Research Article

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New records and species of *Verosphacela* (Onslowiales, Phaeophyceae): *Verosphacela ebrachia* from Greece, *Verosphacela henryi* sp. nov. from Rapa Nui and *Verosphacela asensii* sp. nov. from Ascension Island

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Abstract: Onslowiales are minute sublittoral marine brown algae found in warm-temperate to tropical regions of the North Atlantic Ocean. Sequences of the plastid-encoded markers *psa*A, *psb*C, *rbc*L and the Rubisco spacer of a strain of *Verosphacela* from Greece, previously identified as *Verosphacela silvae*, were highly similar to sequences of *Verosphacela ebrachia* from Florida, extending the distribution of the species to the Mediterranean and suggesting re-examination of the morphologically similar *V. silvae* described from Italy. Two other species were grown from substratum collected in the subtidal of Rapa Nui (Easter Island, subtropical Southeast Pacific) and Ascension Island (tropical Atlantic). According to sequences of the aforementioned markers, they also belong to the Onslowiales, forming a clade with *V. ebrachia*. They are described here as *Verosphacela henryi* sp. nov. and *Verosphacela asensii* sp. nov., respectively. In culture, they consisted of creeping branched filaments growing by means of an apical cell, and subterminal filament cells dividing once longitudinally. *V. henryi* formed four- to five-celled propagules without apical cells, resembling those of *V. ebrachia*, whereas *V. asensii* remained vegetative. *V. asensii* extends the distribution of the Onslowiales to the central tropical Atlantic, and *V. henryi* is the first record of the order in the Pacific Ocean.

Keywords: biogeography; circalittoral; germling emergence; molecular-assisted identification; sciaphilic algae; taxonomy

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1 Introduction

The brown algal order Onslowiales so far contains four species (Table 1); it is based on *Onslowia endophytica* Searles, a microscopic prostrate brown alga from the Atlantic coast of North America, detected as endophyte in the red alga *Halymenia floridana* J. Agardh (Henry 1987a; Searles and Leister 1980). Additional taxa are *Onslowia bahamensis* Henry, *Verosphacela ebrachia* Henry (Henry 1987b), and *Verosphacela silvae* Alongi, Cormaci *et* G. Furnari (Alongi et al. 2007), all being microscopic and generally found as endophytes or epiphytes on green, brown or red macroalgae (Table 1). Except for *V. silvae* from the central Mediterranean, the known species of *Onslowia* and *Verosphacela* are from tropical to warm-temperate waters of the Northwest Atlantic Ocean (Figure 1); all species of the Onslowiales, except for *V. ebrachia*, which was obtained from drift material (Henry 1987b), were detected in deep water (25–90 m), i.e. the circalittoral.

Because of growth from a prominent apical cell, presence of numerous small discoid plastids without pyrenoids, occasional longitudinal divisions in filaments, and formation of propagules, this group of algae was initially tentatively classified in the Sphacelariales. However, their apical cells do not show blackening after treatment with eau de

Table 1: The species of Onslowiales, with geographic distribution, nomenclature, habitat, propagule cell number, and availability of cultures.

Site (cf. Figure 1)	Species and strains	Authorities and dates	Origin	Substratum	Depth (m)	Cells per propagule	Culture	Comment 1	Comment 2
1	*Onslowia endophytica*	Searles and Leister (1980)	Off Onslow Bay, North Carolina, USA	Endophyte in *Halymenia floridana* J. Agardh	26–32	9	Not isolated into culture	Type species of *Onslowia*	Type locality of *O. endophytica*
2	*O. endophytica* SGAD143		Fort Pierce Inlet, Florida, USA	Endophyte in *Halymenia* sp.	Drift	9	Culture no longer available		
3	*Onslowia bahamensis*	Henry (1987)	Off The Bahamas	Epiphyte on *Johnson-se-linkia profunda* Eiseman et S.A. Earle and *Lobophora variegata* (J.V. Lamouroux) Womersley ex E.C. Oliveira	60–90	9	Culture no longer available		Type locality of *O. bahamensis*
4	*Verosphacela ebrachia* SGAD144	Henry (1987)	Vero Beach, Florida, USA	Endophyte in *Spatoglossum schroederi* (C. Agardh) Kützing	Drift	9–13	Culture no longer available	Type species of *Verosphacela*	Type locality of *V. ebrachia*
5	*Verosphacela silvae*	Alongi, Cormaci et G. Furnari (2007)	Grotta Racina, Salina Island, Italy	Endophyte in *Peyssonnelia rubra* (Greville) J. Agardh	25	7	Not isolated into culture		
6	*V. ebrachia* GR11-s#8-3[a]		Off Tsolis, Gulf of Corinth, Greece, 38.3217 °N, 21.973 °E	Developed in culture from incubated small pebble	15	4–13	CCAP 1336/1		
7	*Verosphacela henryi* IP14-27	Present paper	Cathedral, in marine cave, off Rapa Nui (Easter Island), Chile, 27.11 °S, 109.424 °W	Developed in culture from incubated coarse sand	7	4–5	CCAP 1336/2		Type locality of *V. henryi*
8	*Verosphacela asensii* ASC11-17	Present paper	Off White Island, Ascension Island, British Overseas Territory; 7.895 °S, 14.395 °W	Developed in culture from incubated coarse sand	23	Propagules absent	CCAP 1336/3		Type locality of *V. asensii*

[a]*Verosphacela ebrachia* from Greece was initially identified as *V. silvae* (Peters et al. 2015) but the present study shows that it is genetically similar to *V. ebrachia* SGAD144.

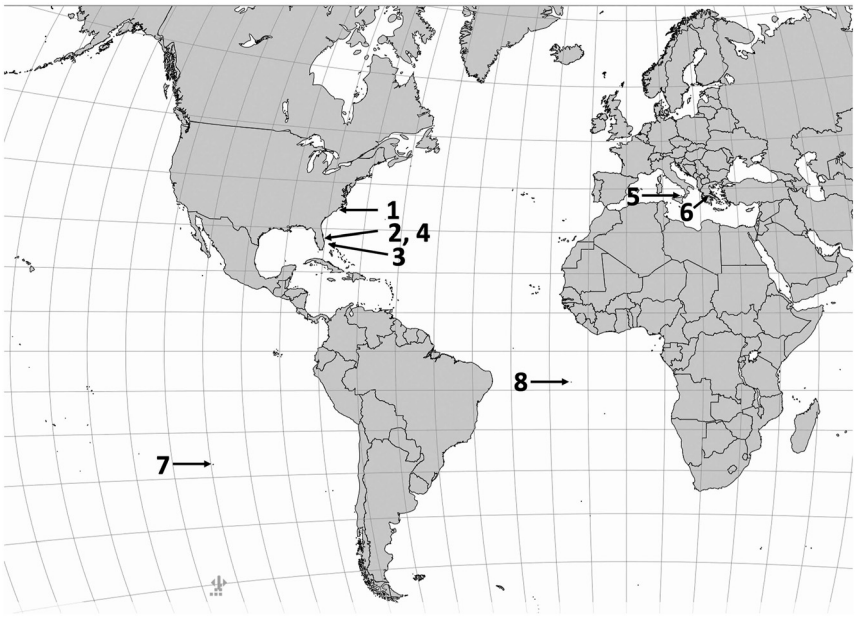


Figure 1: Collecting sites of Onslowiales. Previous collections of *Onslowia* spp. at 1–3 and of *Verosphacela* spp. at 4–6. Additional sites for the present study were at Rapa Nui (7) and Ascension Island (8). See Table 1 for details. Created with a world map from mapchart.net.

Javel, which is typical for the Sphacelariales (Henry 1987b; Prud'homme van Reine 1974), and subsequent molecular studies showed that a separate order Onslowiales was required to accommodate *Onslowia* and *Verosphacela* (Phillips et al. 2008).

Henry (1987b) suspected that more species of the Onslowiales would be detected in the tropical Atlantic and the Mediterranean because such small subtidal benthic marine algae are easily overlooked in field collections. However, they may appear when substratum is cultivated, and by this method, also referred to as "germling emergence", we have previously isolated a strain of *Verosphacela* from the subtidal in Greece (eastern Mediterranean; Peters et al. 2015). Because the COI sequence generated for that isolate did not permit comparison with the sequences of markers available for *V. ebrachia* from the type locality in Florida, we tentatively identified the strain from Greece as *V. silvae*, which is the only species to be recorded so far in the Mediterranean. Here we correct that identification because of sequence similarity of the Greek isolate to *V. ebrachia* in the plastid-encoded molecular markers *psa*A, *psb*C, *rbc*L, and the Rubisco spacer, and we provide the essential morphological characters of the strain from Greece, which were not given in Peters et al. (2015).

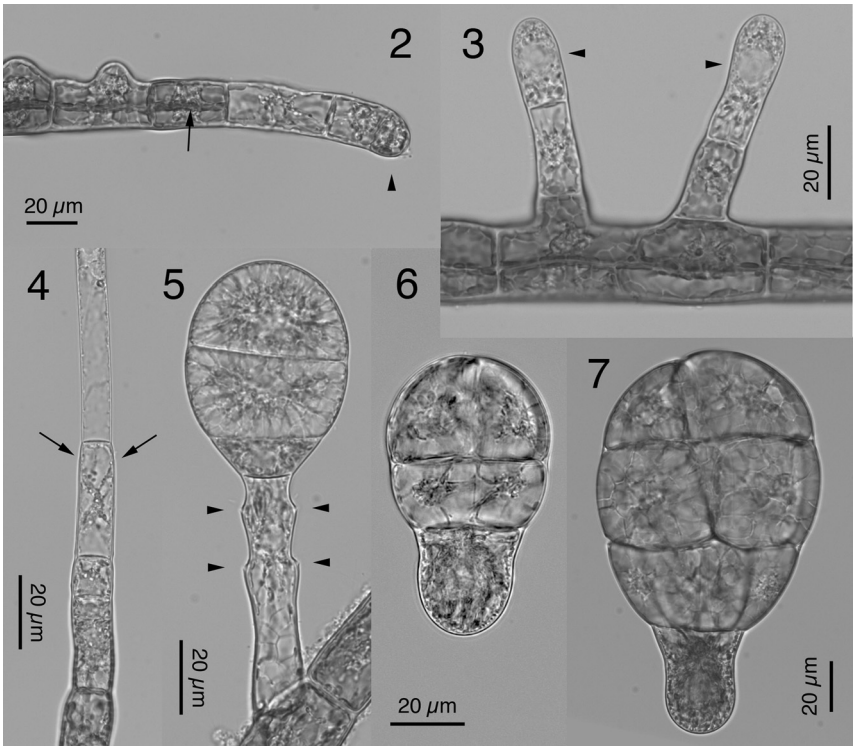
Moreover, we describe two new species of *Verosphacela* based on two strains obtained in cultures from substratum collected in the subtidal of the oceanic islands Rapa Nui in the Southeast Pacific Ocean and Ascension Island in the central Atlantic Ocean. Morphology and sequences from the plastid-encoded markers mentioned in the preceding paragraph revealed that these algae belong to the Onslowiales, wherein they cluster with *V. ebrachia*.

2 Materials and methods

Collection, isolation and culture. Coarse sand was collected by F. Küpper from two sites off Rapa Nui and Ascension Island (Table 1, Figure 1), during SCUBA dives. The samples were posted to A. Peters for incubation and isolation of cultures as described previously (Muñoz et al. 2018; Peters et al. 2015). Two strains of brown algae presenting multiple discoid plastids, filamentous growth from apical cells, and longitudinal divisions leading to biseriate filaments, thus putative members of the Onslowiales, were selected for further scrutiny. The two selected strains and the previously (Peters et al. 2015) isolated strain GR11-s#8-3, identified originally as *V. silvae*, were incubated in 8- or 35-ml polystyrene Petri dishes in half-strength Provasoli-enriched (Coelho et al. 2012) sterilised natural sea water from Roscoff, under dimmed ($\leq$30 µmol m^{-2} s^{-1}) natural light at 14–25 °C and the natural change of daylengths. In addition, to simulate the conditions in the subtidal of tropical regions, incubation was also made at 25 (±1) °C and 5–10 µmol m^{-2} s^{-1} in an incubator equipped with artificial white light and 12-h daylength.

Herbarium abbreviations follow Thiers (2023), and CCAP designates the Culture Collection of Algae and Protozoa at Oban, Scotland, UK (Gachon et al. 2013), where the strains studied were deposited.

Sequences. As listed in Supplementary Table S1, there are 12 published sequences of *Onslowia*, all of which are from strain SGAD143 of the generitype (Henry 1987a). In the genus *Verosphacela*, five sequences are so far published for *V. ebrachia* (strain SGAD144 from Florida), and one for a Greek isolate originally identified as *V. silvae*, which we re-



Figures 2–7: *Verosphacela ebrachia* strain from Greece, culture: (2) vegetative filament showing apical cell (arrowhead) and longitudinal division in second subterminal cell (arrow); (3) young erect filaments, with nuclei discernible in apical cells (arrowheads); (4) phaeophycean hair, arrows pointing to end of sheath; (5) developing propagule at 3-cell stage, on thin pedicel, arrowheads indicating scars from two previously released propagules; (6) released propagule consisting of possibly 9 cells, of which 5 are visible; (7) released propagule consisting of possibly 13 cells.

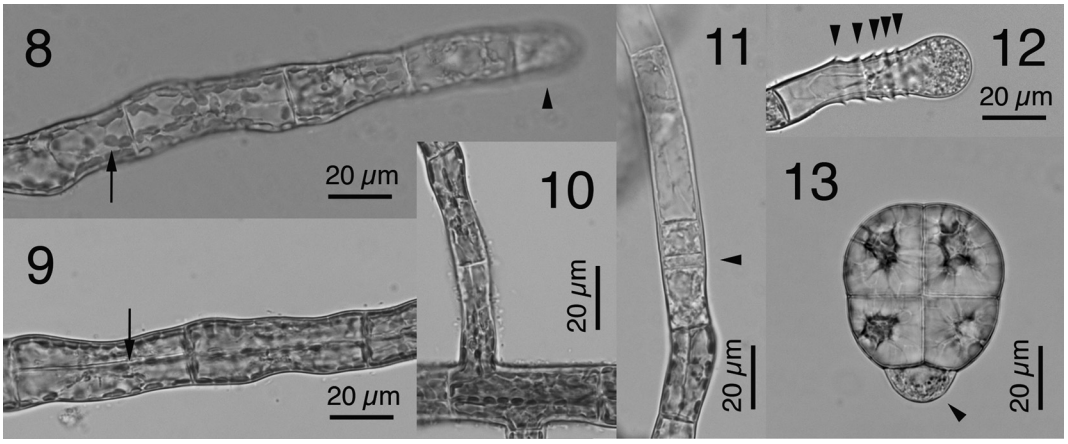
identify as *V. ebrachia* in the present work. There are no published sequences of *O. bahamensis* or of *V. silvae* from its type locality. To allow phylogenetic analyses with our three strains of interest, we generated sequences of partial nrSSU, the plastid-encoded protein-coding markers *psa*A, *psb*C and *rbc*L, and of the *rbc*L-*rbc*S intergenic spacer, also referred to as the Rubisco spacer. Sequences were generated as described in Muñoz et al. (2018), i.e. by PCR using oligonucleotide primers (Supplementary Table S2), followed by commercial Sanger sequencing with the same primers (Source Biosciences, Cambridge, UK). Sequences were deposited in the public data base NCBI/GenBank (www.ncbi.nlm.gov).

Alignments and phylogenetic analyses. Sequences of the three plastid-encoded genes *psa*A, *psb*C, and *rbc*L of our three strains were aligned using MAFFT (Katoh and Standley 2013) with homologous sequences of representatives of most of the recognised orders of brown algae (Bringloe et al. 2020; Guiry and Guiry 2024), with *Schizocladia ischiensis* (Schizocladiophyceae) as outgroup. Phylogenetic reconstructions were performed with RaXML version 8.2.11, utilizing 1000 bootstrap replications (Stamatakis 2014), as well as with MrBayes version 3.2.6 under default settings with four MCMC chains (Ronquist et al. 2012). These analyses were carried out in Geneious v11 (Kearse et al. 2012), with each gene being analyzed individually, followed by analysis of the combined alignments.

3 Results

3.1 Morphology in culture and new species descriptions

Verosphacela ebrachia from Greece. Filaments were approximately 20 μm in diameter, new cells being added by transverse divisions of an apical cell of 20–60 μm in length. Subterminal cells – frequently the second subterminal cell – divided longitudinally to render the filament biseriate (Figure 2). Branches, often issued at right angles, could have the same diameter (not shown) or less (Figure 3), and they remained creeping and attached to the substratum, or they grew upright into the space. The thinner laterals produced either phaeophycean hairs (Figure 4) or propagules, which were formed on a weakly pigmented pedicel, on the sides of which scars testified to previous propagules formed on the same pedicel (Figure 5). Propagules divided once laterally; the basal cell subsequently remained undivided, while the upper cell underwent one or two transverse divisions, with the resulting 2–3 cells dividing once or twice longitudinally or remaining undivided. Released propagules consisted of 4–13 cells (Figures 6 and 7). The propagules sedimented to the bottom of the dish, to which they did not adhere. Germination of propagules was usually from the basal cell, but any upper cell could also form a filament, the resulting thallus presenting the same morphology as the parent thallus.



Figures 8–13: *Verosphacela henryi* sp. nov. from Rapa Nui, culture: (8) vegetative filament, cells contain discoid plastids (arrow), apical cell discernible albeit out of focus (arrowhead); (9) longitudinal divisions in filament (arrow); (10) laterals disposed at approximately right angles; (11) phaeophycean hair, arrowhead pointing to basal meristematic region; (12) immature propagule at 1-cell stage, with the scars (arrowheads) of 5 previous propagules formed on the same stalk cell; (13) released propagule consisting of four body cells and a basal cell (arrowhead).

Reproductive structures (unilocular or plurilocular sporangia) were absent in our cultures, and filaments showing groups of four cells, resulting from two consecutive longitudinal divisions, were not seen either. The strain, original code GR11-s#8-3, was deposited as CCAP 1336/1, and a permanent mount of this culture as herbarium specimen (PC0643630).

Verosphacela henryi L.A. Muñoz, L. Ridehalgh, F.C. Küpper *et* A.F. Peters sp. nov.

Type locality: Submarine cave at 7 m depth, Cathedral, Rapa Nui, 27.11°S, 109.424°W, leg. F.C. Küpper, 29 April 2014 (holotype PC0643626, isotype PC0643627). Sequence accessions: nrSSU PQ571205, *psa*A PQ576428, *psb*C PQ576431, *rbc*L-Rubisco spacer-*rbc*S PQ655435. Authentic strain, original code IP14-27, deposited as CCAP 1336/2.

Etymology: The specific epithet honours Eric C. Henry, acknowledging his contributions to phycology, which included description of the genus *Verosphacela*.

Description: Microscopic brown alga, which emerged in culture from field-collected abiotic substratum. Thalli are composed of branched creeping filaments, their growth being achieved by transverse divisions of a meristematic apical cell. Filament cells 40–50 µm in length and 20 µm in diameter, with multiple discoid plastids devoid of pyrenoids (Figure 8). Filament cells divided once longitudinally, the resulting two cells not increasing in width (i.e. 10 µm; Figure 9). Branching of filaments usually at right angles (Figure 10). Phaeophycean hairs present but rare, with a basal meristem and a thin basal sheath, terminating few-celled thinner filaments (Figure 11). Propagules formed terminally on little pigmented pedicels of 10 µm width, scars showing that several propagules could be released consecutively from the same pedicel (Figure 12). Propagules with an undivided basal cell and up to 4 body cells (Figure 13), heavier than sea water and not adhering to Petri dish. Germination of propagules from each upper cell and usually not from the basal cell (not shown), leading to the development of the same kind of thallus as the parent. Reproductive structures (unilocular or plurilocular sporangia) not observed in our cultures.

Verosphacela asensii L.A. Muñoz, L. Ridehalgh, F.C. Küpper *et* A.F. Peters sp. nov.

Type locality: 23 m depth, off White Island, Ascension Island, 7.895°S, 14.395°W, leg. F.C. Küpper, 11 February 2011 (holotype PC0643628, isotype PC0643629). Sequence accessions: nrSSU PQ571206, *psa*A PQ576429, *psb*C PQ576432, *rbc*L-Rubisco spacer-*rbc*S PQ655436. Authentic strain ASC11-17 deposited as CCAP 1336/3.

Etymology: The specific epithet honours Aldo O. Asensi, acknowledging his contributions to phycology.

Description: Microscopic brown alga, which emerged in culture from field-collected abiotic substratum. Thallus composed of branched creeping filaments (Figure 14), the growth of which is achieved by transverse divisions of a meristematic apical cell of up to 80 µm in length (Figures 14 and 15). Filament cells 40–50 µm in length and 20 µm in diameter, with multiple discoid plastids devoid of pyrenoids. Subterminal cells divided once longitudinally (Figure 15). Branching leading to laterals of the same morphology

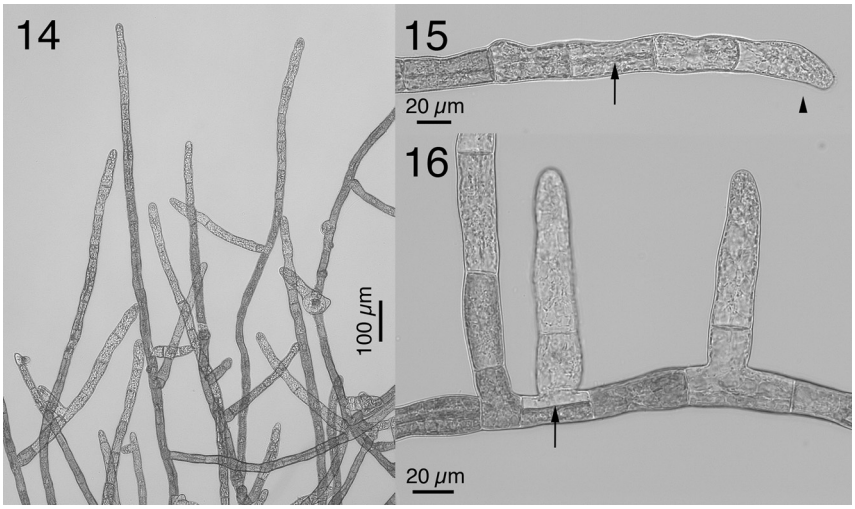


Figure 14–16: *Verosphacela asensii* sp. nov. from Ascension Island, culture: (14) general aspect of thallus; (15) tip of filament with apical cell (arrowhead) and longitudinal division in second subterminal cell (arrow); (16) laterals disposed at approximately right angles, arrow pointing to longitudinal division.

(Figure 16), either creeping or erect; thinner laterals, hairs, propagules or reproductive structures not known.

Both *V. henryi* and *V. asensii* were obtained only once in culture, emerging from substratum collected at their respective type localities. The propagules of *V. henryi* and of our strain of *Verosphacela* from Greece were formed in all culture conditions and seasons.

3.2 DNA sequence analyses

For a first tentative positioning of the *Verosphacela* strain from Greece and the two isolates from Rapa Nui and Ascension Island, we generated 526 bp in the 3′-end of the nrSSU sequence, which included three loops supposed to be slightly more variable than the highly conserved stem and root sectors. For 439 bp, a homologous sequence was available for *O. endophytica* but none has so far been published for *V. ebrachia*. The sequences of our strains were closest to *O. endophytica* and to members of the Sphacelariales (*Heribaudiella, Halopteris, Bodanella, Cladostephus*), with genetic identities between 98.41 and 98.63 % (details not shown), pointing to a systematic position in the subclass Dictyotophycidae (Guiry and Guiry 2024), which is also referred to as SSDO clade (Bringloe et al. 2020) because it comprises the orders Sphacelariales, Syringodermatales, Dictyotales, and Onslowiales (cf. Figure 17). All members of these orders show growth by means of meristematic apical cells.

For our strain of *Verosphacela* from Greece, sequences of *psa*A (1566 nt), *psb*C (1343 nt), and a small part of *rbc*L (199 nt at the 3′-end) were highly similar (identities of 99.33, 99.92, and 100 %, respectively, Supplementary Table S1) to the published homologous sequences of *V. ebrachia* from Florida. The Rubisco spacer, which is a sector known to evolve faster than coding genes, of the strain from Greece had the same length (143 nt) as in *V. ebrachia* from Florida, with a single substitution, i.e. it had 99.3 % identity (Supplementary Table S1, Supplementary Figure S1).

Sequences of the two strains from Rapa Nui and Ascension Island were different from *V. ebrachia* GR11-s#8-3 in SSU (Supplementary Table S3) and in *psa*A, *psb*C and *rbc*L (Supplementary Table S1). The two strains from Rapa Nui and Ascension Island were also different from each other, although to a lower degree (Supplementary Tables S3 and S4). In molecular phylogenies of the brown algae based on sequences of these three coding markers, either single or concatenated, the two strains from Rapa Nui and Ascension Island consistently clustered with high statistical support with each other and with *V. ebrachia* (Figure 17).

4 Discussion

There exists a large undetected biodiversity of small organisms like marine algae, in particular from the less studied parts of the globe and from ecosystems difficult to sample (De Vargas et al. 2015; Mora et al. 2011). All species of the Onslowiales so far described are small algae of the sciaphilic community (growing in shadow; Table 1), for which the classical approach of identification requires trained phycologists who take their time to meticulously study field-collected algae (Alongi et al. 2007; Henry 1987a,b; Searles and Leister 1980).

Raising algae in culture from substratum collected in the field (e.g., Hajiya Hasan et al. 2023; Peters et al. 2024; Rizouli et al. 2020) may likewise generate isolates of small species difficult to detect in nature, and can provide more than just DNA from environmental samples. Our previous isolate of

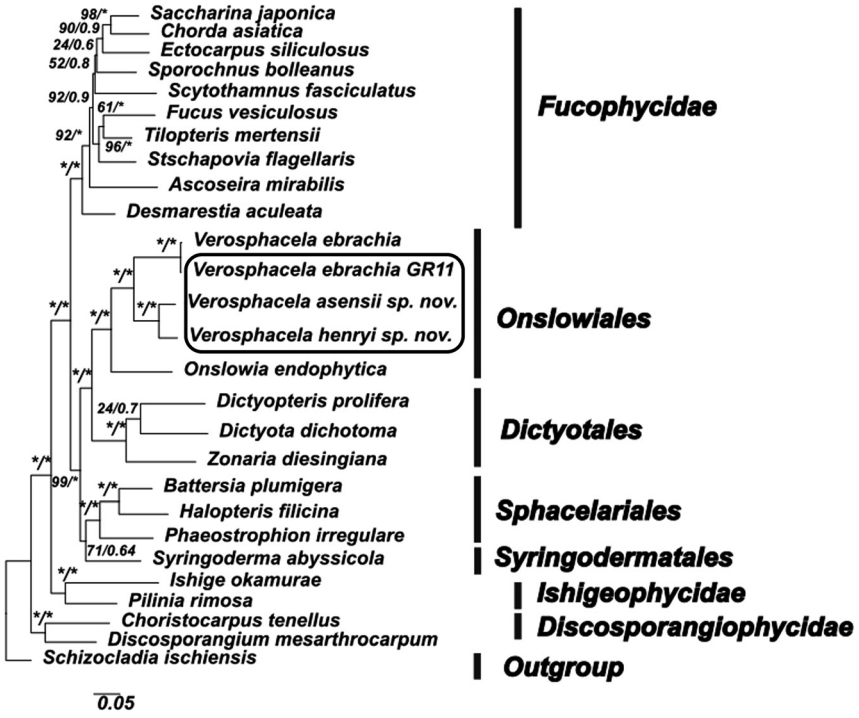


Figure 17: Position of the strains studied in the present paper (rectangle) in a phylogenetic tree of the Phaeophyceae based on concatenated *psa*A, *psb*C and *rbc*L sequences (4,844 bp), *Schizocladia ischiensis* serving as outgroup, from Bayesian inference (BI) analysis. The tree from maximum likelihood (ML; not shown) had essentially the same topology. Support values from ML (bootstrap percentages, bp, from 1000 bootstraps) and BI posterior probabilities (pp) are provided at the nodes (MLbp/BIpp), a star (*) indicating full support. The scale represents the number of substitutions per site. The strains studied in the present paper cluster with *Verosphacela* in the order Onslowiales, which in turn is associated in a clade with the Dictyotales, Sphacelariales and Syringodermatales. Subclasses, provided for more distant taxa representing the majority of brown algal orders, are named according to Guiry and Guiry (2024).

Verosphacela obtained in this way from the eastern Mediterranean (Peters et al. 2015) agreed morphologically with both *V. ebrachia* from Florida (Henry 1987b) and *V. silvae* from Italy (Alongi et al. 2007), however, we did not see tetraseriate filament sectors, which were described for *V. ebrachia* (Henry 1987b) and which may be difficult to spot in the light microscope, or any sporangia. The present molecular-assisted identification based on *psa*A, *psb*C and *rbc*L of our Greek strain was therefore decisive because the sequences were highly similar (99.33–100 %, Supplementary Table S1) to published homologous sequences of *V. ebrachia* from the type locality. Molecular data do not exist for *V. silvae* from Italy.

In the absence of comparable sequences, we had previously identified our strain of *Verosphacela* from Greece as *V. silvae* because of geographic proximity (Peters et al. 2015). According to Alongi et al. (2007), *V. silvae*, described from the central Mediterranean based on field material, showed minor morphological differences to *V. ebrachia* from Florida (Henry 1987b). Sporangia were borne terminally on laterals instead of sessile on laterals and main axes, and propagules, composed of seven cells instead of 9–13 in *V. ebrachia*, were formed on three-celled pedicels instead of a single-celled pedicel in *V. ebrachia*. Our strain of *V. ebrachia* from Greece showed more variation in the number of cells per propagule than in the original description of the species (Table 1), and the three-celled pedicels reported for *V. silvae* may have been a misinterpretation of the scars of propagules formed previously on the same pedicel (compare our Figure 5 with Figure 7 in Alongi et al. 2007). Both morphology and sequences thus suggest that *V. silvae* may not be significantly different from *V. ebrachia*. Recollection of material of *V. silvae* in the central Mediterranean, preferably at the type locality, and the generation of molecular data for *V. silvae* would clarify the situation. In any case, the geographic distribution of *V. ebrachia*, previously restricted to the NW Atlantic, now also includes the Mediterranean.

Two isolates from Rapa Nui and Ascension Island, also obtained via germling emergence, presented multiple discoid plastids without pyrenoids, a branched filamentous morphology with occasional longitudinal divisions, and growth by means of a meristematic apical cell, suggesting that they could also belong to the Onslowiales. In culture, the strain from Rapa Nui formed propagules devoid of apical cells, resembling those of *V. ebrachia* (Figures 12 and 13), whilst the strain from Ascension Island remained vegetative (Figures 14–16). Molecular data from *psa*A, *psb*C, and *rbc*L, and from the highly variable *rbc*L-*rbc*S intergenic spacer consistently showed that the two isolates from RapaNui and Ascension represent so far unknown species of the Onslowiales, forming a clade that clustered with *V. ebrachia* (Figure 17).

Because of the similarity of the thallus morphology and of the propagules with homologous structures of *V. ebrachia*, we

propose accommodation of the isolate from Rapa Nui in *Verosphacela*. The clustering of the isolate from Ascension Island with that from Rapa Nui (Figure 17) consequently makes us classify it likewise in *Verosphacela* despite the lack of propagules in our culture. However, the isolate from Ascension Island does not belong to the same species as the Rapa Nui strain because the two are too different genetically. They are different in the SSU (Supplementary Table S3), have Rubisco spacer sequences that are difficult to align and have different lengths (Supplementary Figure S1, Supplementary Table S1), and show genetic distances of 3.5–6.5 % in the coding genes (Supplementary Table S4). In brown algae, SSU sequences do not show differences between individuals of the same species (e.g. data for *Desmarestia* spp. in Yang et al. 2014), and Rubisco spacer sequences show very small intraspecific variation and are thus perfectly alignable (Rizouli et al. 2020; Stache-Crain et al. 1997); in the coding genes we used, intraspecific genetic distances are usually <1 % (Cho et al. 2004; Rizouli et al. 2020; Yang et al. 2014), also exemplified by our data for *V. ebrachia* from two geographically distant isolates (Supplementary Table S1). In conclusion, we propose *V. henryi* sp. nov. and *V. asensii* sp. nov. for the isolates from Rapa Nui and Ascension Island, respectively.

Sexuality and a haploid-diploid life history of morphologically similar generations were described in *O. bahamensis* showing that this complete life history is principally present in members of the order (Henry 1987b). However, our strains of *V. ebrachia* and *V. henryi* did only show direct replication by propagules, despite varying the temperatures between 15 and 25 °C, whereas unilocular and plurilocular sporangia were described for *V. ebrachia* from Florida (Henry 1987b) and *V. silvae* from Italy (Alongi et al. 2007). It is possible that our strains require particular combinations of abiotic and biotic conditions for the formation of reproductive structures. Similarly, our strain of *V. asensii*, which remained vegetative in our cultures, may need special cues to become fertile or produce propagules.

In *O. bahamensis*, only the sporophytes produced propagules in culture (Henry 1987b); it is possible that our strain of *V. asensii* is a gametophyte in a haploid-diploid life history. It is a shortcoming of the germling emergence technique that, in algae with a more complex life history, it may provide only one generation and sex. For instance, in a previous report of *Phrix spatulata* (E.Y. Dawson) M.J. Wynne, M. Kamiya *et* J.A. West, a red alga isolated from the same substratum as *V. henryi*, we isolated only a male gametophyte (Muñoz et al. 2018).

In field-collected algae, species of the Onslowiales were usually found as endo-epiphytes of macroalgae (Table 1). The germling emergence technique, with small pebbles and sand grains as initial substratum, does not provide such ecological information; close scrutiny of macroalgae from the sublittoral of Rapa Nui and Ascension Island could reveal that possibly *V. henryi* and *V. asensii* in nature also grow in association with host species.

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