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Parviphycus felicinii sp. nov. (Gelidiales, Rhodophyta) from South-East Italy

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Abstract – The marine red alga *Parviphycus felicinii* sp. nov. (Gelidiellaceae, Gelidiales) is described from Italy. The species shows a predominantly creeping habit and forms small, dark purplish red turfs on smooth calcareous substrata at the supra-littoral level. It is distinguished by the following combination of features: size and shape of the outermost cortical cells in the upright thallus, number of tetrasporangia per tier, an extreme habitat. In culture, *Parviphycus felicinii* retains its creeping habit. In the presence of a suitable substratum, stolons are soon attached by rhizoids and growth is similar to that of field-collected plants. Tetrasporangial sori form in LD photo-regimes, in both floating and attached thalli. In the attached plants, sori only form at the upright apices, whilst in floating plants they form especially in lateral stichidium-like branchlets. Cultured tetraspores show high vitality and germination potential. The presumed gametophytes develop regularly, although they remain vegetative for six months in both SD and LD conditions.

Gelidiales / Gelidiellaceae / *Parviphycus* / taxonomy

Résumé – ***Parviphycus felicinii* sp. nov. (Gelidiales, Rhodophyta) du Sud-est de l'Italie.** L'algue rouge *Parviphycus felicinii* sp. nov. a été recoltée en Italie, sur la côte adriatique meridional. L'espèce montre habituellement un habitus rampant et forme des gazons rouges foncés sur des rochers calcaires plats au niveau supralittoral. Elle est caractérisée par : la taille et la forme des cellules corticales extérieures du thalle dressé, le nombre de tétrasporanges par rangée, et un habitat extrême. En culture, *Parviphycus felicinii* conserve son habitus rampant. Sur un substrat approprié, les stolons se fixent par leurs rhizoïdes et se développent de la même façon que dans la nature. Les sores tétrasporangiales se forment en rythme d'éclairement à jours longs dans les thalles flottants comme dans les thalles fixés. Dans les plantes fixées les sores sont produit seulement aux sommets des axes dressés, tandis que dans les plantes flottantes ils sont produits surtout dans de petits rameaux latéraux ressemblant à des stichidies. Les tétraspores cultivées sont très viables et germent, en produisant, vraisemblablement, des gamétophytes qui, pendant six mois en culture, demeurent stériles quelque soit le rythme de l'éclairement.

Gélidiales / Gelidiellaceae / *Parviphycus* / taxonomie

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INTRODUCTION

During an extensive exploration of the south-eastern Italian coasts, with the intention of making an inventory of the agarophytes and carrageenophytes of the Apulian region, a previously undescribed species belonging to the family Gelidiellaceae (agarophytes) was found. It is described herein as *Parviphycus felicinii* sp. nov.

The attribution of the new Mediterranean taxon to the recently established genus *Parviphycus* Santelices (Santelices, 2004) is supported by both a long-term study of the same population and laboratory cultures. The new genus was proposed to accommodate those species previously assigned to *Gelidiella* that bear tetrasporangial sori arranged in transverse rows (the so-called *Pannosa*-type stichidia) and have sub-apical cells that undergo a distichous pattern of division. Furthermore, in *Parviphycus* axial and periaxial cells are arranged in a distinctive transverse row that remains evident throughout the thallus. *Gelidiella adnata* E.Y. Dawson, *Gelidiella antipae* Celan, *Gelidiella tenuissima* J. Feldman *et* Hamel and *Gelidiella womersleyana* Kraft *et* I.A.Abbott have been transferred to *Parviphycus* (Santelices, 2004).

Biological surveys intended to document the benthic flora of the south-eastern coasts of Italy have rarely been conducted. From the 1960s to the present day, true marine floristic research is restricted to a few papers dealing with the flora of the Gargano promontory and the Tremiti Islands (Adriatic Sea) (Pignatti *et al.*, 1967; Rizzi Longo *et al*., 1967), and to the Gulf of Taranto (Ionian Sea) (Cecere *et al*., 1991, 1992, 1994; Perrone & Cecere, 1994; Cecere & Perrone, 2002). The remaining coastline has seldom been explored (Felicini, 1965; Solazzi, 1968; Giaccone, 1978; Cormaci & Furnari, 1991; de Gregorio *et al*., 1995; Lapenna & Perrone, 1999; Cormaci *et al.*, 2001; Delle Foglie *et al*., 2003; Furnari *et al*., 2003).

MATERIALS AND METHODS

Vegetative and tetrasporic plants of *Parviphycus felicinii* have been collected since 1995 in the Grotta della Regina at Torre a Mare (Bari), South-East Italy, Adriatic Sea. Voucher specimens and materials used in the morphological study were preserved in 4% formalin-seawater or pressed on herbarium sheets, and deposited in the Herbarium Orti Botanici Barensis (BI).

The following material was also examined: 1) Fresh material: *Parviphycus antipae* (Celan) Santelices, *Parviphycus tenuissimus* (Feldmann *et* Hamel) Santelices and *Gelidiella lubrica* (Kützing) J. Feldmann *et* Hamel, frequently collected from the Apulian coasts (Adriatic and Ionian Seas). 2) Liquid preserved material: *G. lubrica* (Kützing) Feldmann *et* Hamel, sterile and tetrasporic; *Parviphycus tenuissimus* (as *G. tenuissima* (Feldmann) Feldmann *et* Hamel), sterile and tetrasporic; *Parviphycus antipae* Santelices (as *G. antipae* Celan), sterile and tetrasporic, all collected by C. Perrone at S. Cesarea Terme (Lecce), Italy, in 1990,1994, 1995, 1997, 1998, 2000; *G. lubrica*, sterile and tetrasporic, 1994, Capo S. Alessio (Sicily); *G. nigrescens* (Feldmann) Feldmann *et* Hamel, sterile; *G. ramellosa* (Feldmann) Feldmann *et* Hamel, tetrasporic; *Parviphycus tenuissimus* (as *G. pannosa*), sterile and tetrasporic, all collected in 1993 at Is. Lachea (Sicily) (sent by M. Cormaci and G. Furnari); *G. ramellosa*, 1993, S. Paolo, Cheradi Islands, Taranto (Italy) (sent by E. Cecere).

Both apical explants (0.5 mm) and isolated tetraspores of *Parviphycus felicinii* were kept in unialgal cultures, arranged in growth chambers and illuminated with cool-white fluorescent tubes at low irradiance (20 μmol m^{-2} s^{-1}, the max. value measured on the cave population), at 18° ± 1°C temperature, 8h:16h and 16h:8h light:dark regimes. Plain seawater, filtered on Millipore, was used as culture medium and replaced weekly. 6ppm GeO_2 was added to the culture medium to limit diatom development.

Cultures on natural calcareous substrata were also prepared to simulate as carefully as possible sub-aerial field conditions: thin slabs of porous calcarenite were arranged in small aquaria and inoculated with explants or tetraspores. Water depth was maintained at the level of the slabs.

Morphological and anatomical observations were carried out on fresh material, liquid preserved specimens and cultured thalli. Sections were cut by hand or on a Leitz Kryomat microtome (Ernst Leitz Gmbh®, Wetzlar, Germany), and stained with 1% aniline blue acidified with 0.5% HCl, or in 1% aqueous Fast Green and observed under a light microscope (Olympus BX – 41) Photographs were taken with a digital camera (Nikon Coolpix 990). Morphometric parameters were measured by means of a micrometric eye piece.

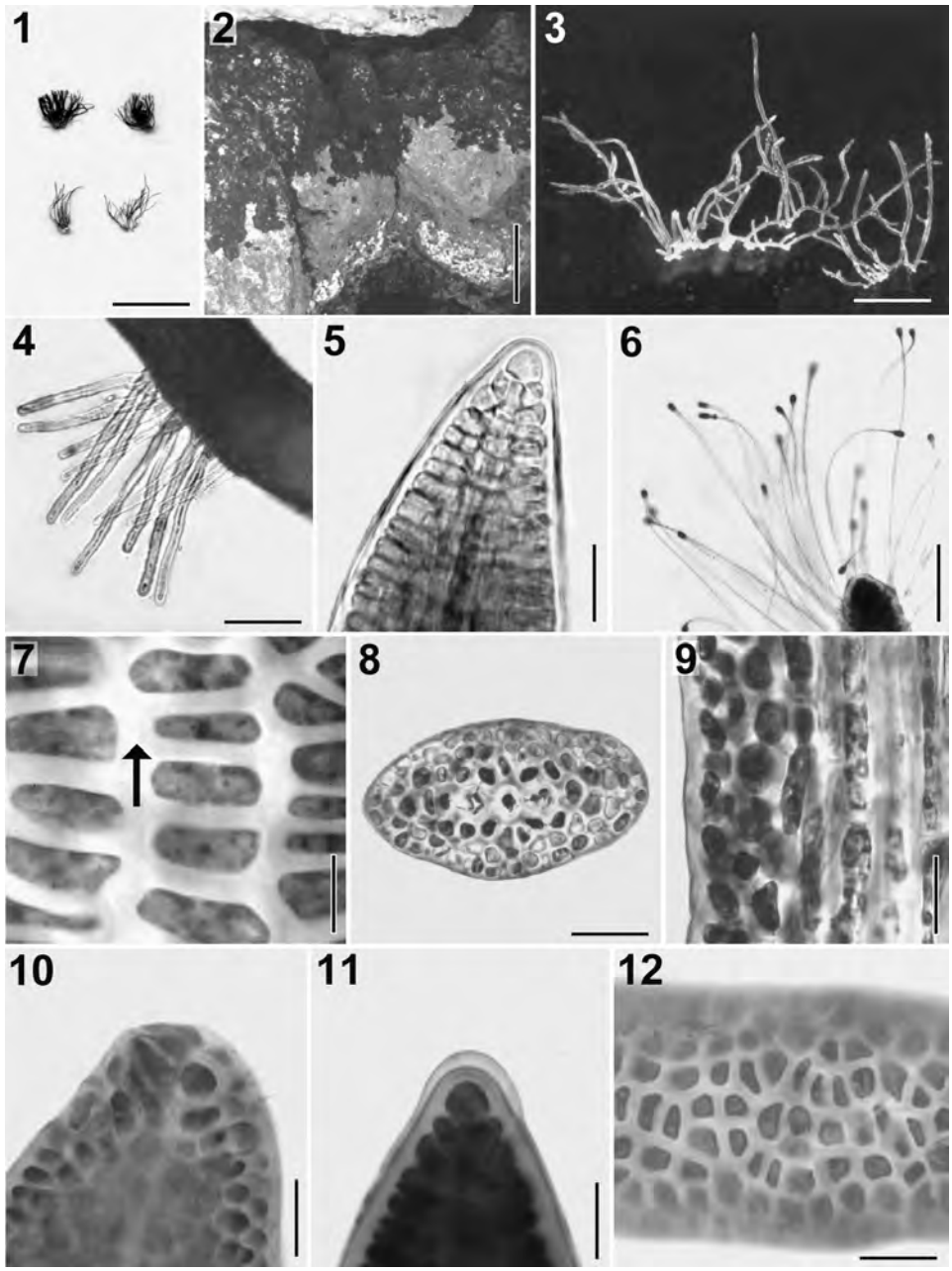
RESULTS

Parviphycus felicinii Perrone *et* Delle Foglie, sp. nov.

Figs 1-3

Thalli rubropurpurei ex teretibus axibus repentibus magis constantes qui per singula unicellularia rhizoidea ex extimis irregulariter ordinatis cellulis corticalibus orientia affixi sunt. Erecti axes compressi cellula apicali se transverse dividenti cellulaque subapicali duas longitudinales divisiones subienti duas cellulas periaxiales facientes quae postea cellulas laterales generant; externae corticales cellulae pro maxima parte transverse elongatae superficiali visu; medulla cum 3-5 cursibus transversalibus cellularum. Rami laterales non multum atque irregulariter ramosi; omnes erecti axes in stolones se convertere possunt. Sori tetrasporangiorum in erectiorum axiorum lateraliumque ramulorum apices, plerumque intercalares ubi veteres vacuique; tetrasporangia parallele seriatimque disposita, 8-9 per seriem, subsphaerica, tetraedrice divisa. Sexualis reproductio ignota.

Thalli dark purplish red forming turfs due to a predominant creeping habit; terete stolons attached to the substratum by means of unicellular independent rhizoidal filaments originating from only the outermost isodiametric and irregularly arranged cortical cells. Compressed uprights with the apical cell transversally dividing; sub-apical cells undergoing two longitudinal divisions to form 2 periaxial cells which later cut off two further lateral cells; outer cortical cells mostly transversely elongated in surface view; 3-5 medullary cells (axial, periaxials and derivates) arranged in one transverse row, as observed in trans-section. Uprights scarcely and irregularly branched; all the axes can grow as stolons reattaching to the substratum. Tetrahedrally divided subspherical tetrasporangia arranged in parallel rows, 4 (+1) per row, 8 (+1) per tier, in the apices of both erect axes and also in lateral sessile stichidium-like ramuli; intercalary when old and almost empty. Sexual reproduction unknown.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Holotype: BI 38.000, 7 June 1995 (Fig. 1).
Paratypes: BI 38.002, 14 March 2001; BI 38.001, 12 December 2000, from Grotta della Regina; BI 38.003, 4 July 2001; BI 38.004, 6 December 2001, from Cala Colombo.
Type locality: Grotta della Regina, Torre a Mare (Bari), southern Italy (Lat 41°13' N, Long 16°78' E).
Distribution: Known only from the type locality, Grotta della Regina, and Cala Colombo (Torre a Mare, Bari, Italy).
Habitat and seasonality

Parviphycus felicinii has been found in a small marine cave, Grotta della Regina, and on a vertical rock at Cala Colombo, about 3 km from the type locality, at Torre a Mare. Inside the cave, it colonizes a horizontal erosion platform, up to 5-10 m from the sea, staying between 80 and 40 cm above the mean sea level, and pushes toward the entrance of the cave always above the level of the sea. The populations are never reached by the high tide, but only by rough sea. Outside the cave, it colonizes about 20 cm^2 at 170 cm above the level of the sea. On the more southern coast of Torre a Mare, at Cala Colombo, *Parviphycus felicinii* grows on a vertical rocky wall up to about 2 m above the level of the sea. Due to the small range of the Mediterranean tides, this habitat should be considered supra-littoral. *Parviphycus felicinii* populations at their lowest level, when occasionally this corresponds to the high tide level, are substituted by *Gelidium pusillum* (Stackhouse) Le Jolis. In these extreme conditions, *Parviphycus felicinii* grows in unialgal populations and only some cyanobacteria share the same habitat at Torre a Mare. This taxon is perennial and slow-growing, both creeping axes and erect branches being present all year round. New tetrasporangia form in spring.
Etymology: "*felicinii*" is in honour of Prof. Gianni P. Felicini, a prominent Italian phycologist, who introduced the first author to the world of algae.
Specimens examined: Holotype, paratypes.
Habit and vegetative morphology

Parviphycus felicinii forms small dark purplish red turfs on smooth calcareous substrata (Fig. 2). The species shows a predominantly creeping habit: indeterminate main axes are terete stolons and are more conspicuous than the uprights (Fig. 3). Stolons are attached to the substratum by means of independent, long, thick-walled, refractive unicellular rhizoids (Fig. 4). Upright axes are only basally terete and otherwise are compressed throughout, 4-6 (–11) mm high, max 140 µm wide, scarcely and irregularly branched and represent determinate lateral branches. Some uprights can continue to grow as stolons, curving downward and re-attaching to the substratum by producing attachment rhizoids from the outermost cortical cells.

Figs 1-12. *Parviphycus felicinii*. **1**. Holotype, BI 38.000. Scale = 2 cm. - **2**. Turf-forming population at 2 m above the mean sea level. Scale = 20 cm. - **3**. Part of thallus detached from the substratum. Scale = 700 µm. - **4**. Independent rhizoidal filaments issuing from a stolon. Scale = 50 µm. - **5**. Apex of an upright branch showing the sub-apical cell distichously dividing. Scale = 10 µm. - **6**. Long apical hairs on upright branches. Scale = 60 µm. - **7**. Outermost cortical cells of upright branches in surface view. Arrow indicates the longitudinal axis of the branch. Scale = 10 µm. - **8**. Cross-section of a compressed upright branch; axial, periaxials and derivates are aligned along the major axis of the section in an evident row. Scale = 20 µm. - **9**. Longitudinal section of branch. Scale = 15 µm. - **10**. Stolon (main axis) apex showing an irregular cell arrangement. Scale = 10 µm. - **11**. Stolon apex with regular cell arrangement and a protective mucilaginous cap. Scale = 10 µm. - **12**. Irregularly shaped and arranged outermost cortical cells of stolons in surface view. Scale = 40 µm.

Both stolons and uprights grow uniaxially from a dome-shaped apical cell giving rise to subapicals dividing distichously (Fig. 5). The erect branches bear long hairs in the apical parts (Fig. 6). In the uprights, surface cells are mostly transversely elongated, as observed in surface view, mean height 7.5 μm and mean width 12.8 μm. (Fig. 7). A row of five cells, consisting of an axial cell, two pericentrals and two derivatives, is always evident in cross sections at any level in both young and mature parts of the erect branches (Fig. 8). Both axial and periaxial cells are the same size, approx. 13 μm in diameter, and have a stratified cell wall approx. 2.5 μm thick. They are aligned along the major axis of the section and are the largest cells of the vegetative structure. Medullary cells measure approx. 7.9 μm in diameter, whilst the outermost cortical cells measure approx. 5 μm (Fig. 8). In longitudinal section, the medulla is composed of cylindrical cells that become ever more elongated as they approach the axial filament. The thallus does not contain thick-walled refractive internal rhizoidal filaments, the so-called hyphae or rhizines in the Gelidiales (Fig. 9).

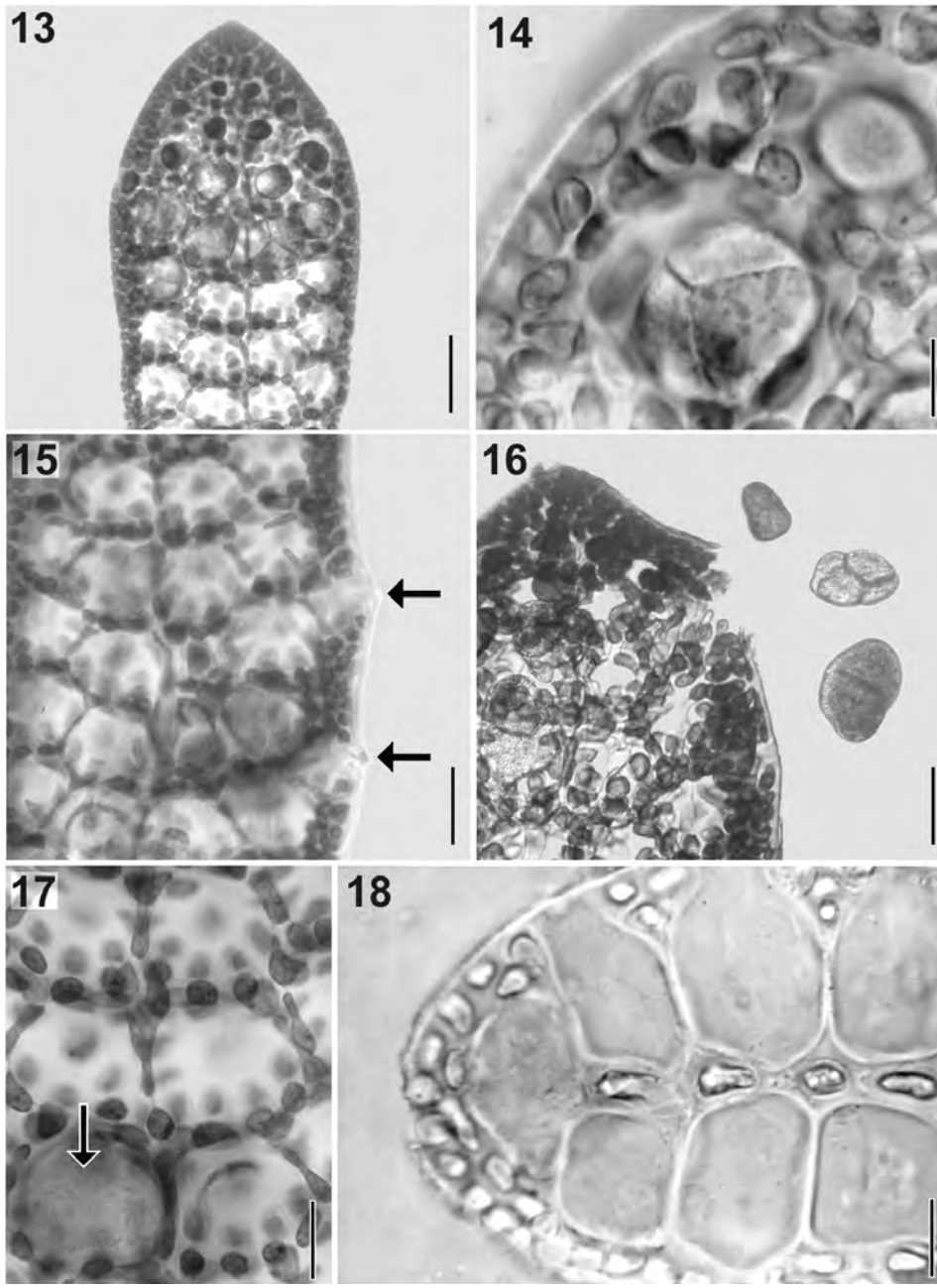
In creeping axes, the apex is organised as in the uprights, but sometimes has an irregular structure in which the apical cell is not evident (Fig. 10). Commonly, stolon apices are protected by a transparent cap (Fig. 11). Stolon surface cells are polygonal or circular in shape (mean diameter 9.6 μm) and are irregularly arranged (Fig. 12). Rhizoidal filaments arise from the outermost cortical cells by budding and tip growth and remain in open connection with their mother cells. Rhizoidal filaments are approx. 4-5 μm wide, with a bi-stratified cell wall, which is 1-2 μm thick and refractive when mature. Rhizoidal filament length exceeds 200 μm, when still unattached to the substratum.

Reproductive morphology

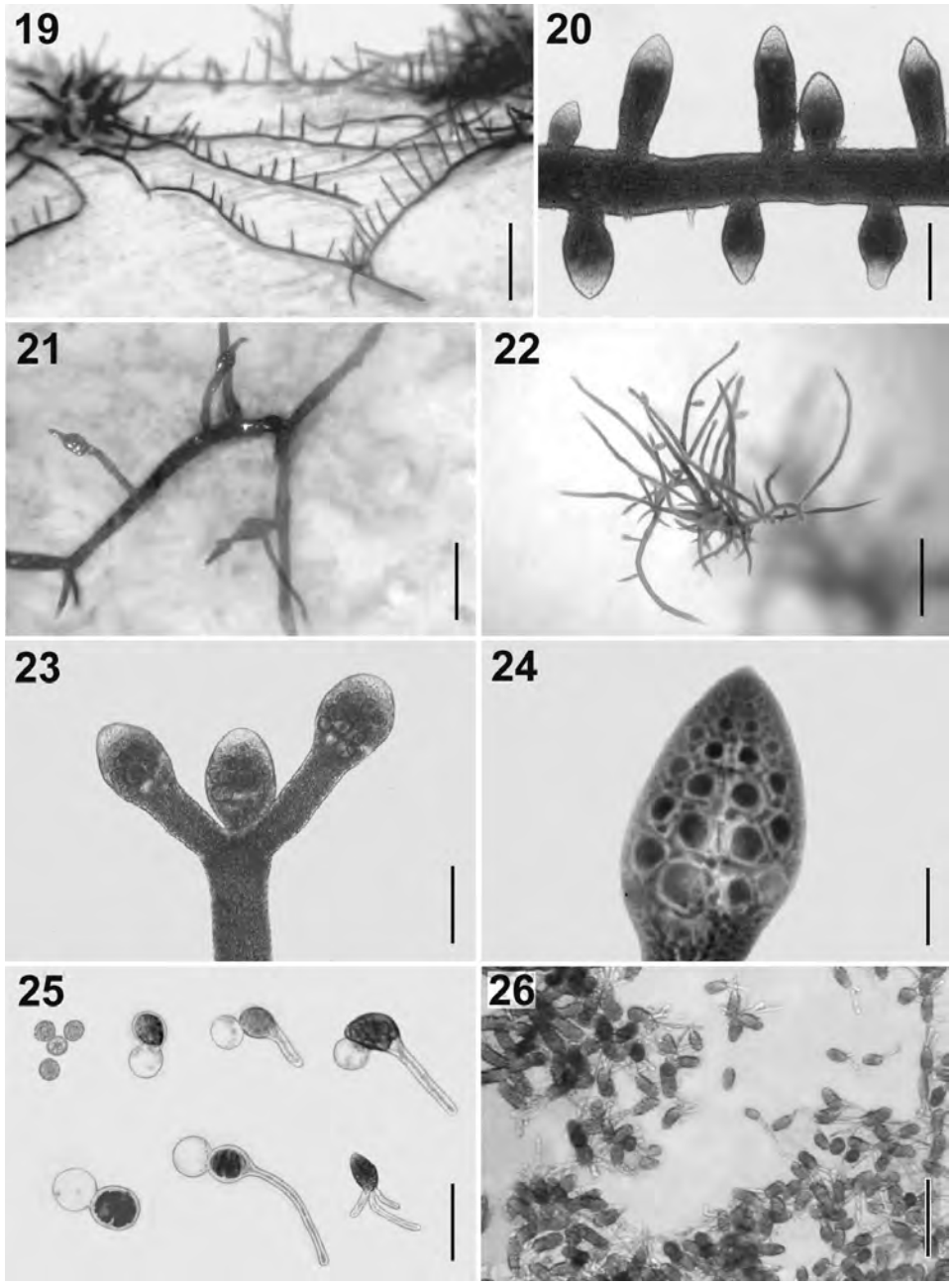
Thalli are mostly tetrasporic, with numerous basal and intercalary partially empty tetrasporangial sori. In the field, tetrasporangial sori begin forming in May-June in both main upright apices and some in lateral stichidium-like ramuli. Tetrasporangial sori consist of a variable number of tiers (2-20) concave towards the apex, with 4 tetrasporangia per row (Fig. 13). Tetrasporangia (up to 35 μm in diameter) mature acropetally from the centre to the periphery (Fig. 13) and are tetrahedrally divided (Fig. 14). Very often a ninth tetrasporangium in the frond margin can alter the sorus symmetry (Fig. 15). Tetrasporangia are released as a unit (Fig. 16). Usually secondary tetrasporocysts are produced (Fig. 15, 17). In cross-section, sori exhibit bilateral symmetry according to either two or a single plan, that is the major axis of the section (Fig. 18).

Cultures

Laboratory cultures have demonstrated that *Parviphycus felicinii* grows well at low irradiance, stops growing in short day (SD) photo-regimes, resumes growth and produces tetrasporocysts in long day (LD) photo-regimes. The creeping habit was retained in culture, growth being in the form of stolons producing long rhizoids from the growing tips and dorsal upright compressed branches. In the sub-aerial cultures, the explants reattach themselves within 5 days by means of rhizoidal filaments arising from the outer cortical cells. Once attached, explants produced numerous long terete stolons everywhere, rarely as regenerates from the proximal cut surfaces, and colonised most of the substratum in 15 days. Subsequently, upright compressed axes arose on the dorsal side of the stolons at quite regular intervals, reached about 1 cm in height and then stopped growing. Lateral branches were rarely produced by the uprights (Fig. 19).



Figs 13-18. *Parviphycus felicinii*. - **13**. Tetrasporangial apical sorus with regularly arranged 4 tetrasporangia per row. Scale = 45 µm. - **14**. Trans-section of a tetrasporangial sorus showing a tetrahedrally divided tetrasporangium. Scale = 10 µm. - **15**. Tetrasporangial sorus with asymmetrically arranged tetrasporangia, 4 + 1 per row (arrows). Scale = 30 µm. - **16**. Tetrasporangia released as units. Scale = 35 µm. - **17**. Secondary tetrasporocyst (arrow). Scale = 20 µm. - **18**. Trans-section of asymmetrical tier of a tetrasporangial sorus. Scale = 15 µm.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In contrast, submerged and unattached explants produced numerous lateral compressed branches from the margins at 90° (Fig. 20), whilst regenerated branches from the explant proximal cut surfaces were formed very rarely. 40-50 day old plantlets became ball-like as a consequence of being unattached (Fig. 22).

In LD photo-regimes, tetrasporangial sorus formation occurred in both floating and attached thalli. In the attached plantlets, sori only formed at the upright apices, contained a low number of tiers, and soon resumed vegetative growth (Figs 21, 23). In the floating thalli, in contrast, tetrasporangial sori formed especially in lateral stichidium-like branchlets and produced more numerous tiers (Figs 22, 24). The number of sporangia per tier, on the contrary, was constant in all cultured explants.

Tetrasporangial sorus formation does not stop apical growth, which continues giving rise to branches with basal and intercalary sori in the subsequent period, as occurs in the field. Mature tetrasporangia were soon released as units and tetraspores separated in a few hours.

Cultured tetraspores showed high vitality and germination potential. Tetraspores attached to both the glass of the Petri dishes and natural calcareous substrata. Germination took place according to a regular *Gelidium*-type pattern (Figs 25-26) and presumed gametophytes grew well for the subsequent six months but did not form sexual organs at all, or tetrasporangia, in either SD or LD conditions.

DISCUSSION

Parviphycus is one of the several gelidiaceous genera occurring as a perennial on rocky Italian shores. Five species belonging to the Gelidiellaceae family have been described from the region and also reported from the Apulian coasts: *P. antipae* (as *Gelidiella antipae*), *P. tenuissimus* (as *Gelidiella pannosa*), *Gelidiella lubrica*, *G. nigrescens,* and *G. ramellosa*, the latter only recorded from the Cheradi Islands (Gulf of Taranto) (Lapenna & Perrone, 1999).

The new species has been ascribed to the genus *Parviphycus* because the subapical cells divide distichously and tetrasporangia are arranged according to the "*pannosa*-type" pattern. *Parviphycus felicinii* exhibits a combination of features unique among the other gelidiellacean species described for the Mediterranean region. It proved to be distinct especially in the number of tetrasporangia per tier and shape of the outermost cortical cells of upright branches. It shares some characters with *P. tenuissimus*, as described (as *Gelidiella*

Figs 19-26. *Parviphycus felicinii*. - **19**. 15 day old cultured explants on the sub-aerial surface of partially submerged calcarenite slabs. The explants colonised the substratum by producing long stolons and orthogonal uprights. Scale = 1.5 cm. - **20**. Floating explants producing at 90° from the margins numerous compressed laterals. Scale = 200 µm. - **21**. Apical tetrasporangial sori formed on cultured explants attached on hard substratum. Scale = 1 mm. - **22**. Lateral stichidium-like tetrasporangial branchlets formed on cultured floating thalli. Scale = 4 mm. - **23**. Tetrasporangial sori on an irregularly branched axis. Scale = 200 µm. - **24**. Tetrasporangial sorus of a floating plant with regular tetrasporangium arrangement. Scale = 45 µm. - **25**. Phase sequences of *Gelidium*-type germination of the tetraspores produced in culture. Scale = 50 µm. - **26**. A comprehensive view of presumed gametophytes grown in culture. Scale = 100 µm.

tenuissima) by both Boudouresque (1969) from Corsica and Rico *et al*. (2002) from Gran Canaria, but has a different medullary structure, tetrasporangium arrangement and habitat characteristics. In contrast, it is very distinct from the other Apulian Gelidiellaceae. *P. antipae* is constantly smaller and bears 2 tetrasporangia per row; *Gelidiella lubrica* has a very developed terete upright thallus approx. 2 –2.5 cm tall with rounded outermost cortical cells irregularly arranged. *Gelidiella ramellosa* from the Cheradi Islands shows a combination of characters which require further investigation in that, in our opinion, it seems quite distinct from the Australian specimens. Finally, *G. nigrescens*, a very ambiguous taxon never found in tetrasporic form, is locally distinguished by its terete, dark, very robust and irregularly branched upright thallus.

Compared to the species from outside the Mediterranean, *Parviphycus felicinii* shares some features with *Gelidiella myrioclada* (Børgesen) Feldmann *et* Hamel, but habitat, branching and both surface cell shape and arrangement do not correspond (Børgesen, 1934; Feldmann & Hamel, 1934).

The very dry supra-littoral habitat of *Parviphycus felicinii* in Apulia is particular to this species: the other species belonging to the Gelidiellaceae being collected from the high inter-tidal to sub-tidal zones, in particular *P. antipae* at 5-10 m depth, *P. tenuissimus* at 0-40 cm on vertical walls and *Gelidiella lubrica* at 0-35 cm on platforms, all being permanently submerged.

In culture, *Parviphycus felicinii* retained its creeping habit. In the presence of a suitable substratum, plants soon attached by rhizoids; floating plants on the other hand only formed compressed laterals. Growth was only normal in the attached cultures; however, more regular formation of tetrasporangial sori in stichidium-like branchlets occurred in the floating cultures, probably due to the immersion conditions.

Cultured tetraspores showed high vitality and germination potential, but the presumed gametophytes did not form sexual organs at all in either SD or LD conditions. Their growth, on the other hand, continued regularly like that of cultured explants.

It can be speculated that *Parviphycus felicinii* has recently colonized its extreme habitat, perhaps due to competition for space, and subsequently adapted to both dessication and low irradiance.

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