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A new contribution to the alien red macroalgal flora of Greece (Eastern Mediterranean) with emphasis on *Hypnea* species

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Abstract – Three alien Rhodophyta, *Botryocladia madagascariensis*, *Hypnea anastomosans* and *Hypnea valentiae*, were recorded and illustrated for the first time from Greece (Aegean Sea and nearby areas). Previously reported from Karpathos Island, *B. madagascariensis* was observed from Korinthiakos Gulf. Reported as *H. esperi* from the Mediterranean coast of Israel, *H. anastomosans* was recorded from the Saronikos Gulf and Rhodes Island. Lastly, the occurrence of *H. valentiae* in the Eastern Mediterranean basin was confirmed. Habitat, taxonomical comments, and geographical distribution were provided for each species. A critical review of *Hypnea* species occurring in the Mediterranean Sea was also provided.

Alien / *Botryocladia madagascariensis* / Greece / *Hypnea anastomosans* / *Hypnea valentiae* / macroalgae / Mediterranean / taxonomy

Résumé – Nouvelle contribution à la macroflore des algues rouges introduites en Grèce (Méditerranée orientale), avec une attention particulière sur les espèces d'*Hypnea*. Trois espèces introduites de Rhodophyta, *Botryocladia madagascariensis*, *Hypnea anastomosans* et *Hypnea valentiae* sont reportées et illustrées pour la première fois de la Grèce (mer Egée et régions voisines). Précédemment récoltée à l'Île de Carpathos, *B. madagascariensis* est décrit du Golfe de Corinthe. Rapporté comme *Hypnea esperi* de la côte méditerranéenne d'Israël, *H. anastomosans* est décrit du Golfe Saronique et de l'île de Rhodes. Enfin, la présence d'*H. valentiae* en Méditerranée orientale est confirmée. L'habitat, des remarques taxonomiques et la distribution géographique sont indiqués pour chaque espèce. Les auteurs proposent une revue critique des espèces du genre *Hypnea*, présentes en Méditerranée.

Espèces introduites / *Botryocladia madagascariensis* / Grèce / *Hypnea anastomosans* / *Hypnea valentiae* / Macroalgae / Mer Méditerranée / Taxonomie

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INTRODUCTION

The Mediterranean Sea is a hot spot of biodiversity but also of species introductions (Lejeusne *et al.*, 2009). With 125 exotic taxa, the alien marine flora of the Mediterranean Sea represents ca. 10% of its total flora (Zenetos *et al.*, 2010; Verlaque *et al.*, in press). Several phycologists, and generally marine biologists, have therefore focused their interest on alien marine macroalgae (Boudouresque & Verlaque, 2002; Schaffelke *et al.*, 2006), particularly those that exhibit invasive behavior, such as *Caulerpa racemosa* var. *cylindracea* (Sonder) Verlaque, Huisman *et al.* Boudouresque, which can monopolize the sea bottom and displace indigenous vegetation (Klein & Verlaque, 2008).

Alien macroalgae have been introduced into the Mediterranean Sea through shipping, aquaculture, aquaria and via the Suez Canal (Cormaci *et al.*, 2004; Verlaque *et al.*, in press). In the latter case, species are known as Lessepsian immigrants, which originate from warm-temperate to sub-tropical regions of the Red Sea and the Indian Ocean (Por, 1978). However, a combination of vectors is also possible for the introduction of an alien species (Schaffelke *et al.*, 2006).

In Greece, as in other Mediterranean regions, there has been an undisputable increase of marine macroalgae introductions since the 1970s (Tsiamis *et al.*, 2008). The acceleration of this phenomenon is worldwide and is attributed to the increase and the diversification of human exchanges (Streftaris *et al.*, 2005). Tsiamis *et al.* (2010) listed more than 30 alien species hitherto recorded along the Greek coasts, where shipping (mainly fouling) seems to be an important vector of marine macrophyte introductions (Tsiamis *et al.*, 2008); however, the Suez Canal also plays a major role since the South Aegean Sea is within the Mediterranean region colonized by the species immigrating from the Red Sea via the Suez Canal (Lessepsian Province sensu Por, 1978, 1990; Pancucci-Papadopoulou *et al.*, 2005). Indeed, all known Greek alien macroalgae have been recorded in the South Aegean Sea, with certain areas, such as Rhodes Island, corresponding to crucial hot spot areas for the occurrence of aliens (Tsiamis *et al.*, 2010). Furthermore, over the past few decades, Red Sea immigrants have been spreading westwards and northwards in the Aegean Sea, possibly in relation to the recent global warming (Galil & Zenetos, 2002).

In the present paper, three alien Rhodophyta, *Botryocladia madagascariensis* G. Feldmann, *Hypnea anastomosans* Papenfuss, Lipkin *et al.* Silva, *H. valentiae* (Turner) Montagne, are described and illustrated for the first time from Greece, with habitat details, taxonomical comments, and geographical distribution for each species. Emphasis is given to the two *Hypnea* species, while a critical review concerning the species of the genus reported so far in the Mediterranean Sea is proposed.

MATERIALS AND METHODS

Field observations and sampling were carried out in 2008-2009, during a general seaweed survey along the coasts of the Aegean Sea and nearby areas (Fig. 1) (Greece, Eastern Mediterranean Sea), as part of the PhD thesis of one of the authors (K.T.). Sampling of midlittoral and upper sublittoral rocky shore assemblages, 0-1 m depth, was seasonally performed by snorkeling. Macroalgal



Fig. 1. Locations of alien macroalgal findings new for Greece: 1 = *Hypnea anastomosans* (Saronikos Gulf and Rhodes Island, Faliraki), 2 = *Hypnea valentiae* (Rhodes Island, port), 3 = *Botryocladia madagascariensis* (Korinthiakos Gulf).

samples were taken through the conventional destructive method of scraping with hammer and chisel. In addition, several macroalgae were retrieved from fishing nets of trawlers, allowing to examine also some deep-water species, down to 40 m depth. All samples were preserved in 4% buffered formalin/seawater for laboratory analyses. Selected species were also pressed, air dried, and prepared as herbarium specimens without formalin treatment.

In the laboratory, specimens were observed under the light microscope, manually sectioned with a razor blade, stained in 1% aqueous Aniline Blue, washed, and then acidified by the addition of a drop of 1 N HCl. Photomicrographs and measurements were made using a Nikon Optiphot-2 (Nikon, Japan) or a Leitz Ortholux (Wetzlar, Germany) microscope equipped with an Olympus μ 1030 SW camera (Putian, China). Herbarium specimens noted "H..." have been deposited in the Verlaque Herbarium, HCOM, Marseille,

France and the others in the personal herbarium of one of us (K.T.). The Herbarium abbreviation follows Thiers (2009). For nomenclature purposes, the following taxonomic databases were used: Index Nominum Algarum (Silva, 2011) and AlgaeBase (Guiry & Guiry, 2011).

RESULTS

***Botryocladia madagascariensis* G. Feldmann**

Figs 2-5

Rhodophyta, Rhodymeniales, Rhodymeniaceae

Type locality: near Irakoka, Madagascar (Feldmann, 1945: 57, figs 3-5)

Studied specimen: H8131, Porto Germeno, Korinthiakos Gulf, (38°08'47.8''N, 23°13'23.1''E), 28 June 2009, 25-40 m depth.

Description

Thallus erect, cartilaginous in texture, dark red in colour, up to 4 cm in height (Fig. 2); axes, terete, up to 0.8 mm in diameter, irregularly branched, bearing lateral vesicles, characteristic of the genus; vesicles ellipsoid, hollow, up to 4 mm long and 2.8 mm broad; alternately disposed all around the axes; outer cortical cells in surface view ovoid, 2.8-7.0 µm in diameter, continuously and randomly arranged (Fig. 3); vesicles in cross section constituted by several layers of cells increasing in size towards the inner side, with 2-3 layers of small rounded pigmented cortical cells and 1-2 layers of large, ovoid, colorless medullary cells, 58-71 µm long and 38-60 µm broad (Fig. 4); gland cells present, ovoid to rounded, 11-15 µm in diameter, occurring solitary or in groups of 2-3, both directly on inner medullary cells and on small special cells protruding into the vesicular cavity (Figs 4-5); reproductive structures not observed.

Habitat

A single thallus was collected from trawler fishing nets from a depth of 25-40 m. The deep water species *Nemastoma dichotomum* J. Agardh, *Osmundaria volubilis* (Linnaeus) R.E. Norris and *Peyssonnelia stoechas* Boudouresque et Denizot were also encountered entangled in the fishing nets.

Distinctive characters

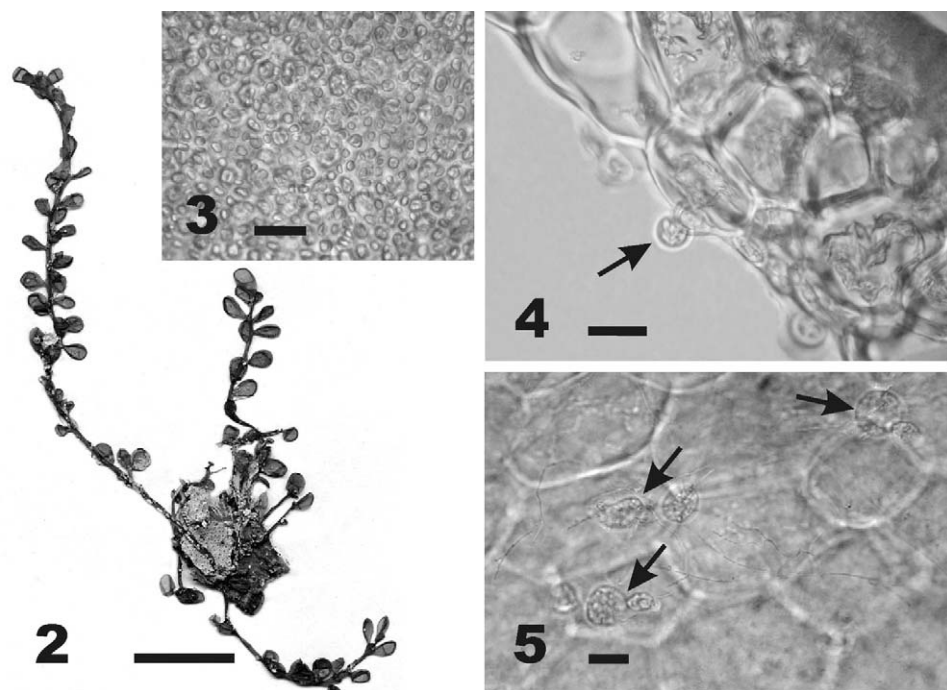
Despite the absence of reproductive structures, *Botryocladia madagascariensis* can be identified by the following features: (i) erect habit, (ii) ellipsoidal vesicles alternately disposed all around the axes, and (iii) gland cells occurring both on inner medullary cells and on special cells protruding into the vesicular cavity.

***Hypnea anastomosans* Papenfuss, Lipkin et P.C. Silva**

Figs 6-19

Rhodophyta, Gigartinales, Cystocloniaceae

Type locality: Scopus Ridge, Museri Island, Dahlak Archipelago, Eritrea, Red Sea (Lipkin & Silva, 2002: 20-21, fig. 5 a-c).

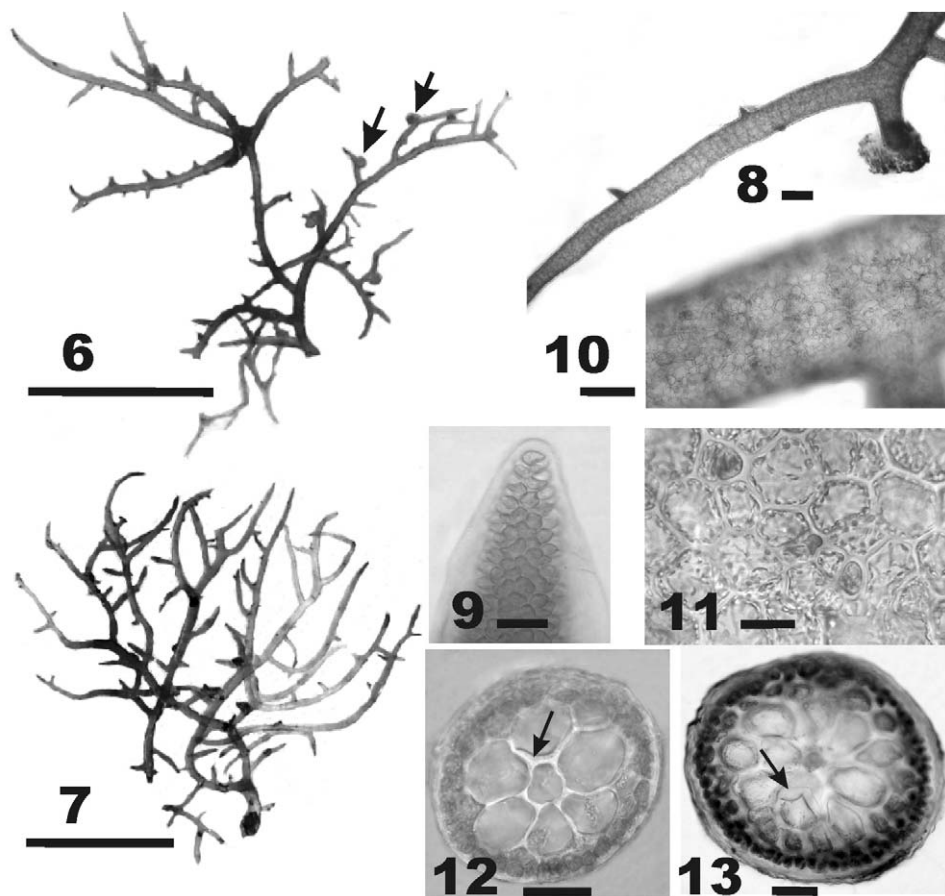


Figs 2-5. *Botryocladia madagascariensis* G. Feldmann (H8131, Korinthiakos Gulf, June 2009). **2.** Habit. **3.** Surface view of cortical cells. **4.** Cross section of a vesicle showing 2 layers of medullary cells, 2-3 layers of cortical cells, and several gland cells; one of these being attached to a special protruding cell (arrow). **5.** Inner side surface view showing gland cells attached singly or in groups on special protruding cells (arrows). Scale bars: Fig. 2 = 1 cm; Fig. 3-5 = 20 µm.

Studied specimens: H8143, female gametophytes, Faliraki, Rhodes Island (36°19'48.9"N, 28°12'42.2"E), 21 July 2009, 0.3m depth; H8144, tetrasporophytes, Agios Kosmas, Saronikos Gulf, Aegean Sea (37°53'35.4"N, 23°43'02.4"E), 29 September 2008, 0.3m depth; Faliraki, Rhodes Island (36°19'48.9"N, 28°12'42.2"E), 6 April 2009, 0.3m depth.

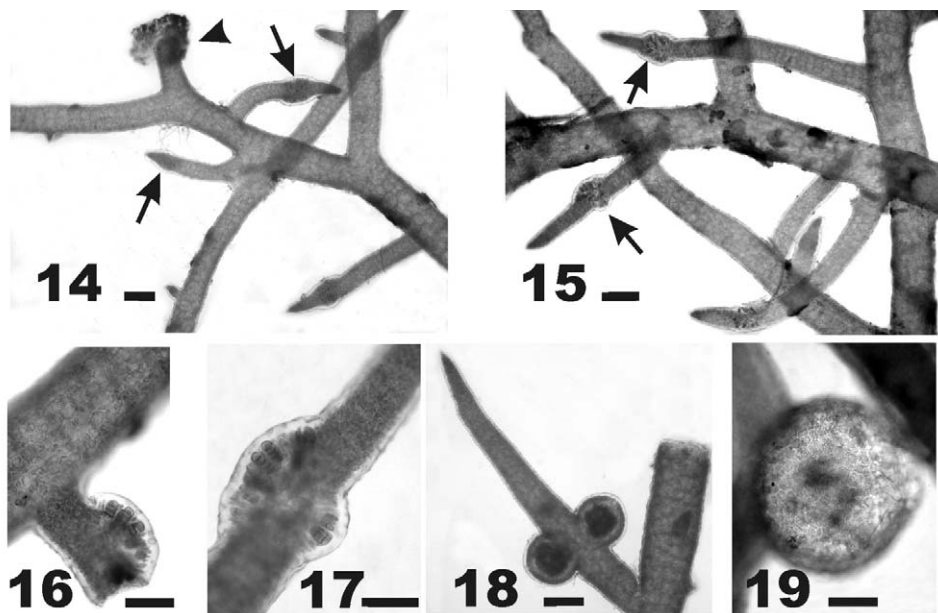
Description

Thalli small, up to 2 cm long, delicate and bushy, soft in texture, and red-brown in colour; axes terete, non-percurrent, up to 400 µm in diameter tapering towards the apices to 100 µm in diameter (Figs 6-8), entangled and often anastomosing when in contact with one another (Figs 14-15); irregular branching pattern; short spines sparse along the axes, becoming branches up to 200-500 µm long and 100-250 µm in diameter; frequent adventitious short discoid holdfasts attaching to the substrate; structure uniaxial; protruding apical cell (Fig. 9); cortical cells in surface view polygonal to irregular, 8.5×10 µm to 25×35 µm (Figs 10-11); in cross section, axial cell 28-45 µm in diameter, surrounded by 5-6 ovoid periaxial cells, 50-60 µm in diameter, a layer of medullary cells, 30-50 µm in diameter in the largest



Figs 6-13. *Hypnea anastomosans* Papenfuss, Lipkin *et* P.C. Silva. **6.** Habit of a fertile female gametophyte bearing mature cystocarps (arrows) (H8143, Rhodes Island, July 2009). **7-13.** Fertile tetrasporophyte (H8144, Saronikos Gulf, September 2009). **7.** Habit. **8.** Apical portion of an axis bearing simple spines and tapering in an acute apex. **9.** Apex showing the conspicuous apical cell. **10-11.** Surface views of cortical cells. **12-13.** Cross sections showing the distinct axial cell surrounded by periaxial cells (arrows: lenticular thickenings in cell walls). Scale bars: Figs 6-7 = 5 mm; Fig. 8 = 200 μ m; Figs 9, 11 = 20 μ m; Fig. 10 = 100 μ m; Figs 12-13 = 50 μ m.

axes, and 1-2 layers of pigmented cortical cells; lenticular cell wall thickenings present in periaxial cells (Figs 12-13); fertile tetrasporophytes morphologically similar to gametophytes, although more branched; tetrasporangial sori, 200-330 μ m long and 190-200 μ m broad, located on the distal parts of simple fertile branchlets, sometimes on median parts, usually all around the branchlet, sometimes not on the entire circumference (Figs 14-17); mature tetrasporangia zonately divided, 40-47 μ m long and 20-25 μ m broad (Figs 16-17); fertile female gametophytes with subspherical cystocarps, 260-360 (-390) μ m in diameter, usually borne, single or rarely in pairs, on the lower or median parts of fertile branchlets (Figs 6, 18-19); fertile male gametophytes not observed.



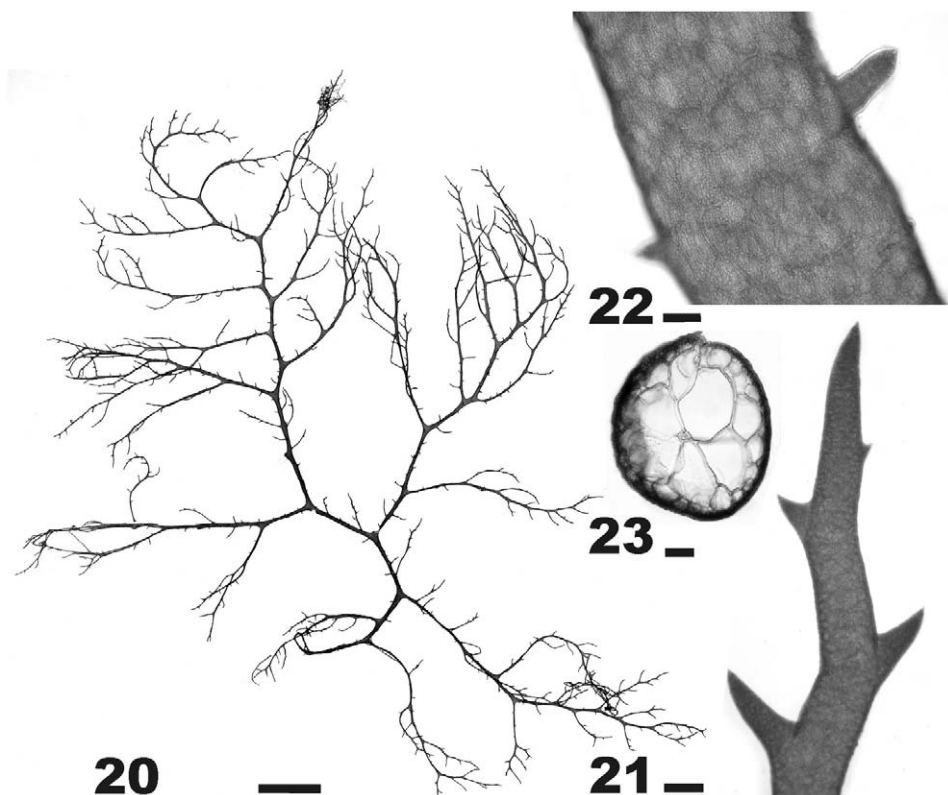
Figs 14-19. *Hypnea anastomosans* Papenfuss, Lipkin *et* P.C. Silva. **14-17.** Fertile tetrasporophyte (H8144, Saronikos Gulf, September 2009). **14-15.** Details showing anastomoses between axes, lateral holdfast (arrow head) and tetrasporangial nemathecia (arrows). **16-17.** Tetrasporangial nemathecia with zonate tetrasporangia. **18-19.** Fertile female gametophyte (H8143, Rhodes Island, July 2009). **18.** Lateral branchlet with two mature cystocarps (H8143, Rhodes Island, July 2009). **19.** Cystocarp. Scale bars: Figs 14, 15, 18 = 200 μ m; Figs 16, 17, 19 = 100 μ m.

Habitat

Several specimens of *Hypnea anastomosans* were found in the upper sublittoral zone of sheltered rocky shores. Plants were epilithic but usually entangled and epiphytic on other algae, such as *Cystoseira compressa* (Esper) Gerloff & Nizamuddin and *Halopteris scoparia* (Linnaeus) Sauvageau. Associated flora included other epiphytic algae, such as *Ceramium* spp., *Herposiphonia secunda* (C. Agardh) Ambronn, *Jania rubens* (Linnaeus) J.V. Lamouroux, and *Polysiphonia* spp. The alien species *Caulerpa racemosa* var. *cylindracea* and *Lophocladia lallemandii* (Montagne) F. Schmitz were also found in the nearby shores.

Distinctive characters

Hypnea anastomosans can be easily identified by the following distinctive features: (i) thalli small and prostrate, (ii) small diameter of axes (< 500 μ m), (iii) absence of percurrent axes, (iv) frequent anastomoses between axes, (v) sparse and simple branchlets; (vi) frequent adventive, short discoid holdfasts; (vii) no sharp difference in size between axial and periaxial cells, and cortical cells smaller than periaxial cells, in cross section, and (viii) tetrasporangia sori located on the distal parts of simple fertile branchlets, sometimes on median parts.



Figs 20-23. *Hypnea valentiae* (Turner) Montagne (H8132, Rhodes Island, July 2009). **20.** Habit. **21.** Apical portion of a percurrent axis. **22.** Surface view of cortical cells. **23.** Cross section of the axis showing the distinct small axial cell surrounded by large periaxial cells. Scale bars: Fig. 20 = 1 cm; Figs 21-23 = 100 μ m.

***Hypnea valentiae* (Turner) Montagne**

Rhodophyta, Gigartinales, Cystocloniaceae

Basionym: *Fucus valentiae* Turner (1808-1809: 17-18, pl. 78)

Type Locality: Red Sea

Synonym: *Hypnea musciformis* var. *valentiae* (Turner) Harvey (1834)

Studied specimen: H8132, Rhodes Port, Rhodes Island (36°26'26.9''N, 28°13'51.5''E), 23 July 2009, 0.2 m depth.

Figs 20-23

Description

Thalli erect, loosely branched, delicate, soft and fragile in texture, dark purple in color, up to 15 cm tall (Fig. 20); axes percurrent, terete, although slightly compressed at branching points, up to 1.3 mm broad, getting thinner towards the apex, to 300 μ m in diameter; branching spirally to irregular with branchlets tapering to acute apices (Fig. 21); spines sparse, simple, to 1.5 mm long and 130 μ m in diameter; cortical cells in surface view, polygonal, 7-15 μ m in diameter

(Fig. 22); structure uniaxial; apical cell protruding; cross section in middle part of axes: axial cell, 30-50 μm in diameter, surrounded by 5-6 large and irregular periaxial cells, 110-250 μm in diameter, covered by 1-2 layers of smaller cells, about 46-60 μm in diameter, giving finally the outer cortical cells, 8.5-12.5 μm in diameter (Fig. 23); reproductive structures not observed.

Habitat

A few thalli of *Hypnea valentiae* were found in the midlittoral zone, epilithic in a sheltered rock pool on a semi-exposed rocky shore. Associated flora included the brown alga *Dictyota implexa* (Desfontaines) J. V. Lamouroux and the green alga *Ulva rigida* C. Agardh. The alien macroalgae *Caulerpa racemosa* var. *cylindracea*, *Lophocladia lallemandii*, and *Stypopodium schimperi* (Buchinger ex Kützinger) Verlaque et Boudouresque were also found on the nearby shore.

Distinctive characters

Despite the absence of reproductive structures, *Hypnea valentiae* can be easily identified by the following features: (i) the erect, rather delicate habit (ii) evident main (percurrent) cylindrical axes, (iii) branching not divaricate, (iv) spines sparse, simple, to 1.5 mm long, and (v) axial cell visible in cross section and distinctly smaller than periaxial cells.

DISCUSSION

In the present work, three alien Rhodophyta, *Botryocladia madagascariensis* G. Feldmann, *Hypnea anastomosans* Papenfuss, Lipkin et Silva, *H. valentiae* (Turner) Montagne, are described for the first time from Greece.

Botryocladia madagascariensis

At least five *Botryocladia* species occur in the Mediterranean Sea: *B. boergesenii* Feldmann, *B. botryoides* (Wulfen) Feldmann, *B. chiajeana* (Meneghini) Kylin, *B. madagascariensis* G. Feldmann, and *B. microphysa* (Hauck) Kylin (Feldmann, 1941; Furnari et al., 2010). *Botryocladia madagascariensis* differs from *B. boergesenii*, *B. chiajeana*, and *B. microphysa*, which are small species with few vesicles, in having an erect, long, and branched axis with numerous vesicles. *B. madagascariensis* is very similar to *B. botryoides* but its gland cells are attached to both inner medullary cells and to special protruding cells, whereas in the latter species gland cells are borne only on medullary cells.

Described first from Madagascar (Feldmann, 1945), *B. madagascariensis* was later reported from Natal (Norris, 1989) and the Canary Islands (Wilkes et al., 2006). In the Mediterranean Sea, *B. madagascariensis* was reported from Italy (Cormaci et al., 1992), Malta (Cormaci et al., 1997), Turkey (Taskin et al., 2008) and Greece (Catra & Giardina, 2009), where it was recorded from the south Aegean Sea (Karpathos Island) but without description or illustration. It is clear

that a comparison between the Mediterranean populations and specimens of *B. madagascariensis* originating from its native region using molecular taxonomic tools will be invaluable to confirm the identity of this *Botryocladia*. The distribution of this species in the Mediterranean Sea might be more extensive than that shown by the literature due to the high risk of confusion with the native *B. botryoides* (see also Ribera & Boudouresque 1995; Turna *et al.*, 2000).

Our specimen agrees well with the descriptions given by previous authors (Feldmann, 1945; Norris, 1989; Cormaci *et al.*, 1992, 2004; Turna *et al.*, 2000), except for the number of gland cells per group that differs according to the authors: 2-5 gland cells per group at Madagascar (Feldmann, 1945), 3-6 in South Africa (Norris, 1989), 2-4 (5-6) in Italy (Cormaci *et al.*, 1992), 2-4 in Turkey (Turna *et al.*, 2000), and 2-3 in Greece (present study).

The genus *Hypnea* J.V. Lamouroux (1813)

The genus *Hypnea* includes 54 currently accepted species (Guiry & Guiry, 2011). Since many species have wide ranges of geographical distribution, critical reassessment of their status is required on the basis of a new study of the type material and/or specimens collected at the type locality (Masuda *et al.*, 1997). Eleven species of *Hypnea* have hitherto been reported in the Mediterranean Sea: two native species, *H. musciformis* (Wulfen) J.V. Lamouroux and *H. furnariana* Cormaci, Alongi *et* Dinaro, and nine exotic species, *H. anastomosans* (previously misidentified as *H. esperi* Bory de Saint-Vincent), *H. cornuta* (Kützinger) J. Agardh, *H. flexicaulis* Yamagishi *et* Masuda, *H. hamulosa* (Esper) J.V. Lamouroux (as *H. hamulosa* J.V. Lamouroux), *H. nidifica* J. Agardh, *H. spicifera* (Suhr) Harvey (as *H. harveyi* Kützinger), *H. spinella* (C. Agardh) Kützinger (as *H. cervicornis* J. Agardh), *H. valentiae* and *H. variabilis* Okamura (Jacquin, 1791; Reinbold, 1898; Forti, 1928; Mayhoub, 1976; Gomez Garreta *et al.*, 1979; Aleem, 1993; Cormaci *et al.*, 1993; Verlaque, 1994, 2001; Lipkin & Silva, 2002; Cecere *et al.*, 2004; Wolf *et al.*, 2011; Zenetos *et al.*, 2011; Zeybec *et al.*, 1993, and present study). However, four of these exotic species *H. hamulosa*, *H. nidifica*, *H. spicifera*, and *H. variabilis*, which are each known in the Mediterranean Sea by only one poorly documented record, must be reconsidered.

Forti (1928) reported *H. nidifica* J. Agardh (1852) from Greece [Simi (Symi) Island] on the base of cast ashore specimens. Described from the Hawaiian Islands, *H. nidifica* has been treated by Abbott (1999) as a synonym of *H. cervicornis* J. Agardh (1852), a taxon described from the Tropical Western Atlantic Ocean and the Indian Ocean, and currently treated as a later synonym of *H. spinella* (C. Agardh) Kützinger (1847), which was described from the Tropical Western Atlantic Ocean as *Sphaerococcus spinellus* C. Agardh (1822). Thereafter, molecular studies showed that Pacific strains of *H. spinella* and *H. nidifica* were distinct from each other and both from the genuine *H. spinella* from the Atlantic Ocean (Geraldino *et al.*, 2006, 2009, 2010). *Hypnea nidifica* has a divaricate branching habit, quite devoid of percurrent axes (Dawson, 1944) whereas the Mediterranean specimens attributed to this species possess clear percurrent axes (see Forti, 1928, pp. 1448-1449, pl. XXI, fig. 1). Consequently, the alga collected in Greece by Forti cannot be *H. nidifica* and might belong to *H. cornuta* or *H. valentiae* as well, two species possessing percurrent axes. It is worth noting that Simi Island is very close to Rhodes Island where *H. cornuta* was observed for the first time in the Mediterranean Sea in 1894 (Reinbold, 1898, misidentified as *H. valentiae*; see Cecere *et al.*, 2004). Consequently, we agree with Cormaci *et al.*

(2004) who excluded *H. nidifica* from their checklist of alien marine macrophytes of the Mediterranean Sea.

Mayhoub (1976) reported *H. hamulosa* J.V. Lamouroux from Syria with the following mention: “= *H. cornuta* (Kützinger) J. Agardh = *H. valentiae* (Turner) Montagne”. The name *H. hamulosa* is associated with a great deal of confusion (Papenfuss 1958; Yamagishii & Masuda, 1997). Lamouroux (1813) entered the name in his list of *Hypnea* as “*H. hamulosa* - *Fucus hamulosus*? Id. [Turner 1809 Historia Fucorum]” for a plant collected in “Indiae Orientalis”. However, the name *F. hamulosus* Turner (1808-1809: 19-20, pl.79), referred to a plant collected in the Red Sea by Viscount Valentia, is a *nomen illegitimum* because it is a later synonym of *Fucus hamulosus* Esper (1800), a species described from India (Malabar Coast). The correct combination for Esper’s taxon should be *Hypnea hamulosa* (Esper) J.V. Lamouroux as pointed by Montagne (1850). Nevertheless, J. Agardh (1852: 447) referred to *H. hamulosa sensu* (Turner) Montagne non *Fucus hamulosus* Esper to indicate an entity from the Red Sea taxonomically distinct from the Indian species. The taxon from the Red Sea, *H. hamulosa* (Turner) Montagne *nom. illeg.* (= *Fucus hamulosus sensu* Turner, 1808-1809: 19-20, not *F. hamulosus* Esper) has been considered by Papenfuss (1968) a taxonomic synonym of *H. valentiae* (Turner) Montagne (Basionym: *Fucus valentiae* Turner, 1808-1809: 17-18). Similarly to Forti’s alga, the description of the Syrian “*Hypnea hamulosa*” by Mayhoub (1976) agrees with *H. cornuta* and *H. valentiae* as well, and this unique Mediterranean record has been attributed to one or the other of these species according to the authors (Verlaque, 2001; Cormaci *et al.*, 2004; Verlaque *et al.*, in press).

Aleem (1993) reported *H. harveyi* Kützinger (1849) from Egypt (Alexandria). Described from South Africa, *H. harveyi* is currently treated as a synonym of *H. spicifera* (Suhr) Harvey, another South African species described as *Gracilaria spicifera* Suhr (1834). However the Egyptian alga differs from *H. spicifera* by (i) the thinner axes (up to 1 mm in diameter versus 3 mm in *H. spicifera*), and (ii) the short and thin branchlets becoming shorter and sparse from base to the tip whereas the spines are only present at the upper parts of axes in *H. spicifera* (Stegenga *et al.*, 1997). With regard to the Mediterranean material, Aleem (1993) added: “The similarity of this species to *H. flagelliformis* Greville ex J. Agardh is not excluded”, and, indeed, the alga from Alexandria agrees well with *H. flagelliformis*, a species described from the Indian Ocean (J. Agardh, 1852). Verlaque *et al.* (in press) attributed provisionally Aleem’s record to *H. flagelliformis* pending further investigations.

Zeybek *et al.* (1993) reported the Japanese species *H. variabilis* from Turkey but the description is insufficient to retain this species among the alien flora of the Mediterranean Sea.

Finally, only 7 (-8) species of *Hypnea* are present in the Mediterranean Sea: *H. anastomosans*, *H. cornuta*, *H. flagelliformis* (to confirm), *H. flexicaulis*, *H. furnariana*, *H. musciformis*, *H. spinella* and *H. valentiae*, which are ranged in two groups according to the absence (*H. anastomosans*, *H. flexicaulis* and *H. spinella*) or the presence (*H. cornuta*, *H. flagelliformis*, *H. furnariana*, *H. musciformis* and *H. valentiae*,) of distinct percurrent axes.

Hypnea anastomosans belongs to the group of *Hypnea* species with no percurrent axes. Our material is in good agreement with the original diagnosis of Lipkin & Silva (2002), although there are some points of difference. The Greek specimens are smaller (diameter of axes up to 400 µm versus up to 500 µm in the Red Sea specimens), less spinose and possess larger cystocarps (usually 260-360 µm in diameter versus 250-280 µm), and smaller tetrasporangia (40-47 µm ×

20-25 μm versus 40-87 $\mu\text{m} \times 22-29 \mu\text{m}$). However, in the first Red Sea description of *H. anastomosans* (Rayss & Dor, 1963, as *H. esperi* Bory), the authors mention the absence of spines and highlight the striking resemblance of young individuals with a *Chondria* species, a characteristic also observed in our material (see Fig. 8). In addition, the presence of lenticular cell wall thickenings in periaxial cells was not mentioned by Lipkin & Silva (2002).

In the Mediterranean Sea, *H. anastomosans* could be confused with the small cushion-like form of *H. spinella* from exposed shores. However, *H. spinella* differs from *H. anastomosans* in having larger axes, 130-600 (-900) μm , regularly decreasing in size upwards, branchlets subulate and thinner than axes, and antlers-like terminal branches (Gomez Garreta *et al.*, 1979; Ribera Siguan, 1983; Cormaci *et al.*, 1993).

Our plant also resembles *H. coccinea* (Clemente) Cremades, a poorly known Atlantic species described from Cádiz (Spain) (Cremades & Perez-Cirera, 1990), but *H. anastomosans* differs from it in having a larger diameter of axes (up to 400 μm versus 133-155 μm in *H. coccinea*) and cortical cells smaller than periaxial cells in cross section (not very different in *H. coccinea*). Other similar prostrate and intricate species of *Hypnea* have been described: *H. arenaria* Kylin (1938), *H. caespitosa* P.J.L. Geraldino & S.M. Boo (2010), *H. cenomyce* J. Agardh (1851), *H. intricata* Kylin (1938), *H. nidulans* Setchell (1924), *Hypnea pannosa* J. Agardh (1847), *H. saidana* Holmes (1896), *H. tenuis* Kylin (1938), *H. viridis* Papenfuss (1947) and *H. yamadae* Tanaka (1960), but they differ all from *H. anastomosans* in having one or several of the following features: larger diameter of axes (> 500 μm), axes subcomplanate, axial cell smaller than periaxial cells in cross section; absence of lenticular cell wall thickenings in periaxial cells, presence of hooked axes and stichidia-like tetrasporangial sori.

According to Lipkin & Silva (2002), *H. anastomosans* is widespread in warm seas where it was previously reported as *H. esperi* Bory de Saint-Vincent *sensu* Kützing (e.g. Børgesen, 1924; Tanaka, 1941; Dawson, 1954). *Hypnea esperi* Bory de Saint-Vincent (1828) was superfluous, and thus illegitimate, when published, being a substitute name for *Fucus nootkanus* Esper (1802), a name applied to a species of uncertain identity (see Papenfuss, 1964; Silva *et al.*, 1987). Dawson's (1961) description of the Kützing's material illustrated as *H. esperi* Bory in Kützing (1868, table 26a-c) agrees rather with *H. anastomosans* (2.3 cm in extent, diameter of axes 100-300 μm , branching pattern divaricate, and accessory attachment discs present) than with Bory de Saint-Vincent's diagnosis ("3-6 inches in height") and supports the hypothesis of a wide distribution range of *H. anastomosans* in the Indo-Pacific Ocean.

Many descriptions of "*H. spinella*" from the Red Sea and Indo-Pacific (including axes $\leq 500 \mu\text{m}$ in diameter and presence of lenticular cell wall thickenings in periaxial cells) (Dawson, 1961 as *H. cervicornis*; Mshigeni, 1978; Natour *et al.*, 1979; Wynne, 1995; Yamagishi & Masuda, 1997; Bangmei & Yongqiang, 1997; Abbott, 1999) might also relate to *H. anastomosans*, since the genuine *H. spinella*, described from the Atlantic Tropical Ocean seems absent from the Pacific Ocean (Geraldino *et al.*, 2006, 2009, 2010).

Hypnea anastomosans is common in the Red Sea and the Suez Canal (see details of records in Lipkin & Silva, 2002). In the Mediterranean Sea, the species was reported for the first time in Israel (Lipkin, 1972, as *H. esperi*). Afterwards, *H. esperi* was described from the Saronikos Gulf in Greece (Diapoulis, 1983; Diapoulis & Haritonidis, 1987a) but the antlers-like branches and the diameter of axes (400-600 μm) agree more with prostrate forms of *H. spinella* than with *H. anastomosans*. In 1987, *H. esperi* was also listed, without description, from the

west coasts of Greece (Diapoulis & Haritonidis, 1987b). *Hypnea anastomosans* is illustrated for the first time from the Mediterranean Sea.

Hypnea valentiae, which belongs to the group with percurrent axes, can be easily distinguished from *H. cornuta* by the absence of stellate spines, from *H. flagelliformis* by its periaxial cells without intercalary small cells, from *H. furnariana* by its branches slightly slender than axes (much more slender than axes in *H. furnariana*), and from *H. musciformis* by the absence of hooked apices.

Described first from the Red Sea (Turner 1808-1809, as *Fucus valentiae*), *H. valentiae* has been reported worldwide in all the tropical and warm-temperate regions of the Atlantic and the Indo-Pacific Oceans (Silva *et al.*, 1987, 1996; Huisman & Walker, 1990; Schneider & Searles, 1991; Abbott, 1999; Haroun *et al.*, 2002; Díaz-Pulido & Díaz-Ruiz, 2003; Suárez, 2005). However, its world distribution should be considered with caution due to numerous confusions and misidentifications (Verlaque *et al.*, in press). In the Mediterranean Sea, *H. valentiae* was reported first in 1896 from Greece (Rhodes Island; Reinbold, 1898), but it turned out to be a misidentification of *H. cornuta* (Kützinger) J. Agardh (see Cecere *et al.*, 2004). Reinbold's erroneous report has been successively cited by different authors (Rechinger, 1943; Aleem, 1948; Gerloff & Geissler, 1974) before to be excluded by Athanasiadis (1987). Afterwards, Mediterranean collections were made in 1945 in Egypt (Alexandria), but the photographed specimen (fig. 6, pl. 48 in Aleem, 1993) does not agree with *H. valentiae*. In Greece, *H. valentiae* was reported from Messolonghi Lagoon (Bogdanos & Diapoulis, 1984), Lesvos Island (Diapoulis & Haritonidis, 1984), and west coasts (Diapoulis & Haritonidis, 1987a), but in the absence of detailed descriptions or illustrations these records were considered as insufficient to establish the presence of *H. valentiae* in Greece (see Tsiamis *et al.*, 2010). In the Mediterranean Sea, the occurrence of *H. valentiae* was clearly established for the first time only in 2000 in France (Lagoon of Salses-Leucate and Thau Lagoon, Verlaque, 2000, 2001), then in Italy, in the Lagoon of Venice (Sfriso *et al.*, 2002) and the Mar Piccolo di Taranto (E. Cecere and A. Petrocelli, pers. com.). Our records from Rhodes Island are in good agreement with the previous descriptions of the species, including those from the western Mediterranean Sea and the Adriatic Sea (Verlaque, 2001; Verlaque *et al.*, in press).

Alien status in the Mediterranean Sea

In the Mediterranean Sea, *Botryocladia madagascariensis* is considered to be an alien species introduced into the basin through shipping or via the Suez Canal (Cormaci *et al.*, 2004; Verlaque *et al.*, in press). Lipkin (1972) considered *Hypnea anastomosans* (as *H. esperi*) as a recent Red Sea immigrant into the Mediterranean Sea and Por (1978) ranged it in the category of high probability Lessepsian immigrants. Lastly, the discovery of *Hypnea valentiae* at Rhodes Island confirms its introduction in the Eastern Mediterranean basin. Taking into account that Rhodes Island is one of the best study areas concerning macroalgae in Greece (Diapoulis *et al.*, 1986; Tsiamis *et al.*, 2007), we believe that our material corresponds to a recent introduction event. Whether it originates, via shipping or shellfish transfer, from the populations already introduced into the Western Mediterranean Basin or the Adriatic Sea (Verlaque *et al.*, 2007) or directly from the Red Sea, via the Suez Canal, remains unknown.

In conclusion, this new contribution to the marine alien flora of Greece attests that the spread of Eastern Mediterranean alien flora is still in progress. It

would now be interesting to investigate, through molecular analyses, the taxonomical link between native and introduced populations.

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