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Formation of secondary connecting filaments, a new post-fertilisation stage in Mediterranean species of *Kallymenia* (Kallymeniaceae, Rhodophyta)

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Abstract – A new post-fertilisation stage, consisting of secondary connecting filaments produced from the primary connecting filament in the vicinity of the auxiliary cell branch system, was observed in Mediterranean monocarpogonial and polycarpogonial species of *Kallymenia*. In the monocarpogonial species the connecting filaments were formed from a little modified primary connecting filament close to the auxiliary cell and in the polycarpogonial species they were produced from a highly modified, swollen, hyaline portion of the primary connecting filament that remained attached to the auxiliary cell. Since most of these connecting filaments were seen clearly linked by pit connections in both instances, they are interpreted as secondary connecting filaments produced in essentially the same way as the primary connecting filaments that derive from the carpogonial branch fusion cell.

***Kallymenia* / Rhodophyta / Mediterranean Sea / monocarpogonial species / polycarpogonial species / secondary connecting filaments**

Résumé – La formation, près du rameau de la cellule auxiliaire, de plusieurs filaments de jonction secondaires à partir du filament de jonction primaire qui fusionne avec la cellule auxiliaire a été observée, pour la première fois, dans deux espèces méditerranéennes du genre *Kallymenia*, l'une monocarpogoniale, l'autre pluricarpogoniale. Dans l'espèce monocarpogoniale, les filaments de jonction secondaires sont émis par un filament de jonction primaire peu modifié ; dans l'espèce pluricarpogoniale, par une portion très modifiée, gonflée, hyaline, du filament de jonction primaire, qui reste attachée à la cellule auxiliaire. La plupart de ces filaments ont été vus liés par des synapses à la portion plus ou moins modifiée du filament de jonction primaire. Ils sont donc interprétés comme étant des filaments de jonction secondaires qui se forment essentiellement de la même façon que les filaments de jonction primaires émis par la cellule de fusion du rameau carpogonial.

***Kallymenia* / Rhodophyta / mer Méditerranée / espèce monocarpogoniale / espèce polycarpogoniale / filaments de jonction**

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INTRODUCTION

The genus *Kallymenia* currently includes 36 species (Guiry & Guiry, 2011) with foliose thalli, a multiaxial structure consisting of a compact cortex of few layers of cells decreasing in size towards the surface and a loose medulla composed of filaments intermixed with more or less refractive stellate cells. The genus is non-procarpic. The carpogonial branch system is either polycarpogonial or monocarpogonial and generates a fusion cell after presumed fertilisation that produces connecting filaments. The auxiliary cell branch systems are distinct and distant from the carpogonial branch system. Following the fusion of a connecting filament with an auxiliary cell branch system, the gonimoblasts develop from the connecting filament (Norris, 1957) or, in some instances, from vegetative cells in the vicinity of the auxiliary cell branch system (Hommersand & Ott, 1970).

Six species of the genus are reported from the Mediterranean Sea: *K. reniformis* (Turner) J. Agardh, *K. feldmannii* Codomier, *K. requienii* (J. Agardh) J. Agardh, *K. lacerata* Feldmann, *K. patens* (J. Agardh) P.G. Parkinson and *K. spathulata* (J. Agardh) P.G. Parkinson. The last three species are endemic to this sea (Furnari *et al.*, 2003, 2010). Recently, Rodriguez-Prieto & Hommersand (2009) questioned the presence of *K. reniformis* in the Mediterranean.

Pre- and post-fertilisation stages in these species were described in detail by Norris (1957), Hommersand & Ott (1970), Irvine (1983) and Hommersand & Fredericq (1990) (*K. reniformis*); Vergés (2001) (*K. reniformis*, *K. feldmannii*, *K. lacerata*, *K. requienii*); Vergés & Rodríguez-Prieto (2006a, *K. lacerata*, 2006b, *K. patens*); Rodriguez-Prieto and Hommersand (2009) (*K. reniformis*, *K. feldmannii*, *K. lacerata, K. patens*, *K. requienii*), except for *K. spathulata* in which reproductive structures are still unknown. The last of these authors particularly investigated the nuclear behaviour during development of the female reproductive system in five species.

In this paper, we present and discuss a post-fertilisation event absent in previous studies, which we observed in two species of *Kallymenia* collected from Lazio (Central Italy, Tyrrhenian Sea) coast.

MATERIAL AND METHODS

Fertile specimens were collected in 2001, by SCUBA, at Lazio coasts (Fig. 1), and are represented by two monocarpogonial specimens (Figs 2-3) found in July at La Botte rock (a rock off shore between the islands of Ponza and Ventotene, 40°50'35''N, 13°06'50''E), at 35 m depth (referred to here as *Kallymenia* sp. 1), and one polycarpogonial specimen (Fig. 8) found on the continental coast, at S. Marinella (42°02'03''N, 11°50'25''E), in December, at 18 m depth (referred to here as *Kallymenia* sp. 2). The thalli fixed in 4% formalin/seawater are kept in our private collection at the Phycological Laboratory of the Dipartimento di Biologia Ambientale of Sapienza University of Rome. In order to investigate the reproductive structures, small square pieces of thallus, 2 cm side, were softened by immersion in distilled water for 2-3 days, then squashed and directly observed.

Abbreviations used in the figures are as follows: ac = auxiliary cell; cc = cortical cell; cp = carpogonium; csc = cortical stellate cell; cw = cross wall; icf = incoming primary connecting filament; icf_s = swollen cellular portions of the incoming primary connecting filament; gf = gonimoblast filaments; hc =

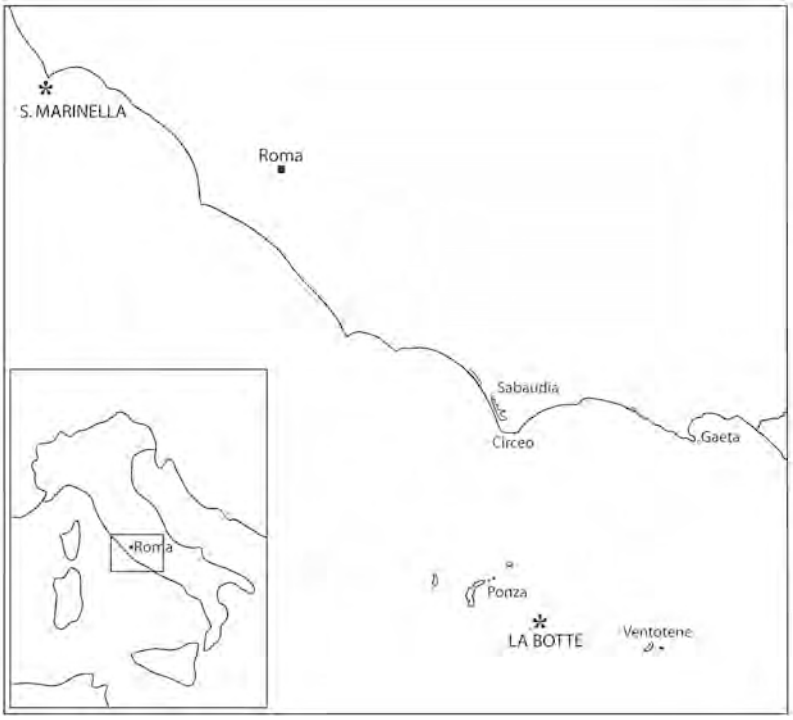


Fig. 1. Continental coast and Pontine Islands (Lazio). Asterisks indicate the sampling stations.

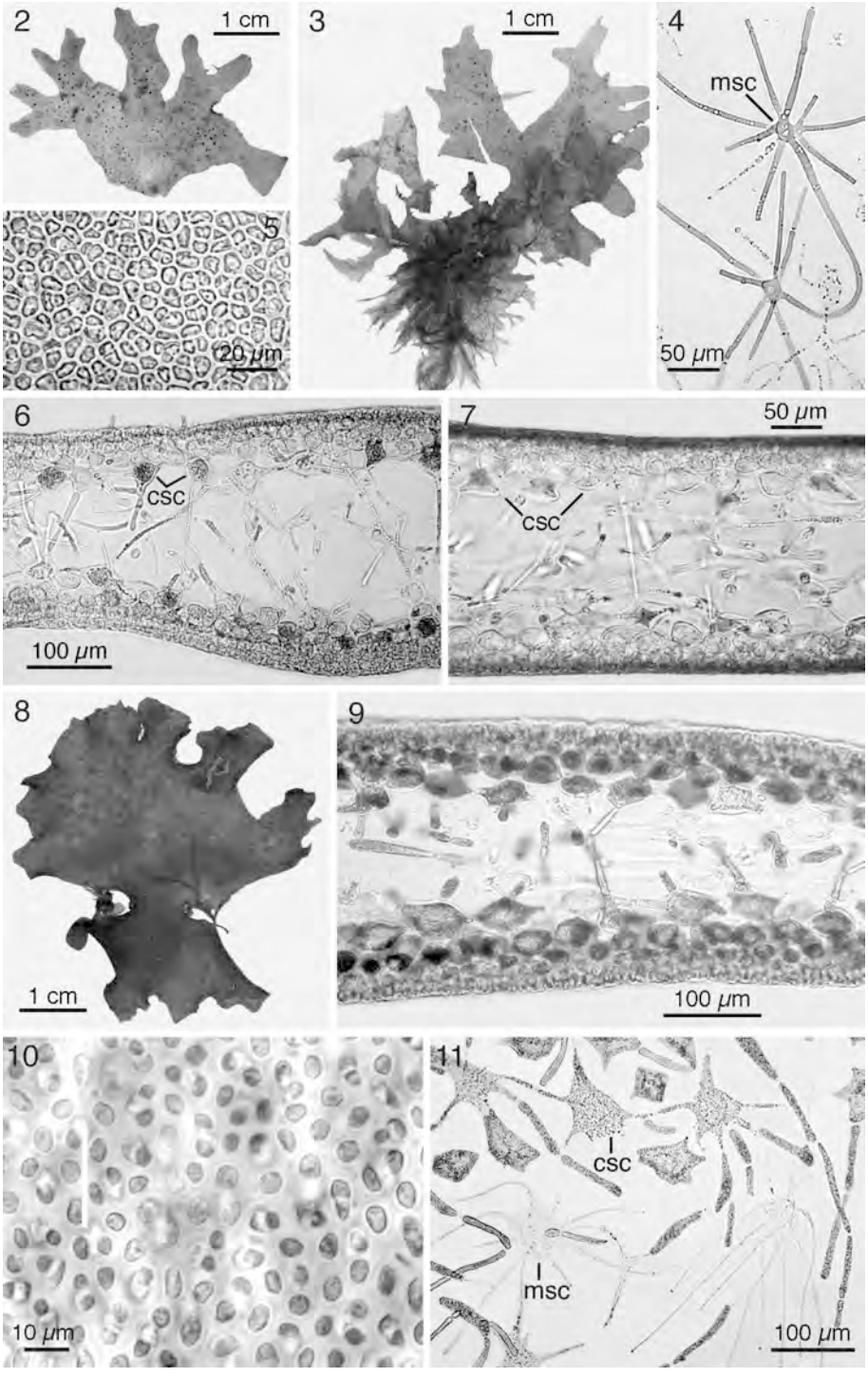
hypogenous cell; msc = medullary stellate cell; ocf = outgoing secondary connecting filament; sbc = subsidiary cell; sc = supporting cell; st = slightly swollen tips of primary connecting filaments; t = trichogyne.

RESULTS

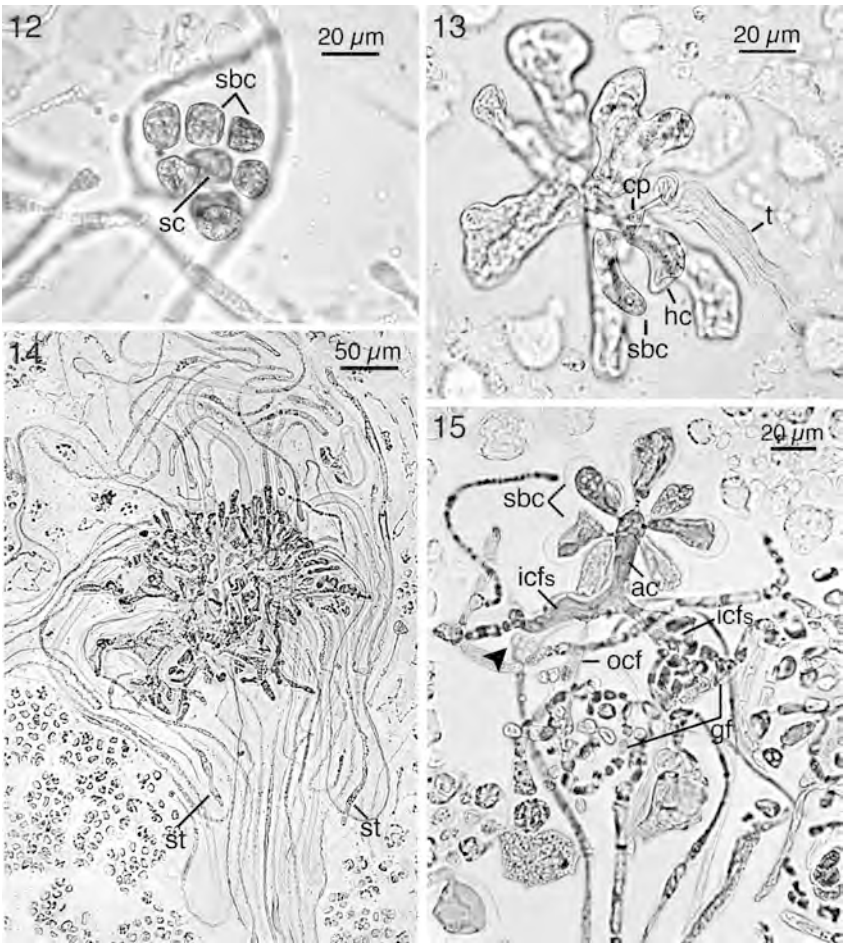
Kallymenia sp. 1 (monocarpogonial specimens)

Habit and structure. The two fertile specimens have membranous fronds up to 4.5 cm wide and 6 cm high, with apices divided pseudodichotomously into segments (Figs 2-3). The cortical cells of the outer layer, 4-10 μm wide, are more or less closely packed and irregularly shaped in surface view (Fig. 5). Transverse sections of thalli show a cortex composed of 4-5 compact layers of cells decreasing in size towards surface (Figs 6-7). Stellate cells of the inner cortical layer have short arms (up to 35 μm in length) and a body up to 37-40 μm in diameter (Figs 6-7). The medulla is trasversed by filaments and refractive stellate cells in which the body is 16-25 μm in diameter and the arms are 30-210 μm in length (Fig. 4).

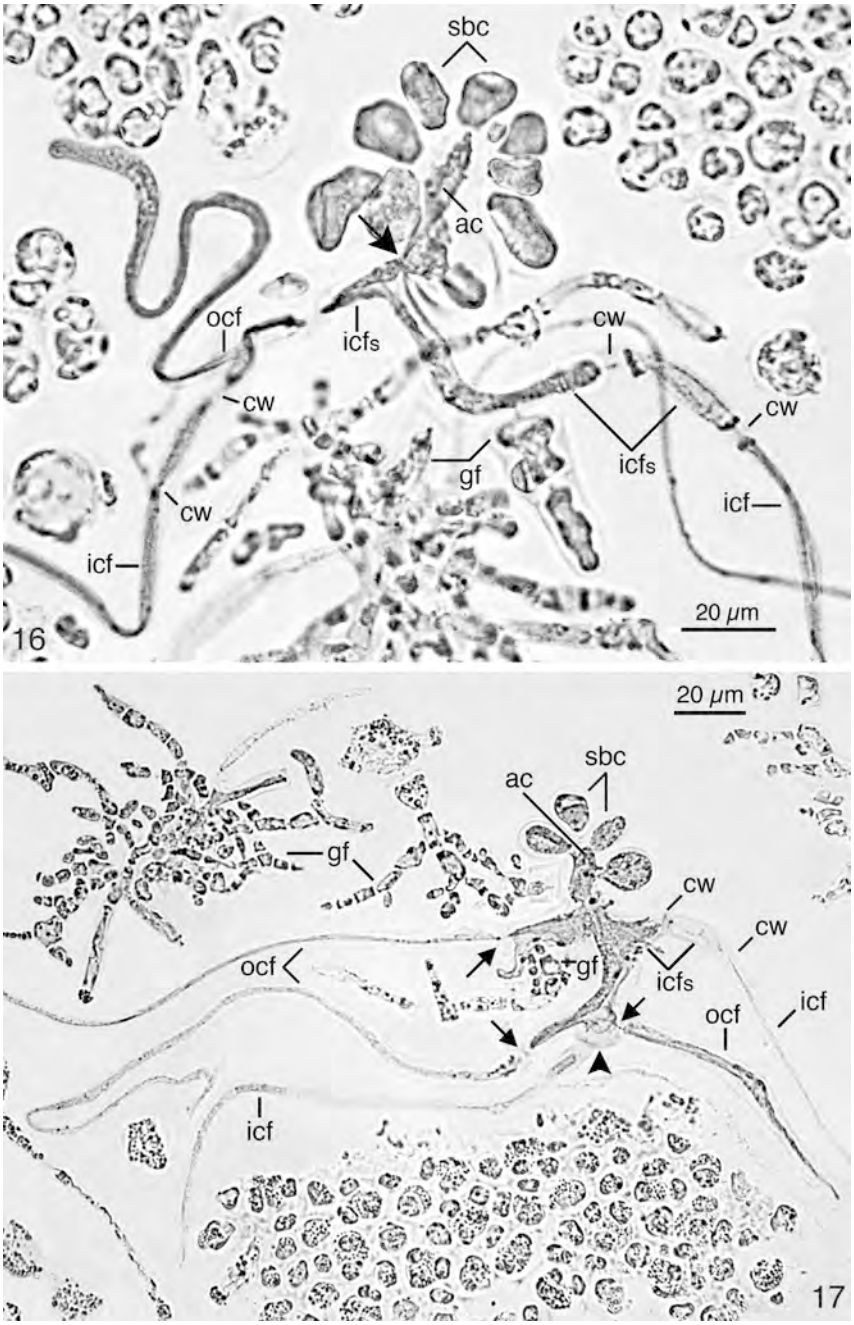
*Female reproductive structures and development of gonimoblasts.*The mature carpogonial branch system consists of 6-7 prominent, clavate, sometimes lobed subsidiary cells and a single 2-celled carpogonial branch, with the hypogenous cell long, curved and similar in shape and length to the basal subsidiary cell that bears the carpogonial branch (Fig. 13). The carpogonium is triangular in shape and has a


2
1 cm
3
1 cm
4
msc
50 µm
5
20 µm
6
csc
100 µm
7
50 µm
csc
8
1 cm
9
100 µm
10
10 µm
11
csc
msc
100 µm

◀ Figs 2-11. *Kallymenia* sp. 1 and sp. 2. Habit and structure. **2-7.** *Kallymenia* sp. 1 (La Botte rock, 35 m, July). Monocarpogonial specimens. **2-3.** Habit. The base of the thallus in Fig. 3 is covered with epiphytes. **4.** Stellate cells in medulla. **5.** Cortical cells in surface view. **6.** Transverse section of blade of specimen in Fig. 2. **7.** Transverse section of blade of specimen in Fig. 3. **8-11.** *Kallymenia* sp. 2 (S. Marinella, 18 m, December). Polycarpogonial specimen. **8**. Habit. **9**. Transverse section of blade. **10**. Cortical cells in surface view. **11**. Stellate medullary and cortical cells.



Figs 12-15. *Kallymenia* sp. 1. Monocarpogonial specimens. Development of carpogonial branch and auxiliary cell branch systems and formation of gonimoblasts and secondary connecting filaments. **12.** Young carpogonial branch system or auxiliary cell branch system. **13.** Mature carpogonial branch system. **14.** Functional fusion cell with numerous, long connecting filaments with slightly swollen tips (st). **15.** Auxiliary cell branch system after fusion with a primary connecting filament. The incoming primary connecting filament that has fused with the auxiliary cell is not detectable in this figure but its swollen parts (icf_s) are clearly visible. Note the secondary connecting filament (ocf) linked by pit connection and on the far left the transparent swollen apex (arrowhead) of the second incoming primary connecting filament.



long trichogyne that is coiled at the base (Fig. 13). The auxiliary cell branch system consists of the auxiliary cell and 4-8 subsidiary cells, which are initially small and isodiametric (Fig. 12) but later enlarge and become clavate (Figs 15-17). Few

◀ Figs 16-17. *Kallymenia* sp. 1. Monocarpogonial specimens. **16.** Fusion of a primary connecting filament with an auxiliary cell branch system. The primary connecting filament (icf) on the right has cut off two cells that are slightly enlarged (icf_s), the proximal cell being hyaline and the distal cell having dense contents. Note the thick cell walls (cw), the point of fusion (arrow) between the distal cell of the primary connecting filament and the auxiliary cell (twisted just above the point of fusion) (ac) and the secondary connecting filament (ocf) coming out laterally. Note also a second primary connecting filament (icf) at the lower left that has cut off two cells approaching the auxiliary cell branch. **17.** A later stage showing a higher degree in swelling of both the dense distal and the hyaline proximal parts (icf_s) of the primary connecting filament with their intercalary cell walls (cw) still visible. Three secondary connecting filaments (ocf) linked by pit connections (arrows) go out from the swollen distal part of the primary connecting filament. Note the second incoming primary connecting filament (icf) with its transparent swollen apex (arrowhead).

functional multilobed fusion cells were observed, bearing numerous long, non-septate connecting filaments with slightly swollen tips (Fig. 14). Fusion between a connecting filament and an auxiliary cell branch system is illustrated in Fig. 16. The incoming primary connecting filament on the right has cut off two cells with thick cell walls, the proximal cell being hyaline and the distal having dense contents. Both cells appear slightly enlarged. The fusion occurs between the distal cell of the incoming primary connecting filament and the auxiliary cell (Fig. 16, arrow). A later stage of development can be seen in Fig. 17, which shows a slightly greater degree of swelling in both the cells cut off by the incoming primary connecting filament. The gonimoblast filaments arise in separate packets from the distal cell of the incoming primary connecting filament that has fused with the auxiliary cell, which produces also one to three outgoing secondary connecting filaments linked by pit-connections (Figs 15-17). A second incoming primary connecting filament was sometimes observed close to an auxiliary cell branch system that had already fused with a first incoming primary connecting filament (Figs 15-17). The second incoming primary connecting filament was seen either in a early stage, having cut off two apical cells that approach the auxiliary cell branch system (Fig. 16), or in a later stage, showing the apex that has failed the fusion with the auxiliary cell stopped against the swollen distal part of the first incoming connecting filament that has fused with the auxiliary cell, and become swollen and depleted (Figs 17 and 15, arrowhead). At maturity, the gonimoblasts form compact masses of carposporangia that form spherical bright red swellings scattered over the surface of the blade on both sides (Figs 2-3). Carpospores were often seen germinating *in situ*.

Kallymenia sp. 2 (polycarpogonial specimen)

*Habit and structure.*The fertile thallus is compressed, rigid, lobed, and up to 5 cm wide and high (Fig. 8). The cortical cells of the outer layer, (3-) 4-7 µm in diameter, are rounded in surface view (Fig. 10). Transverse sections of the thallus show a cortex composed of five layers of cells decreasing in size towards the surface (Fig. 9). Stellate cells of the inner cortical layer have a body up to 75 (-82) µm in diameter and arms up to 45 µm in length (Figs 9, 11). Medullary stellate cells are colourless, and have a body 52-60 µm in diameter and radiating arms up to 230 µm in length (Fig. 11).

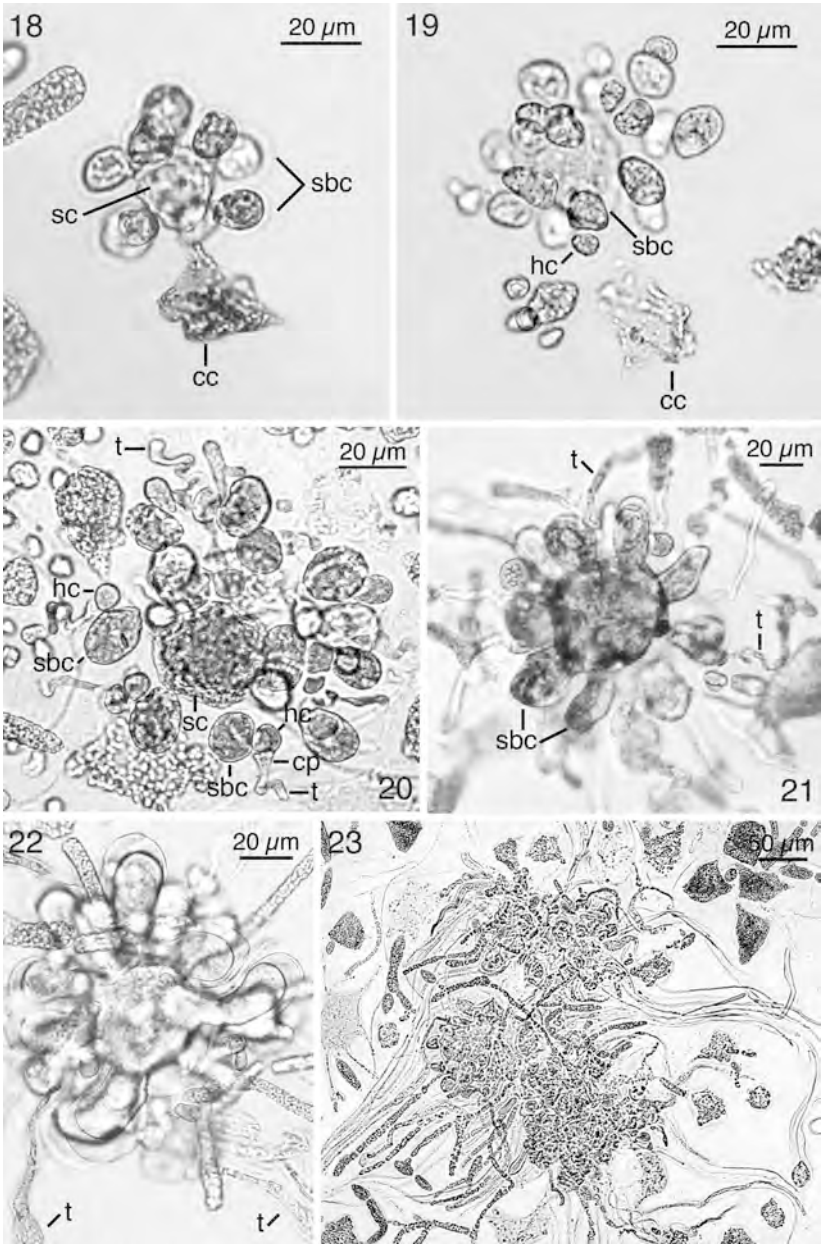
Female reproductive structures and development of gonimoblasts. The carpogonial branch system consists of 8-15 (-20) subsidiary cells, almost all of which enlarge

and give rise to 2-celled carpogonial branches with small, spherical hypogenous cells. The carpogonia are triangular or irregular in shape and the trichogynes are coiled one-two times at the base (Figs 20-21). Fusion of the first cell of each carpogonial branch with the supporting cell leads to the formation of large fusion cells (Figs 21-22), most of which appeared to be non-functional since only a few were seen bearing connecting filaments (Fig. 23). The auxiliary cell branch system contains rounded or ovoid subsidiary cells that enlarge only slightly when mature (Figs 24-25). Near the point of fusion with the auxiliary cell the connecting filament undergoes swelling in two-three cellular portions separated by short constrictions (Fig. 24). Packets of gonimoblast filaments are produced from these swollen areas (Fig. 24). A strong increase in the swelling occurs at a later stage as seen in Fig. 25 which shows the large, swollen hyaline connecting filament attached at one side to the auxiliary cell and producing four-five outgoing secondary connecting filaments. Clusters of gonimoblast filaments are seen attached to the swollen connecting filament or separated from it by squashing. A few gonimoblast filaments are also produced by the narrow part of the primary connecting filament (Fig. 25).

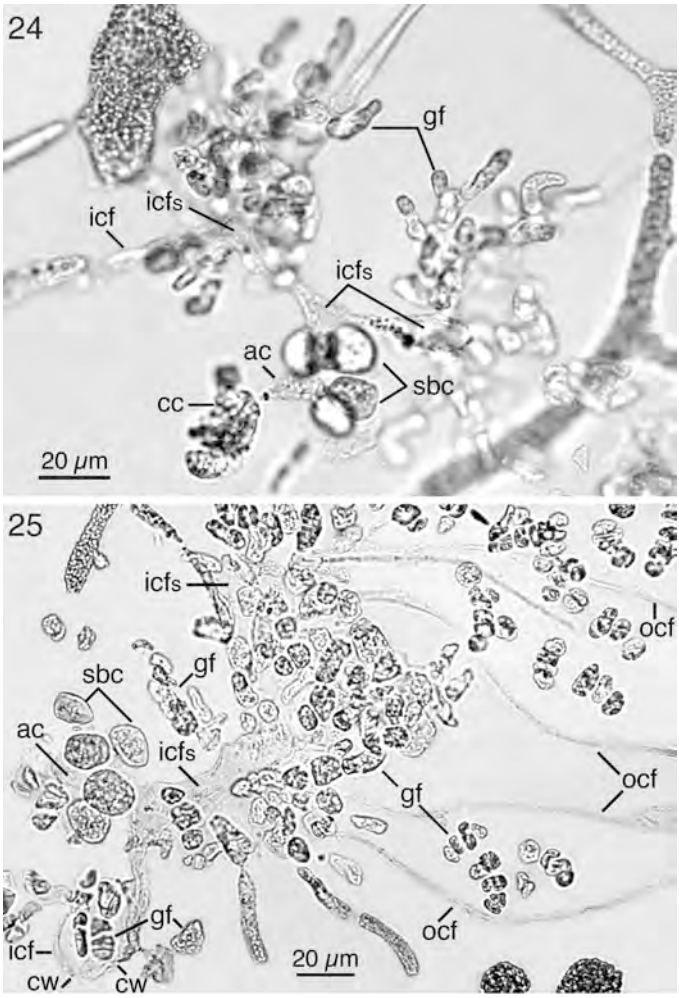
DISCUSSION

Since the early studies by Norris (1957) there was evidence that the primary connecting filament swells to varying degrees in *Kallymenia* near the point of fusion with the auxiliary cell, and that the gonimoblasts develop from these swollen areas which provide the bulk of food material for the growing gonimoblasts. In the monocarpogonial species studied here the primary connecting filaments undergo a lesser degree of swelling than in the polycarpogonial species. In this study we could clearly observe that the cellular portions of the primary connecting filament that undergo swelling are cut off by cross walls from the remaining filament, especially in the monocarpogonial specimens. We also observed that, in both monocarpogonial and polycarpogonial species of *Kallymenia,* the swollen portions of the primary connecting filament that had fused with the auxiliary cell gave rise to multiple secondary outgoing connecting filaments as well as to gonimoblasts. These secondary connecting filaments are produced by the primary connecting filaments in the same way as those that are produced from the carpogonial branch fusion cell (see Rodriguez-Prieto and Hommersand, 2009: 141, 151), as indicates the presence of a pit connection at their point of origin. Secondary connecting filaments, linked by pit connections to the auxiliary cell, have already been observed in other genera of red algae (for example *Grateloupia*, see Wei Wang *et al.*, 2000, Fig. 14; De Clerck *et al.*, 2005, Fig. 6F) but are observed here for the first time in the genus *Kallymenia*. Once the filament reaches the auxiliary cell branch system in *Kallymenia* it stops further increase in length (Norris, 1957, Rodriguez-Prieto & Hommersand, 2009). An exception is represented by *K. cribrogloea* Womersley & Norris in which the primary connecting filament bifurcates close to the auxiliary cell forming a branch that continues on after the other branch has fused with the auxiliary cell (Womersley & Norris, 1971, Fig. 11; Womersley, 1994, Fig. 73H).

In red algae, secondary connecting filaments are generally produced singly, either as continuing elements or as branches that form prior to fusion with



Figs 18-23. *Kallymenia* sp. 2. Polycarpogonial specimen. Development of carpogonial branch and auxiliary cell branch systems. **18.** Young carpogonial branch system or auxiliary cell branch system. Note the undifferentiated cortical cell linked to the apparatus. **19-20.** Development of two polycarpogonial branch systems. **21-22.** Fusion of the subsidiary cell of each carpogonial branch with the supporting cell. Trichogynes are still visible. **23.** Large fusion cell with connecting filaments.



Figs 24-25. *Kallymenia* sp. 2. Polycarpogonial specimen. Formation of gonimoblasts and secondary connecting filaments. **24.** Primary connecting filament (icf) with three swollen cellular portions (icf_s), separated by short constrictions, from two of which packets of gonimoblast cells (gf) are developing. The fusion has occurred between a swollen cellular portion (icf_s) of the primary connecting filament and the auxiliary cell (ac). The narrow point of fusion is hidden by subsidiary cells. **25.** A later stage showing an increase in the size of the swelling of the primary connecting filament (icf_s) The enlarged portion, attached at one side to an auxiliary cell branch system, has produced gonimoblasts and 4-5 outgoing secondary connecting filaments. Note, at bottom left, the narrow part of the primary connecting filament is cut off by cell walls (cw) in two places.

an auxiliary cell. Multiple secondary connecting filaments have been reported occasionally in the Dumontiaceae, as in *Dudresnaya hawaiensis* (Robins & Kraft, 1985) and *D. crassa* (Taylor, 1950); however, in these instances the secondary connecting filaments originate close to the auxiliary cell. Most of the fusion cells observed had aborted in our material and were devoid of connecting filaments. The production of multiple secondary connecting filaments from auxiliary cell

branch systems could be a way of overcoming the low number of successful fertilisations, ensuring the transfer of derivative diploid nuclei to many auxiliary cells. The high number of primary connecting filaments formed by the few functional fusion cells observed could have the same function.

Vegetative morphology and anatomy as well as the carpogonial branch system of our polycarpogonial specimen correspond to those of *K. feldmannii* as described by Codomier (1971, 1973), Vergés (2001) and Rodriguez-Prieto & Hommersand (2009), except for the presence of secondary connecting filaments in our material. It could be regarded as a variant of that species or a determination could await confirmation from molecular studies. The identification of the monocarpogonial specimens is less certain. Their morphology and anatomy fit well with the description given by Codomier (1971, 1973, 1978) and Vergés (2001) for sterile plants of *K. patens* but are different from those of fertile plants as described by Vergés & Rodríguez-Prieto (2006b, Figs 4-6). Female thalli observed by those authors have small spathulated proliferations on which the gonimoblasts are located, inner cortical cells up to 70 µm in diameter and a polycarpogonial branch system. Although there have been some detailed studies of Mediterranean species of *Kallymenia*, there is still a need for further work. Among Mediterranean *Kallymenia* species, a monocarpogonial branch system is present in *K. requienii* (see Codomier, 1971, Vergés, 2001, Rodriguez-Prieto & Hommersand, 2009), to which species however our specimens cannot be referred since they differ by the pseudodichotomously branched habit (simple or lobed in *K. requienii*) and by the length of the radiating arms of the medullary cells (up to 1000 µm in *K. requienii* and up to 210 µm in our specimens).

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