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Epulo multipedes gen. et sp. nov. (Corallinaceae, Rhodophyta), a coralline parasite from Australia

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Epulo multipedes gen. et sp. nov. is described for a coralline red alga growing parasitically on *Jania* collected from eastern Australia. *Epulo* is a monospecific genus with vegetative filaments that invade host cells and totally disrupt them, a phenomenon not seen before in the Corallinaceae. The new genus comprises two phases: an unconsolidated vegetative portion that is endophytic within the host tissue, and reproductive conceptacles formed at the surface of the host. Vegetative cells are uninucleate and form haustoria within host cells. Reproductive conceptacles are formed when outgrowths of the parasite consolidate at the surface. Tetrasporangial conceptacles are multiporate, with zonate tetrasporangia. Sexual conceptacles are uniporate. *Epulo* is included in the tribe Austrolithoideae and has affinities with *Austrolithon,* but differs in being parasitic, having uninucleate rather than multinucleate cells, and having conceptacles formed externally on the host.

INTRODUCTION

The vast majority of red algae are photosynthetic autotrophs, essentially living independently of other organisms. Also included in the division, however, are a small number of genera and species that are parasitic on various hosts. The existence of these algae has been known since 1875, when Reinsch (1875) described the presence of colourless, host-specific plants in nature (Goff 1982). Setchell (1905) described parasitic red algae, although Goff (1982) indicates it was in Setchell's later work (Setchell 1918) that he first used the phrase 'parasitic red alga'. Within the red algae, parasitic taxa are found in several orders, including the Ceramiales, Corallinales and Gigartinales. The first coralline parasite, described only a few years after Reinsch (1875) was *Melobesia thuretii* Bornet (*in* Thuret & Bornet 1878), which was a parasite on *Jania rubens* (Linnaeus) Lamouroux. During the 20th century, numerous parasitic taxa were described (Goff 1982). These descriptions were essentially morphological (e.g. Goff 1982; Goff & Zuccarello 1994; Zuccarello & West 1994; Broadwater & Lapointe 1997; Chamberlain 1999) and it was not until the latter years of the century that the evolutionary relationships between parasite and host and between parasites were examined using molecular tools (Goff *et al.* 1996, 1997).

Apparently, autotrophic endophytes are also commonly observed within the thalli of coralline red algae. These endophytes are from a diverse range of organisms: there are records of lower fungi (Townsend *et al.* 1994; Sim & Townsend 1999), filamentous red algae (Woelkerling & Irvine 1982), brown algae and blue-green algae (R.A. Townsend, personal observation), as well as other coralline algae such as *Lithophyllum cuneatum* Keats (Keats 1995) and *Austrolithon intumescens* Harvey & Woelkerling (Harvey & Woelkerling 1995).

In a review of coralline red algal interactions, Morcom & Woelkerling (2000) discuss the terms 'parasitic' and 'endophytic'. The recognition of true parasites, as opposed to non-parasitic endophytes (which can appear parasitic), has been controversial. Morcom & Woelkerling (2000, p. 6) define a parasite as a living organism that adversely affects another (the host) as a consequence of obtaining nutrients (from the host). The presence of parasitic taxa within the coralline algae has been assumed (Goff 1982), then questioned (Woelkerling & Ducker 1987; Woelkerling 1988; Harvey & Woelkerling 1995) and finally observed at an ultrastructural level, to the extent that it is now accepted that in at least one coralline species, *Choreonema thuretii* (Bornet) Schmitz, a truly parasitic association exists with its host (Broadwater & Lapointe 1997). Four genera, *Choreonema* Schmitz (Broadwater & Lapointe 1997), *Ezo* Adey, Masaki & Akioka (Adey *et al.* 1974), *Lesueuria* Woelkerling & Ducker (Woelkerling & Ducker 1987) and *Kvaleya* Adey & Sperapani (Adey & Sperapani 1971), possess haustoria (Morcom & Woelkerling 2000) and have been classified as true parasites (e.g. Chamberlain 1999). All but one of the coralline parasites and endophytes described in the literature (Goff 1982; Chamberlain 1999) infest other coralline algae.

A collection of *J. verrucosa* Lamouroux was made in late winter, at Long Reef Point, near Dee Why, a northern suburb of Sydney, Australia. This collection of *Jania* Lamouroux bore numerous parasitic thalli, which superficially appeared to belong to the genus *Choreonema.* Upon sectioning the *Jania,* however, it became apparent that the parasite was not *Choreonema* but a species with multiporate conceptacles, which is here described as a new genus and species of Corallinaceae, *Epulo multipedes* Townsend & Huisman.

MATERIAL AND METHODS

Selected pieces of specimens were placed in vials and fixed in a solution of 3% glutaraldehyde in buffer (0.025 M potassium phosphate buffer), pH 6.8, for 60 min at room temper-

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ature. Decalcification was carried out in a solution of 3% ethylenediaminetetraacetic acid in buffer (0.025 M potassium phosphate buffer). The vials of material were kept at 4°C and the solutions were changed daily until decalcification was complete. Postfixation in 2% OsO_4 (v/v) in buffer (0.025 M potassium phosphate buffer) at room temperature was carried out for 1 h. Dehydration was carried out using a graded acetone series of 10% increments each for 10 min, with two 30 min changes at 100% acetone. Specimens were embedded in Spurr's resin. Sections for light microscopy were stained in Richardson's stain (Richardson *et al.* 1960).

The terminology used in this paper is that used for algal–algal interaction by Morcom & Woelkerling (2000), for vegetative growth by Woelkerling *et al.* (1993), for cell shapes by the Systematics Association (1962) and for tetrasporangial formation by Townsend & Adey (1990). Herbarium abbreviations follow Holmgren *et al.* (1990).

RESULTS

Epulo Townsend & Huisman, *gen. nov.*

Genus characteribus familiae Corallinaceae subfamiliae Austrolithoideae, sed a generibus cognatis habitu parastico et conceptacula ad paginam hospitis posita inter alia differt.

A genus with the characteristics of the Corallinaceae subfamily Austrolithoideae, but differs from related genera among other ways in being parasitic and having conceptacles at host surface.

TYPE SPECIES: *Epulo multipedes* Townsend & Huisman, *sp. nov.*

Epulo multipedes Townsend & Huisman, *sp. nov.*

Figs 1–17

Thallus parasiticus, diffusus, partim pseudoparenchymatus, monomerus. Filamenta vegetativa ex cellulis uninucleatis 10–30 μm longis, 5–7 μm diametro constantia, unumquidque ad paginam hospitis multoties ramificans et conceptaculum formans. Conceptacula tetrasporangialia multiporata, 100–130 μm alta, 190–230 μm diametro. Tetrasporangia zonata. Tectum conceptaculi ex 1–3 stratis cellularum constans. Conceptacula sexualia uniporata. Conceptacula carpogonialia 140–190 μm alta, 150–170 μm diametro. Ramus carpogonialis 3-cellularis. Conceptacula carposporangialia 200–230 μm alta, 190–210 μm diametro. Catenae carposporangiorum e margine et prope marginem paginae superae cellulae magnae coniungentis convolutae formatae.

Thallus parasitic, diffuse, partly pseudoparenchymatous, monomerous. Vegetative cell filaments composed of uninucleate cells 10–30 μm long, 5–7 μm in diameter, each branching many times at the host surface and forming a conceptacle. Tetrasporangial conceptacles multiporate, 100–130 μm high, 190–230 μm in diameter. Tetrasporangia zonate. Conceptacle roof of one to three layers of cells. Sexual conceptacles uniporate. Carpogonial conceptacles 140–190 μm high, 150–170 μm diameter. Carpogonial branch three-celled. Carposporangial conceptacles 200–230 μm high, 190–210 μm diameter. Chains of carposporangia formed from the edge and periphery of the upper surface of a large convolute fusion cell.

HOLOTYPE: **Australia.** NSW 409336 (Figs 9, 11–14), tetrasporangial thalli on *J. verrucosa,* collected by *P. Farrant,* 22 August 1981, prepared for microscopy, embedded in resin. It is impossible to know whether the gathering of *Jania* by Farrant consists of a single individual of *Jania* that has been infected by both gametophytes and sporophytes of *Epulo,* or a number of individuals of *Jania* each infected by a different life-phase of *Epulo.* Tetrasporangial, female and cystocarpic thalli of *E. multipedes* are found on the gathering of *Jania.* The entire gathering of *Jania* constitutes original material and is housed at NSW.

TYPE AND ONLY KNOWN LOCALITY: **Australia.** New South Wales, Long Reef Point, subtidal on the north side.

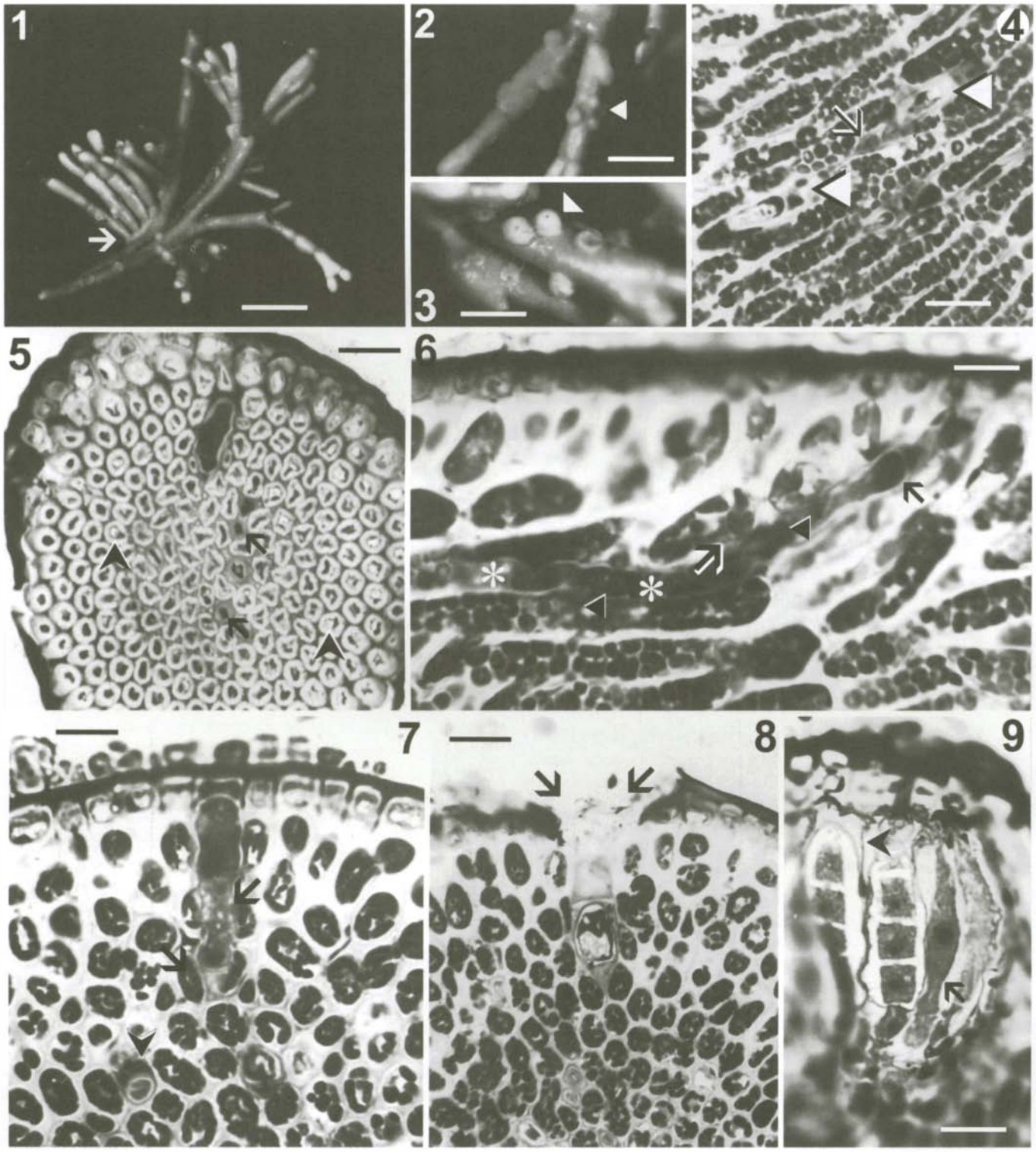
ETYMOLOGY: The generic name refers to the parasitic nature of this taxon, from the Latin *epulo*; guest at a banquet; gender: masculine. The species epithet '*multipedes*', from the Latin *multi* – many and *pes* – foot, describes the numerous 'anchoring points' of parasitism extending into the host thallus along the base of the conceptacles.

HABIT: *Epulo multipedes* appears on the surface of the host as white calcified conceptacles along the intergenicula of *Jania* (Figs 2, 3). The parasite exists as two distinct phases, vegetative and reproductive, which are described below. The parasite occurs throughout the host as indicated by the presence of the parasite reproductive structures along the host intergeniculae (Figs 2, 3) and the proliferous branching produced by the host (Fig. 1).

VEGETATIVE STRUCTURE: Spore germination and initial penetration of the host was not observed. The thallus consists of unconsolidated filaments that meander through the medulla and cortex of the host (Figs 4–6). The level of calcification of the filaments is unknown. Vegetative cells of *E. multipedes* are of similar diameter to those of the host medulla and cortex, but are distinguishable from those of the host by their lack of floridean starch grains and their lack of obvious plastids (Figs 4–6). In infected intergenicula there is often a 'barrier reaction' in the cells of the host, which produces heavily stained and thickened middle lamellae adjacent to the cells of the parasite (Figs 5–7, 10). In the geniculum of *Jania,* the host cells have thick-layered walls with vacuolate cytoplasm (Fig. 5). The cells of *E. multipedes* in this area are easily observed because they have dense cytoplasm and the walls surrounding the parasite cells (originally cell walls of the host in some cases) stain much darker (Fig. 5). It appears that changes occur in the 'fibrillar' portion of the wall due to the presence of the parasite, resulting in this portion staining in a similar fashion to the 'outer cuticle-like layer' (Johansen 1981, p. 61). All vegetative cells of *E. multipedes* are uninucleate (Figs 4, 6, 7, 10). Secondary pit connections and lateral fusions between vegetative cells of *E. multipedes* have not been observed.

Evidence of the parasitic nature of *E. multipedes* is shown in Figs 6, 7, 10; the large cell illustrated (Figs 7, 10) has invaded four cortical cells of the host. Adjacent cells in all areas of the host can be subsumed into the parasite, producing a large, densely cytoplasmic cell (Fig. 5). The parasite vegetative cells make contact with cells of the host by fusion (Figs 6, 7, 10) and dissolution of the contiguous walls. The cell contents of the host are subsequently subsumed as a result of the encounter (compare Figs 7, 8, 10). The contents of host cells under attack can remain discernible for a time (Figs 7, 10), but ultimately all plastids and starch grains in the host cells are lost (Figs 5, 7, 8, 10). The walls of the host cells in the region of initial contact remain faintly visible for a time, but eventually become totally undermined (Figs 7, 10).

The formation of conceptacles is the only time the *Epulo* thallus appears at the surface of the host. Initiation of conceptacles begins when a filament of the parasite thallus approaches the surface of the host thallus (Figs 5, 7). The apical cell of this filament is similar in form to the primary apical cells in all coralline red algae. The cell, which is broadly oblong with rounded ends in longitudinal section, has a large nucleus situated posteriorly and the cytoplasm is evacuolate (Figs 5–7, 10). When the parasite appears at the surface of



Figs 1–9. *Jania verrucosa* being parasitized by *E. multipedes,* showing the gross habit, vegetative morphology and tetrasporophyte of the parasite.

Fig. 1. Habit showing a 'witches-broom' effect (arrow). Scale bar = 1 mm.

Fig. 2. Branch of *Jania* with tetrasporangial parasite (arrowhead). Note that the conceptacles to the rear are not *Epulo* but a 'mastophoroid' epiphyte. Scale bar = 600 μm.

Fig. 3. Branch of *Jania* with carposporangial parasite (arrowhead). Scale bar = 300 μm.

Fig. 4. Longitudinal section (LS) of intergeniculum of *Jania.* Note the cells of *Epulo* (arrowhead) and the single nucleus within the *Epulo* cell (arrow). Scale bar = 22 μm.

Fig. 5. Transverse section (TS) of geniculum of *Jania.* Note the cells of *Epulo* with dense-staining evacuolate cytoplasm (arrow), and the large cell of *Epulo* nearing the surface of the geniculum. The walls of the *Jania* cells deep in the geniculum have been undermined by the presence of *Epulo.* This is indicated by the decrease in thickness of the host cell wall where *Epulo* has invaded. The cortical cells of the host are indicated by arrowheads. Scale bar = 25 μm.

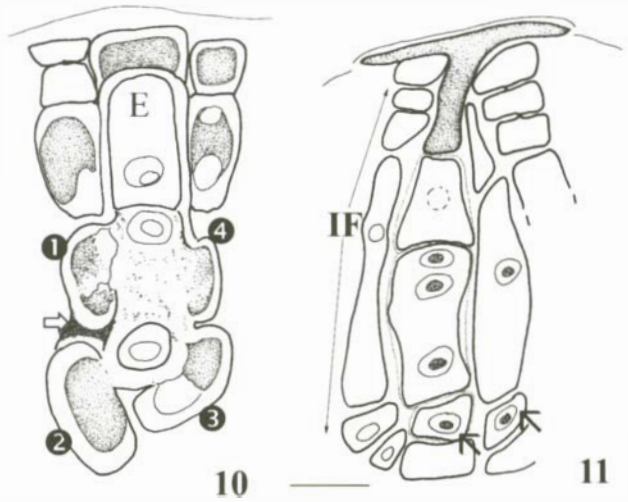


Fig. 10. Line drawing of Fig. 7. Note the epithallial cell of *Jania* above the apical cell of *Epulo* (E) which has a single nucleus, the invasion of the cortical cells of *Jania* (numbered 1–4) by *Epulo,* and a barrier reaction (arrow). See Fig. 7 for scale.

Fig. 11. Partial LS of a semimature sporangial conceptacle. Note the premeiotic sporocyte (right), the postmeiotic sporocyte with nonsynchronous cytokinesis (centre), stalk cells (arrows), interspersed filament (IF) and a pore plug that is continuous with an outer mucilage layer. Scale bar = 30 μm.

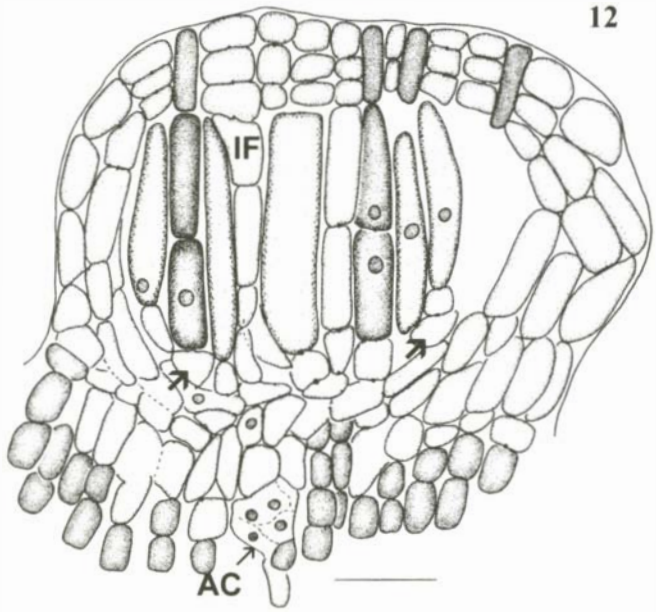


Fig. 12. The LS of a semimature sporangial conceptacle. The anchoring cell (AC) is equivalent to the invasive cell in Fig. 7 below the apical cell of *Epulo.* Unparasitized *Jania* is densely stippled, parasitized *Jania* is stippled and the *Epulo* conceptacle is lightly stippled. Note the sporocytes, each subtended by a proliferative stalk cell (arrows), and an interspersed filament (IF) between the developing sporangia. Scale bar = 40 μm.

the host, its primary apical cell is 'capped' by an epithallial cell of the host (Figs 7, 10). The natural sloughing of the host epithallium results in the parasite reaching the surface. The parasite's presence at the surface disrupts the normal cell formation of the host only minimally, in that only the epithallial cells and the intercalary initials and upper cortical cells of the host are affected, and then only as the conceptacle matures (Figs 8, 12, 15, 17). The host intergeniculum is not flattened in the area of the conceptacle (Figs 15, 17); the parasite conceptacles are a surface feature on the host.

The production of conceptacles begins by the proliferation of a single filament. In the earliest stages of conceptacle formation the apical initial of the parasite is subtended by a large cell that invades a number of host cells (Figs 7, 10). This cell appears to be an anchoring cell for the conceptacle; it remains visible throughout the life of the conceptacle (Figs 8, 12, 15–17) and is the cell that remains when the conceptacle is sloughed at senescence (Fig. 8). This cell shows similarities to the syncytium described for *Gardneriella tuberifera* Kylin by Goff & Zuccarello (1994, p. 698). The filament produced from the apical initial proliferates laterally and radially to produce growth reminiscent of the applanate growth of some mastophoroids (Turner & Woelkerling 1982; Townsend & Adey 1990) and forms the conceptacle base and contents. The cells of the parasite conceptacle contiguous with the host surface become involved in further parasitizing the host tissue (Figs 12, 16, 17). In young conceptacles this may involve only the epithallial cells of the host, but in mature conceptacles the underlying initials and upper cortex of the host also become parasitized (compare Figs 12, 16). This invasion eventually leads to a parasite conceptacle with multiple points of disruption into the host cortex; i.e. the multiple 'feet' or *multipedes* (Figs 12, 15). A similar pattern is observed below the carpogonial and cystocarpic conceptacles (Figs 16, 17). When the conceptacle senesces it is sloughed and leaves a wound in the *Jania* cortex (Fig. 8). Past wound healing by the host often results in a 'witches-broom' (Purdy & Schmidt 1996) reaction in the vegetative growth of the *Jania* (Fig. 1).

REPRODUCTIVE STRUCTURES: Only tetrasporangial, carpogonial and cystocarpic conceptacles have been found in this study.

Tetrasporangial conceptacles, in longitudinal section, are transversely oblong with rounded corners. The conceptacle wall is two or three cell layers thick and consists of one or

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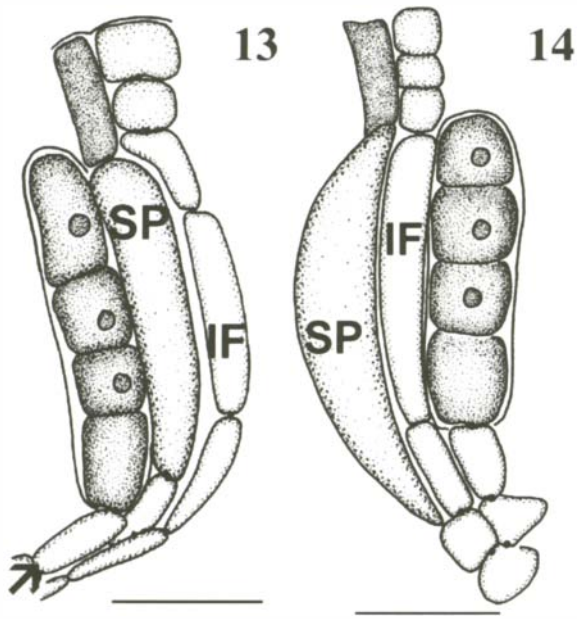
Fig. 6. LS of intergeniculum of *Jania* with filament of *Epulo* (*) (single nucleus in each *Epulo* cell; arrowheads). Note the apical cell of the *Epulo* filament (left-pointing arrow), the barrier reaction between this cell and the adjacent host cell and invasion of a *Jania* cortical cell by *Epulo* (right-pointing arrow). Scale bar = 10 μm.

Fig. 7. TS of intergeniculum of *Jania* with filament of *Epulo* (compare with Fig. 10). Note the epithallial cell of *Jania* above the apical cell of *Epulo* (which has a single nucleus), the invasion of the cortical cells of *Jania* by *Epulo* (arrows) and other cells of *Epulo.* A barrier reaction (arrowhead) between *Epulo* and *Jania* is clearly illustrated. Scale bar = 10 μm.

Fig. 8. TS of intergeniculum of *Jania* with region of *Epulo* where a conceptacle has sloughed (arrows). Note *Epulo* cells in host thallus, especially the large cell remaining after the sloughing. Scale bar = 25 μm.

Fig. 9. LS of sporangial conceptacle. Note the thick cuticle covering the conceptacle, the pit plugs between the roof cells, the crushed interspersed filaments (arrowhead), the sporocyte (arrow) and the subtending stalk cell. Scale bar = 30 μm.



Figs 13, 14. Illustrations showing interspersed filaments (IF), sporocytes (SP) and tetrasporangia with their subtending stalk cells (arrow). Scale bar = 30 μm.

two inner layers of cells giving rise to an outer epithallial layer covered by a cuticle, which stains darkly with Richardson's stain (Figs 9, 11, 15). The walls of the conceptacle are formed by divisions of the apical cell of the parasite filament, which branches laterally and upwardly from the original zone of eruption at the host surface (Fig. 12). The conceptacle roof is formed from the interspersed filaments in a manner similar to the morphogenesis described for *Austrolithon* Harvey & Woelkerling (Harvey & Woelkerling 1995, figs 34–37, see below). Initially the roof of the sporangial conceptacles consists solely of epithallial cells subtended by initials, but the mature conceptacle has up to three cell layers, including the epithallial cell layer (Figs 9, 11–15). As the conceptacle matures, the parasite cells abutting the surface epithallial cells of the host progressively invade the host (Fig. 12).

An initial at the base of the future conceptacle lumen divides laterally to form an interspersed filament consisting of elongate cells and three squat cells that will help form the lumen and roof of the conceptacle, respectively (Figs 11–14). The elongate cells become squashed by the subsequent expansion of the sporangia (Fig. 9). The pit plugs between the roof cells of the tetrasporangial conceptacle are more easily observed than those between the roof cells of the female and cystocarpic conceptacles (compare Figs 9, 16, 17).

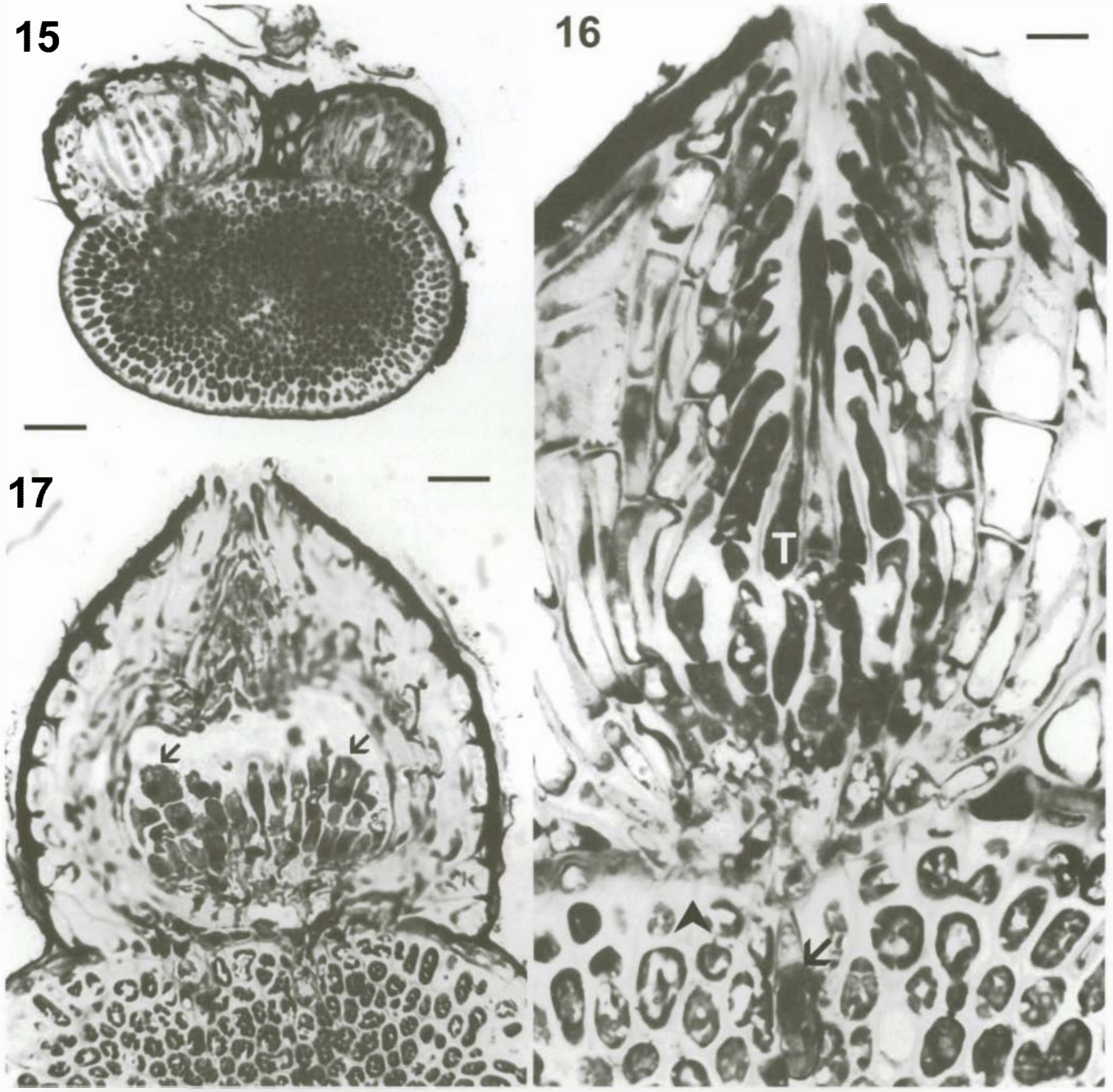
A single cell subtends the sporangial initial and the interspersed filament (Figs 11–14). A similar pattern of development was observed in *Austrolithon* (Harvey & Woelkerling 1995, figs 35, 36, note central initials). Sporangial initials are indistinguishable from the elongate cells of the interspersed filaments in very young conceptacles. Later, the sporangial initials become granular and slightly iridescent when stained with Richardson's stain. The sporangial initials divide to form the stalk cell and sporocyte (Fig. 9). At this stage the walls of all cells stain blue. Meiosis follows but cytokinesis is not necessarily synchronous and one of the three crosswalls may be completed before the other two (Figs 11, 12); cytokinesis produces a zonate pattern of spores in the tetrasporangium (Figs 9, 15). The wall surrounding the four tetraspores stains purple. Bisporangia were not observed. Formation of the extracellular plug material (Figs 9, 12–15) commences at the stage of the sporangial initial (Figs 11, 13) and ends before cytokinesis is complete (Figs 11–14). Thus, a pore is provided for each sporangium. Stalk cells appear to be proliferative and produce further sporangial initials and subsequent sporocytes (Fig. 12).

Carpogonial conceptacles are ovate in longitudinal section. Their walls and roof are derived from lateral and radial proliferations of an emergent vegetative filament of the parasite. The central fertile area is a disc, four to six cells in diameter, from which carpogonial branches arise (Fig. 16). The roof of the conceptacle is minimal at this stage and is formed by the slight ingrowth of the wall filaments (Fig. 16). The ends of these filaments stain darkly and presumably exude the mucus that fills the cavity and pore through which the trichogynes grow. The carpogonial branch consists of a basal cell (at the level of the conceptacle floor), a hypogynous cell and a carpogonium that at first is elliptical but later becomes narrowly triangular as the trichogyne extends to the conceptacle pore (Fig. 16).

The earliest postfertilization stage observed was a small fusion cell at the base of the fertile area; at later stages this fusion cell increases in both diameter and height. The fusion cell is formed initially from the union of the basal cells and later the support cells, but the carpogonia are not involved. In mature cystocarps there is a large convoluted fusion cell, with peripheral carposporangial chains of up to four carposporangia arising from its upper surface (Fig. 17). The wall of the cystocarp becomes thinner with the expansion of the carposporangial chains, which squash the inner wall cells. The squashed cells of the wall form a distinct boundary around the cystocarp (Fig. 17).

DISCUSSION AND CONCLUSION

Harvey & Woelkerling (1995) proposed the subfamily Austrolithoideae for those coralline red algae with multiporate conceptacles and vegetative cells without contiguous cell fusions or secondary pit connections. *Epulo* displays these features and is clearly a member of this subfamily, which now contains two endophytic members, *Austrolithon* and *Epulo,* and a free-living genus, *Boreolithon* Harvey & Woelkerling. *Austrolithon* has unconsolidated vegetative growth and multiporate tetrasporangial conceptacles, character states also found in *Epulo. Epulo,* however, seemingly unlike *Austrolithon,* is parasitic. These two genera are separated, not only by habit, but also by characters such as nuclear number and differentiation of the conceptacle (Table 1). The cells of *Epulo* in their normal state are always uninucleate; the multinucleate anchor cells arise only as a result of full fusion with host cells to produce the 'anchoring' cell below the conceptacles. All other host–parasite interactions, for example below the conceptacle floor, result in the uninucleate state being retained. This represents a distinct difference between *E. multipedes* and *A. intumescens* (Harvey & Woelkerling 1995), the type species of *Austrolithon. Austrolithon* is one of two coralline



Figs 15–17. Reproductive morphology of *E. multipedes.*
Fig. 15. LS of sporangial conceptacles of *Epulo.* The left-hand conceptacle is at a more mature phase than the right-hand conceptacle as evidenced by the presence of tetrasporangia in the left-hand conceptacle. Note the multiple invasions into the host by *Epulo.* Scale bar = 100 μm.
Fig. 16. LS of a carpogonial conceptacle of *Epulo.* Note the anchoring cell of *Epulo* (arrow), the trichogyne (T) subtended by a support cell and basal cell and the floor cells of the conceptacle starting to parasitize the epithallial cells of the host (arrow head). Scale bar = 15 μm.
Fig. 17. LS of a young cystocarpic conceptacle of *Epulo.* Note the original anchoring cell and a secondary parasitism of *Jania,* the central unstained fusion cell and the chains of carposporangia (arrows). Scale bar = 30 μm.

red algae in which more than one nucleus is observed in vegetative cells that have not undergone vegetative fusion. *Choreonema* also has multinucleate vegetative cells (Broadwater & Lapointe 1997). We believe that the difference in the number of nuclei in the vegetative cells is an important generic character, which is consistent with the separation of other red algal genera based on the same feature [e.g. the separation of *Callithamnion* Lyngbye and *Aglaothamnion* Feldmann-Mazoyer on the basis of their vegetative cells being multinucleate and uninucleate, respectively (Maggs & Hommersand 1993)].

The formation of sporangial initials in *Epulo* is lateral and this also appears to be the case in *Austrolithon.* The state of this character in *Boreolithon* is unknown. Lateral development of sporangial initials has also been recorded in the Lithophylloideae (Townsend & Adey 1990). The use of this character at the subfamily level to distinguish the Austrolithoideae

Table 1. Comparison of *Austrolithon intumescens* and *Epulo multipedes.*

Character	*A. intumescens*	*E. multipedes*
Habit	endophytic	endophytic
Haustoria	absent	present
Number of nuclei per vegetative cell	one to many	one
Number of filaments involved in conceptacle construction	one	one
Tetrasporangial conceptacle roof	two-celled	three-celled
Carpogonial disc (diameter)	seven-celled	four- to six-celled

from other members of the Corallinales must await its recognition in *Boreolithon.*

There are six monotypic genera of Corallinaceae that are endophytic or parasitic. Although these are mostly not closely related to *Epulo,* we have included a comparison of their tetrasporangial conceptacle structure, vegetative thallus construction and the presence or absence of haustoria (Table 2). *Epulo,* like *Choreonema* (Woelkerling 1987; Broadwater & Lapointe 1997), does not cause a visible host reaction but does alter the anatomy of the host cells. In contrast, when *Austrolithon* invades *Haliptilon* (Decaisne) Lindley (compare our Figs 1, 8 with Harvey & Woelkerling 1995, figs 3, 4, 23) there is a proliferation of cortical filaments producing gall-like structures. The response of *Jania* to *Epulo* follows the sloughing of mature parasite conceptacles and involves only renewed meristematic activity in the host cortex. This is a typical coralline wound response (see Townsend *et al.* 1994). A 'witches-broom' effect may result (see Purdy & Schmidt 1996). A similar response is not seen in *Jania* infected with *Choreonema* (Woelkerling 1987; Broadwater & Lapointe 1997).

There are only two cases in the Corallinaceae where an endophyte appears to alter the anatomy of the host cells: *Epulo* and *Choreonema* (Broadwater & Lapointe 1997). Broadwater & Lapointe (1997) showed that *C. thuretii* forms parasitic connections with cells of the host plant *Jania.* Although the ultrastructure of *Epulo* has not been investigated, it is clear that *Epulo* also forms parasitic connections with *Jania* (Figs 6, 7, 10), but the situation does not match the interaction between *Jania* and *Choreonema* in that *Epulo* does not have lenticular cells that disrupt and finally destroy the host cells.

In *Choreonema,* as interpreted by Broadwater & Lapointe (1997), each conceptacle represents an individual thallus, consisting of an area of pseudoparenchymatous cells below the conceptacle with a single filament radiating from this pseudoparenchymatous area into the host. The vegetative cells are 'usually multinucleate' (Broadwater & Lapointe 1997, p. 397). Thus, the germination scenario is that germlings of *Choreonema* invade the host from the surface and each produces a single conceptacle and a single filament. This is not the case in *Epulo,* where filaments of the parasite, coursing through the medulla and cortex of the host, eventually grow towards the surface and, after erupting, form a conceptacle. Although we have not observed filaments connecting one surface eruption to the next in serial section, we did observe the deep filaments in Fig. 6 continuing in the inner cortex of the *Jania,* and lateral branches from these filaments turning towards the host thallus surface. *Epulo,* therefore, does not exhibit the single reproduction event seen in *Choreonema.*

Vegetative growth in coralline algae consists of a set of apical initials that produce the medulla or the upright portions in geniculate taxa (Johansen 1981), or secondary cortex as is the case in a wound-healing response (Townsend *et al.* 1994). These apical cells have a consistent anatomy, with an evacuolate cytoplasm, large basal nucleus and broadly oblong shape. The initial seen in *Epulo* as the parasite nears the surface of the host thallus is an apical initial as defined above. Apical cells were not described by Harvey & Woelkerling (1995) for *A. intumescens,* although their figs 12 and 33 may represent such cells.

Harvey & Woelkerling (1995) discuss the diagnostic features of the subfamilies of Corallinaceae. The character that separates the Austrolithoideae from the Choreonematoideae is 'roof poration' (Table 2). The conceptacle roof formation in the Austrolithoideae, Lithophylloideae and Melobesioideae involves sterile filaments interspersed between sporocytes (Woelkerling 1988; Townsend & Adey 1990; Woelkerling & Campbell 1992; Harvey & Woelkerling 1995). Sterile filaments are not observed during conceptacle formation in the Choreonematoideae (Woelkerling 1987). Presence of sporangial plugs is a character of the Melobesioideae, Austrolithoideae and Choreonematoideae (Table 2). They are absent from the Lithophylloideae (Woelkerling 1988). Cladistic analyses using 18S ribosomal RNA (Bailey 1999, fig. 1; Harvey *et al.* 2002, fig. 40a) show that the Melobesioideae and Lithophylloideae have numerous synapomorphies separating them. Based on these cladograms, the loss of sporangial plugs appears to be apomorphic, and conceptacle formation in the Lithophylloideae and Melobesioideae, although involving

Table 2. Comparison of endophytic or parasitic species of Corallinaceae in terms of tetrasporangial conceptacle structure, location of vegetative thallus and habit. (Data from Woelkerling 1988; Harvey & Woelkerling 1995; this paper.)

Species	Subfamily	Tetrasporangial conceptacles	Sporangial plugs	Vegetative thallus	Haustoria
Austrolithon intumescens	Austrolithoideae	multiporate	present	internal	absent
Epulo multipedes	Austrolithoideae	multiporate	present	internal	present
Kvaleya epilaeve Adey & Sperapani	Melobesioideae	multiporate	present	external	present
Choreonema thuretii (Bornet) Schmitz	Choreonematoideae	uniporate	present	internal	present
Ezo epiyessoense Adey, Masaki & Akioka	Lithophylloideae	uniporate	absent	external	present
Lesueuria minderiana Woelkerling & Ducker	Mastophoroideae	uniporate	absent	internal	present

sterile filaments in both cases, is not homologous. The most parsimonious hypothesis is that the Austrolithoideae, with its sporangial plugs and sterile filaments, is closely related to the Melobesioideae, but what of the Choreonematoideae? Does *Choreonema* represent an austrolithoid still with sporangial plugs but with a loss of the sterile filaments involved in conceptacle roof formation? It might be that the Austrolithoideae will eventually be subsumed into the Choreonematoideae, but further study is required before a definitive statement can be made.

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