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Development of conceptacles in *Amphiroa* (Corallinales, Rhodophyta)

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ABSTRACT

Here, we describe the development of sporangial and gametangial conceptacles for *Amphiroa beauvoisii* and *A. vanbosseae*; sporangial conceptacles only for *A. misakiensis*; and gametangial conceptacles only for *A. cryptarthrodia* and *A. rigida*. The descriptions are based on the observation of histological preparations obtained from 112 specimens collected from the Gulf of California, in Mexico, and the Azores archipelago of Portugal. Information on the development of the sporangial conceptacle pore and conceptacle senescence is here described and illustrated for the first time. Four development patterns were observed: two for sporangial conceptacles; one for spermatangial conceptacles; and one for carposporangial conceptacles. The phases of development of the sporangial conceptacle were found to be useful in delimiting species within the genus. Based on the sporangium location on the cavity floor and the pore canal anatomy, the species *A. beauvoisii, A. misakiensis* and *A. vanbosseae* can be distinguished from each other.

Key words: carposporangial filament origin, sporangium location, sporangial pore canal anatomy, senescence, taxonomy

Introduction

Information concerning the reproduction of articulated coralline algae has been provided by several authors, thus expanding knowledge of the Corallinaceae family. Nevertheless, the reproductive structures are still poorly described for many genera of articulated coralline algae. In general, all members of the Corallinaceae produce sporangia, carposporangia and spermatangia within uniporate conceptacles, whose development undergoes a number of stages (Johansen 1968). Although the transformation from vegetative to fertile tissue is not fully understood in the Corallinaceae, it is known to involve the local destruction of cells, which, when combined with continued accelerated growth of surrounding tissue, results in the depression of peripheral cells (Johansen 1972), a process that differs among genera (Johansen 1968). Historically, the morphology and anatomy of conceptacles, together with other features, have been used to distinguish genera within the geniculate Corallinaceae.

A description of the genus *Amphiroa*, encompassing a summary of the diagnostic characteristics, is provided by Womersley (1996) and Harvey *et al.* (2009). Johansen (1972), in a revision of Corallinaceae conceptacles, described a general pattern for the development of tetrasporangial conceptacles in *Amphiroa* in which the roof is formed by intersporangial growth and the chamber is opened via a single pore. Additional information on the development of sporangial and gametangial/carposporangial conceptacles was published by Johansen (1968), Murata & Masaki (1978) and Srimanobhas & Masaki (1987). Several other taxonomic studies have included general information on the developmental phases of *Amphiroa* (Ganesan 1968; Norris & Johansen 1981; Riosmena-Rodríguez & Siqueiros-Beltrones 1991, 1996; Womersley 1996; Moura & Guimarães 2002; Harvey *et al.* 2009). Aspects of the reproduction of a few species within *Amphiroa* have been described: by Segawa (1940a,b) for *A. rigida* J.V. Lamouroux and *A. misakiensis* Yendo; by Johansen (1968) for *A. ephedraea* (Lamarck) Decaisne; by Murata & Masaki (1978) for *A. zonata* Yendo; and by Srimanobhas & Masaki (1987) for *A. itonoi* V. Srimanobhas & T. Masaki. As a result, different types/patterns of development have been described for *Amphiroa* species. A sporangial origin has been recorded in the center/periphery (Johansen 1968; Harvey *et al.* 2009) or in the center and periphery separately (Ganesan 1971; Segawa 1940b; Srimanobhas & Masaki 1987). Two types of development have been described for the sporangial pore canal anatomy, one in which there is a ring of large block-shaped cells (Norris & Johansen 1981;

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Harvey *et al*. 2009) and another in which there are no block--shaped cells (Johansen 1968). Two patterns have also been described for the origin of the carposporangial filaments in the fusion cell: from the margins (Ganesan 1968; Riosmena--Rodríguez & Siqueiros-Beltrones 1996) or from the entire surface (Ganesan 1971). Despite all of these data, there is as yet no agreement across studies. For example, the origin of the carposporangial filaments in the fusion cell *A. rigida* is reported to be from the margins (Riosmena-Rodríguez & Siqueiros-Beltrones 1996) and from the entire surface (Segawa 1940b). This indicates the need for further studies on the development of the reproductive structures within *Amphiroa*.

In this study, we describe the patterns of development for sporangial and gametangial conceptacles of *Amphiroa beauvoisii* J.V. Lamouroux and *A. vanbosseae* Me. Lemoine; sporangial conceptacles of *A. misakiensis*; and gametangial conceptacles for *A. cryptarthrodia* Zanardini and *A. rigida*. Furthermore, comparisons are made among species and with published information and the taxonomic value of conceptacle development for species segregation is discussed.

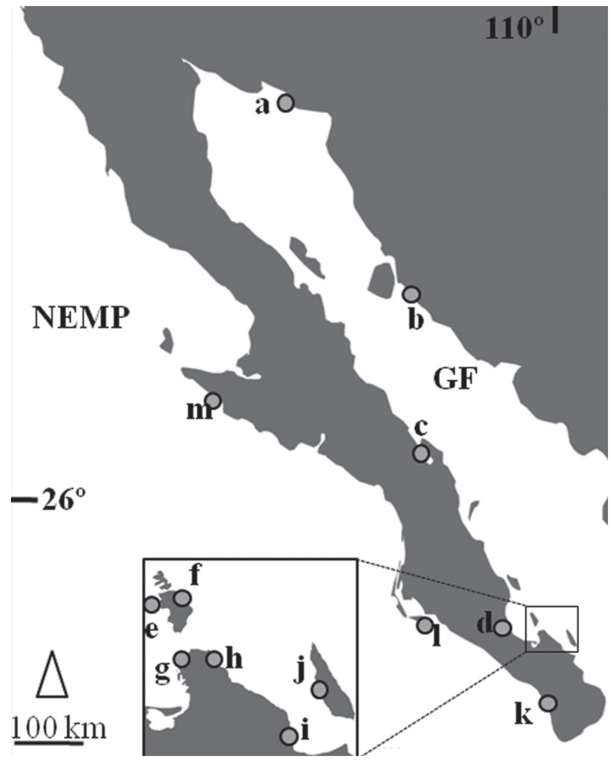


Figure 1. Location of the collecting sites: Gulf of California - (a) Puerto Peñasco, (b) Kino Nuevo, (c) Requesón, (d) San Juan de la Costa, (e) La Ballena, (f) I. Espíritu Santo, (g) Balandra, (h) Calerita, (i) El Sargento, (j) Isla Cerralvo; Northeastern Mexican Pacific - (k) Cerritos, (l) Isla Margarita, (m) Bahía Asunción.

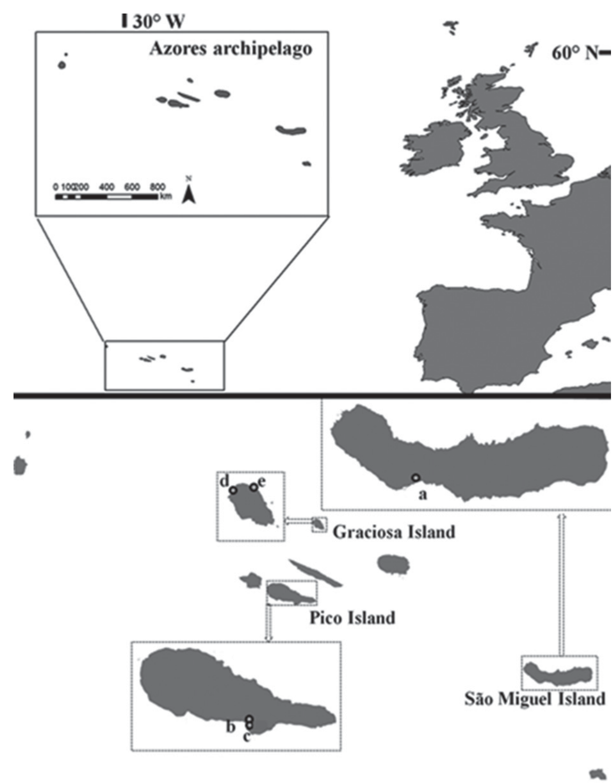


Figure 2. Location of the Azores archipelago and of the collecting sites: São Miguel Island - (a) Ilhéu São Roque; Pico Island - (b) Lajes do Pico, (c) Cais do Pico; Graciosa Island - (d) Baia da Vitória, (e) Santa Cruz.

Material and methods

A total of 112 specimens were studied, including fresh specimens and historical collections: sporangial/gametangial plants of *Amphiroa beauvoisii* J.V. Lamouroux (40 specimens) and *A. vanbosseae* Me. Lemoine (44 specimens); sporangial plants of *A. misakiensis* Yendo (10 specimens); and gametangial plants of *A. cryptarthrodia* Zanardini (10 specimens) and *A. rigida* J.V. Lamouroux (eight specimens). Fresh specimens were collected intertidally and subtidally, at a depth of 30 m, at 18 locations; 10 in the Gulf of California, in Mexico, three in the northeastern Mexican Pacific (Fig. 1) and five in the Azores archipelago of Portugal (Fig. 2). Collecting sites and voucher specimens are listed in Tab. 1. Algae were removed from the substrate with a hammer and chisel, brought to the laboratory and fixed in 4% formalin (v/v) in seawater. Specimens were stored in the Phycological Herbarium of the Autonomous University of Baja California Sur, in Baja California Sur, Mexico (code, FBCS) and in the Ruy Telles Palhinha Herbarium of the University of the Azores, in Ponta Delgada, Portugal (code, AZB). Historical collections encompass dried specimens of *A. cryptarthrodia* and *A. beauvoisii* from the Dutch oceanographic CANCAP V expedition housed at the National Herbarium Nederland, Leiden University branch, in Leiden, the Netherlands (code, L). Herbarium abbreviations are as in Holmgren & Holmgren (1998).

Permanent microslides from longitudinal sections in the sagittal region of bi-tetrasporangial and gametangial conceptacles were obtained following the histological technique of Riosmena-Rodríguez *et al*. (1999). The number of evaluated conceptacles was 130 for *Amphiroa beauvoisii*, 80 for *A. vanbosseae*, 50 for *A. misakiensis*, 30 for *A. cryptarthrodia* and 30 for *A. rigida*. Representative developmental

Table 1. Sample collection information for the studied species of *Amphiroa* from the Gulf of California, the Northeastern Mexican Pacific and the Azores archipelago.

Region	Collecting sites	Species	Vouchers
Gulf of California*	Puerto Peñasco	*A. vanbosseae*	134
	Kino Nuevo	*A. beauvoisii*	654
	Requesón	*A. vanbosseae*	8485
	San Juan de la Costa	*A. beauvoisii*	158
		A. valonioides	no number
		A. vanbosseae	no number
	Islote, La Ballena	*A. beauvoisii*	9490, 9497
		A. misakiensis	9496
		A. rigida	9499
	Isla Espíritu Santo	*A. rigida*	452
	Balandra	*A. vanbosseae*	1886
	Calerita	*A. beauvoisii*	9494
		A. misakiensis	526, 1568
		A. vanbosseae	9495
	El Sargento	*A. misakiensis*	526
		A. vanbosseae	7951-7958, 7960-7964, 8340, 8351, 8358
	Isla Cerralvo	*A. misakiensis*	5889
		A. rigida	456
	Cerritos	*A. misakiensis*	123
Northeastern Mexican Pacific*	Isla Margarita	*A. beauvoisii*	7543
	Bahía Asunción	*A. beauvoisii*	7300, 7306, 7325, 7328, 7331, 7345, 7356, 7378, 7384
Azores archipelago**	Ilhéu São Roque	*A. cryptarhrodia*	L 0650053, L 0650056
		A. beauvoisii	SMG-94-332
	Lajes do Pico	*A. beauvoisii*	PIX-07-09
	Cais do Pico	*A. beauvoisii*	L 0650058
	Baia da Vitória	*A. cryptarhrodia*	GRW-06-154
			GRW-06-192
	Santa Cruz	*A. cryptarhrodia*	GRW-06-92

*Phycological Herbarium of the Autonomous University of Baja California Sur (code, FBCS).
**Ruy Telles Palhinha Herbarium of the University of the Azores (code, AZB).

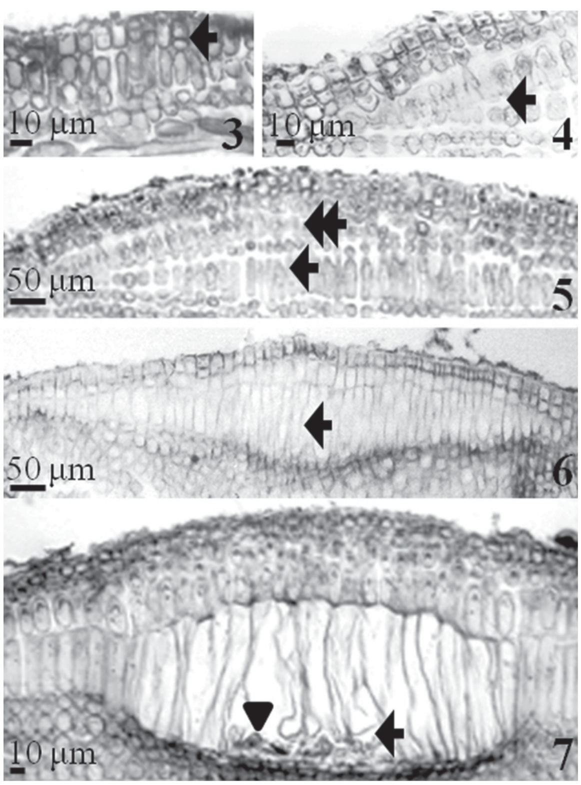
stages were described and photographed with a digital camera (model C5060; Olympus, Tokyo, Japan) attached to a compound microscope (BX50F; Olympus) and edited with Photoshop 6.0.1 software (Adobe Systems Incorporated, San Jose, CA, USA). Additional data were obtained from Segawa (1940b), Ganesan (1968; 1971), Johansen (1968), Murata & Masaki (1978), Norris & Johansen (1981), Srimanobhas & Masaki (1987), Riosmena-Rodríguez & Siqueiros-Beltrones (1991; 1996), Womersley (1996), Moura & Guimarães (2002), Harvey *et al.* (2009), and Rosas-Alquicira *et al.* (2010). Vegetative anatomical terminology follows that of Woelkerling (1988), and reproductive terminology follows that of Johansen (1968; 1981), Murata & Masaki (1978) and Srimanobhas & Masaki (1987).

Results

Sporangial conceptacle development

We investigated the development of the sporangial conceptacles prior to conceptacle pore formation in three species (*Amphiroa beauvoisii*, *A. misakiensis* and *A. vanbosseae*) and found it to be identical all three. Therefore, the various stages are illustrated by selected examples of these taxa. The youngest stage observed is the formation of a conceptacle is the appearance of an initial conceptacle dome with a cellular cap (Johansen 1968). A group of peripheral cells in a subsurface stratum elongates anticlinally; these cells are designated cavity cells (Johansen 1968), as shown in Fig. 3 and 4. The cavity cells elongate and divide to form rows of short overlying cells (Fig. 5). These layers of small cells, derived from the cavity cells, are pushed upward by further elongation of the cavity cells and eventually become the conceptacle roof (Fig. 6). Before cavity cells reach their full length, the destruction of some of them begins. Atrophy of the cavity cells forms a cavity, the so-called conceptacle chamber, followed by the development of the reproductive structures.

For all three of the species evaluated (*Amphiroa beauvoisii*, *A. misakiensis* and *A. vanbosseae*), atrophy of the cavity cells creates space for the sporangial initials (Fig. 7). Sporangial initials (Fig. 8-15) develop in the periphery of the chamber floor (*A. beauvoisii*, Fig. 8) or in the periphery and center (*A. misakiensis* and *A. vanbosseae*, Fig. 12 and 14). In all three species, while the initials are still very small, they elongate and divide transversally to form the sporangia mother cells and their stalk cells (Fig. 11 and 13). Autolysis continues until the adjacent cavity cells are largely destroyed. Further growth mostly involves tetrasporangial or bisporangial maturation (Fig. 14 and 15, respectively) and cavity cell destruction. An exception occurs in *A. beauvoisii* (Fig. 8, 10 and 15), in which cavity cells are still present in conceptacles with mature sporangia.



Figures 3-7. Early stages in the formation of sporangial conceptacles. *Amphiroa beauvoisii* J.V. Lamouroux (3-6) and *Amphiroa vanbosseae* Me. Lemoine (7): 3. Cell elongation in the peripheral region (arrow) of the intergeniculum on a sporangium forming an initial conceptacle dome; (4). Cell division and formation of new layers beneath the initial conceptacle dome (arrows); (5). The initial conceptacle cavity (arrow) covered by several layers of cells (double arrow); (6). Progressive elongation of the cavity cells (arrow); (7). Destruction of the cavity cells (arrow). Also note sporangia initials (arrowhead).

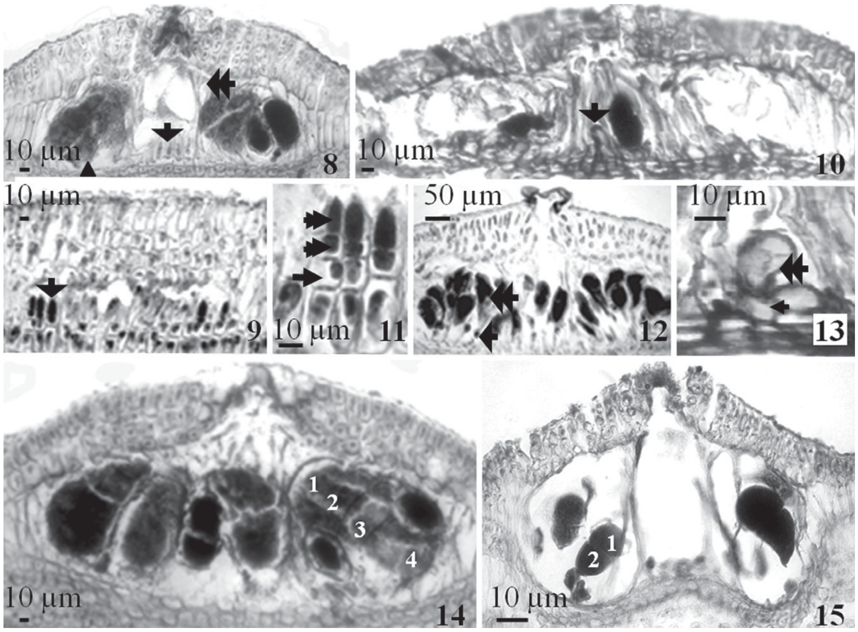
The conceptacle pore (Fig. 16-22) is initiated by an increase in the volume of subepithallial cells in the central region of the roof cavity (Fig. 16), to form large block-shaped cells (Fig. 19). The cells in the roof and immediately below the block-shaped cells elongate centripetally and migrate towards the future location of the pore (Fig. 17 and 18), compressing the cavity cells. Their continuous elongation causes the autolysis of the large block-shaped cells and the formation of the conceptacle pore (Fig. 19). A different pattern occurs in *Amphiroa misakiensis*, in which no large--block shaped cells appear. Instead, the cells located in the center of the roof elongate (Fig. 20) and those closest to the cavity show signs of atrophy (Fig. 21). The decalcification and atrophy of the cells immediately below the subepithallial cells results in the formation of a canal leading to the exterior (Fig. 22). This canal soon becomes lined with small, centripetally oriented elongated roof cells.

The final stage of development observed was senescence of the conceptacle. Two patterns were observed; the first, observed in the sporangial conceptacles of *Amphiroa misakiensis* (Fig. 23 and 24), was initiated by the downward, lateral and upward growth of the cells of the conceptacle floor and the second pattern, observed in the sporangial conceptacles of *A. beauvoisii* and *A. vanbosseae*, was initiated solely by the downward and lateral growth of the cells of the conceptacle floor (Fig. 25 and 26). For all conceptacle types, senescence occurred in the presence of tetrasporangia (Fig. 26).

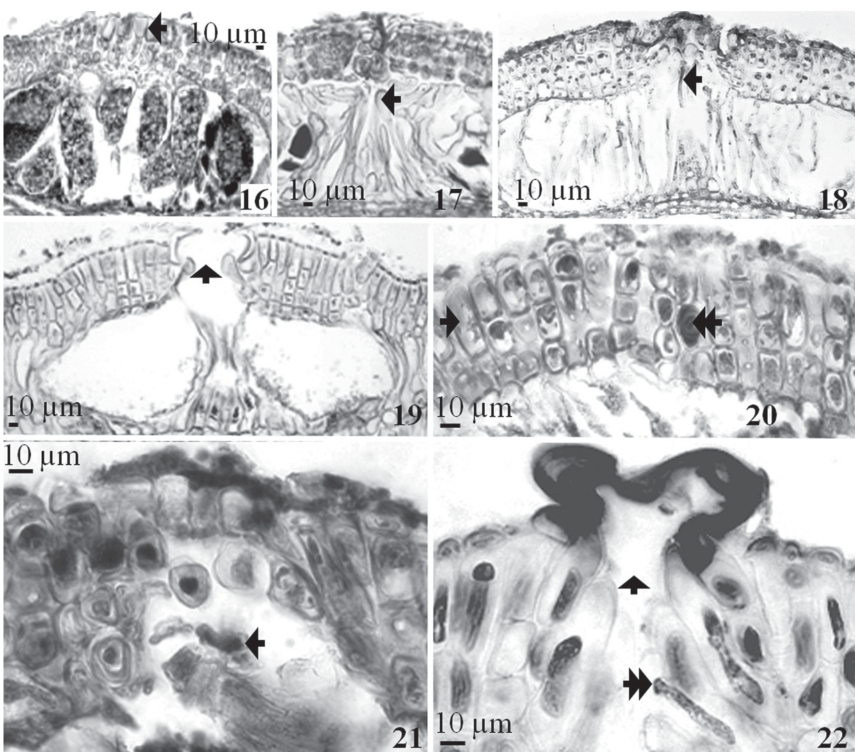
Gametangial and carposporangial conceptacle development

We evaluated the development of male and female conceptacles in *A. beauvoisii, A. cryptarthrodia*, *A. rigida* and *A. vanbosseae*. Because conceptacle development was identical in all of those species, the various stages are illustrated with examples selected from among those taxa. The first stage in the development of male and female conceptacles is characterized by the anticlinal elongation and division of a group of peripheral cells in a subsurface stratum of the intergenicula. Those cells are designated cavity cells (Johansen 1968). The continued elongation and delayed cell division result in the formation of an initial conceptacle dome with a cellular cap (Johansen 1968), as shown in Fig. 27. The cavity cells secrete a mucilaginous material from their distal portion, the cap, formed by copious amounts of light, clear material (Fig. 27). Due to the presence of the cap, the epithallial cells and the central peripheral cells of the dome become detached (Fig. 28).

Cavity cell dissolution begins above the male gametangial initials and progresses upward toward the cavity cells and the conceptacle dome (Fig. 28). Following this destruction, the surrounding tissue grows over the fertile layer, forming the conceptacle roof (Fig. 29 and 30). Because the reproductive initials are still relatively small when this growth occurs, the lack of filling material within the cavity allows the roof to

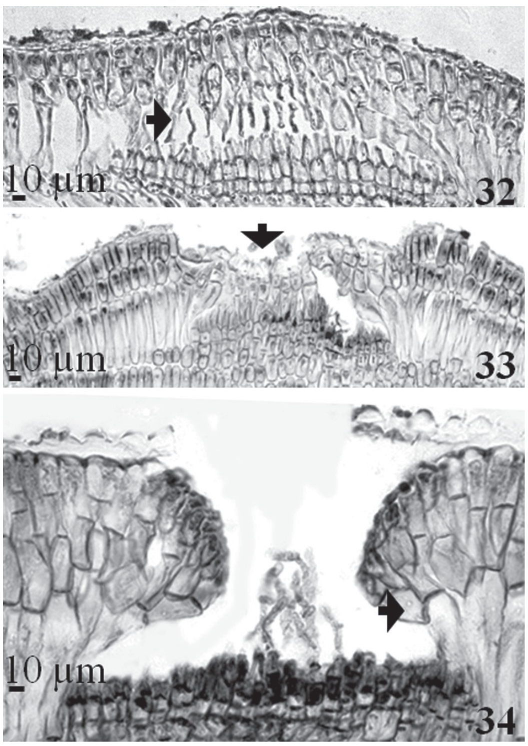


Figures 8-15. Development of sporangia in *Amphiroa beauvoisii* J.V. Lamouroux (8, 10 and 15), *Amphiroa misakiensis* Yendo (9, 11-13) and *Amphiroa vanbosseae* Me. Lemoine (14): (8). Undeveloped sporangia mother cells (arrow), interspersed cavity cells (double arrow) and peripheral mature tetrasporangia (arrowhead); (9). Sporangia mother cells and their stalk cell development on the periphery of the cavity floor (arrow); (10). Sporangium mother cell and its stalk cell development on the center of the cavity floor; (11). One stalk cell (arrows) and bisporangia mother cells (double arrow); (12). One stalk cell (arrows) and mature tetrasporangia (double arrow); (13). Single stalk cells (arrow) and sporangia mother cell (double arrow); (14). Mature tetrasporangia with four zonately arranged spores (numbered); (15). Mature bisporangia with two zonately arranged bispores (numbered).

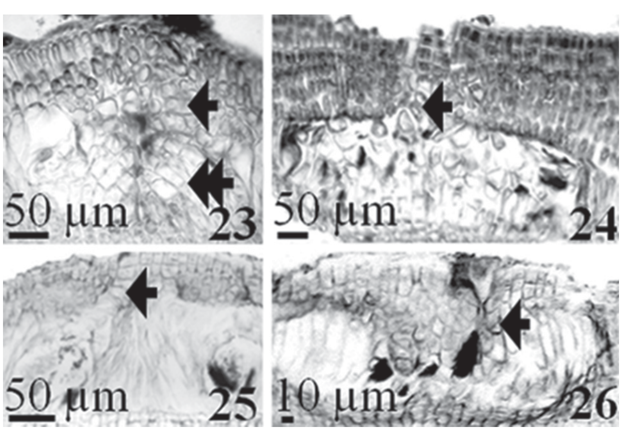


Figures 16-22. Development of sporangial conceptacle pores of *Amphiroa beauvoisii* J.V. Lamouroux (16-19) and *Amphiroa misakiensis* Yendo (20-22): (16). Increase in volume of central subepithallial cells (arrow); (17 and 18). Initial pore development by compression of the cavity cells (arrow); (19). Conceptacle pore (arrow); (20). Elongation of the roof cavity central cells (arrow), and decalcification of the roof cavity central cells (darkly staining, double arrow); (21). Atrophy of the roof cavity central cells (arrow); (22). Pore canal lined by small centripetally-oriented filaments (double arrow) and conceptacle pore (arrow).

grow downward as well as centripetally. The terminal part of the roof elongates and divides transversely (Fig. 31), providing new cells that repeat the process until the pore canal formation is completed. In the female gametangial initials, the process follows the same pattern, with cavity cell dissolution (Fig. 32 and 33), followed by the formation of a conceptacle roof and pore canal (Fig. 33 and 34).



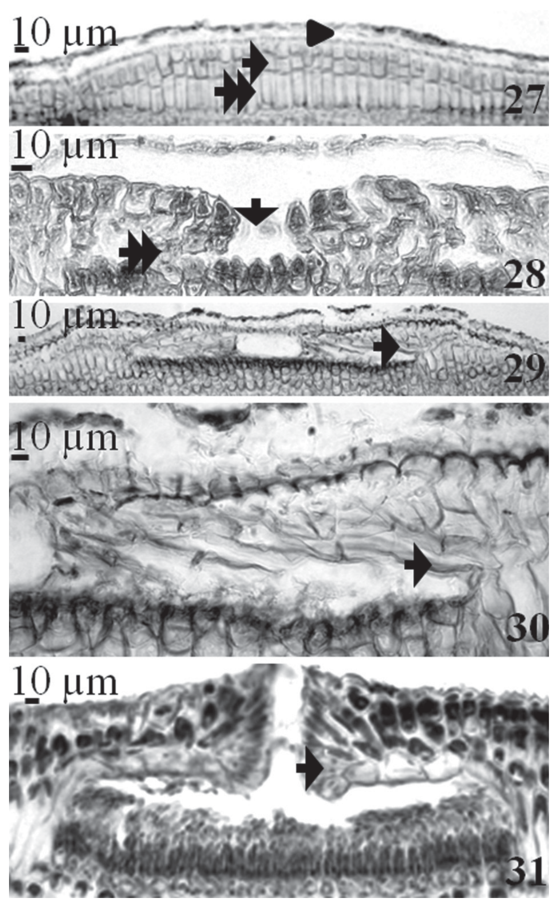
Figures 32-34. Early stages in the formation of the female conceptacles of *Amphiroa cryptarthrodia* Zanardini (32), *Amphiroa beauvoisii* J.V. Lamouroux (33) and *Amphiroa vanbosseae* Me. Lemoine (34): (32). Cavity cells dissolution (arrow) above the gametangial initials; (33). Initial conceptacle dome cells dissolution (arrow) above the gametangial initials; (34). Surrounding cavity cells (arrow) grow over the fertile area forming the conceptacle roof.



Figures 23-26. Senescence of sporangial conceptacles of *Amphiroa misakiensis* Yendo (23 and 24) and *Amphiroa beauvoisii* J.V. Lamouroux (25 and 26): (23). Growth of the cells of the central region of the roof cavity (arrow) and the conceptacle floor (double arrow); (24). Complete senescence of the pore (arrow); (25 and 26). Downward growth of the cells of the central region of the roof cavity (arrow).

Male gametangia were observed only for *Amphiroa beauvoisii* and *A. vanbosseae*. Their development begins with the formation of basal cells from the transversal basal division of the cavity cells (Fig. 35). After cavity cell dilution, each basal cell forms two spermatangial mother cells and the spermatangia continuously derive from the spermatangia mother cells (Fig. 36-38) to form simple and unbranched filaments. The spermatangia in turn, liberate spermatia that fill the chamber (Fig. 38).

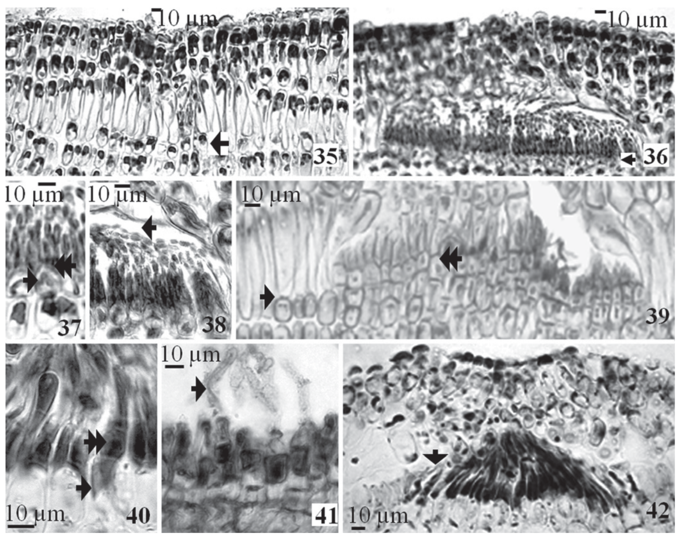
Female gametangia were observed for *Amphiroa beauvoisii, A. cryptarthrodia*, *A. rigida* and *A. vanbosseae.* Their development initiates with the elongation and transverse division of the basal region of the central cavity cells (Fig. 39). As a consequence, three new layers of cells cover the central region of the cavity floor; the elongation of cells from the terminal layer gives rise to the procarp initials (Fig. 39). Each of these, by elongation and transverse division, leads to the formation of a supporting cell and carpogonia branch initial (Fig. 40). The elongation and further division of the latter form the trichogyne (Fig. 41 and 42).



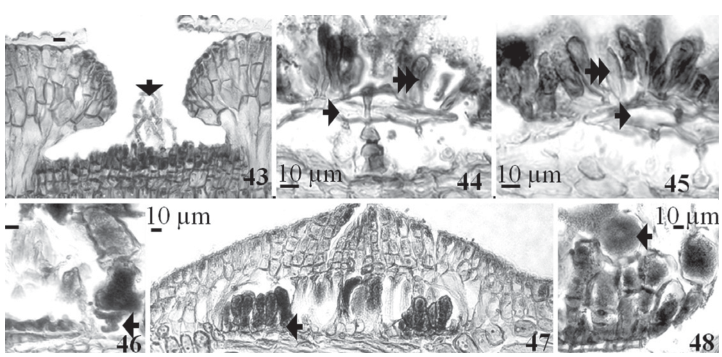
Figures 27-31. Early stages in the formation of the male conceptacles of *Amphiroa vanbosseae* Me. Lemoine (27) and *Amphiroa beauvoisii* J.V. Lamouroux (28-31): (27). Conceptacle cap (arrowhead) and formation of an initial conceptacle dome (arrow) above the developing conceptacle primordium (double arrow); (28). Cavity (double arrow) and protective dome cells (arrow) dissolution above the gametangial initials; (29 and 30). Surrounding cavity cells (arrow) grow over the fertile area forming the conceptacle roof; (31). Transverse division on the terminal part of surrounding cavity cells (arrow).

The fact that the basic reproductive features are similar to those in other coralline red algae (Lebednik 1977) suggests that spermatia fuse with trichogynes and that male and female haploid nuclei then combine to form a diploid nucleus. The development of carposporangial filaments was observed for *Amphiroa cryptarthrodia*, *A. rigida* and *A. vanbosseae*. This phase initiated when the trichogynes were well developed (Fig. 43). The presence of a carpogonial branch and a discontinuous flat disc-like fusion cell, supporting a few residual carpogonia on its dorsal part (Fig. 44 and 45), provided evidence that fertilization had occurred. In *A. vanbosseae*, the carposporangial filaments arose solely from the margins of the fusion cell (Fig. 46), whereas in *A. cryptarthrodia* and *A. rigida*, they arose over the entire surface of the fusion cell (Fig. 47). The cells of the carposporangial filaments increase in size, the upper being relatively large and constituting the carposporangium (Fig. 48).

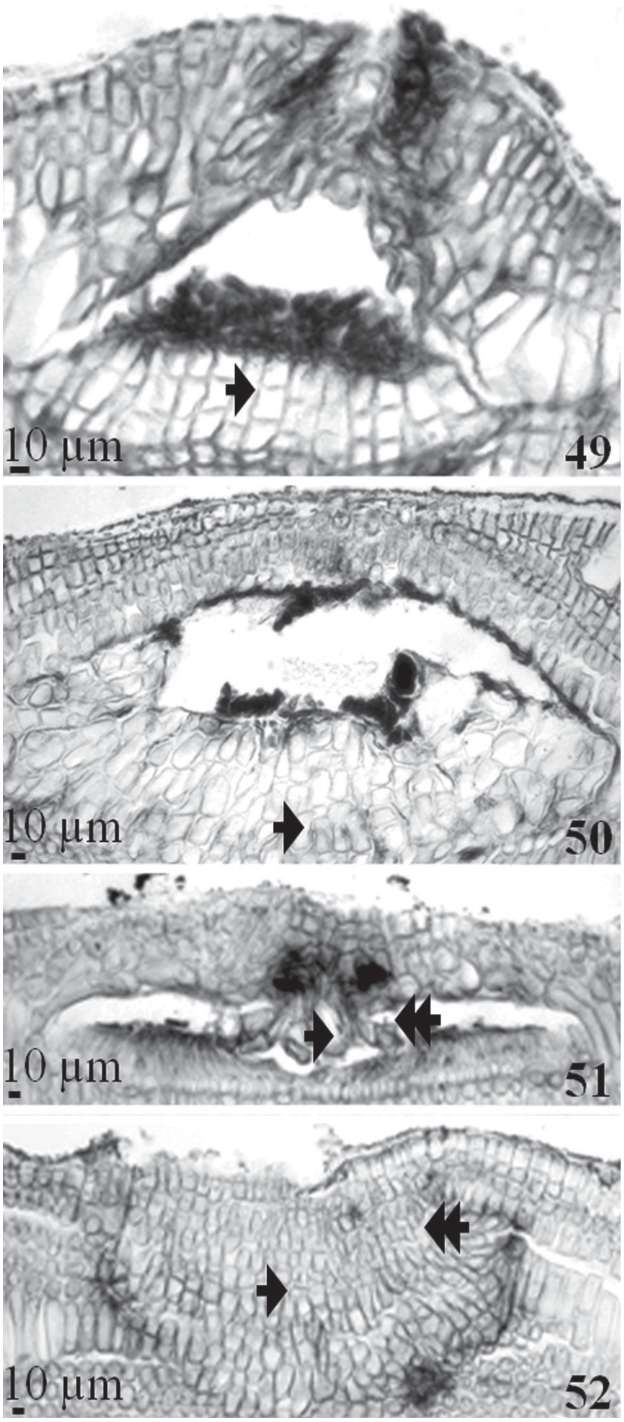
Two patterns were observed for the senescence of gametangial/carposporangial conceptacles. The first, observed in carposporangial conceptacles (Fig. 49 and 50), was initiated by the downward, lateral and upward growth of the cells of the conceptacle floor. The second pattern, observed in the gametangial conceptacles (Fig. 51 and 52), was initiated only by downward and lateral growth. For all conceptacle types, senescence occurred in the presence of carposporangia (Fig. 49) and gametangia (Fig. 51).



Figures 35-42. Development of male gametangia of *Amphiroa vanbosseae* Me. Lemoine (35-38), female gametangia of *Amphiroa vanbosseae* Me. Lemoine (40-42) and female gametangia of *Amphiroa beauvoisii* J.V. Lamouroux (39): (35). Anticlinal elongation and basal division of the central cavity cells giving rise to the basal cells (arrow); (36) Spermatangia mother cells on the cavity floor (arrow); (37). Basal cells (arrow) and spermatangia mother cells (double arrow); (38). Mature spermatangia branch reaching the cavity roof (arrow); (39). Elongation and successive transverse divisions of the basal region of the central cavity cells (arrow), forming the procarp initials (double arrow); (40). Supporting cell (arrow) and carpogonia branch initial (double arrow), scale bar, 10 µm; (41 and 42). Carpogonial branches with trichogynes (arrow).



Figures 43-48. Development of carposporangial filaments of *Amphiroa vanbosseae* Me. Lemoine (43-47) and *Amphiroa rigida* J.V. Lamouroux (48): (43). Well developed trichogynes (arrow). Also note the remnants of the protective dome (arrowhead); (44 and 45). Discontinuous flat disc-like fusion cell (arrow) supporting a few residual carpogonia (double arrow) on its dorsal part; (46). Carposporangial filaments arising from the margins of the fusion cell (arrow); (47). Carposporangial filaments arising from the entire surface of the fusion cell (arrow); (48). Mature carposporangial filament with a carposporangium (arrow).



Figures 49-52. Senescence of the gametangial/carposporangial conceptacle of *Amphiroa vanbosseae* Me. Lemoine (49-51) and *Amphiroa beauvoisii* J.V. Lamouroux (52): (49 and 50). Growth of the cavity floor cells (arrow); (51 and 52). Downward and lateral growth (arrow and double arrow respectively) of the cells of the conceptacle pore.

Discussion

The present study describes the conceptacle development of *Amphiroa* species. Our findings clearly demonstrate the importance of conceptacle anatomy in distinguishing between species within *Amphiroa*, the most diverse geniculate genus of the subfamily Lithophylloideae.

The development of the conceptacle pore is described and illustrated here for the first time, as is conceptacle senescence, for which four patterns were observed: two for sporangial conceptacles, one for spermatangial conceptacles and one for carposporangial conceptacles. As first described for *Amphiroa* by Johansen (1972), Murata & Masaki (1978) and Srimanhobas & Masaki (1987), we confirmed that conceptacle roof development was by intersporangial growth for sporangial conceptacles and by vegetative filament growth up and over the chamber for the gametangial conceptacles. The development of the sporangial conceptacle cavity by elongation and atrophy of cavity cells described for the genus by Johansen (1972) was also confirmed. Nevertheless, the presence of intact cavity cells among mature sporangia described and illustrated for *Amphiroa zonata* by Murata & Masaki (1978) was observed only for *A. beauvoisii* (cf. Fig. 15). The presence of cavity cells in mature sporangial conceptacles in this species was previously reported by Harvey *et al*. (2009).

The development of the carposporangial filament, previously suggested to be important for species identification within the genus *Fosliella* (Chamberlain 1977), was also found to be important for the genus *Amphiroa*. In *A. cryptarthrodia* and *A. rigida*, we found that the carposporangial filament arose from the surface of the fusion cell. This finding was previously reported for *A. rigida* by Segawa (1940b); for *A. foliacea* by Ganesan (1968); for *A. zonata* for Murata & Masaki (1978); and for *A. beauvoisii* and *A. valonioides* by Riosmena-Rodríguez & Siqueiros-Beltrones (1996). It is of note that Riosmena--Rodríguez & Siqueiros-Beltrones (1996) reported a different pattern for *A. rigida* from the Gulf of California. In *A. vanbosseae*, we found that the carposporangial filament arose only from the margins of the fusion cell, as previously reported by Riosmena-Rodríguez & Siqueiros--Beltrones (1996) and Moura & Guimarães (2002) and by Riosmena-Rodríguez & Siqueiros-Beltrones (1996) for *A. misakiensis* (Tab. 2).

We confirmed the diagnostic importance of the sporangial pore canal anatomy in distinguishing between species within the subfamily Lithophylloideae, namely in *Lithophyllum* (Woelkerling & Campbell 1992; Keats 1997; Harvey *et al*. 2005) and *Amphiroa* (Harvey *et al*. 2009). The type of sporangial pore canal anatomy reported in this study for *Amphiroa beauvoisii* and *A. vanbosseae* was also previously reported by Harvey *et al*. (2009), Norris & Johansen (1981) and Ganesan (1971). However, the present study reports and illustrates, for the first time, the complete occlusion of the pore canal by large block-shaped cells in both species (Fig. 8).

Based on the pore canal anatomy and the location of sporangia on the cavity floor, we created the following key, by which three of the five studied *Amphiroa* species can easily be distinguished:

Table 2. Events in sporangial/carposporangial conceptacle development for *Amphiroa* species.

Species	Reference	Tetrasporangial conceptacles		Carposporangial conceptacles
		Sporangia location on the cavity floor	Block-shaped cells	Origin of the carposporangial filament in the fusion cell
Amphiroa anceps (Lamarck) Decaisne	Ganesan (1968)		No	Margins
	Womersley (1996)	Periphery		
	Harvey *et al.* (2009)	Periphery	No	
Amphiroa beauvoisii J.V. Lamouroux	Present study	Periphery	Yes	
	Rosas-Alquicira *et al.* (2010)	Periphery	Yes	
	Riosmena-Rodríguez & Siqueiros-Beltrones (1996)			Entire surface
	Harvey *et al.* (2009)	Periphery	Yes	
Amphiroa currae Ganesan	Ganesan (1971)	Center and periphery		Entire surface
Amphiroa cryptarthrodia Zanardini	Present study			Entire surface
	Rosas-Alquicira *et al.* (2010)			Entire surface
Amphiroa ephedraea (Lamarck) Decaisne	Johansen (1968)	Periphery	No	Margins
Amphiroa foliacea J.V. Lamouroux	Ganesan (1968)			Entire surface
Amphiroa gracilis Harvey	Harvey *et al.* (2009)	Center and periphery	No	
Amphiroa itonoi V. Srimanobhas & T. Masaki	Srimanobhas & Masaki (1987)	Center and periphery	Yes	Entire surface
Amphiroa klochkovana A.S. Harvey, W.J. Woelkerling & A.J.K. Millar	Harvey *et al.* (2009)	Center and periphery	No	
Amphiroa misakiensis Yendo	Present study	Center and periphery	Yes	Entire surface
	Segawa (1940a)	Periphery		
	Riosmena-Rodríguez & Siqueiros-Beltrones (1991; 1996)			Margins
Amphiroa rigida J.V. Lamouroux	Present study	Center and periphery	Yes	Entire surface
	Segawa (1940b)	Center and periphery		Entire surface
	Riosmena-Rodríguez & Siqueiros-Beltrones (1996)			Margins
Amphiroa valonioides Yendo	Norris & Johansen (1981)		Yes	
	Riosmena-Rodríguez & Siqueiros-Beltrones (1996)			Entire surface
Amphiroa vanbosseae Me. Lemoine	Present study	Center and periphery	Yes	Margins
	Moura & Guimaraes (2002)			Margins
	Ganesa (1971)		Yes	
	Riosmena-Rodríguez & Siqueiros-Beltrones (1996)			Margins
Amphiroa zonata Yendo	Murata & Masaki (1978)	Center and periphery	Yes	Entire surface

1. Large block-shaped cells in sporangial conceptacle canal (Fig. 8)
   2. Sporangia location only in the periphery of the cavity floor (Fig. 8) .... 1. ***A. beauvoisii***
   2. Sporangia location in the center and periphery of the cavity floor (Fig. 14) .... 3. ***A. vanbosseae***
1. No large block-shaped cells in sporangial conceptacle canal (Fig. 12) .... 2. ***A. misakiensis***

Amphiroa vanbosseae exhibits the same pattern reported for *A. zonata* by Murata & Masaki (1978) and for *A. itonoi* by Srimanobhas & Masaki (1987); *A. beauvoisii* and *A. misakiensis* are closely related to the Australian species *A. gracilis* and *A. klochkovana* (Harvey *et al*. 2009). *A. cryptarthrodia* and *A. rigida* are not included in this key, because no sporangial conceptacles were observed in those species.

The consistency of the observed reproductive development patterns was found to be diagnostic for species within *Amphiroa*. It would be interesting to evaluate the importance of this feature in the closely-related genus from the *Lithophyllum-Titanoderma* complex included in the Lithophylloideae by Harvey *et al*. (2003), based on phylogenetic studies.

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References

Chamberlain, Y.M. 1977. Observations on *Fosliella farinosa* (Lamour.) Howe (Rhodophyta, Corallinaceae) in the British Isles. **British Phycological Journal 12**: 343-58.

Ganesan, E.K. 1968. Studies on the morphology and reproduction of the articulated Corallines-III. *Amphiroa* Lamouroux emend. Weber van Bosse. **Phykos 6**: 7-28.

Ganesan, E.K. 1971. *Amphiroa curare* (Corallinaceae), a new species of marine algae from Venezuela. **Phycologia 10**: 155-68.

Harvey, A.S.; Broadwater, S.T.; Woelkerling, W.J. & Mitrovski, P. 2003. *Choreonema* (Corallinales, Rhodophyta): 18S rDNA phylogeny and resurrection of the Hapalidiaceae for the subfamilies Choreonematoideae, Austrolithoideae and Melobesioideae. **Journal of Phycology 39**: 988-98.

Harvey, A.S.; Woelkerling, W.J.; Farr, T.; Neill, K. & Nelson, W. 2005. **Coralline algae of central New Zealand. An identification guide to common 'crustose' species**. Wellington, NIWA.

Harvey, A.S.; Woelkerling, W.J. & Millar, A.J.K. 2009. The genus *Amphiroa* (Lithophylloideae, Corallinaceae, Rhodophyta) from the temperate coasts of the Australian continent, including the newly described *A. klochkovana*. **Phycologia 48**: 258-90.

Holmgren, P.K. & Holmgren, N.H. 1998 [continuously updated]. **Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium**. http://sweetgum.nybg.org/ih/ (Accessed on 10 Sept 2011).

Johansen, H.W. 1968. Reproduction of the articulated coralline *Amphiroa ephedraeea*. **Journal of Phycology 9**: 141-48.

Johansen, H.W. 1972. Conceptacles in the Corallinaceae. **Proceedings of the 7th International Seaweed Symposium** 114-119.

Johansen, H.W. 1981. **Coralline Algae: A first Synthesis**. Boca Raton, CRC Press.

Keats, D.W. 1997. *Lithophyllum insipidum* Adey, Townsend et Boykins and *L. flavescens* sp. nov.: two flat lithophylloid coralline algae (Corallinales, Rhodophyta) abundant in shallow reef environments in Fiji. **Phycologia 36**: 351-365.

Lebednik, P.A. 1977. Postfertilization development in *Clathromorphum*, *Melobesia* and *Mesophyllum* with comments on the evolution of the Corallinaceae and the Cryptonemiales (Rhodophyta). **Phycologia 16**: 379-406.

Moura, C.W. & Guimarães, S.M.P. 2002. *Amphiroa van-boseae* (Corallinales, Rhodophyta) no Atlântico tropical americano. **Hoehnea 29**: 267-273.

Murata, K. & Masaky, T. 1978. Studies of reproductive organs in articulated coralline algae of Japan. **Phycologia 17**: 403-412.

Norris, J.N. & Johansen, H.W. 1981. Articulated coralline algae of the Gulf of California, Mexico, I: *Amphiroa* Lamouroux. **Smithsonian Contributions to the Marine Sciences 9**: 1-29.

Riosmena-Rodríguez, R. & Siqueiros-Beltrones, D.A. 1991. First report of sexual conceptacles of *Amphiroa misakiensis* (Yendo) in the Gulf of California. **Revista de Investigación Científica Serie Ciencias Marinas. Universidad Autónoma de Baja California Sur 2**: 8-12.

Riosmena-Rodríguez, R & Siqueiros-Beltrones D.A. 1996. Taxonomy of the genus *Amphiroa* (Corallinales, Rhodophyta) in the southern Baja California Peninsula, México. **Phycologia 35**: 53-65.

Riosmena-Rodríguez, R.; Woelkerling, W.J. & Foster M.S. 1999. Taxonomic reassessment of rhodolith-forming species of *Lithophyllum* (Corallinales, Rhodophyta) in the gulf of California, Mexico. **Phycologia 38**: 401-17.

Rosas-Alquicira, E.F.; Riosmena-Rodríguez, R. & Neto, A.I. 2010. Segregating characters used within *Amphiroa* (Corallinales, Rhodophyta) and taxonomic reevaluation of the genus in the Azores. **Journal of Applied Phycology 23**: 475-488.

Segawa, S. 1940a. Systematic anatomy of the articulated corallines. I. *Amphiroa rigida* Lamouroux. **Journal of Japanese Botany 16**: 219-25.

Segawa, S. 1940b. Systematic anatomy of the articulated corallines. II. *Amphiroa misakiensis* Yendo. **Journal of Japanese Botany 16**: 488-94.

Srimanobhas, V. & Masaki, T. 1987. *Amphiroa itonoi* (Corallinales, Rhodophyta), a new species of marine algae from Japan. **Japanese Journal of Phycology 35**: 1-9.

Woelkerling, W.J. 1988. **The Coralline red algae: An analysis of the genera and subphamilies of nongeniculate Corallinaceae**. London, Oxford University Press. Woelkerling, W.J. & Campbell, S.J. 1992. An account of southern Australian species of *Lithophyllum* (Corallinaceae, Rhodophyta). **Bulletin of the British Museum (Natural History). Botany series 22**: 1-107.

Womersley, H.B.S. 1996. **The marine benthic flora of southern Australia. Part III B**. Canberra, Union Offset, Australian Biological Resources Study.

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