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Hassallia littoralis sp. nov. (Cyanobacteria, Microchaetaceae) from Mexico's marine supralittoral based on morphological and molecular evidence

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Abstract

A new species of *Hassallia* (Cyanobacteria, Nostocales, Microchaetaceae) from a supralittoral tropical marine biotope is described. *Hassallia littoralis* is a false-branched nostocalean cyanobacterium with caespitose free filaments or with fasciculated individual filaments not in a common sheath. Filaments are mainly heteropolar, bearing mono- and bi-pored heterocysts and isopolar or heteropolar hormogonia. The sheath is often widening, with pronounced rounded terminals. This new species is defined according to molecular, morphological and ecological criteria, considering data from different stages of its life cycle as well as the 16S rRNA partial gene sequence.

Introduction

In recent decades, many important revisions have occurred for Cyanobacteria, applying the polyphasic approach that generated a modern classification system (Hoffmann *et al*. 2005). This strategy has led to major advances in taxonomy and the phylogenetic cyanobacterial classification by combining the traditional morphological characterization with the inclusion of molecular and ecological data from cultured, and especially field material. This new approach has provided essential support for the modern classification system of cyanobacteria (Komárek 2006). Key studies such as those of Flechtner *et al*. (2002), Gugger *et al*. (2002), Iteman *et al*. (2002), Rajaniemi *et al*. (2005), and Komárek *et al*. (2012), among others, have contributed to support the relevance of this approach, that has produced the description of numerous new taxa, as well as species being transferred into different generic entities.

The family Microchaetaceae (Nostocales) has been under intensive revision, with several new species and even genera being described, such as *Spirirestris* Flechtner & Johansen in Flechtner *et al*. (2002: 6), *Rexia* Casamatta, Gomez & Johansen (2006: 23), *Streptostemon* Sant'Anna, Azevedo, Kastovský & Komárek (2010: 220), *Ophiotrhix* Sant'Anna, Azevedo, Kastovský & Komárek (2010: 218), *Godleya* Novis & Visnovsky (2011: 14), *Toxopsis* Lamprinou & Pantazidou in Lamprinou *et al*. (2012: 2872), and *Calochaete* Hauer, Bohunická & Mühlsteinová (2013: 38). Currently, the family Microchaetaceae contains 13 genera (Table 1).

Hassallia Berkeley ex Bornet & Flahault (1886–1888: 115), *Coleodesmium* Borzì ex Geitler (1942: 154) and *Tolypothrix* Kützing ex Bornet & Flahault (1886–1888: 118) are genera that show some morphological overlapping traits, and therefore have delimitation problems. Even *Hassallia* and *Coleodesmium* have not been accepted in some identification manuals (Komárek *et al*. 2012), for instance Starmach (1966) included several species of *Hassallia* within *Tolypothrix*, and similar criteria were used by Bourrelly (1969, 1970). Similar morphotypes of *Hassallia* have been sometimes identified as members of genus *Tolypothrix*, such as

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T. bouteillei (Brébisson & Desmazières ex Bornet & Flahault) Lemmermann (1910: 219), which was recently included within genus *Hassallia* (Komárek *et al*. 2012) and has been recorded as the marine *Hassallia bouteillei* Brébisson & Desmazières ex Bornet & Flahault (1886–1888: 116) by Bárbara *et al*. (2005). In addition, populations of *T. byssoidea* (Berkeley) Kirchner in Engler & Prantl (1898: 80) are reported by Whitton & Potts (1979) as endolithic and chasmolithic in the Aldabra supralittoral, these probably belong to *Hassallia byssoidea* Hassall (1845: 233) in Bornet & Flahault (1888: 116).

The supralittoral zone is a distinctive extreme biotope from the intertidal zone to the limit of terrestrial vegetation (Garbary 2007). This zone is inhabited by few organisms, and is considered to have one of the toughest physical conditions (Ramírez-Reinat, 2010). Mexican marine cyanobacteria are poorly known, with just a few studies describing populations of this group of organisms (León-Tejera & Montejano 2000, López-Cortés *et al*. 2001, Montejano & León-Tejera 2002, León-Tejera *et al*. 2003, 2005). Previous reports from the supralittoral zone in Oaxaca coasts are few, reporting dark green-brown crusts or dark green filamentous mats. For the supralittoral of the same region (San Agustín, Huatulco area), *Scytonema* cf. *insulare* Sant'Anna (1988: 528), a heterocystous cyanobacteria, which grows over a crust of *Kyrtuthrix* cf. *maculans* (Gomont 1901: 210) Umezaki (1961: 85) and *Cyanodermatium gonzalezii* León-Tejera, Montejano & Cantoral-Uriza (2003: 365) have been reported.

Marine species of *Hassallia* have not been recorded previously from Mexico; however, we have found conspicuous growths of epilithic black mats of this genus as the dominant taxon in the rocky supralittoral zone of Tangolunda bay (Tropical Pacific Mexico). The aim of this work is to describe a new species of *Hassallia* from field material collected in a particular and extreme marine biotope, using a polyphasic approach that combines a detailed morphological characterization of our populations as well as genotypic characteristics determined by 16S rRNA analysis.

Materials and Methods

Site description and sample collection:—This study was conducted with material collected from a granitic coastal cliff on the east side of Tangolunda bay (15° 46' 24.79" N, 96° 5' 28.32" W), within Huatulco National Park, located in the Tropical Mexican Pacific (TMP). Climate is warm subhumid with summer rains and low rainfall in winter, with an annual average temperature of 26.9 ˚C. This region has a torrential rainfall regime with annual values from 800 to 1500 mm, concentrated from June to October. Seawater temperature range is 20.5–34.4 ˚C, and salinity values 17.3–40.1 PSU, average 34.6 PSU (Granja & López-Pérez 2008). Tidal regime is mixed, with semidiurnal dominance. The dominant swell comes from the West and a component from the South during summer. 92.5% of the incident waves arrive with heights in the range of 0.3 to 2.4 m (highest values in summer, CONANP 2003). Three samples of conspicuous microbial mat growths, found on nearly vertical rock walls 7 m above sea level, were collected in February 2011 with hammer and chisel. Each sample was divided into two subsamples, one preserved dry and the other in 4% formaline in seawater. Even though cultures have not been obtained, we have made an ecological, molecular and morphological characterization to describe these populations.

Morphology:—For the morphological characterization of field populations, we have analysed micrographs obtained with an Olympus DP12 digital camera in an Olympus CX51 microscope (DIC and light field); epifluorescence micrographs were obtained with an Olympus Provis AX70 microscope. Transmission electron microscopy (TEM) images were obtained in a JEOL mod JEM 1010 microscope from live material after fixation in 6% glutaraldehyde in 0.1 M PBS and subsequent postfixation in 1% osmium tetroxide, dehydration in ethanol series and embedding in epoxy resin. Ultrathin sections were contrasted with acetate uranil and lead citrate according to Reynolds (1963). Morphology measurements were obtained using SigmaScan© automated image analysis software (Jandel Scientific, Sausalito, California) and expressed as means ± standard deviation of 20–40 measurements. Morphological identification was done in accordance with new and traditional reference works (Bornet & Flahault 1886–1888, Gardner 1927, Frémy 1930, Flechtner *et al*. 2008, Komárek *et al*. 2012). Komárek & Hauer (2013) was used for the systematics.

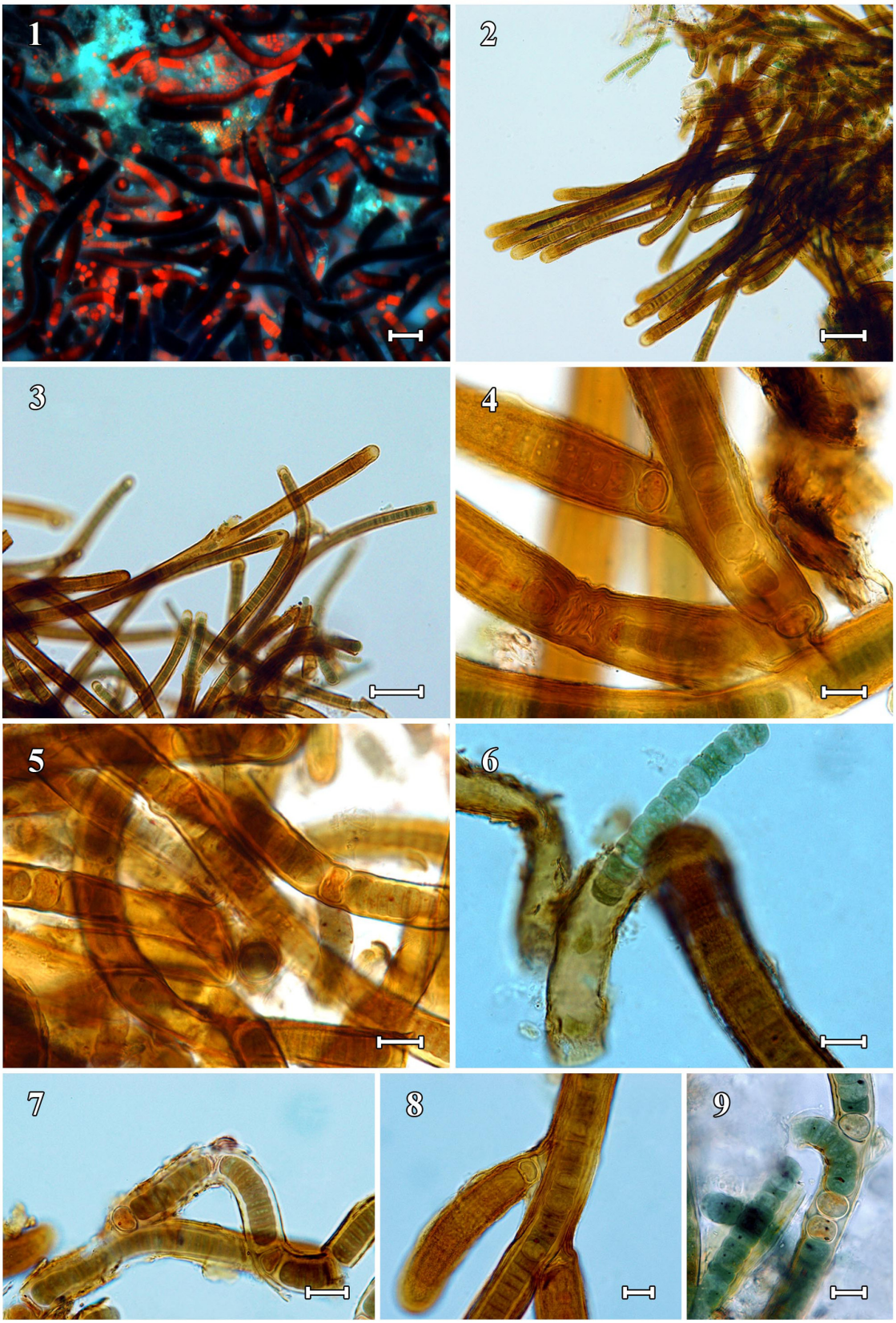
Molecular analysis:—Total DNA was extracted from natural populations using the MoBio UltraClean Soil DNA Isolation Kit (MOBIO Laboratories, Inc., Carlsbad, California), according to manufacturer's instructions. Quality and quantity of extracted DNA were checked on a 1% agarose gel. The 16S rRNA gene was partially amplified using the cyanobacteria specific primers proposed by Nübel *et al*. (1997) (106F, CYA781R, CYA781F) and Neilan *et al*. (1997) (CYA359F, 1494Rc). PCR conditions used for the 16S rRNA gene cyanobacterial amplification were as follows: 50 µl PCR reaction mix containing 1x reaction buffer, 1 µl of each primer (36 µmol), 1 µl of a stock solution of dNTPs (10 mM dNTPs mixture) 5 µl of $MgCl_2$ buffer (1 × 2.5 mM) and 1 µl of DNA polymerase (5U/µl) (Amplificasa, Biogénica, Mexico), as well as 1 µl of genomic DNA (10 ng DNA). Cycling conditions had an initial denaturation step of 95 °C for 7 min, followed by 30 cycles of denaturation at 94 °C for 0.5 min, annealing at 58 °C for 1 min, extension at 72 °C for 1 minute and final extension at 4 °C. PCR products were visualized on a 1% agarose gel. After amplification, DNA was cleaned using the QIAquick® PCR purification kit (QIAGEN). Sequencing was conducted using a BigDye Terminator Cycle Sequencing Reaction Kit (Applied Biosystems, California) on an ABI PRISM3100 Genetic Analyzer (Applied Biosystems, California). The same primers were used for sequencing.

Phylogenetic analysis:—Sequences were obtained for both DNA strands, assembled and corrected into 1307 bp fragments using Bioedit software version 7.0.9.0 (Hall 1999). Representative taxa from available sequences of Microchaetaceae and Scytonemataceae taxa from GenBank were used as ingroups and outgroups. Phylogenetic analysis was based on 20 sequences obtained from GenBank (>1100 bp fragments of the 16S rRNA gene) except *Hassallia byssoidea* type species sequence (1057 bp). These sequences included all those of the *Hassallia* species described up to date as well as sequences of representative genera of the family Microchaetaceae (*Microchaete*, *Fortiea*, *Tolypothrix*, *Spirirestris*, *Toxopsis*, *Calochaete* and *Coleodesmium*). Additionally, we added three sequences considered as outgroups, two belonging to *Brasilonema* and one to *Scytonema* (family Scytonemataceae). All sequences were aligned using the CLUSTAL W Multiple Sequence Alignment Program (Thompson *et al*. 1994) and manually edited in PhyDE® version 0.9971 (Müller *et al*. 2010). Phylogenetic relationships were inferred with Winclada (Nixon 1999–2002) and TOPALi v2 (Milne *et al*. 2009). Maximum parsimony (MP) trees were constructed using the Ratchet search (Nixon 1999). The model used in the Bayesian analysis (B) was the Hasegawa-Kishino-Yano model of nucleotide substitutions with invariant sites and gamma distributed rates for the variable sites (HKY+I+G). This model was selected based on the Maximum Likelihood (ML) ratio test implemented by TOPALi version 2 software with a significance level of 0.01. For the Bayesian analysis, we ran five chains of the Markov chain Monte Carlo (one hot and four cold), sampling one tree every 1000 generations for $5{\cdot}\times10^6$ generations starting with a random tree. ML and MP analyses were subjected to bootstrap resampling (1000 replicates with 10 random additions) to estimate robustness (Felsenstein 1985). The range of 16S ribosomal RNA gene divergence values within and among species was calculated using uncorrected "p" distances using PAUP* version 4.0b10 (Swofford 2002).

Results

Hassallia littoralis González-Resendiz & León-Tejera, *sp. nov*. (Figs. 1–25)

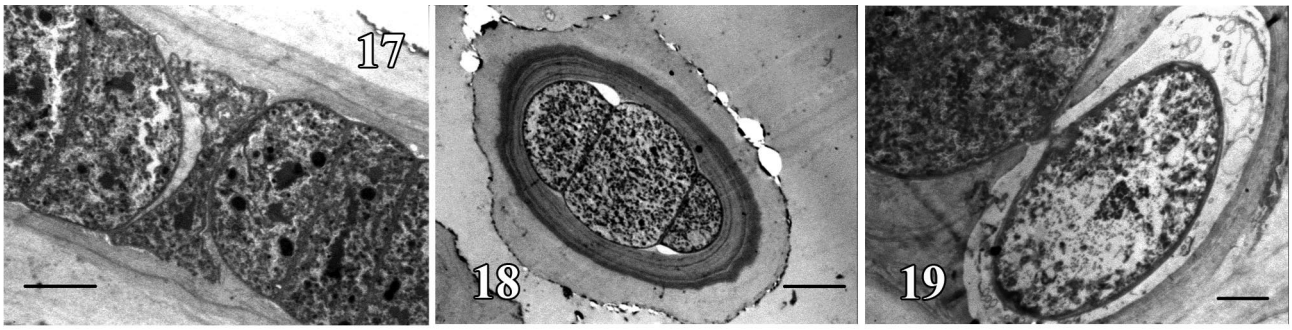
Cespitose short mats brown to blackish (Figs. 1, 2), filaments cylindrical (Fig. 13), straight or curved; parallely oriented or intermingled, sometimes forming fascicles 200–300 µm long and 5–12 µm in diameter, without a common sheath (Figs. 2, 3, 20, 21). Falsely branched, branches tightly joined or irregularly divaricated to the main filament (Figs. 4, 8, 11, 21, 24). Trichomes 3–6 µm wide (Figs. 3, 5), constricted at cross-walls (Figs. 15, 16). Cells cylindrical to barrel-shaped (Figs. 15, 16), shorter than wide, 3–6 µm wide, 1–3 µm long, with slightly granulated content. Terminal cells widely rounded, sometimes nearly spherical, hyaline to greenish or yellowish (Figs. 12, 15, 16) (2-4 µm long × 3–6 µm wide). Sheaths firm, stratified, slender, hyaline (Fig. 16) to thick and amber or dark yellowish-brown, often widening with pronounced, rounded terminals (3–7 µm in diameter) (Figs. 11, 12, 16), generally closed and rarely opened at the apex (Figs. 10, 14). Heterocytes spherical, ovoid to cylindrical (Figs. 4, 5, 9, 11), 4–6 µm in diameter, 4–8 µm long (Fig. 5), one basal (Figs. 4, 8, 11, 19), rarely two (Fig. 9), and less frequently intercalary. Akinetes not detected. Reproduction by hormogonia (Figs. 6, 7, 18, 22, 23).



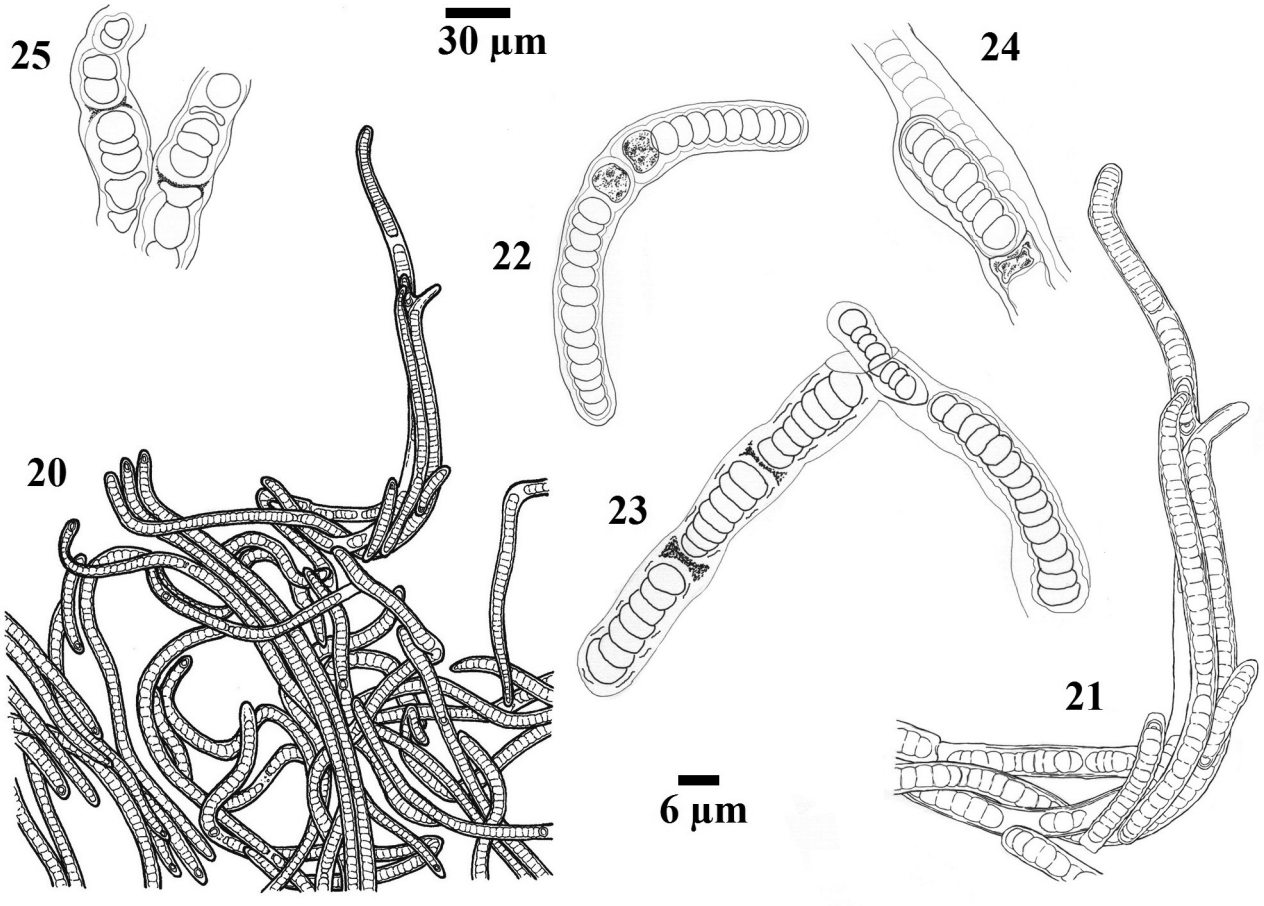
FIGURES 1–9. Morphological features of *Hassallia littoralis*. Fig. 1. Intermingled filaments as seen with epifluorescence. Fig. 2. Erect fascicle growth form with lighter terminal parts. Fig. 3. Straight or curved filament growth forms. Fig. 4. False branching with basal heterocytes. Fig. 5. Trichomes with evident intercalar heterocytes and polar nodules. Fig. 6. Release of short isopolar trichome with evident nodal constrictions. Fig. 7. Series of heteropolar and isopolar hormogones. Fig. 8. Divaricated pseudobranch with basal heterocyte and stratified sheath. Fig. 9. False branching with single and paired heterocytes. Scale bars: Figs 1–3: 30 µm, Figs 4–9: 6µm.

FIGURES 10–16. Morphological features of *Hassallia littoralis*. Fig. 10. Terminal widening of the sheath. Fig. 11. Branched filament with evident cytoplasmic granules. Fig. 12. A trichome with variations in diameter bearing a darker, shortened necridial cell. Fig. 13. Plane view of a cylindrical trichome and filament. Fig. 14. Intense terminal EPS production by highly granulosed trichome with an open sheath. Fig. 15. Darkening of hyaline sheath produced probably by gelatinization. Fig. 16. A trichome of a young filament with necridic cells and sheath widening. Scale bars: 6 µm.

Type:—MEXICO. Oaxaca: Tangolunda bay, 15° 46' 24.79" N, 96° 5' 28.32" W. Marine supralittoral, *León-Tejera, 2-2-2011* (holotype FCME! PTM9586, GenBank sequence access number KF017617, isotype UAMIZ! 1225, paratypes FCME! PTM9587, PTM9588).



FIGURES 17–19. *Hassallia littoralis* TEM images. Fig. 17. Hormogonia formation through a necridic cell. Fig. 18. Young isopolar hormogonium with a wide stratified sheath. Fig. 19. Evident polar nodule in a basal heterocyte. Scale bars: Fig. 17: 3 µm, Fig. 18: 5 µm, Fig. 19: 1 µm.



FIGURES 20–25. *Hassallia littoralis* habitus. Figs 20, 21. Fasciculated growth form. Figs 22, 23. Formation of hormogonia. Fig. 24. Hormogonia and branch formation. Fig. 25. Production of monocyte-like cells. Scale bars: Figs 20, 21: 30 µm, Figs 22–25: 6 µm.

Etymology:—The species epithet was selected according to the specific environment (marine littoral).

Habitat:—In Tangolunda Bay, supralittoral black mats of *Hassallia* dominate this habitat and the three collected populations were epilithic mats from the rocky marine littoral. They were found growing on nearly vertical igneous rocks 7 m from the seaside and approximately 4–5 m above sea level, facing the sea. The rocks are only slightly wetted by the sea breeze and totally exposed to sunlight. Considering the maximal tidal heights of 2.4 m, probably they are never covered by the sea but grains of salt were found intermingled among filaments due to the sea breeze water evaporation. This species was found growing with several undescribed

coccoid and LPP species (Fig. 1) but not with common filamentous non-heterocystous (*Oscillatoria*, *Lyngbya*, *Microcoleus*, *Phormidium*, *Schizothrix*) or heterocystous genera (*Rivularia*, *Calothrix*, *Scytonema*) typically described for intertidal biotopes (Brito *et al*. 2012).

Observations:—Cells appearing as monocytes and short hormogonia of 2 to 10 (15) cells long (Fig. 25), are sometimes liberated. Young filaments and hormogonia are either isopolar (Figs. 6, 7, 24) or heteropolar (Figs. 9, 22). Isopolar hormogonia formation through necridic cells (Fig. 23) develop later terminal/basal heterocytes (Figs. 6, 7, 9, 17, 18, 22). Sheath becomes lighter or darker toward the extremes, probably due to terminal gelatinization (Figs. 10, 14, 15). Populations morphologically characterized are C76 (PTM9586 and UAMIZ1225), C77 (PTM9587) and C78 (PTM9588). The sequence was obtained from sample C76.

Molecular and phylogenetic analyses:—A total of 24 sequences of representative taxa from available sequences of Microchaetaceae and Scytonemataceae deposited at GenBank were analyzed, including *Brasilonema bromeliae* DQ486055, *Scytonema* sp. AB093483 and *Brasilonema tolantongensis* JN676147 as outgroups and our strain. The three approaches used (MP, B and ML), produced similar clustering, therefore only the ML tree, with support values of ML, B and MP is shown in Fig. 26. Topology of the ML tree showed that the *Hassallia* assemblages including seven taxa are segregated into two clades supported by modest bootstrap values. The earliest diverging clade included *H. byssoidea*. The second clade included two sister groups: one defined by *H. antarctica* Komárek, Nedbalová *et* Hauer (2012: 770) assemblage, and the other by *H. littoralis, H. andreassenii* Komárek, Nedbalová *et* Hauer (2012: 768) and *Coleodesmium* sp. ANT (Fig. 26). *Hassallia littoralis* is moderately supported by bootstrap values in ML and MP topologies, but with a higher value in Bayesian analysis (0.97), forming a distinct sister clade to *H. andreassenii* and *Coleodesmium* sp. ANT. Molecular data confirmed the distinctiveness of our tropical populations that show similarity values of 98.4% with *H. andreassenii*, 97.8-98.1% with the *H. antarctica* assemblage, 97.8% with *H. byssoidea*, and finally 98.5% with *Coleodesmium* sp. ANT.

Discussion

In the last decade the family Microchaetaceae has had important changes; the recent addition of new generic-level taxa indicates a great activity derived from the study of new or not well known habitats, such as tropical or extreme ones (Flechtner *et al*. 2002, Cassamata *et al*. 2006, Sant'Anna *et al*. 2010, Novis & Visnovsky 2011, Lamprinou *et al*. 2012, Hauer *et al*. 2013). These taxa were described mainly using a polyphasic approach including molecular methods, but when this was not possible new taxa description has been based on a detailed morphological and ecological characterization of populations (Sant'Anna *et al*. 2010). However, whenever possible, new species descriptions must be carried out with combined molecular, morphological and ecological data. The development of a logical and robust classification system is essential, this being a baseline for diversity estimations and ecological, taxonomical or ecophysiological interpretations (Komárek 2006, 2010). It is considering this recommendation that we describe this new species. According to the revised taxonomic classification system of Komárek & Anagnostidis (1989) and Komárek & Hauer (2013), in terms of morphology, our populations clearly belong to the family Microchaetaceae, and correspond phenotypically to genus *Hassallia,* as they show many typical characters attributed to this genus either in its original description or in recent studies (Komárek *et al*. 2012) such as growth form, cell morphology, trichome polarity, type of false branching, sheath type and color, as well as reproductive structures.

There are eleven valid and recognized *Hassallia* species (Table 1) (Komárek & Hauer 2013) including four new species described from Antarctica and desert soils biotopes (Flechtner *et al*. 2008, Komárek *et al*. 2012) but none had been recorded from a similar biotope (tropical marine supralittoral) and geographical area (Table 1). In general, the lack of reports of cyanobacterial genera such as *Hassallia* in many geographical regions, and specially in marine habitats from tropical zones, is not due to its rare distribution or low abundance in natural communities, but to the lack of studies in habitats of very specific environmental conditions where cyanobacteria form frequently dominant multispecific assemblages.

TABLE 1. Phenological characters of *Hassallia* species. Information gathered from Bornet et Flahault (1886–1888), Frémy (1927, 1929–1933, 1930), Gleitler (1932), Gardner (1927), Fletchner *et al*. (2008), and Komárek & Hauer (2007). NA: Not avalaible. *: Data from drawings, not included in the original descriptions.

	H. littoralis sp. nov.	*H. byssoidea*	*H. bouteillei*	*H. andreassenii*	*H. antarctica*	*H. californica* Johansen & Flechtner in Flechtner *et al*. (2008: 417)	*H. pseudoramosissima* Johansen & Flechtner in Flechtner *et al*. (2008: 417)	*H. granulata* Gardner (1927: 82)	*H. discoidea* Gardner (1927: 83)	*H. usambarensis* Hieronymus in Engler & Prantl (1895: 10)	*H. manginii* Frémy (1927: 57)	*H. pulvinata* Frémy (1930: 294)
Ecology	Epilithic on marine supralittoral	Subaerophytic, epilithic, calcareous humid rocks, in fissures, mosses and humid soil and tree trunk	On wet rocks	Epilithic, calcareous precipitates (Antarctica)	Stone crevices, in water among stones or on dripping rocks. (Antarctica)	Arid soil	Arid soil	Bark of tree	On rocks in association with Hepaticae	Bark of tree	On limestone rocks	On stones in streams
Thallus habit	Cespitose mats	Cespitose mats 1 mm	Rounded mat	Flat mats	Clusters of filaments	Bushy	Bushy on parallel filaments	Flocculent	Sparse filaments	Mat of filaments	Cushion crust	Cushion shaped rounded, semi-lenticular, reminiscent of *Rivularia.*
Thallus color	Brown to Black	Dark-green, Black	Dark-brown	Yellow brown to brown	Dark brown up to blackish	Dark brown	Green	Pale-green	NA	Dark-green	Black-brown	Blue-green or black-green
Filaments	Heteropolar, isolated straight and curved, or parallely oriented filaments, 200–300 µm long	Heteropolar uniseriate	Heteropolar, filaments easily separated	Heteropolar, fasciculated	Heteropolar, fasciculated and cylindrical	Heteropolar, frequently singly pseudo–branched	Heteropolar, abundantly pseudo–branched	Heteropolar, straight or somewhat arcuate. Diameter uniform	Heteropolar, relatively short, compara–tively straight	Heteropolar, fragile, slightly flexuous to nearly straight. 200–250 µm long	Heteropolar, straight-sided translucent regularly branched	Heteropolar, filaments radiating from the center, up to 2 cm long
Filament width (µm)	5–12	10–15	5–7	12.4–20	11.2– 17.5	14–16	11–20	11–13.5	22–25	5–7	9–11	13–14
Sheath	Firm, stratified, slender, hyaline, to thick and amber or dark yellowish-brown	Thin without lamellation, yellow, gold to brownish	Thin, narrow, colorless or yellow gold	Firm, thick, laminated, dark brown, cylindrical	Firm, relatively thick, laminated, yellowish to yellow-brown	Firm, unlamella–ted, colorless	Sheath thin, colorless, unlamella–ted	Very thin, smooth, homogeneous, golden yellow	Smooth, homoge–neous, hyaline to yellowish, unlamella–ted	Thin, homogeneous and colorless	Gelatinous, colorless and cylindrical, yellow to brown and irregularly thickened	Gelatinous, narrow, thin, smooth outside, irregular
Sheaths at apex	Generally closed rarely opened, often with pronounced rounded terminal widenings	Closed, narrowing towards the ends	Closed*	Firstly closed, later open, or gelatinized at the ends	Closed	Open*	Open*	Closed	Closed	NA	Open*	Open*
False branching	Branches tightly joined or irregularly divaricated to the main filament	Irregular, solitary, short and fragile lateral branches	Short	Branches tightly joined to the main filaments	Slightly and irregularly divaricated	Fragmenting easily into very short filaments	Single, double	Sparse	Moderately frequent	Fragile	Branches erect spreading, moderately long, curved or rarely straight	Repeatedly branched translucent. Upright branches spreading

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TABLE 1. (Continued)

	H. littoralis sp. nov.	*H. byssoidea*	*H. bouteillei*	*H. andreassenii*	*H. antarctica*	*H. californica* Johansen & Flechtner in Flechtner *et al.* (2008: 417)	*H. pseudoramosissima* Johansen & Flechtner in Flechtner *et al.* (2008: 417)	*H. granulata* Gardner (1927: 82)	*H. discoidea* Gardner (1927: 83)	*H. usambarensis* Hieronymus in Engler & Prantl (1895: 10)	*H. manginii* Frémy (1927: 57)	*H. pulvinata* Frémy (1930: 294)
Trichomes	Cylindrical, constricted at cross-walls	Constricted at the cross-walls, not attenuated towards ends	NA	Cylindrical, not constricted at cross-walls (very rarely slightly constricted) sometimes slightly narrowed at the ends	Cylindrical, not constricted up to slightly constricted at cross-walls	Constricted at the cross- walls	Constricted to very slightly constricted	Very slightly constricted	Constricted at the dissepi–ments, uniform throughout the entire length	NA	Slightly constricted at the cross- walls	Constricted at the cross-walls
Trichoma width (µm)	3–6	8–12	NA	7.5–13	(7.4) 8.6–12.3	11–12	8–14	10–12	17–20	NA	4–5	10–12
Cytological features	Cylindrical to barrel shaped, sometimes granulated	Short, barrel-shaped	Barrel-shaped, slightly shorter than wide	Cylindrical	Cylindrical to shortly barrel-shaped	One-seventh to one-fourth as long as broad	Shorter than wide	Densely granulated	Discoidal, finely granulated	Little shorter than the diameter or almost	Cells almost square	Barrel-shaped*
Cell color	Hyaline to greenish or yellowish	Olive-green	NA	Blue-green or olive-green	Blue-green or olive-green	Blue-green to dark olive	Bright blue-green	Golden yellow	Bright blue-green or methyl green	Green	Olive green	Pale blue-green or pale yellow-brown
Cell length (µm) or length/width rate	3–6 × 1–3	8–11 wide; 2–3 × wider than long	4–5	NA	NA	1.6–3.2	(2)–3–9	One-third to one-fourth the diameter long	4–6 or slightly longer in the oldest parts	4.5–6.0	Length is half the width	Cells 0.3–0.6 usually ½ times as long as wide
Heterocytes	Spherical, ovoid to cylindrical. Basal one, rarely two. Intercalary rare	Hemispherical to oval. Basal, one rarely two	Hemispherical to oval. Basal rare intercalar, single	Spherical, hemispherical, cylindrical, ovoid or slightly oval. Basal intercalary, solitary rarely up to 2 (3) in a row	Hemispherical, rarely up to oval sometimes slightly complanated. Mainly basal, intercalary, (usually before the false branching), solitary or in pairs	Oval. First developing intercalary, single or paired	Compressed rectangular. Basal and intercalar	Subglobose to compressed. Mostly terminal	Discoidal, golden yellow, numerous. Basal and intercalary*	Subglobose or depressed-globose	Solitary, rarely in pairs. Nearly spherical or hemisphe–rical. Basal	Hemispherical or disc-shaped. Basal rarely intercalar
Heterocytel ength × width (µm)	4–6 ×4–8	11 × 5–6	NA	NA	NA	NA	9–11 × 7–9	NA	Same size as the cells	Slightly larger than the vegetative cells	NA	NA

This new species of *Hassallia* is phenotypically distinguished from all other species of the genus (Table 1) mainly by growth form, cell and trichome dimensions and distinctive sheath features: terminal part widening, funnel-like or "wing-like" (Figs. 15, 16, 24, 25), with evident gelatinization. In adittion, it differs from the rest of species of this genus in its specific habitat. Only few species of *Hassallia* had been sequenced to date (*H. byssoidea*, *H. andreassenii* and *H. antartica* assemblage); the difference of 16S rRNA genetic similarity among these strains and our population was 1.6–2.2%. The morphological and biotope differences, as well as the high levels of genetic variation between our populations from those three species, support the proposal of our population as a new taxon within genus *Hassallia.*

In our tree topology, *H. littoralis* formed a distinctive and well-supported clade within the *Hassallia* genus using Bayesian analysis. *Hassallia byssoidea* (type species) appears as a basal group, and *H. andreassenii* and *Coleodesmium* sp. ANT, form a sister clade to *H. littoralis*. The phylogenetic position of *Coleodesmium* sp. ANT (Taton *et al*. 2006) is within the *Hassallia* cluster, and phylogenetically close to *H. andreassenii* (Fig. 26), similar to results obtained by Komárek *et al*. (2012). Interestingly, both *Coleodesmium* sp. ANT and *H. andreassenii* have been isolated from Antarctica, therefore further studies are needed to compare these two strains.

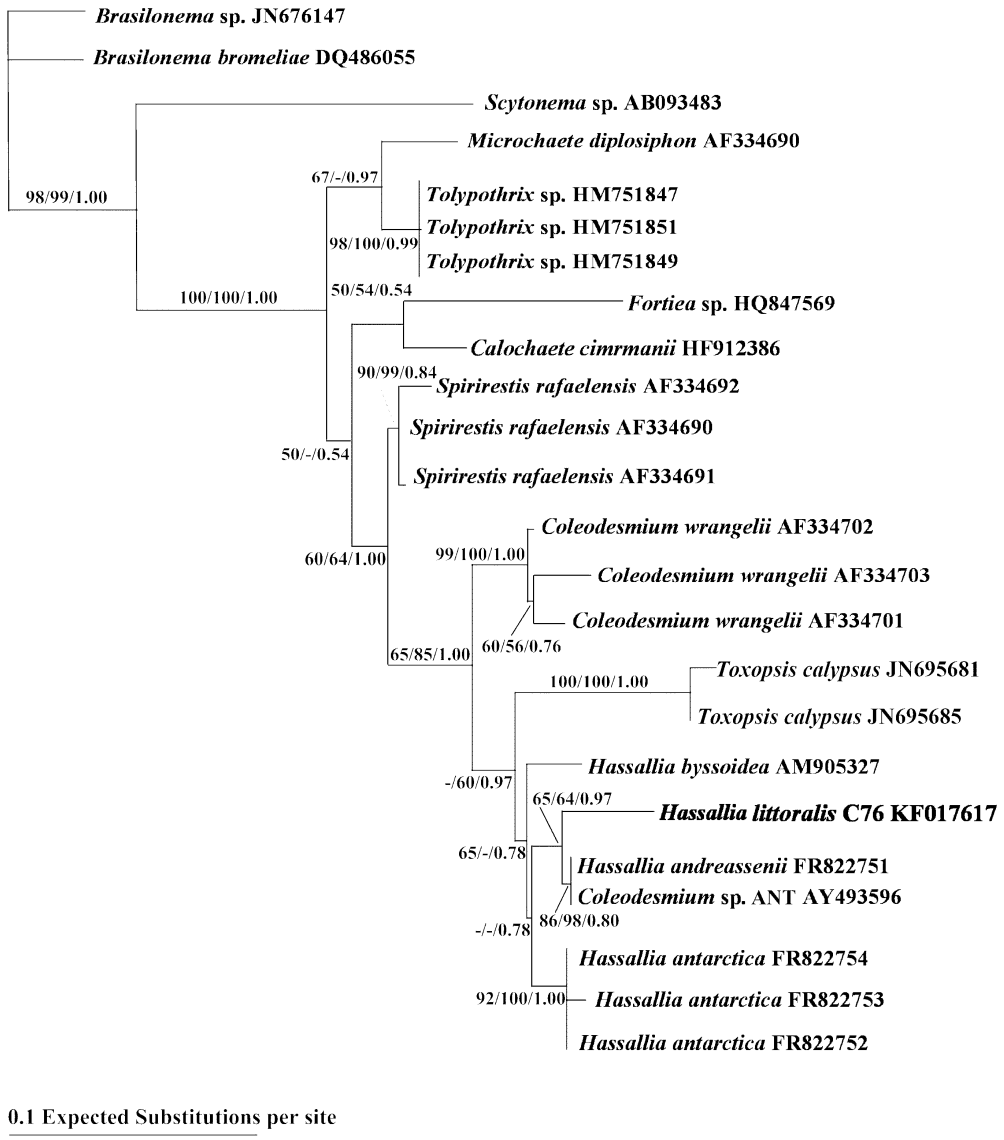

FIGURE 26. Maximum-likelihood tree based on the analysis of 16S rRNA gene of representatives of the families Scytonemataceae and Microchaetaceae showing the position of the sequence obtained in the present study (in bold). Numbers at nodes indicate bootstrap values ≥ 50% for Maximum Parsimony (left), Maximum Likelihood analysis (medium) and Bayesian posterior probabilities (right) values.

The identity of *H. littoralis* is moderately supported by bootstrap values in ML and MP topologies. Similar modest support values have been documented in Microchaetaceae by Cassamatta *et al*. (2006); Berrendero *et al*. (2011), Komárek *et al*. (2012), Lamprinou *et al*. (2012) and Hauer *et al*. (2013), the scarce number of sequences available in this group probably influences these values. According to Berrendero *et al*. (2011) and Hauer *et al*. (2013), the Microchaetaceae family needs a more detailed analysis of its members. Our analysis supports this opinion, we consider that further inventory studies are still necessary, particularly in tropical and extreme areas, which have shown to have a notorious diversity of previously undescribed cyanobacteria (Komárek & Komárková 2007).

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