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Grateloupia huertana sp. nov. (Halymeniaceae, Rhodophyta), a peculiar new prostrate species from tropical Pacific Mexico

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Grateloupia huertana sp. nov. is newly described from Oaxaca, Pacific Mexico on the basis of comparative morphology and *rbc*L sequence analysis. It is distinguished from other *Grateloupia* species by its entirely prostrate, fleshy-cartilaginous, highly polymorphic habit ranging from single, irregularly spreading and perforated lobes with marginal proliferations, to rosette-like clumps of overlapping blades up to 5 cm in diameter. The entire lowermost surface acts as a single expanded holdfast conforming to the topography of the substratum. Globose and deeply immersed cystocarps are scattered and restricted to the dorsal side. Auxiliary cell and carpogonial ampullae are of the simple *Grateloupia*-type. The medulla varies from compact and cellular to a network of predominantly periclinal filaments producing varying degrees of secondary cells as the thallus expands laterally. The new species is embedded within the *Grateloupia* clade in *rbc*L trees, but its phylogenetic position in relation to other members of the genus remains equivocal. A systematic revision of *Grateloupia sensu lato* is called for.

INTRODUCTION

During continuing investigations assessing the diversity of the benthic marine macroalgae of the state of Oaxaca, tropical Pacific Mexico, an undescribed prostrate red alga with simple auxiliary cell and carpogonial ampullary filaments typical of the *Grateloupia*-group in the Halymeniaceae (Chiang 1970) was recently collected. The Halymeniaceae consists of 20 genera and at least 160 species dispersed worldwide, from cold-temperate through tropical waters (Womersley & Lewis 1994). The genus *Grateloupia* was established by C. Agardh in 1822 based on the generitype *G. filicina* (Lamouroux) C. Agardh from the Mediterranean Sea (Agardh 1822–1823), and with 51 described species is the largest in the family. In the western Pacific 19 species of *Grateloupia* and several forms and varieties have been reported (Silva *et al*. 1987; Wang *et al*. 2000), whereas for the coast of Pacific Mexico 13 species are recorded (Dawson 1954).

This genus is ill-defined, and includes taxa with a wide variety of habits, ranging from finely pinnate (e.g. *G. filicina*), foliose (e.g. *G. turuturu* Yamada) to hollow tubular blades [*G. intestinalis* (Hooker f. & Harvey) Setchell *ex* Parkinson]. The recent synonymy of *Prionitis* J. Agardh with *Grateloupia* (Wang *et al*. 2001) highlights the need for a critical reassessment of this genus. Recent studies combining molecular and morphological analyses are starting to clarify the taxonomic status of some entities (Wang *et al*. 2000; Kawaguchi *et al*. 2001; Gavio 2002; Gavio & Fredericq 2002) but many taxa are still in need of a thorough revision.

On the basis of morphological and anatomical observations and comparative *rbc*L sequence analyses, we describe the new taxon as belonging to the genus *Grateloupia*.

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MATERIAL AND METHODS

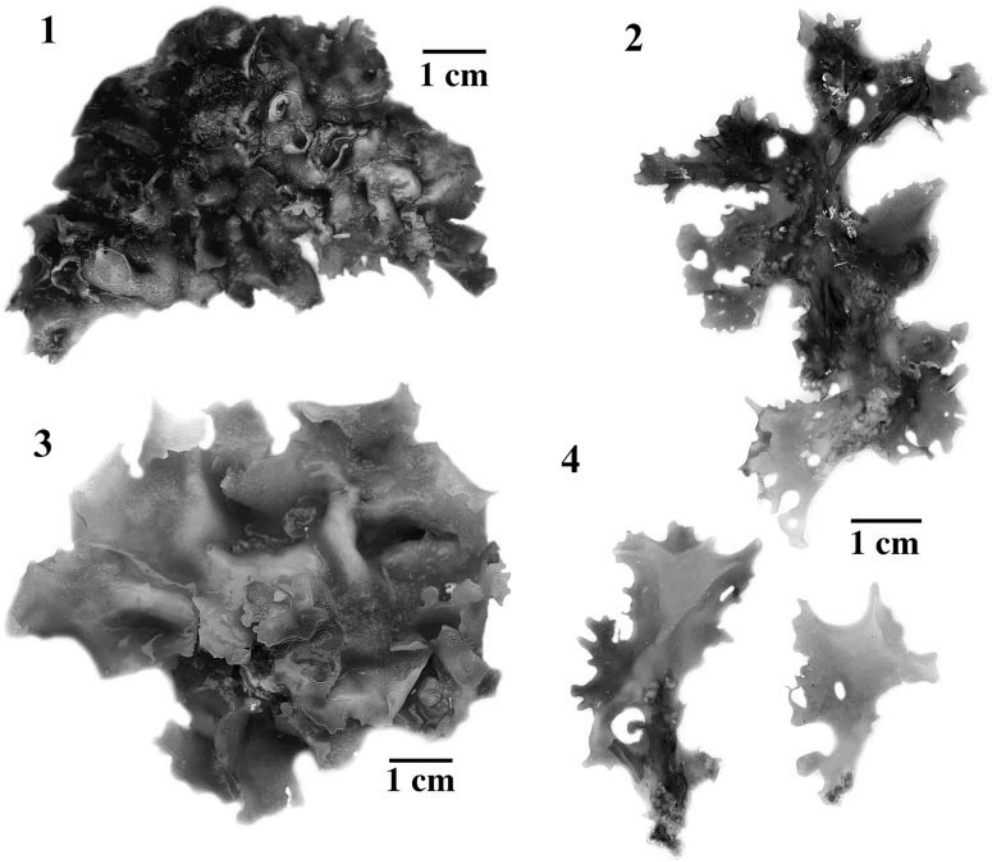
Morphological analysis

Tetrasporangial and cystocarpic specimens were collected from six localities in Oaxaca, tropical Pacific Mexico. Slides prepared for microscope observations were made from material preserved in 4–5% formalin–seawater. Whole-mount slides, cross-sections and longitudinal sections were made by hand with a razor blade, stained in 1% aniline blue (following the method of Papenfuss 1937) and permanently added on microscope slides in a mixture of 50% Karo Syrup–distilled water with 1% phenol added. Photographs of sections were taken on a BX60 photomicroscope (Olympus, Melville, NY, USA) with a DMC Ie digital camera (Polaroid, Cambridge, MA, USA). Habits of specimens were scanned using a Scanmaker III (Microtek, Redonda beach, CA, USA). Digital images were edited and assembled on plates using Photoshop 5.0 (Adobe, www.adobe.com).

Herbarium specimens are deposited in the herbarium at The National School of Biological Sciences Mexico City, Mexico (ENCB) and the University of Louisiana at Lafayette (LAF).

Molecular analysis

Algal samples for molecular analyses were desiccated in the field in silica gel. Chloroplast-encoded *rbc*L sequences were produced from 17 recently collected samples of Halymeniaceae, of which 15 are placed in *Grateloupia,* one in *Pachymenia* J. Agardh and one in *Aeodes* J. Agardh. The new taxon was also included in a global phylogenetic analyses of ~ 100

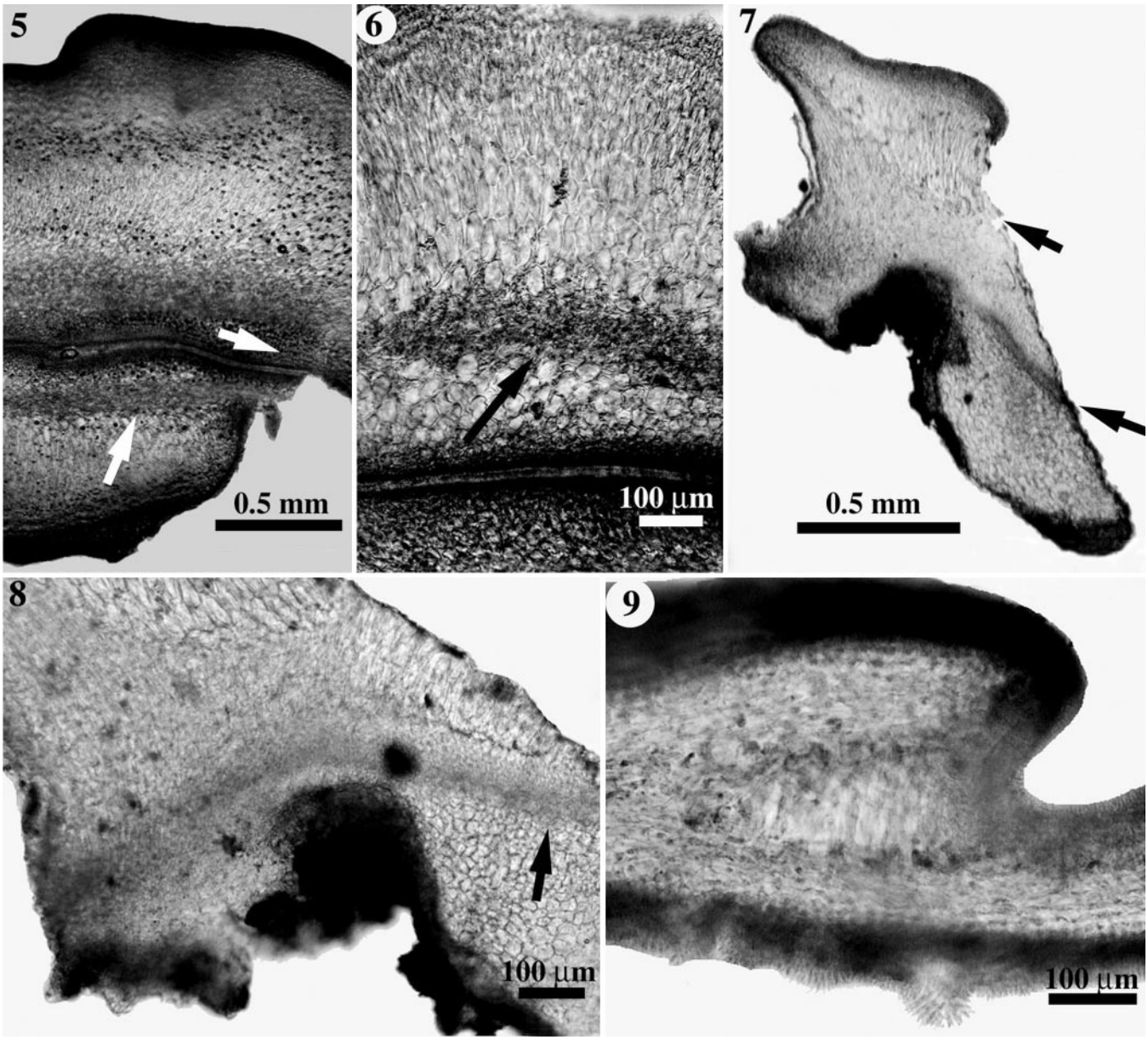


Figs 1–4. *Grateloupia huertana*. Habit.
Fig. 1. Holotype. Cystocarpic specimen, Masunte, Oaxaca, Mexico, ENCB 15807.
Fig. 2. Paratype, tetrasporophyte, Bahía Santa Cruz Huatulco, Oaxaca, Mexico, ENCB 15808.
Figs 3, 4. Paratype, cystocarpic specimen, Playa Agua Blanca, Oaxaca, collected by *C. Mendoza-Gonzáles & L.E. Mateo-Cid,* 27 February 1996, LAF.

taxa of Halymeniaceae including representative species of *Carpopeltis* Schmitz, *Halymenia* C. Agardh and *Polyopes* J. Agardh but proved to be unrelated to any of these genera (Gavio 2002). Collection information used in this study is listed in Table 1 and includes specimen locality, date and collector's name, percentage of *rbc*L sequenced, and Genbank accession numbers. Two species of *Cryptonemia* J. Agardh (Halymeniaceae) were used as the outgroup because in global *rbc*L analyses of the Halymeniaceae, the genus *Cryptonemia* was consistently the closest sister group to *Grateloupia sensu lato* (Gavio 2002).

Protocols for DNA extraction, gene amplification and cycle sequencing are as listed in Gavio & Fredericq (2002, 2003). Algal samples for molecular analyses were desiccated in the field in silica gel. Total genomic DNA was extracted using the DNeasy Plant Mini Kit (QIAGEN, Valencia, CA, USA) following the manufacturer's instructions or by grinding a small sample (4–6 mm^2) with liquid nitrogen, and placing it in 60 µl extraction buffer, prepared with 6 µl 10× polymerase chain reaction (PCR) buffer (Perkin Elmer, Foster City, CA, USA), 3 µl 10% polyvinyl polypyrrolidone, 0.6 µl proteinase K (at 100 µg ml^{-1}), 50.4 µl nanopure water. The samples were vortexed and placed in a waterbath at 65°C overnight; they were then heated at 95°C for 5 min, 140 µl nanopure water was added and then the sample was centrifuged at maximum speed for 10 min. The supernatant was transferred to a new tube and used immediately for *rbc*L amplification. Silica gel–dried specimens and extracted DNA samples are deposited in the Seaweed Laboratory at the University of Louisiana at Lafayette and stored at −20°C.

The gene selected was chloroplast-encoded *rbc*L; primers used for gene amplification include the following primer combination: F7-R753, F577-R1381, and F993-*Rrbc*Sstart (Gavio & Fredericq 2002, 2003). For *rbc*L amplification, 1–4 µl of the resulting extractions were used as templates for a 50 µl PCR consisting of 10 µl 5 M betaine, 6 µl 10× PCR buffer (Perkin Elmer), 6 µl 25 mM $MgCl_2$ solution, 8 µl of 500 mM deoxynucleoside triphosphate (dNTP) stock, 2 µl each of the appropriate 10 mM primers and 0.3 µl Amplitaq DNA Polymerase (PE Applied Biosystems, Foster City, CA, USA). Amplification conditions consisted of 4 min at 96°C for denaturation, followed by 35 cycles of 60 s at 94°C, 60 s at 42°C and 90 s at 72°C, with a final 10 min extension cycle at 72°C and soak cycle at 10°C. The PCR was performed on a GenAmp PCR system 9700 or 2400 (PE Applied Biosystems). For automated gene sequencing, the amplified products were cleaned using the Prep-A-Gene DNA Purification Kit (BioRad, Hercules, CA, USA) following the manufacturer's recommendation. The concentration of the template was then estimated using either a TKO 100 fluorometer (Hoefer Scientific Instru-



Figs 5–9. *Grateloupia huertana*. Vegetative structure.
Figs 5, 6. Longitudinal section through overlapping blades with cuticle among blades remaining distinct. A core of small-sized subcortical cells (arrows) is present on lowermost part of blade.
Figs 7, 8. Longitudinal section through lower part of blade portion showing two distinct boundaries (arrows); the region above the top arrow (Fig. 7) is surmounted by dedifferentiated cell derivatives that have become meristematic.
Fig. 9. Longitudinal section showing repair of perforation by production of short-celled secondary filaments.

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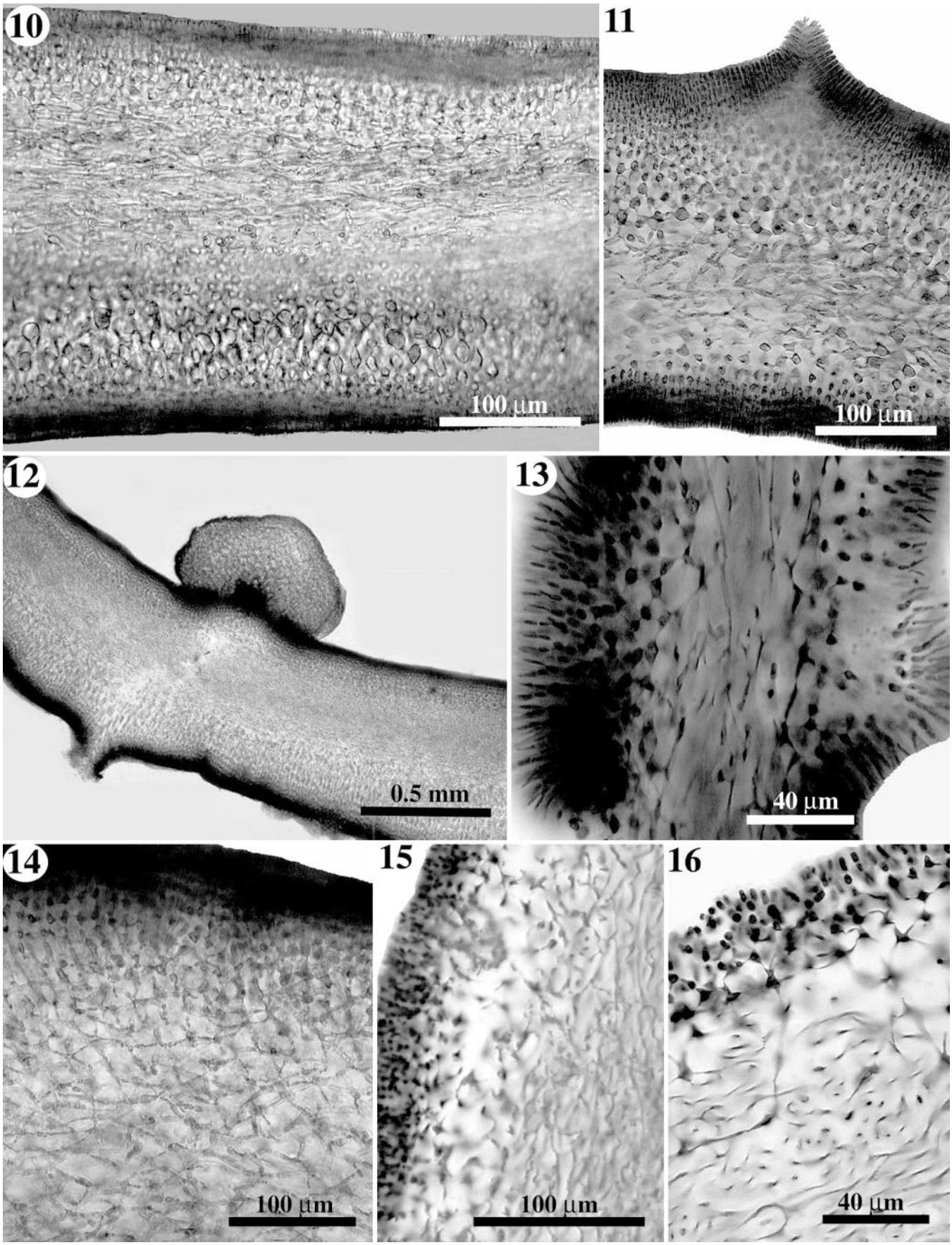
Figs 10–16. *Grateloupia huertana*. Vegetative structure.
Figs 10–13. Longitudinal section through blades showing varying degrees of compactness of predominantly periclinal medullary filaments and darkly staining subcortex.
Fig. 10. Longitudinal section showing compact cortex and medulla.
Fig. 11. Blade protuberance.
Fig. 12. Protuberance and new bladelet.
Fig. 13. Longitudinal section through marginal pinnule showing loose arrangement of filiform medullary filaments and cortex.
Figs 14, 15. Longitudinal section through young blade showing regular medullary network with varying degrees of intercellular spaces.
Fig. 16. Transverse section through young blade showing elongate secondary cells.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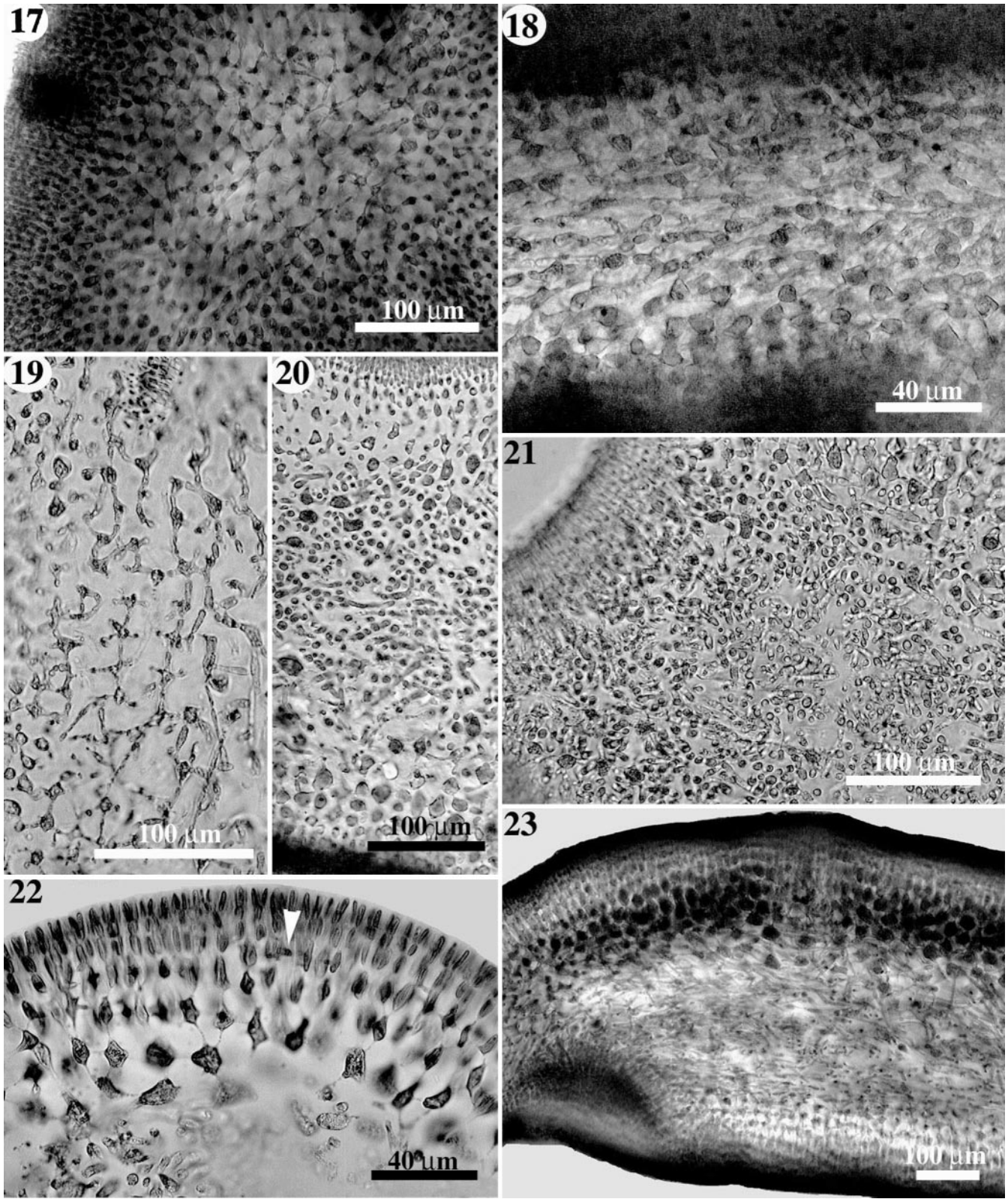


Figs 17–23. *Grateloupia huertana*. Vegetative structure.
Fig. 17. Longitudinal section; one or two segments below surface in actively growing region, intercalary cortical cells cut off conjunctor cells forming secondary pit connections with neighbouring cells resulting in a primary network.
Fig. 18. Longitudinal section, just below area shown in Fig. 17; subcortical cells resemble medullary cells in shape.
Fig. 19. Longitudinal section; young medullary network with large intercellular spaces.
Figs 20, 21. Transverse section through compact medulla and cortex of young blades.
Fig. 22. Transverse section through cortex of mature blade. Note abundant formation of conjunctor cells (arrowhead).
Fig. 23. Longitudinal section through mature blade.

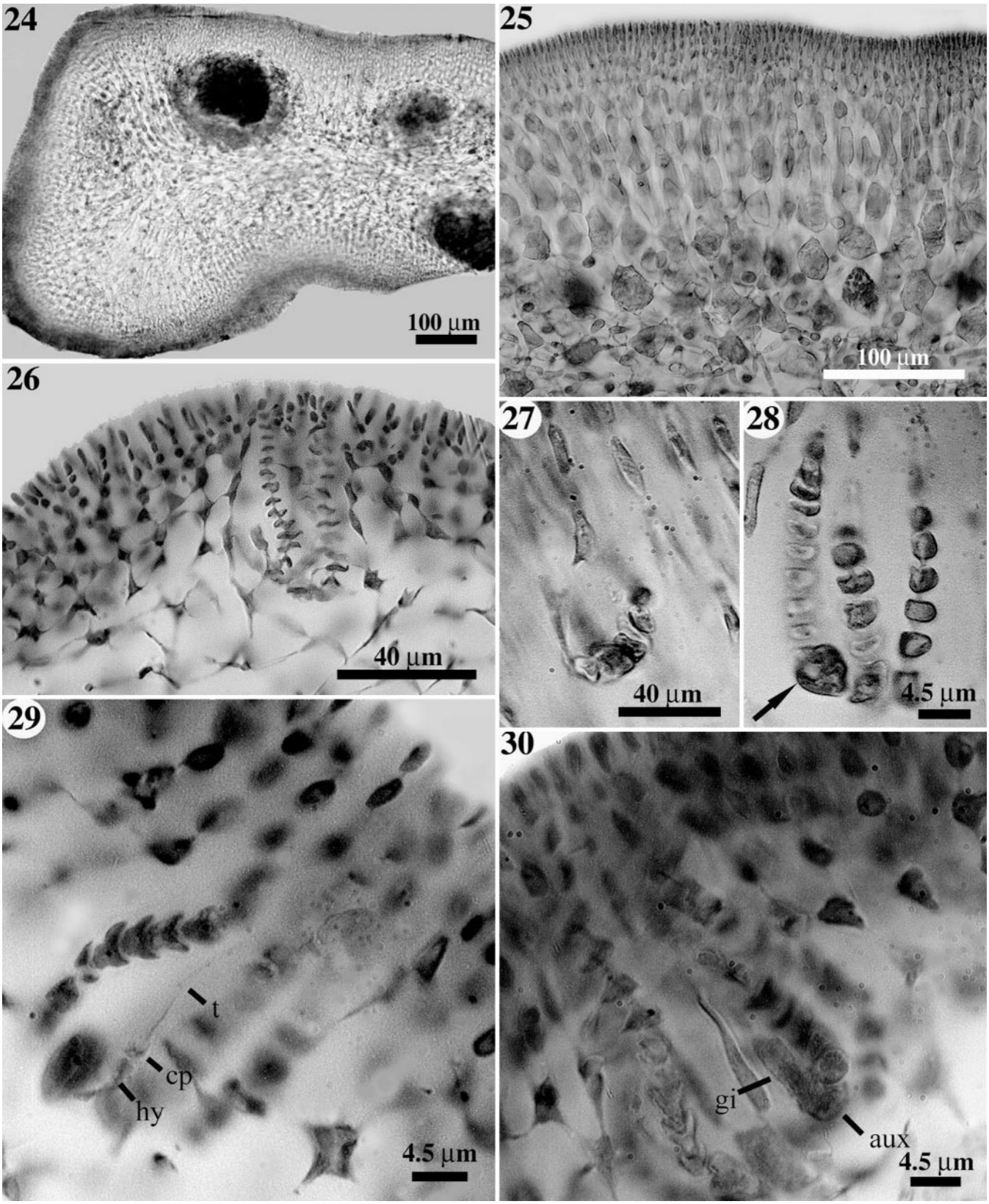
Table 1. List of species used in *rbc*L analysis with GenBank accession numbers.

Species	Location	Collector, collection date	*rbc*L portion sequenced	GenBank accession number
Aeodes nitidissima J. Agardh	Hangliton Bay, North Island, New Zealand	W. Nelson, 24 Mar. 1993	41-947, 976-1467 (95.36%)	AF385637
Cryptonemia bengryi Taylor	Long Bay Point, Isla Colon, Caribbean Panama	B. Wysor, 19 Oct. 1999	614-1467 (58.21%)	AY178766
Cryptonemia luxurians (C. Agardh) J. Agardh	Praia Rasa, Rio de Janeiro, Brazil	C.F. Gurgel, 12 Dec. 1998	52-1467 (96.52%)	AF488813
Grateloupia acuminata Holmes	Katase, Fujisawa, Kanagawa Prefecture, Japan	s.n.	107-1365 (85.82%)	AB055480[1] (Kawaguchi *et al*. 2001)
Grateloupia americana Kawaguchi & Wang	Whan Park, near Sitka (Baranof Island), Alaska, USA	S. Lindstrom, 21 Apr. 2000	38-1467 (97.37%)	AF488814
Grateloupia angusta (Okamura) Kawaguchi & Wang	Schichirigahama, Kamakura, Kanawaga Prefecture, Japan	s.n.	107-1365 (85.82%)	AB061378[1] (Wang *et al*. 2001)
Grateloupia asiatica Kawaguchi & Wang	Qingdao, Shadong Province, China	S. Fredericq, 29 Jun. 1994	8-1467 (99.52%)	AY178763
Grateloupia asiatica Kawaguchi & Wang	Qingdao, Shadong Province, China	S. Fredericq, 29 Jun. 1994	9-1467 (99.45%)	AY178762
Grateloupia dichotoma J. Agardh	Marataizes, Espiritu Santu, Brazil	S. Guimarães & M. Fujii, 15 Sep. 2001	9-1467 (99.45%)	AF488823
Grateloupia divaricata Okamura	Oshoro, Hokkaido, Japan	S. Fredericq, 5 Sep. 1995	40-1467 (97.34%)	AY178764
Grateloupia doryphora (Montagne) Howe	Playa de San Francisco, Bahia de Ancon, Ancon, Lima, Peru	P. Carbájal, 15 Sep. 2001	9-1467 (99.45%)	AF488817
Grateloupia filiformis Kützing	Marataizes, Espiritu Santu, Brazil	S. Guimarães & M. Fujii, 15 Sep. 2001	11-1467 (99.31%)	AF488822
Grateloupia huertana sp. nov.	Playa Agua Blanca, Oaxaca, Mexico	C. Mendoza-Gonzáles, 27 Feb. 1996	9-1467 (99.45%)	AY178761
Grateloupia huertana sp. nov.	Puerto Angel, Oaxaca, Mexico	C. Mendoza-Gonzáles, 27 Feb. 1996	70-1467 (95.29%)	AY178760
Grateloupia livida (Harvey) Yamada	Muroran, Hokkaido, Japan	S. Fredericq, 6 Sep. 1993	40-1467 (97.34%)	AF488815
Grateloupia longifolia Kylin	Kommetjie, Cape Peninsula, South Africa	M.H. Hommersand, 9 Nov. 1999	9-1467 (99.45%)	AY178765
Grateloupia ramosissima Okamura	Ho Ping Island, Keelung, North Taiwan	S. Fredericq & S.M. Lin, 12 Aug. 1993	40-1467 (97.34%)	AF488810
Pachymenia carnosa (J. Agardh) J. Agardh	Kommetjie, Cape Peninsula, South Africa	O. De Clerck, 9 Nov. 1999	41-1467 (97.27%)	AF385640

[1] Sequence downloaded from GenBank.

ment, San Francisco, CA, USA), following the manufacturer's instructions, or by running 1 μl of the template on a 1% agarose minigel and comparing the brightness of the band with known DNA concentrations. The sequences were determined over both strands using an ABI Prism 310 or 3100 Genetic Analyzer (PE Applied Biosystems) with the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems). The same primers used for gene amplification were used for sequencing. The reactions were performed in 20 or 10 μl, and comprised the following: 2 μl Terminator Ready reaction mix, 2 μl X buffer, 1.6 μl 1 mM primer, 18–20 ng of template and nanopure water up to a total volume of 10 μl; for the 20 μl reactions, all the volumes were doubled. The cycle sequencing reactions were performed on a GenAmp PCR system 9700 or 2400 (PE Applied Biosystems) for 28 cycles (96°C for 10 s, rapid thermal ramp to 50°C, 50°C for 5 s, rapid thermal ramp to 60°C, 60°C for 4 min, rapid thermal ramp to 10°C). Resulting products were then purified using Centri-Sep spin columns (CS-901; Princeton Separations, Adelphie, NJ, USA). For most samples, the entire *rbc*L-coding region, 1467 bp, was newly sequenced except for the first 8–10 bp (Table 1).

The generated sequence data were compiled and aligned with Sequencher (Gene Codes, Ann Arbor, MI, USA) and exported for phylogenetic analysis in PAUP (v. 4.0b10, Swofford 2002) and MacClade (Maddison & Maddison 2000). Phylogenetic analyses were performed using the Maximum Parsimony (MP), Neighbour Joining (NJ) and Maximum Likelihood (ML) algorithms available in PAUP. For ML the aligned sequences were first analysed with the software Modeltest v. 3.0 (Posada & Crandall 1998) which compared different models of DNA substitutions in a hierarchical hypothesis-testing framework to select a base substitution model that best fits the sequence data. The optimal model found was a GTR + I + G evolutionary model (General Time Reversible model + Invariable sites + Gamma distribution). The param-



Figs 24–30. *Grateloupia huertana*. Female reproduction.
Fig. 24. Longitudinal section showing cystocarps immersed in medulla and surrounded by vegetative envelope.
Fig. 25. Transverse section in vicinity of developing cystocarps showing secondary production of vegetative cells in the centre of the branch bearing the cystocarps.
Figs 26–30. Transverse section through ampullae.

eters were as follows: assumed nucleotide frequencies A = 0.3152; C = 0.1263; G = 0.1966; T = 0.3620; substitution rate matrix with A–C substitutions = 1.0093, A–G = 6.8815, A–T = 2.5136, C–G = 1.5167, C–T = 18.4103, G–T = 1.0000; proportion of sites assumed to be invariable = 0.5256; rates for variable sites assumed to follow a gamma distribution with shape parameter = 1.1625. These values were imported into a ML analysis using heuristic search (PAUP).

Support for nodes was determined by calculating bootstrap proportion values (Felsenstein 1985) using NJ (5000 bootstrap resamplings), MP (5000 bootstrap resamplings) and ML (100 bootstrap resamplings) methods.

RESULTS

Grateloupia huertana Mateo-Cid, Mendoza-González & Gavio, *sp. nov.*

Figs 1–42

Plantae prostratae, usque ad ~ 5 cm, cartilagineae et lubricae, lobis 1.5–2.0 cm latis. Laminae compressae; ramificatio simplex aut irregularis, pinnulis lateralis frequentis. Pagina ventralis laminae ad substratum per discos basales parvos affixa. Thallus bistratus: internum stratum ex filis intricatis cellularum medullosarum elongatarum 12.5–15 μm diametro constans, filis cellulis irregularibus stellatis circumcinctis; externum stratum ex 4–7 seriebus cellularum obovatarum 6–7 μm × 17–3.1 μm constans. Inter medullam et corticem stratum ex cellulis irregularibus aut stellatis 18–37 × 31–69 μm compositum. Tetrasporangia cruciate divisa 25–38 × 6.2–9.8 μm, apud cellulas corticales dispersa. Spermatangia non visa. Carpogonia et cellulae auxiliares in separatis ampullis. Ramus carpogonialis bicellularis. Ampullae cellularum auxiliarium copiosae, 3–4 (5) filis ampullariis instructae. Cystocarpia matura 150–450 μm diametro ostiolata, carposporis 18–25 × 31–56 μm.

HOLOTYPE (designated here): Fig. 1. Masunte, Oaxaca, 15°30′50″N, 96°32′59″W, Mexico, *Mateo-Cid & Mendoza-González,* 13 May 1999, ENCB-15807, cystocarpic.

PARATYPES: Fig. 2. Bahia Santa Cruz Huatulco, Oaxaca, 15°44′12″N, 96°08′39″W, *Mateo-Cid & Mendoza-González,* 15 May 1999, ENCB-15808. Tetrasporophyte. Fig. 3. Playa Agua Blanca, Oaxaca, 15°43′58″N, 96°48′50″W, *Mendoza-González & Mateo-Cid,* 27 February 1996, ENCB-5809, cystocarpic.

DISTRIBUTION: Restricted to the State of Oaxaca, tropical Pacific Mexico.

ADDITIONAL SPECIMENS STUDIED: Bahia el Maguey, Oaxaca, 15°43′53″N, 96°08′58″W, *Mendoza-González & Mateo-Cid,* 14 May 1999, ENCB-15810, tetrasporophyte; Bahia San Agustin, Oaxaca, 15°41′02″N, 96°14′17″W, *Mateo-Cid & Mendoza-González,* 26 September 1992 and 3 May 1996, ENCB-15 811, ENCB-15 812, tetrasporophyte; Puerto Angel, Oaxaca, 15°39′46″N, 96°29′41″W; *Mateo-Cid & Mendoza-González,* 8 December 1994, vegetative, 26 February 1996, ENCB-15 813, female, and 7 May 1997, ENCB-15814, female, ENCB-15 815, tetrasporophyte; Puerto Angelito, Oaxaca, 15°51′25″N, 97°04′20″W, *Mendoza-González & Mateo-Cid,* 9 May 1997, ENCB-15816, tetrasporophyte.

HABITAT: Intertidal to upper sublittoral, epilithic on rocks and attached to dead coral, 0.5–3.0 m depth.

ETYMOLOGY: The species epithet is named in honour of Prof. Laura Huerta Muzquiz, a pioneer in floristic studies of the seaweeds from Mexico.

HABIT: The thallus is greenish to red-purple, lacking a stipe, entirely prostrate (Figs 1–5), fleshy-cartilaginous and slippery in texture. Older thalli vary in thickness and are of indefinite outline, consisting of overlapping blades, 1.5–2 cm broad, forming rosette-like clumps ~ 5.0 cm in diameter (Figs 1, 3). The entire ventral blade surface acts as a single expanded holdfast conforming in shape to the topography of the substratum to which it attaches by means of small basal discs. Single juvenile blades comprise irregularly spreading lobes that become perforated, 0.5(1) cm broad and 3(5) cm long (Figs 2, 4). The margins are generally thinner and may produce a few to numerous pinnules or rounded proliferations (Figs 2, 4). Cystocarps are scattered and restricted to the dorsal surfaces (Fig. 1). Thalli become cartilaginous upon drying.

VEGETATIVE ANATOMY: A longitudinal section through overlapping blades reveals tightly appressed blades consisting of a compact cellular medulla and cortex, with the cuticle between the overlapping blades remaining distinct (Figs 5, 6). Surface cells have typically ceased dividing when cortical and medullary cells are thick-walled and mostly devoid of cytoplasm (Figs 5, 6). The outer cortex is composed of four to seven rows of obovate cells, 6–7 μm × 17–31 μm. A core of small-sized, rounded subcortical cells (Figs 5–8, arrows) remains present on the lowermost part of each blade. This boundary represents the former outer cortex of a juvenile blade that has dedifferentiated and resumed growth, resulting distally in medullary cells that have elongated vertically and become inflated. This zone of tightly appressed medullary cells lacking intercellular spaces may be surmounted by a second boundary comprising locally dedifferentiated surface cells and their derivatives that have radiated and likewise become inflated and tightly appressed to one another (Fig. 5). The distalmost surface layer may cease growth altogether (Fig. 5) or become meristematic (Fig. 7). In thalli in which blades do not overlap, the two boundaries are readily recognizable (Fig. 7, arrows).

The outline of the ventral-most region is irregular and adapted to the contour of the substratum (Fig. 8). Perforations within a blade are common (Figs 2, 4) and seemingly the result of obstacles in substratum topography. These perforations are usually repaired by the formation of short secondary filaments produced by subcortical cells, filling the spaces (Fig. 9).

The width of the cortex may vary depending on the rate of thallus expansion laterally. In mature blades the cortex usually consists of an inner zone characterized by rounded darkly staining cells, and an outer region (Fig. 10). Medullary filaments tend to be tightly packed and predominantly periclinal, but pronounced anticlinal filaments bridging the cortical regions on both sides are absent.

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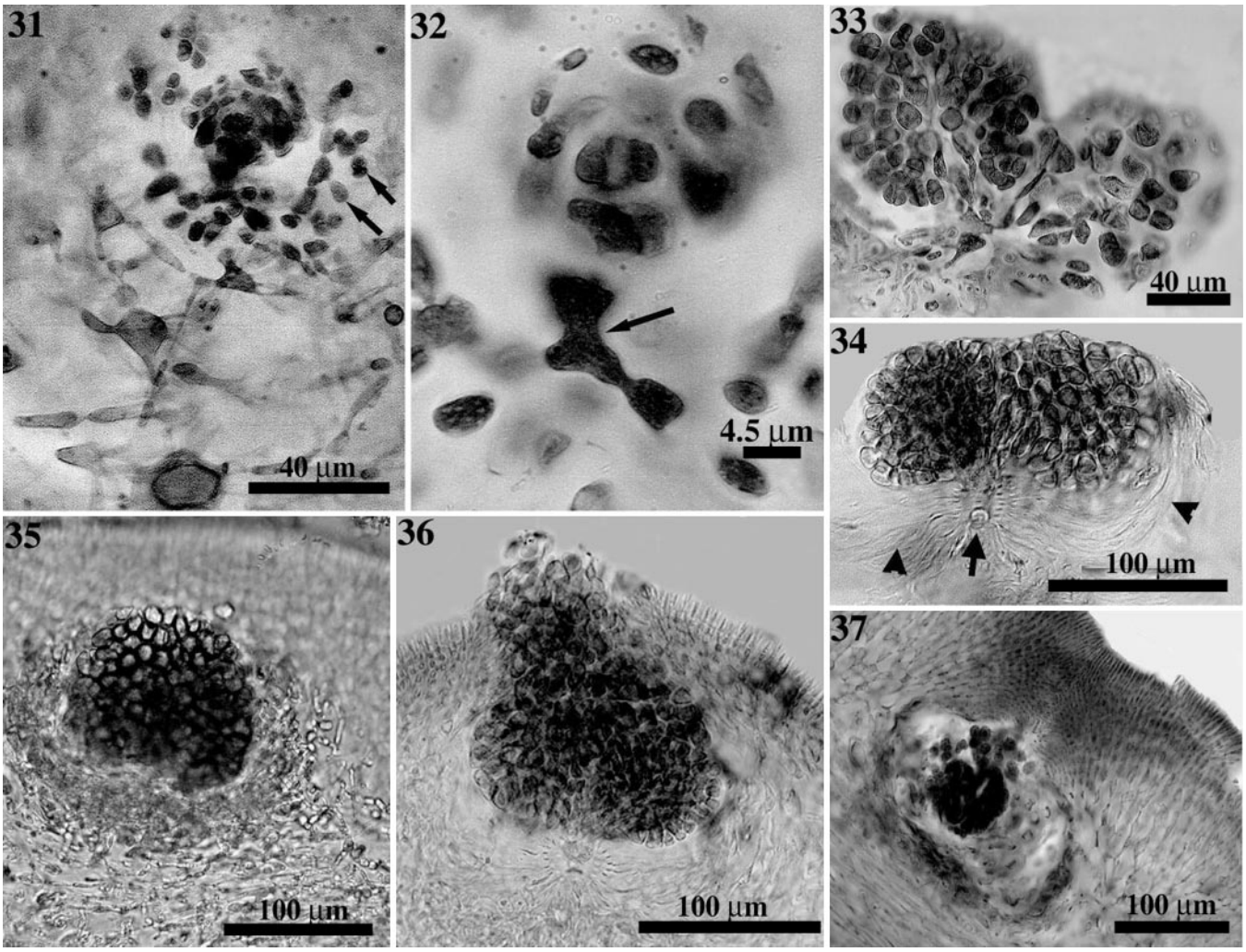
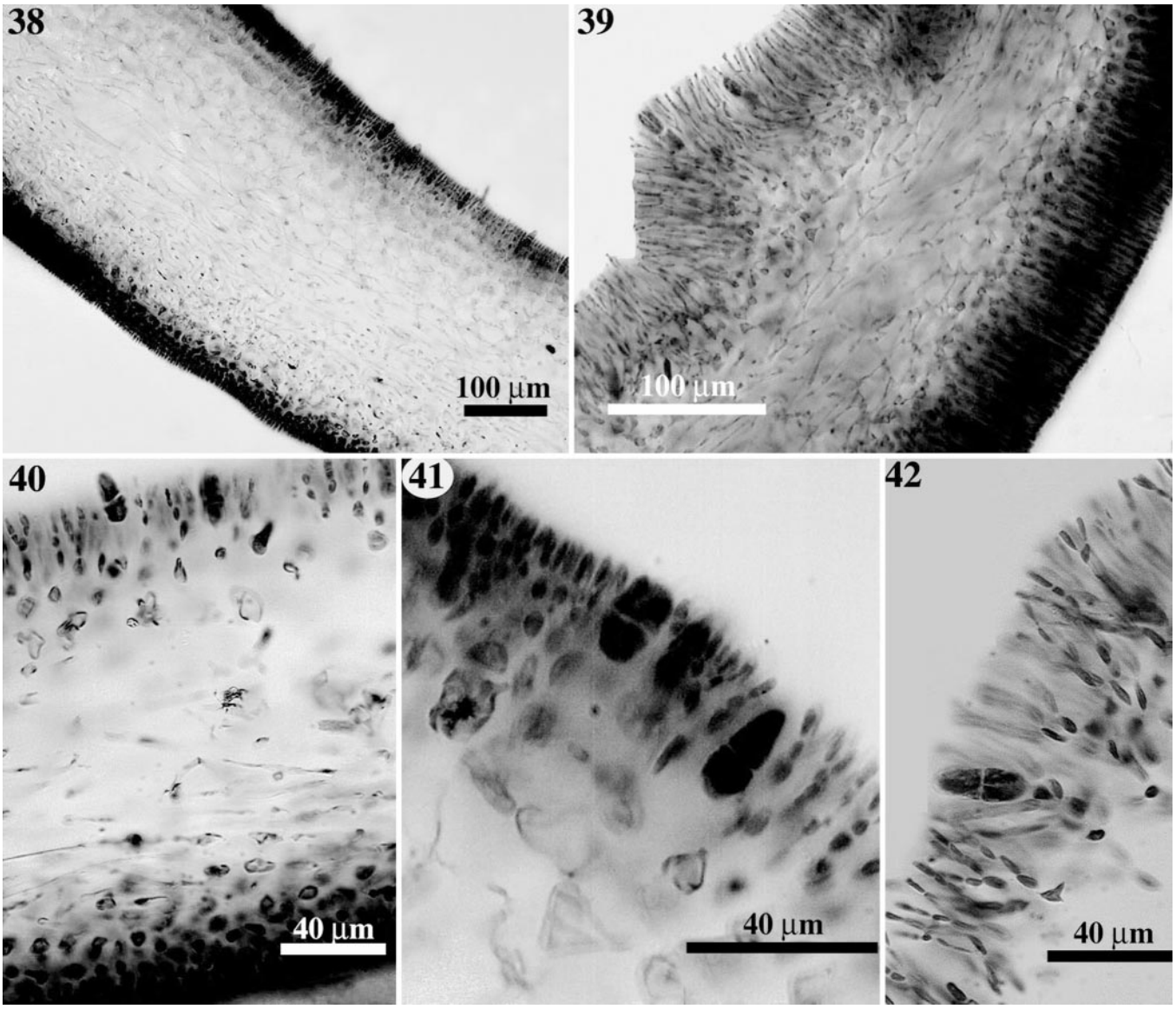
Fig. 26. Ampullary filaments composed of sagittate cells.
Fig. 27. Initiation of ampulla.
Fig. 28. Ampulla with auxiliary cell (arrow).
Fig. 29. Ampulla with two-celled carpogonial branch consisting of a basal hypogenous cell (hy) and terminal carpogonium (cp) with straight trichogyne (t).
Fig. 30. Elongate gonimoblast initial (gi) cut off from auxiliary cell (aux).



Figs 31–37. *Grateloupia huertana*. Female reproduction.
Fig. 31. Carposporophyte with young gonimoblasts and primary ampullary filaments bearing short lateral initials (arrows). Note the production of irregular secondary vegetative cells below the carposporophyte.
Fig. 32. Fusion cell product (arrow) between auxiliary cell and basalmost contiguous ampullary cells.
Fig. 33. Gonimoblasts consisting of a pair of gonimolobes.
Fig. 34. Mature cystocarp showing broadened pit connection (arrow) between auxiliary cell and gonimoblast initials and flattened remnants of ampullary filaments (arrowheads).
Fig. 35. Maturing cystocarp surrounded by well-developed filamentous envelope.
Fig. 36. Mature cystocarp with remnants of filamentous envelope.
Fig. 37. Mature cystocarp with overlying pericarp.

In actively growing blade regions, the subcortex is less darkly staining (Fig. 11) than in mature thalli, and intercellular spaces among cortical and medullary cells are more prevalent. Surface cells may divide outwardly forming pinnules or hemispherical outgrowths (Fig. 12), which may contact the substratum or overhanging blade portions. Medullary filaments in pinnules are filiform and loosely periclinal, and the outer cortical cells are ovoid and separated from one another (Fig. 13). The young cortex consists of four to five rows of pigmented cells resulting from transverse division followed by longitudinal concavo-convex division of surface cells, which become arranged in tightly packed anticlinal filaments when growth ceases (Fig. 14). In longitudinal sections through young blades, the initial medulla comprises a regular network of filiform cells, 12.5–15 µm in diameter, separated by large intercellular spaces, and becomes more compact (Figs 15, 16) as the thallus matures. Eventually the cortex becomes constant in thickness and the medullary filaments cease elongation.

At one or two segments below the surface in actively growing regions, intercalary cortical cells cut off conjunctor cells that, upon fusion, form secondary pit connections with neighbouring cells, resulting in a primary network (Fig. 17). Just below this outer cortical zone, the shape of subcortical cells resembles those of medullary cells (Fig. 18). As the thallus expands laterally, the medullary filaments comprising the primary network stretch, becoming narrow (Fig. 19) when viewed in a longitudinal section, and the boundary between cortex and medulla becomes evident (Fig. 20). In transverse sections, the narrow filiform medullary filaments are shown as small, rounded cells (Figs 20, 21). Subsequently, the cor-



Figs 38–42. *Grateloupia huertana*. Tetrasporophyte.
Fig. 38. Tangential section through tetrasporophytic blade with tetrasporangia restricted to dorsal side.
Figs 39–42. Longitudinal sections showing a loosely arranged outer cortex bearing tetrasporangia in various stages of development. Each tetrasporangium is a transformed surface cell.

tical cells elongate distally (Figs 21, 22), and numerous conjunctor cells continue to be produced by intercalary cortical cells (Fig. 22, arrowhead) forming secondary pit connections. Elongate secondary medullary cells are also produced in abundance, and most grow obliquely (Figs 20, 21) among the periclinal filaments with which they connect by means of terminal conjunctor cells. The number of cortical cell files may vary within a blade and be more numerous on the dorsal than the ventral side (Figs 20, 21, 23). Inner cortical cells measure 18–37.5 μm × 31–69 μm and are more irregular in shape than outer cortical cells (Figs 20, 23). Mature individual blades are typically 0.70–4.0 mm thick in the deeper portions, but only 250–400 μm thick at the margins. The mature medulla, 80–900 μm thick, consists of a compact zone of intertwined, periclinal, filiform filaments surrounded by a subcortex of refringent cells and a tightly packed, small-sized outer cortex (Figs 10, 23).

REPRODUCTIVE ANATOMY: Cystocarps are globose and immersed within the thallus (Fig. 24). Subcortical cells in the vicinity of developing cystocarps typically cut off elongated and rounded cells and conjunctor cells (Fig. 25) that become incorporated in a localized network. The cystocarps are surrounded by an envelope of secondarily formed vegetative filaments (Fig. 24). These vegetative filaments include the remnants of ampullary filaments (Fig. 26). Ampullary filaments are produced from an intercalary subcortical cell that cuts off derivatives bilaterally and anticlinally (Fig. 27) to produce three to four (rarely five) unbranched filaments that grow straight towards the surface (Fig. 26). Each ampulla bears a

single auxiliary cell or a carpogonium. A basal intercalary ampullary cell cuts off a single cell that enlarges and becomes transformed into an auxiliary cell (Fig. 28). A carpogonial ampulla (Fig. 29) bears a two-celled carpogonial branch consisting of a hypogynous cell and a terminal carpogonium with an elongated straight trichogyne (Fig. 29), cut off laterally from an intercalary ampullary cell. Auxiliary cell ampullae are more abundant than carpogonial ampullae. Fertilization and connecting filaments were not observed.

Primary filaments of an auxiliary cell ampulla (Fig. 28) consist of up to 10 ellipsoidal (Fig. 28) or sagittate (Fig. 26) cells. Auxiliary cells are roundish, and each cuts off a single oblong gonimoblast initial towards the surface (Fig. 30). During early stages of gonimoblast development, a few cells of the primary filaments may give rise to rounded initials laterally (Fig. 31, arrows) which continue growing as secondary ampullary filaments and roughly converge towards a point below the thallus surface. Tertiary filaments were not observed. Because the pit connection between the auxiliary cell and its generative ampullary cell becomes dislodged, a fusion cell product results and the fusion process extends among the basalmost contiguous ampullary cells (Fig. 32). The gonimoblast initial divides longitudinally to form a pair of gonimolobes, which continue to produce the same-sized gonimoblasts (Fig. 33). All gonimoblast cells transform into carposporangia. As the gonimoblasts mature, the pit connection between the auxiliary cell and the gonimoblast initial broadens and becomes refringent. The cytoplasm in the ampullary cells is consumed during gonimoblast maturation, with the result that the cells flatten and become compressed (Figs 34, 36). Mature cystocarps measure 150–450 μm in diameter (Figs 34–37) and may be surrounded by a rather thick (Figs 24, 35) or barely developed (Figs 36, 37) filamentous envelope. Carpospores (18–25 μm × 31–56 μm) are released through a central ostiole in the overlying pericarp (Fig. 37).

Mature tetrasporophytes bear cruciate tetrasporangia, 25–38 μm long by 6.2–9.8 μm in diameter (Figs 38–42), restricted to the dorsal surface of the blade and embedded in the outer cortex. Typically, few secondary pit connections are formed between cortical cells in the vicinity of tetrasporangia. Cells in such cortical filaments remain cylindrical (Figs 39–42). Each tetrasporangial initial is a transformed surface cell resulting from a concavo-convex longitudinal division and it becomes slightly buried as neighbouring cells continue to grow the thallus outward. Male plants were not observed.

Molecular analysis

The NJ, MP and ML analyses were performed, with only the ML-based tree shown here (Fig. 43). The difference in topology between the three analyses lies in the unsupported position of *G. doryphora* from Peru.

Grateloupia huertana falls within the *Grateloupia* clade, a clade that has strong bootstrap support (87–94%). The two samples of the new species cluster strongly together, but their position within the *Grateloupia* clade is equivocal with low bootstrap support varying from < 50% to 60%. As in other species of *Grateloupia,* the intraspecific *rbc*L divergence in the two vouchers of *G. huertana* is low based on uncorrected pairwise distances as calculated in PAUP (data not shown), with the two sequences differing by 8 bp, corresponding to 0.57% sequence divergence (1397 total bp/8 = 0.57%). All the nucleotide substitutions are synonymous.

DISCUSSION

The formation of a generative auxiliary cell within a specialized ampullary branch system in our new species clearly indicates that it is a member of the Halymeniaceae. The new species resembles and may be closely related to *Pachymenia saxicola* Taylor (Taylor 1945, pl. 65, figs 1, 2) described from Isla Santa María, the southeasternmost island of the Galapagos Archipelago. Examination of the sterile type specimen of *P. saxicola* reveals a red sessile blade bearing many marginal proliferations and a distinct single holdfast. In contrast, our new species does not form a recognizable holdfast because the entire ventral side acts as one attachment zone. Furthermore, the southern Galapagos island species is affected by the cold Humboldt current, whereas *G. huertana* thrives in the warm waters of the tropical Mexican Pacific averaging 26°C annually (30°C in August) that is affected by the Costa Rican and North Equatorial currents. The distant position in the phylogenetic tree of *G. huertana* from *P. carnosa* and *Aeodes nitidissima,* two taxa having *Aeodes*-type ampullae (Chiang 1970), clearly indicates that the new species is not a member of the *Pachymenia* assemblage.

Chiang (1970) considered the comparative development and structure of the auxiliary cell ampulla to be a good character for separating genera within the Halymeniaceae. In his system of classification, simple ampullae, with a single primary ampullary filament and few secondary filaments are characteristic of the *Grateloupia*-type. Our taxon possesses typical *Grateloupia*-type ampullae. The new alga is unique among species of *Grateloupia* in its completely prostrate, compressed, cartilaginous and slippery habit, and in its unusually complex vegetative structure. The pinnate to foliose habit of many *Grateloupia* species and their lax thallus construction distinguishes our new species from other members of the genus.

According to Chiang (1970), four genera have *Grateloupia*-type ampullae: *Grateloupia, Prionitis, Pachymeniopsis* Yamada *ex* Kawabata and *Phyllymenia* J. Agardh. Both *Pachymeniopsis* and *Prionitis* have recently been merged in the genus *Grateloupia* (Kawaguchi 1997; Wang *et al*. 2001). Kraft (1977) distinguished *Phyllymenia* from its allied genus *Grateloupia* by the relatively deep cortex and the more developed medulla, but *rbc*L sequence data indicate that the monospecific *Phyllymenia* should follow the same fate as *Prionitis* and *Pachymeniopsis* (B. Gavio, unpublished data). The genus *Carpopeltis* was not treated in depth by Chiang (1970), and therefore its systematic position has remained uncertain. Womersley & Lewis (1994) analysed the Australian species of *Carpopeltis* (including the type species *C. phyllophora* Schmitz) and concluded that the ampullae in these specimens are simple, branching only up to the second order, and therefore fall in the *Grateloupia*-type class. In *Carpopeltis,* tetrasporangia are confined to nemathecia near the branch ends, whereas in *G. huertana* they are scattered on the dorsal portion of the thallus. Although our new species superficially resembles a species of *Carpopeltis* due to its cartilaginous thallus (see Womersley & Lewis 1994), the typical thin medulla in mature

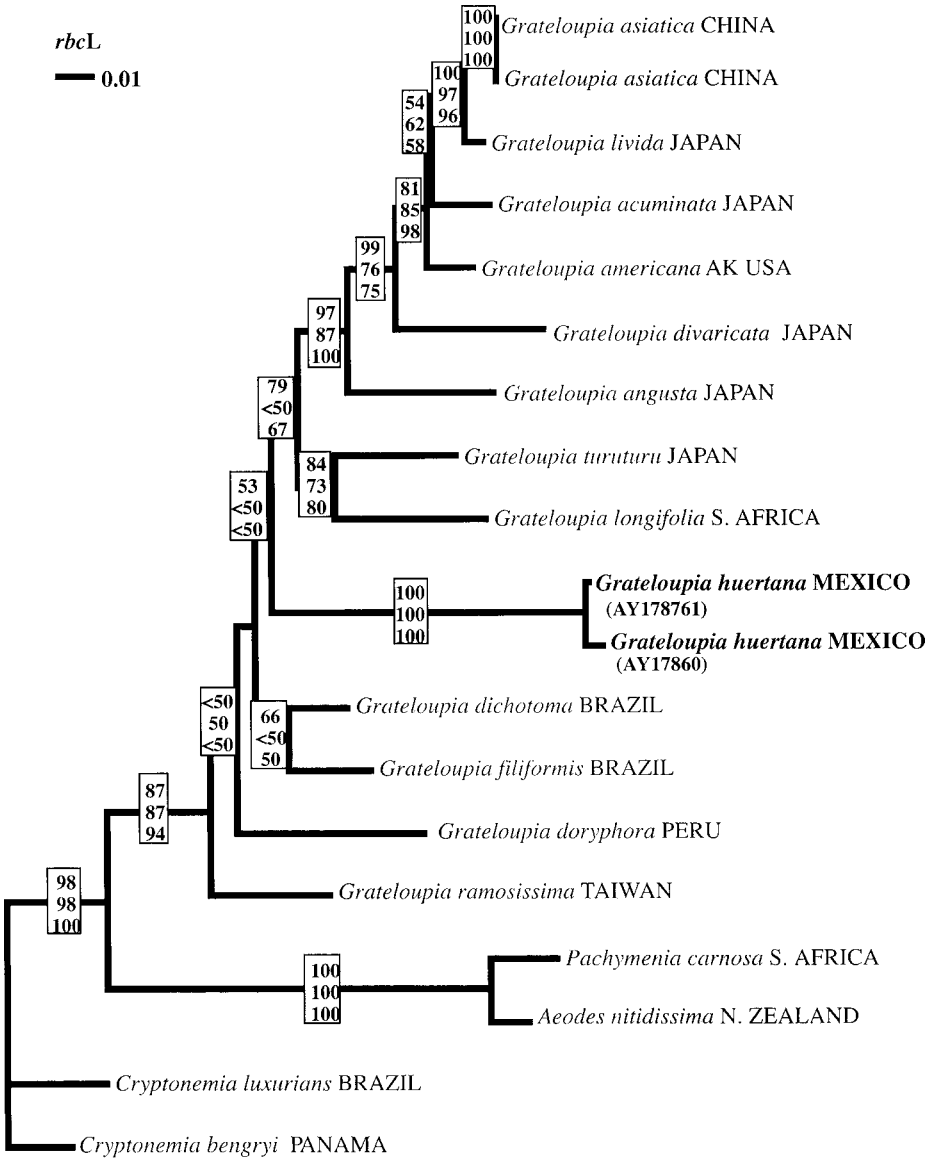


Fig. 43. Maximum likelihood tree for *rbc*L sequences of Halymeniaceae inferred from the GTR + I + G distribution using *Cryptonemia* spp. as the outgroup. Bootstrap proportion values (> 50%) for MP (top, 5000 replicates), NJ (middle, 5000 replicates) and ML (bottom, 100 replicates) are shown at the nodes. Branch lengths are proportional to the amount of sequence change.

specimens of *Carpopeltis,* coupled with the distant position between *C. maillardii* (Montagne & Millardet) Chiang from Taiwan and our new species in *rbc*L trees (Gavio 2002) precludes the latter from being a member of *Carpopeltis*.

Our molecular data support the placement of this taxon within the genus *Grateloupia,* although the closest sister relationship is equivocal (low bootstrap support). Intraspecific *rbc*L variation is very low in *Grateloupia* when compared to other taxa (McIvor *et al*. 2001), whereas interspecific variation can be very high (up to 12%) (Gavio 2002; Gavio & Fredericq 2002).

The auxiliary cell ampullary arrangement of *G. huertana* is almost identical to that illustrated by Kylin (1930, fig. 10d) for *G. filicina,* and it is very similar to that illustrated for other genera with '*Grateloupia*-type' ampullae (Chiang 1970; Kawaguchi 1989). In his taxonomic treatment of the problematic *G. intestinalis,* Kraft (1977) downgraded the importance of the ampullary structure as the key character to separate genera in the Halymeniaceae. In contrast, we agree with Chiang's proposition of the ampullary structure as an important taxonomic character in the family, and the results of this and yet-to-be published studies support his proposition. We therefore place this enigmatic species in the genus *Grateloupia* rather than creating a novel genus for it.

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