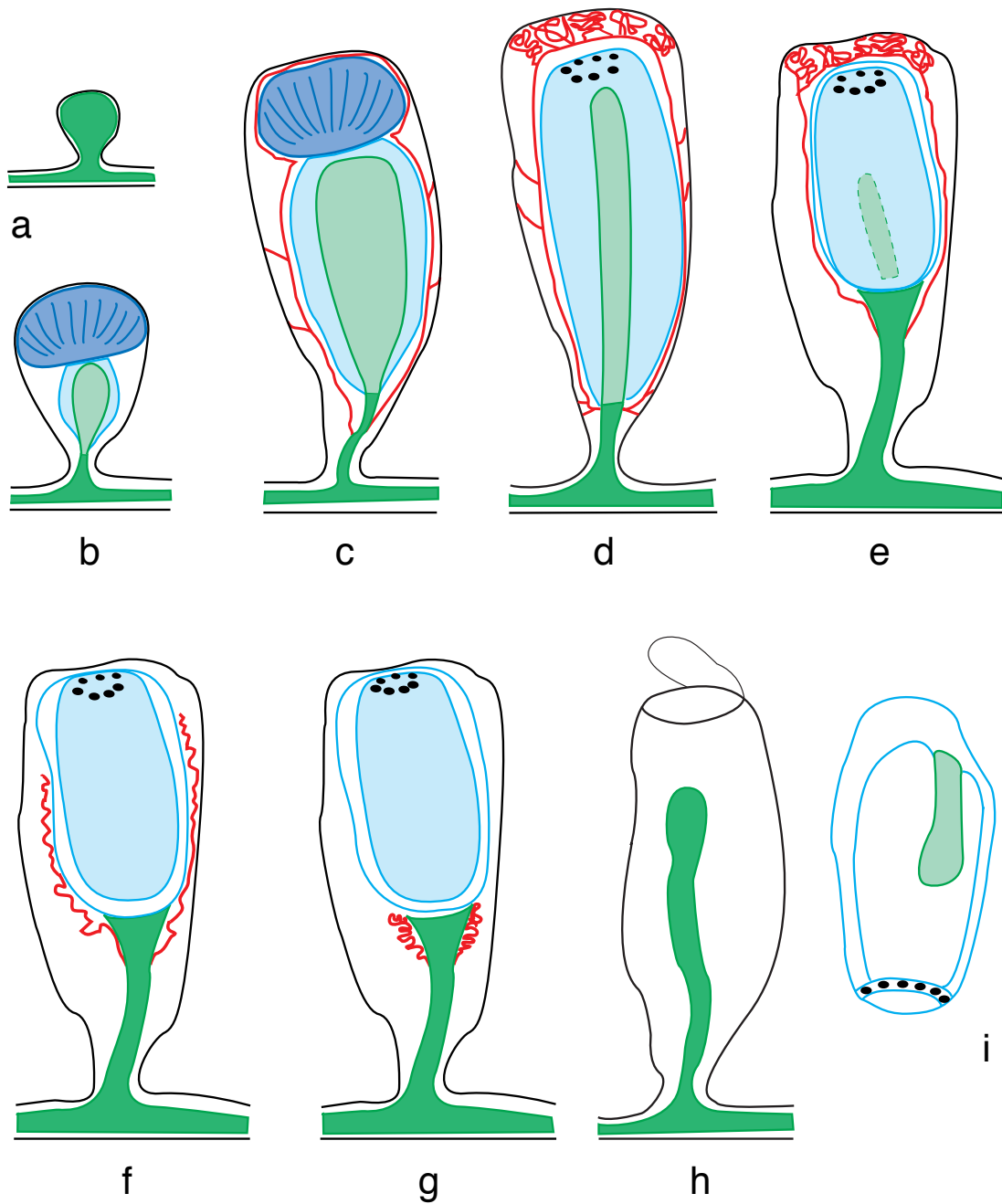
Observations at La Réunion were undertaken using a stereomicroscope (Leica M420) and a light microscope (Leitz Diaplan). Photographs were taken with a mono CCD camera connected to a SVHS magnetoscope (Panasonic NV-FS100), a monitor (Sony Trinitron), a computer (Power Macintosh 8500), and a video printer (Sony UP-3000). Photographs have been treated with Adobe Photoshop and drawings by Adobe Illustrator. Experiments altering the day-night rhythm were carried out in order to record the release on videotape. The migrating and stripping phases of the medusoids occurred at very low speed and were only perceivable by time-lapse photography and by fast motion video recording.

Most of the specialized terms used here are clearly defined in the glossary proposed by Cornelius (1995) except the following two. The word “mantle”, already used by Harris (1990) and Bouillon (1986; “manteau”), for the “ectoderm lining” of Millard (1975), is a double layer of ectoderm which covers the inner side of the perisarc and surrounds other coenosarc structures: hydranths into hydrothecae, gonophores into gonothecae and coenosarc into hydrocladia and stems. “Plateau terminal” (apical plate), used by Bouillon (1986) in describing the gonophore of *N. lighti*, is as a large, hollow vesicle at the terminal part of the gonangium, being covered by the mantle.

## RESULTS

Colonies collected without gonangia developed them in the laboratory within 8 to 10 days either on gonochoric or hermaphroditic colonies. At La Réunion island, the colonies collected were either only male or female, or both; only gonochoric colonies were seen in São Sebastião. The successive developmental phases observed are summarized below and illustrated by schematic drawings (Fig. 1) and photographs (Fig. 2).

From the small bud that appeared from the hydrorhiza and hydrocauli internodes (gonothecae grew only from hydrocauli internodes in the well ramificated colonies from Brazil), there was an initial *growing phase* (phase 1; Fig. 1 a-c). The gonangium gradually elongated, assuming its definitive shape and maximal size after 4 to 5 days. Concomitantly a large mass of cells, the apical plate, formed at the apex of the gonotheca. Both the apical plate and the gonophore below were covered by the mantle, which



**LEGEND**









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|--|--|--|--|
|  colony coenosarc & pedicel |  gametogenetic ectoderm |  "plateau terminal" |  corpuscule |
|  spadix (manubrium)         |  mantle                 |  medusoid           |  perisarc   |

FIG. 1 – Development of the gonangium of *Nemalecium lighti* and medusoid release (schematic drawings). a-c, growing phase; d, ripening phase; e, migrating phase; f-g, stripping phase; h, empty gonotheca with operculum; i, spent male medusoid.

was discontinuously connected with the perisarc of the gonotheca and provided with many nematocysts. At this phase the manubrium was usually thicker than the layer of ectoderm that enclosed the gametes.

The second phase was the *ripening phase* (Fig.1d). The apical plate had completely disappeared, but the gonophore remained surrounded by the mantle where nematocysts, especially pseu-

dostenoteles, were in particular packed at the apex of the gonangium, below the top of the gonotheca. The manubrium became narrower than previously, and the ectoderm enveloping the gametes became wider. This ectoderm included not only the gametes but also nematocysts, glandular cells (like the ones at the base of the tentacles of the hydranth described by Bouillon, 1986) and peculiar needle shaped and crystalline elements (with a small angle of extinction under polarized light) that were distributed radially from the manubrium and could be excretive products. At this stage the presence of a ring of refringent corpuscles encircling the region to the future aperture of the bell was clear.

Observing live gonangia gave us almost no clue about the phases of the gametogenic processes. From the beginning of oogenesis, the oocytes – usually arranged in mosaic shape and with well visible darker nuclei – were opaque and white in color, being difficult to perceive any change inside the gonads. Conversely, during ripening, the male gonad underwent a change in color from translucent to white opaque, probably indicating the transformation of spermatids into spermatozoa. The duration of the ripening phase was 2 to 3 days.

During the third phase (Figs. 1e, 2a) – *migrating phase* – the gonophore, which previously almost completely filled up the gonotheca, became concentrated at the distal part, leaving the base empty. Simultaneously, the peduncle that linked the gonophore and the coenosarc of the colony elongated. This was a relatively slow process, taking about 6 hours.

During the fourth phase (Figs. 1f-g, 2b-d) – *stripping phase* – the gonophore became detached from the mantle. The event began at the distal end (oral) of the gonophore, where, as a first consequence, the circle of refringent corpuscles appeared clearly. Then, at low speed, the mantle withdrew towards the base until the gonophore remained uncovered inside the gonotheca. Concomitantly the oocyte nucleus became invisible and the shape of the oocytes changed from polygonal to round, probably indicating the beginning of their detachment from the manubrium. At this stage, the top of the gonotheca looked more convex under the pressure of the medusoid. This phase took half a day.

The medusoid was released from the top of the gonotheca, where there was an indistinct operculum that could either remain closed or open after medusoid release. The medusoids pulsed during and after release from the gonotheca. Spawning

occurred during the process of release from the gonotheca or shortly after. Therefore, the fifth and sixth phases – *liberation* and *spawning phases* –, occurred simultaneous or near so, and were relatively short compared with the other phases.

Spent medusoids (Fig. 2e-g) lived up to two hours in the culturing dishes. A short time after spawning, the peduncle was still present within the empty gonotheca (Fig. 1h), being longer than before medusoid release. Occasionally some medusoids reached the outside water and spawned while still attached to the colony by the peduncle (Fig. 2e). The peduncle could have a role in pushing the medusoid out; at least the medusoid remained linked by the peduncle during the whole process of release from the gonotheca, detaching from it only when on the outside.

At La Réunion island, medusoid release took place about dawn. Experiences of leaving fertile colonies or isolated gonothecae under light during the night, in order to record the events on videotape, prevented the release from occurring. Conversely, colonies kept in darkness did not delay medusoid release.

Male and female medusoids were very similar, but the spent male gonophore (Figs. 1i, 2f-g) was more ovoid than the more spherical female gonophore (Fig. 2e). Both were 1050 to 1400  $\mu\text{m}$  high and 420 to 550  $\mu\text{m}$  in maximum diameter. They lacked mouth, tentacles and tentacle bulbs, radial and circular canals, ocelli and true statocysts. The bell had an aperture delimited by a velum (Fig. 2g). The remaining part of the manubrium was generally not at the center of the bell (Fig. 2f). Microbasic mastigophore nematocysts (14.5-17 x 5-6  $\mu\text{m}$ ) were more densely distributed on the basal half of the exumbrella, but absent in a narrow band near the margin (there were no pseudostenoteles in medusoids). The exumbrellar and subumbrellar epidermis – the latter densely packed with concentric striated muscular fibers (Fig. 2g) – joined at the umbrella margin where there was a ring of large cells enclosing refringent corpuscles of various shape into wide vacuoles (Fig. 2h). There were either one or several corpuscles of different sizes in one cell and these cells were provided with long cilia (seen by phase contrast microscopy). Under a polarizing microscope, the corpuscles appeared to be composed by an isotropic substance that did not polarize. They presented concentric layers indicating growing stages, and under pressure they broke from the center along more or less perpendicular

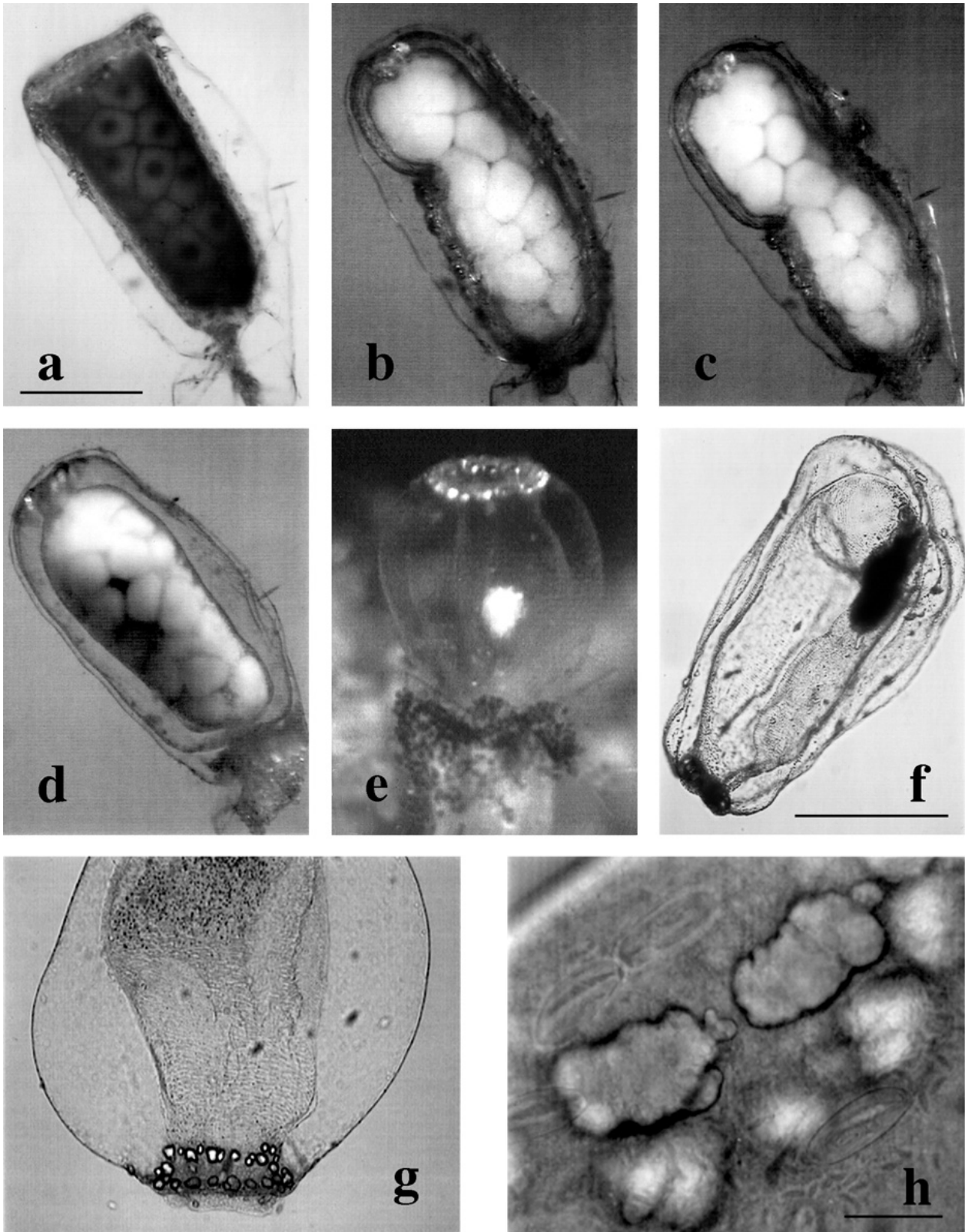


FIG. 2 – Medusoid of *Nemalecium lighti*. a-d, female medusoid inside the gonotheca: a, migrating phase with the mantle surrounding completely the medusoid; b-d stripping phase with retraction of the mantle (beginning, middle and end); e, spent female medusoid still attached to the gonotheca, with well visible circle of refringent corpuscles; f, spent male medusoid soon after liberation, with eccentric manubrium; g, spent male medusoid on slide, clearly showing extruded velum, corpuscles, and circular muscle fibers in the subumbrellar ectoderm; h, corpuscles and nematocysts (pseudostenoteles and microbasic mastigophores) visible at the top of the gonotheca before the release of the medusoid. Bar scales: a-e, 0.25 mm; f-g, 0.25 mm; h = 0.05 mm.

lines. They were quickly destroyed by fixatives (Bouin, alcohol, formaldehyde) but were conserved in seawater.

Each medusoid spawned 40-62 milky-white, spherical eggs, measuring 120-135  $\mu\text{m}$  in diameter. The spermatozoa were rounded and each had a long flagellum. The larval development occurred rapidly; in about 12 hours planulae were already formed, alternating periods of swimming actively in the water column with periods of slowly gliding on the bottom of the culture dish. The planula was elongated with the anterior pole larger and rounder, and with thicker ectoderm than the posterior.

Fully developed male and female gonothecae were identical: curving and tapering basely, elongated, with walls weakly divergent and maximum diameter at the truncated top, and perisarc either smooth or with a few undulations.

The specimens from São Sebastião and La Réunion were apparently identical, except in shape and size of the refringent corpuscles of the medusoid, which differed slightly.

## DISCUSSION

The morphology of the medusoid of *Nemalecium lighti* is very similar to those described for species of Sertulariidae, Plumulariidae and Aglaopheniidae (Migotto, 1998; Migotto and Marques, 1999). They all share distinctive features, including behavior, shape of the bell, eccentric manubrium and distinct velum; and lack mouth, tentacles and tentacle bulbs, radial and circular canals, and sensory structures like ocelli and statocysts. But the presence of striated muscle fibers in the subumbrellar epidermis, typical of a true medusa, which enables swimming and expulsion of gametes, leads us to consider the medusoids as reduced medusae instead of fixed gonophores which became released (as proposed by Boero and Bouillon, 1989 and named swimming gonophores). Such fibers have been already reported for the medusoid of *Plumularia obliqua* (Johnston, 1847) (= *Monothecha obliqua*) (see Motz-Kossowska, 1907). According respectively to Motz-Kossowska (1907) and Bouillon (1986) there is a vestigial circular canal in the gonophores of *Monothecha obliqua* and *Nemalecium lighti*, but this canal was not described for the other similar medusoids, possibly because they were seen in histological preparations and are not visible in live material. However, in histological sections from mature male gonangia from La Réunion, no vestigial circular canal was

observed at the top of the gonophore but two lateral cavities, probably due to the dissolution of the corpuscles into fixatives, were present.

The refringent corpuscles at the margin of the medusoid is a character already described only for species of Plumularioidea: the aglaopheniid *Macrorhynchia philippina* (Kirchenpauer, 1872) by Gravier (1970) and Migotto (1996), and the plumulariid *Dentitheca bidentata* (Jäderholm, 1920) by Migotto (1997) and Migotto and Marques (1999). These corpuscles, previously misinterpreted as lipid droplets by Boero and Bouillon (1989) and Migotto (1998), play the role of a ballast, maintaining the medusoid in a vertical position and carrying it towards the bottom (Gravier, 1970), an observation confirmed recently (Migotto and Marques, 1999). The presence of cilia in the cells enclosing the refringent corpuscles indicates they have a sensorial function, maybe being homologous to statoliths.

The refringent corpuscles are also present in the fixed gonophores of the aglaopheniid *Aglaophenia latecarinata* Allman, 1877 and in the gonophores (possibly short-lived medusoids) of the kirchenpaueriid *Ventromma halecioides* (Alder, 1959) and of the plumulariid *Monothecha margaretta* Nutting, 1900 (Migotto and Marques, unpublished data). In the case of *Aglaophenia latecarinata* the presence of an equilibrium organ seems unnecessary, except if the fixed gonophores could alternatively become free medusoids in response to seasonal environmental changes. Some species of campanulariids, for instance, can have colonies that produce either fixed gonophores or free medusoids if settled, respectively, in calm or rough waters (Stefani, 1956; Gili and Hughes, 1995).

Marginal corpuscles have been noted on the medusoid of *Gymnangium fergusi* (Billard, 1901) (unpublished data of N. Gravier-Bonnet). For Aglaopheniidae, they are known to be present in eleven species: nine cited by Gravier (1970), plus *Gymnangium hians* (Busk, 1852) from La Réunion and *Macrorhynchia* sp. from Madagascar, where, as in *M. philippina*, their shape is rounder and more regular than in *N. lighti* (unpublished data of N. Gravier-Bonnet). Corpuscles have to be confirmed in two species of Plumularioidea that are respectively known to produce short-lived medusoids: *Aglaophenia* sp. referred by Boero and Bouillon (1989) and *Monothecha obliqua* referred by Motz-Kossowska (1907).

These findings indicate that these structures are probably more widespread within the families

Aglaopheniidae, Kirchenpaueriidae, Plumulariidae and Haleciidae, either associated with free medusoids or not. As the corpuscles disappear in fixed material, their presence can only be ascertained by working with living specimens.

About the role of the “plateau terminal”, questioned by Bouillon (1986), it is possible, at the light of these new results, to eliminate any sort of implication in fertilization. This mass of cells, present during the growing phase and absent in the mature gonangium, is at least partly involved in the gonotheca formation.

Sexual dimorphism of *N. lighti* medusoids is very weak, which seems to be usual in hydroids and common among Leptomedusae (Gili and Hughes, 1995). Conversely hermaphroditism has probably been underestimated, at least for some families. Already checked among aglaopheniids (Gravier, 1970; the term dioecious has been wrongly used in the text of that article instead of hermaphrodite, however well documented by the description and illustration) and plumulariids (Gili and Hughes, 1995; Migotto, 1997; Migotto and Marques, 1999), it has been seen in several species of these families (unpublished data of Gravier-Bonnet from Indian Ocean specimens) and it is here and already reported for the haleciids (Gravier-Bonnet and Mioche, 1996). No data are still available on an eventual role of environmental parameters on the sex differentiation.

The diagnosis of the genus *Nemalecium*, recently reviewed by Calder (1991), has to be emended concerning the production of short-lived medusoids instead of fixed gonophores. Up to now the genus is monotypic. But the differences in shape and size of the corpuscles between specimens from São Sebastião and La Réunion could indicate that we are not dealing with one widespread or near circumtropical species, but rather with two or more sibling species. Some characters – like the shape of the gonotheca and of the hydrotheca, and the number and position of the tentacles – differ slightly between specimens from La Réunion and those described by Bouillon (1986) and Calder (1991). In order to clarify this issue, material from different localities should be morphologically compared, taking into account the whole characters of the skeleton and of the coenosarc (hydranth, gonangium, medusoid), and a detailed study of the nematocysts.

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