

*PSEUDOCODIUM MUCRONATUM*, A NEW SPECIES FROM NEW CALEDONIA,  
AND AN ANALYSIS OF THE EVOLUTION OF CLIMATIC PREFERENCES  
IN THE GENUS (BRYOPSIDALES, CHLOROPHYTA)<sup>1</sup>

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A new species, *Pseudocodium mucronatum*, is described from the Chesterfield platform off the west coast of New Caledonia. The species differs from its congeners in having mucronate utricles. A phylogenetic analysis of *rbcL* and *tufA* sequences showed that *P. mucronatum* is most closely associated with *P. natalense* De Clerck, Coppejans et Verbruggen and *P. devriesii* Weber Bosse, with which it shares compressed axes, depressed apices, and plastids in the utricles and the medullar siphons. We studied the evolution of climatic and ecological preferences in the genus using an interdisciplinary approach consisting of relaxed molecular clock analysis, extraction of macroecological data from satellite imagery in a geographic information system (GIS) framework, and ancestral character state estimation. It was shown that the genus originated in tropical waters during the Early Mesozoic. Whereas the *P. floridanum-okinawense* lineage remained tropical, the lineage including *P. natalense*, *P. devriesii*, and *P. mucronatum* gradually invaded more temperate waters during Cenozoic times. Except for *P. devriesii*, which occurs in shallow and intertidal habitats, all *Pseudocodium* species grow in deep-water habitats, and this ecological preference appears to be ancestral.

**Key index words:** Bryopsidales; climatic affinities; evolution; macroecology; molecular phylogenetics; morphology; *Pseudocodium mucronatum*; taxonomy

**Abbreviations:** AIC, Akaike information criterion; GIS, geographic information system; GTR, general time reversible; ML, maximum likelihood; SST, sea-surface temperature

*Pseudocodium* is a rare green algal genus occurring in tropical and subtropical marine habitats. Like other Bryopsidales, it has a siphonous thallus struc-

ture, meaning that it consists of a single giant tubular cell (Vroom and Smith 2003). This tubular cell constricts and expands, branches, and adheres to form the macroscopic thallus. Individual branches of the tubular cell are called siphons. *Pseudocodium* species have bright green dichotomously branching thalli of between 1 and ~10 cm long. The internal structure of a *Pseudocodium* thallus consists of a medulla of a few interwoven siphons and a cortex composed of inflated siphons called utricles. The utricles adhere to one another laterally.

The genus was described over a century ago for the South African intertidal species *P. devriesii* (Weber-van Bosse 1896), but the remainder of the species was not discovered until the exploration of deeper waters using scuba diving and submarines (Dawes and Mathieson 1972, De Clerck et al. 2008, Faye et al. 2008). There are presently five recognized *Pseudocodium* species: *P. devriesii*, *P. australasicum*, *P. floridanum*, *P. okinawense*, and *P. natalense*. However, the strongly deviant anatomy of *P. australasicum* indicates that it is not a genuine member of the genus (De Clerck et al. 2008).

The phylogenetic affinities of the genus have been debated extensively in the past, some authors hypothesizing that *Pseudocodium* was closely related to the morphologically similar genus *Codium* (Gepp and Gepp 1911, Dawes and Mathieson 1972, Vroom et al. 1998), others postulating a relationship with *Halimeda* based on similar anatomical structure (Weber-van Bosse 1896, Feldmann 1946). DNA sequence analysis has firmly established the position of *Pseudocodium* in the suborder Halimedineae, proving that the morphological similarity to *Codium* resulted from convergent evolution (Lam and Zechman 2006). In some cases, the convergent evolution is striking, such as in the case of *Codium reversum* (Kraft 2007). Nonetheless, the presence of amyloplasts, the absence of utricle hairs and pit plugs in medullar siphons, and the lateral cohesion of utricles allow unambiguous identification of *Pseudocodium*.

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The phylogenetic relationships among *Pseudocodium* species have been studied by De Clerck et al. (2008). These authors showed the existence of a tropical clade containing *P. floridanum* and *P. okinawense*, and a subtropical clade containing *P. natalense* and *P. devriesii*, and identified a suite of morphological characters that correlated with the phylogenetic relationships.

Recent collections of a taxon from the Chesterfield platform off the west coast of New Caledonia that belongs to *Pseudocodium* but could not be identified at the species level invited us to explore the *Pseudocodium* diversity of New Caledonia in more detail. The first goal of this study was to assess the identity of several New Caledonian collections. The second goal was to infer the evolution of climatic affinities in the genus. DNA sequence data are presented for two New Caledonian species, including a new species, and the phylogenetic relationships among *Pseudocodium* species are discussed. The evolution of climatic preference was studied using an interdisciplinary approach consisting of relaxed molecular clock analysis, extraction of macroecological data from satellite imagery in a GIS framework, and ancestral character state estimation.

#### MATERIALS AND METHODS

**Study sites.** Collections were made by SCUBA in two different areas belonging to New Caledonia. The Loyalty Islands and Bellona-Chesterfield platform are located in the eastern and western part off the main island (Grande-Terre), respectively. Fresh samples were pressed onto herbarium sheets, and subsamples were in 5% formalin-seawater preserved for morphological study and in silica gel for molecular analysis. Voucher specimens were deposited in the herbaria of PC and NOU-IRD (Phycological Herbarium, Institut de Recherche pour le Développement, Nouméa, New Caledonia). Herbarium abbreviations are in accordance with Holmgren et al. (1990).

**Morphological examination.** Photomicrographs were obtained using an Olympus BH2 compound microscope fitted with an Olympus C-5050 digital camera (Olympus Optical Co. Ltd., Tokyo, Japan). Drawings were made using a microscope with a camera-lucida attachment, and herbarium specimens were scanned.

**Molecular phylogenetics.** DNA was extracted from silica-gel-preserved samples of the specimens from New Caledonia and a selection of specimens belonging to other *Pseudocodium* species. DNA extraction, amplification, and sequencing of the *tufA* and *rbcl* genes followed standard protocols (De Clerck et al. 2008). The new *tufA* and *rbcl* sequences were added to alignments of previously published sequences (Table S2 in the supplementary material). Following concatenation of the *rbcl* and *tufA* data sets, an appropriate model of sequence evolution was determined using the Akaike information criterion (AIC; Posada and Buckley 2004), as implemented in the MrAIC.pl program (Nylander 2004).

Phylogenetic inference was carried out with maximum likelihood (ML) and Bayesian phylogenetic inference. ML phylogenetic analysis was performed with PhyML (Guindon and Gascuel 2003), using the model suggested by the AIC and starting from a BioNJ tree (Gascuel 1997). One thousand nonparametric bootstrap replicates were computed to assess statistical branch support (Felsenstein 1985). A Bayesian phylogenetic analysis was carried out with MrBayes v.3.1.2

(Ronquist and Huelsenbeck 2003). Two independent Markov chain Monte Carlo (MCMC) runs, each consisting of four incrementally heated chains, were run for 2 million generations with default priors, chain temperature increments, and other settings. Convergence of the runs was checked visually with Tracer v.1.4 (Rambaut and Drummond 2007), and an appropriate burn-in was determined with the automated method proposed by Beiko et al. (2006). A majority-rule consensus tree was calculated from the post-burn-in trees with MrBayes' sumt command. Sequences of the related genera *Caulerpa*, *Halimeda*, and *Tydemania* were used to root the trees.

**Evolution of climatic preferences.** We performed several analyses to study the evolution of climatic preferences in a phylogenetic and temporal time frame. First, relaxed molecular clock techniques were applied to calibrate the phylogenetic tree in absolute time. Second, a data set of verified species occurrence records of *Pseudocodium* was compiled. Third, a macroecological (climatic) data set was derived from satellite imagery. Fourth, the climatic preferences of species were extracted using GIS techniques and analyzed in a phylogenetic framework.

Relaxed molecular clock methods smooth out differences in rates of molecular evolution among lineages by assuming that rates of molecular evolution change gradually throughout the phylogeny. The result is a chronogram (chronometric phylogram) in which branch lengths are proportional to evolutionary time instead of amounts of molecular evolution. We used PhyloBayes (Lartillot et al. 2007) to infer ages of the nodes in the ML tree using a relaxed molecular clock model that assumes that rates of molecular evolution evolve according to the Brownian motion model (Thorne et al. 1998). An MCMC of 50,000 generations was carried out, sampling every 100th generation. A general-time-reversible (GTR) +  $\Gamma_4$  model of sequence evolution was used. Because *Pseudocodium* does not have a trustworthy fossil record (see Discussion), we used node ages inferred in a previous relaxed molecular clock analysis of the Bryopsidales and Dasycladales (Verbruggen et al. 2009) to calibrate the chronogram in time. Four nodes in the tree for which the ages had been previously inferred were constrained. These nodes are indicated with letters in the phylogenetic tree (see Results) and were constrained as follows: (a) 355–254 million years ago (mya), (b) 327–233 mya, (c) 273–170 mya, and (d) 165–81 mya.

In order to obtain an estimate of climatic affinities of the five *Pseudocodium* species under study, two geographically explicit data sets were generated. The first is a vector GIS file containing the geographic coordinates of localities where *Pseudocodium* species have been found (all collections are vouchered in herbariums). The second data set is a raster GIS file containing annual mean sea surface temperature (SST) information derived from Aqua MODIS satellite imagery. The procedures used to generate this data set are specified in Verbruggen et al. (2009). Annual mean SST data were extracted for each collecting locality with DIVA GIS (Hijmans et al. 2001). Subsequently, an average SST value was calculated for each species. To reduce the effect of geographical sampling bias, weighted averaging was used. The Cartesian distance from the sample point to the center of gravity of the species was used as the weight. The center of gravity was determined by averaging the Cartesian coordinates of the sample points. It is known that tropical *Pseudocodium* species mainly occur on fore-reef slopes at depths ranging from 25 to 80 m. Even though the temperature data we used applies to surface waters, it can be used as a proxy for the temperature experienced by the algae because it has been shown that temperature declines very slowly with depth in tropical seas until below 100 m, where temperature drops more steeply (Littler et al. 1985, Colin et al. 1986).

Climatic affinities of ancestral nodes were estimated using ML optimization (Schluter et al. 1997) implemented in the

APE package (Paradis et al. 2004). This method assumes that the trait being analyzed evolves along the phylogenetic tree in a Brownian motion fashion. We used the time-calibrated phylogeny for the optimization. The evolution of SST affinities was visualized on the chronogram with TreeGradients v.1.03 (Verbruggen 2008).

#### RESULTS

*Pseudocodium mucronatum* Payri et Verbruggen sp. nov.

A speciebus congeneribus differt utriculis mucronatis.

Differs from congeneric species in having mucronate utricles.

*Holotype*: PC0062775 (New Caledonia, Chesterfield atoll, 19°58.305 S, 158°27.292 E, at 30 m in the lagoon, C. E. Payri, 8 July 2008).

*Specimens examined*: CH08-270 a-c (NOU-IRD-A) (New Caledonia, Chesterfield atoll, 19°58.305 S, 158°27.292 E, 30 m, C. E. Payri & J. L. Menou, 8 July 2008).

*Distribution and habitat*: The species is only known from the Chesterfield atoll (New Caledonia), where it is confined to deep water (30 m) in the lagoonal part. The species has been collected in only one site and was locally abundant, growing on coarse sand and coral debris at the bottom of coral pinnacles.

*Habit and anatomy*: Plants erect, olive green in color when fresh, becoming darker when dried. Axes translucent at the periphery, showing a more colored medullar part when fresh. Several plants arising from a developed filamentous base anchored in coarse sandy substratum, up to 8 cm high, consisting of complanate unstricted axes, (1.5) 2–3 (4) mm wide and 1 mm thick (Fig. 1a). Axes dichotomous to irregular branched up to four times, slightly tapering or not toward the apices; apices truncate with a depressed apical pit (Fig. 2c), sometimes showing evidence of grazing. Thallus composed of longitudinally arranged interwoven and irregularly swollen medullar siphons (65–108  $\mu$ m wide) giving rise to a single peripheral layer of conspicuously mucronate utricles, giving the surface thallus a roughened (spiky) aspect (Fig. 2a). Utricles polygonal in surface view (92–) 100–142 (152)  $\mu$ m diameter (Figs. 2b and 3c), subclavate in lateral view, 120–186  $\mu$ m wide and 200–335  $\mu$ m high with a flat to round apex bearing a distinctive mucron (Figs. 2, a and b, and 3, a and b); adhering laterally to neighboring utricles; utricule stipe central (not eccentric), 1.5 to 3 times the length of the swollen utricule part, often lightly swelling at the basal part. Plastids present in the utricles and medullar siphons. Reproductive structures immersed in the thallus, produced laterally from the medullar siphon, close to the base of utricule stipe, mostly spherical, 140–170  $\mu$ m in diameter, lacking a basal cross wall separating it from the rest of the thallus (Figs. 2, d and e; 3, d and e).

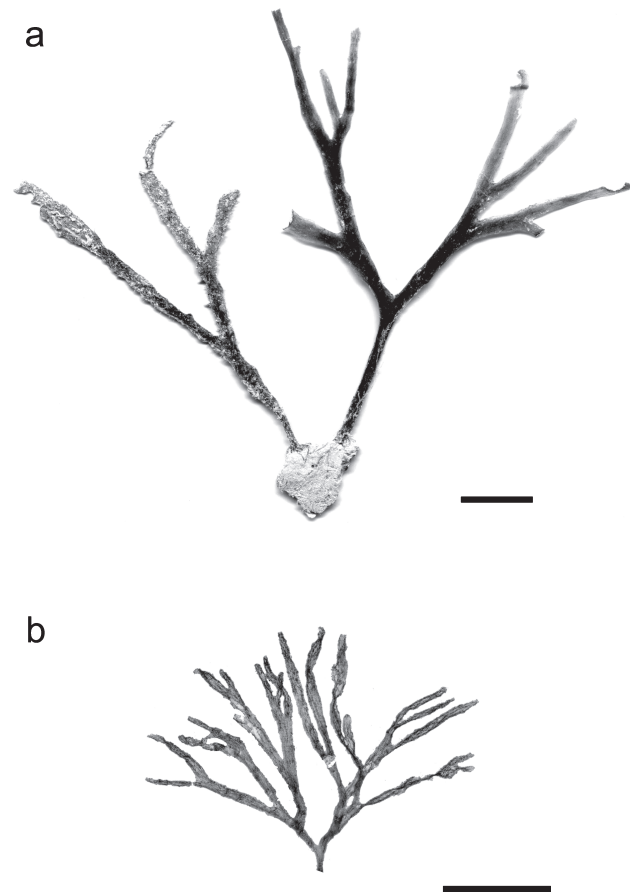


FIG. 1. Habit of (a) *Pseudocodium mucronatum* bars (holotype PC0062775) and (b) *P. okinawense*. Scale bars = 1 cm.

*Pseudocodium okinawense* E. J. Faye, M. Uchimura et Shimada, 2008: 105.

*Specimens examined*: NC05-394b (NOU-IRD-A) (New Caledonia, Loyalty Islands, Lifou, 20°53.517 S- 167°16.437 E, at 45 m on outer reef slope, C. E. Payri, 24 March 2005).

*Distribution and habitat*: The species is uncommon in New Caledonia; it has been collected at a single site in Lifou, Baie de Chateaubriand (Loyalty Islands), in a relatively deep habitat (45 m) growing on coarse sand and coral debris on the outer reef slope.

*Habit and anatomy*: Plants erect, bright green in color, arising from filamentous base anchored in coarse sandy substratum, up to 2.0 cm high, consisting of slightly complanate unstricted axes up to 1.0 mm wide (Fig. 1b). Axes mostly dichotomously branched up to 4 (–5) times, slightly tapering toward the apices; apices round. Thallus composed of longitudinally arranged interwoven and irregularly swollen medullar siphons (25–95  $\mu$ m wide) giving rise to a single peripheral layer of rounded utricles (Fig. 4a). Utricles polygonal to roundish in surface view (90–) 132 (–150)  $\mu$ m diameter (Fig. 4b), isodiametric in transversal view, 120–186  $\mu$ m wide and (106–) 144 (–173)  $\mu$ m high with a round apex;

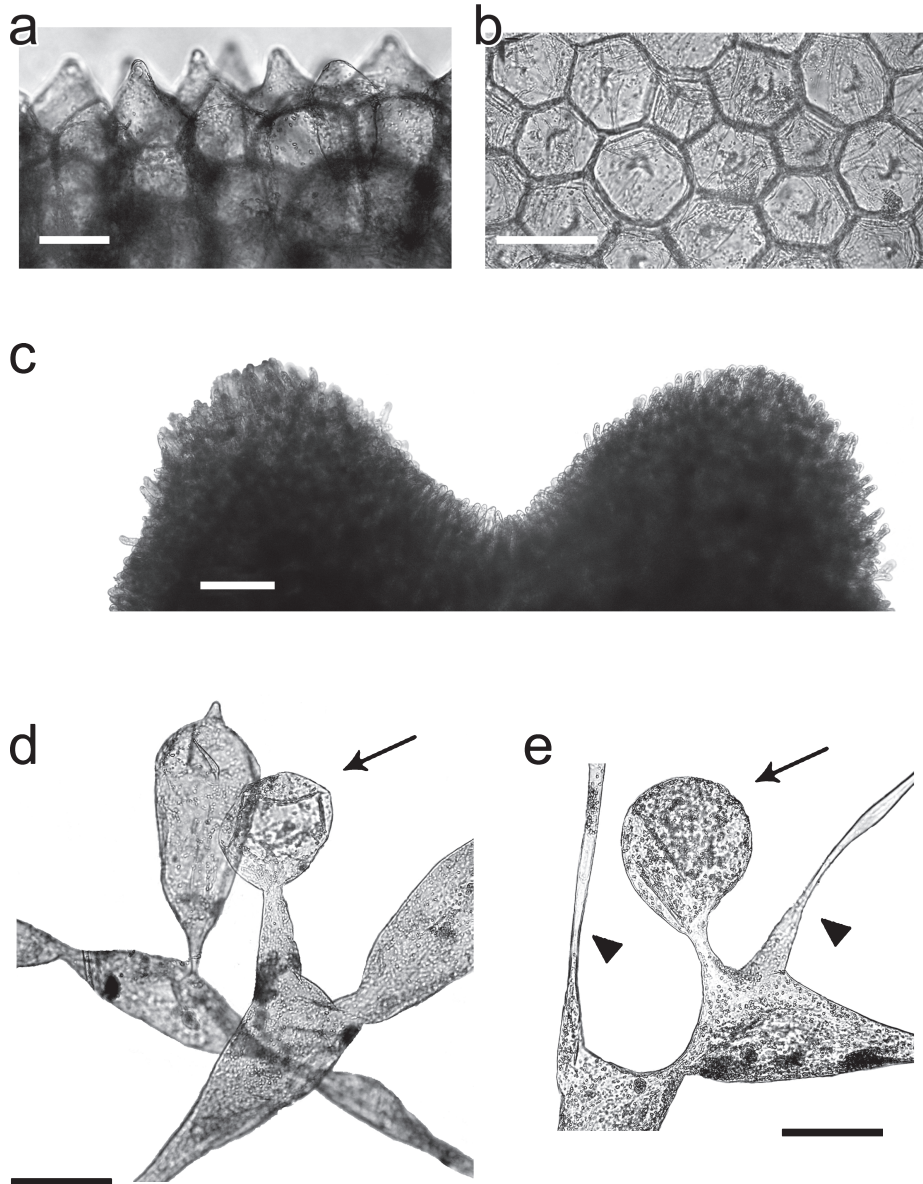


FIG. 2. Morphology of *Pseudocodium mucronatum*. (a) Microphotographs of mucronate utricles in lateral view; (b) microphotographs of polygonal utricles in surface view showing lateral adherence; (c) morphology of depressed apex; (d) microphotographs of utricles and reproductive structure (arrow) in lateral view; (e) microphotographs of reproductive structure (arrow) in lateral view, developing near the base of the utricule stipe (arrowhead). Scale bars: (a, b, d, and e) = 100  $\mu$ m; (c) = 200  $\mu$ m.

adhering laterally to neighboring utricles except at the corners; utricule stipe not eccentric, long, 1 to 1.5 times the length of the swollen utricule part (Fig. 4a). Plastids present mostly in medullar siphons. Reproductive structures not observed.

**Molecular phylogenetics:** The *tufA* alignment included 17 sequences of 905 bases long and the *rbcL* alignment 12 sequences of 1,335 bases long. The concatenated data set used for phylogenetic analysis included 18 specimens and 2,240 characters. The model selection procedure selected a GTR +  $\Gamma_4$  model (Table 1). The Bayesian analysis reached a likelihood plateau very rapidly, and

parameter estimates were consistent among runs. The burn-in was determined at 92,000 generations. The ML tree was identical to the Bayesian consensus tree, and we present only the former with branch support values from both analyses.

The phylogenetic tree (Fig. 5) shows the presence of five clearly delimited species clusters. The New Caledonian specimen G.221 (first black box) clusters tightly with the Japanese specimen of *P. okinawense*, confirming our morphological identification. Specimen G.220 (second black box) forms a separate lineage distinct from the presently recognized species of the genus, validating our recognition of this entity as

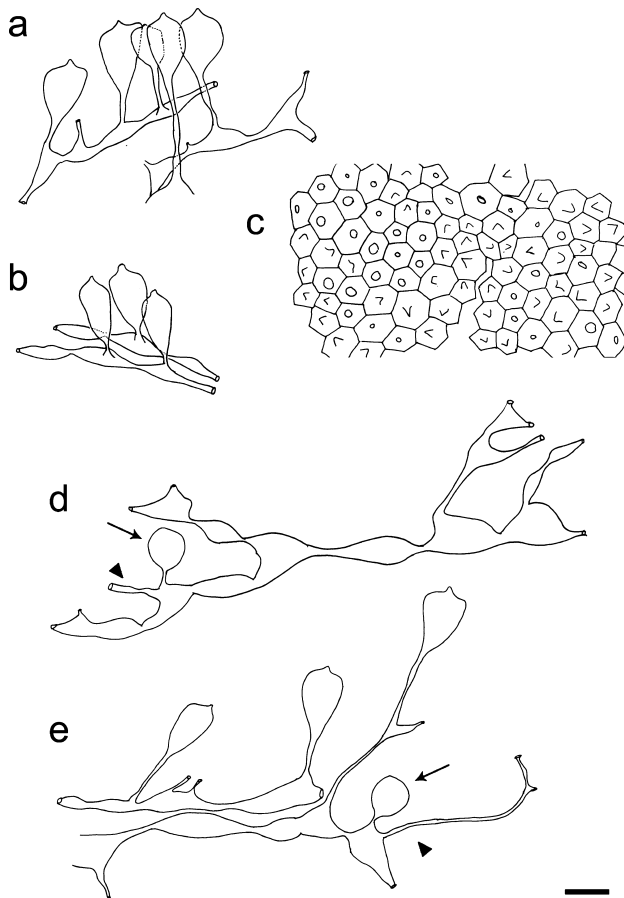


FIG. 3. Morphology of *Pseudocodium mucronatum*. (a, b) Cortex with mucronate utricles in lateral view; (c) surface view of polygonal utricule; (d) medullary siphon with roundish reproductive structure (arrow); (e) both utricles (mucronate) and reproductive structure (arrow). In (d, e), reproductive structures are developing near the base of the utricule stipes (arrowhead). Scale bar = 160  $\mu$ m.

a new species. Phylogenetic relationships among the species are strongly supported by ML bootstrap values as well as Bayesian posterior probabilities (Fig. 5). A first clade contains *P. okinawense* and *P. floridanum*. The new species *P. mucronatum* is sister to the clade comprising *P. natalense* and *P. devriesii*.

TABLE 1. Model selection using the Akaike information criterion (AIC).

Rate matrix	Among-site rate heterogeneity parameters			
	None	+I	+ $\Gamma_4$	+I+ $\Gamma_4$
JC69	1061.5	630.6	422.8	424.8
F81	896.2	416.5	192.4	194.4
K2P	1022.6	588.3	375.9	377.9
HKY	827.5	305.7	59.6	61.6
SYM	757.7	399.5	209.3	211.3
GTR	645.4	224.9	0.0	2.0

The listed values represent differences in AIC scores of a variety of models relative to the best scoring model (GTR +  $\Gamma_4$ ), which had an AIC score of 17,178.3. Lower values indicate a better fit of the model to the data. GTR, general time reversible.

*Evolution of ecological preferences:* A data set of the geographic origin of 49 vouchered *Pseudocodium* specimens resulted in 34 unique collecting localities (Table S1 in the supplementary material) for which annual mean SSTs were derived from Aqua MODIS satellite data (Fig. 6a).

Relaxed molecular clock analyses revealed *Pseudocodium* to have originated in the early Mesozoic and to have diversified into its extant lineages during the late Mesozoic and Cenozoic (Fig. 6b). The lineage leading to the new species *P. mucronatum* diverged from its sister lineage (*P. natalense* and *P. devriesii*) during the Eocene: 44 mya (95% CI: 58–32 mya). The nodes that were constrained to calibrate the tree in time are indicated by the letters a–d in Figure 6b (see also Materials and Methods).

Estimated ancestral states for climatic preference (SST) indicate a tropical origin of the genus: 25.7°C (95% CI: 23.6°C–27.7°C) (Fig. 6c). The *P. okinawense*–*floridanum* lineage appears to have remained in tropical habitats throughout its evolutionary history. A single, apparently gradual invasion of subtropical waters has occurred relatively recently in the *P. natalense*–*devriesii* clade. Whereas *P. natalense* occurs on subtropical coral reefs (weighted average SST = 24.7°C), *P. devriesii* grows in colder water (weighted average SST = 22.7°C). The invasion of

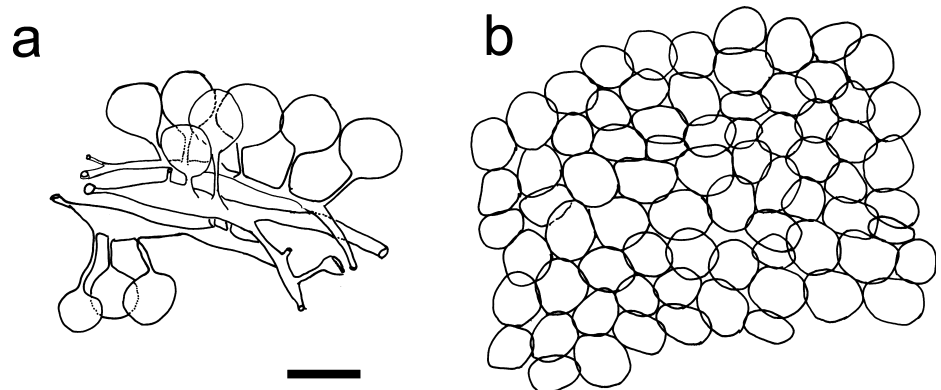


FIG. 4. *Pseudocodium okinawense*. (a) Cortex with roundish to oval utricles in lateral view; (b) surface view of utricles. Scale bar = 160  $\mu$ m.

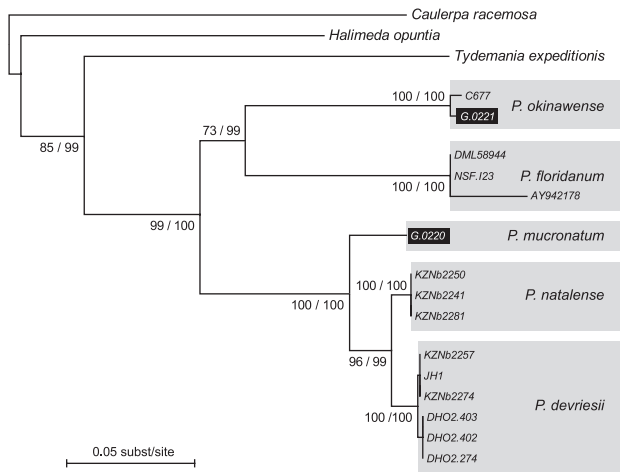


FIG. 5. Phylogenetic tree of *Pseudocodium*, including the new species *P. mucronatum*. The phylogram was inferred from an alignment of *rbtL* and *tufA* sequences using maximum likelihood (ML). Numbers at nodes represent ML bootstrap values (before slash) and Bayesian posterior probabilities (after slash). Specimens recently collected in New Caledonia are in a black box.

colder waters happened during the Cenozoic, a period known for decreasing global temperatures (Fig. 6c). Except for the derived species *P. devriesii*, which occurs in shallow and intertidal habitats, all *Pseudocodium* species grow in deep-water habitats, and this ecological preference appears to be ancestral (Fig. 6c).

#### DISCUSSION

Our data provide strong evidence for the existence of two *Pseudocodium* species in New Caledonia, *P. okinawense* and the new species *P. mucronatum*. The new species possesses a feature that allows immediate distinction from other species in the genus: its utricles terminate in a distinctive mucron. Otherwise, the species is very similar to other *Pseudocodium* species in its overall morphology and its vegetative and reproductive anatomy. In external appearance, the species is most similar to *P. devriesii* in that they both have relatively large, flattened thalli.

The utricles of *P. mucronatum* are more elongated than those of other *Pseudocodium* species. Whereas those of most species are about the same height and diameter (De Clerck et al. 2008), the utricles of *P. mucronatum* are 2 to 2.5 times as long as they are broad. *P. devriesii* is intermediate in this respect, having slightly elongated utricles (Weber-van Bosse 1896, De Clerck et al. 2008). In being elongated, *P. mucronatum* utricles show a striking similarity with *Codium* utricles, especially those of some mucronate *Codium* species (e.g., *C. fragile*, *C. tomentosum* var. *mucronatum*). Mucronate utricles are also present in one *Halimeda* species (*H. scabra*; Howe 1905). The

observation of mucrons in some but not all species of these three evolutionary distinct genera suggests that this character evolves relatively quickly (see also Verbruggen et al. 2007) and may result from parallel selective forces in the three genera.

The genus *Pseudocodium* was previously recorded from New Caledonia without identification at the species level (Garrigue and Tsuda 1988), but we have been unable to locate voucher specimens for identification. The discovery of *P. okinawense* in the Loyalty Islands (New Caledonia) marks a considerable range extension of this species (Fig. 6a). It was previously known from the Ryukyu Islands in Japan (Faye et al. 2008) and from Indonesia and Papua New Guinea, where it was originally reported as *P. floridanum* (Verheij and Prud'homme van Reine 1993, Coppejans et al. 2001, De Clerck et al. 2008). The species was also collected in Vanuatu (Espiritu Santo Island) in 2006 (C. E. Payri, unpublished data). The present range extension into the southwest Pacific Ocean indicates that *P. okinawense* is widespread in the tropical western Pacific Ocean. Due to its diminutive size and deep-water habitat, it may have been overlooked in many localities. In most of the areas, the species was uncommon even if it was locally abundant growing in patches of several individuals.

De Clerck et al. (2008) reported that certain morphological characters correlate with phylogenetic affinities. Whereas species in the *P. floridanum*–*okinawense* clade have terete axes with rounded apices and plastids confined to the medulla, species of the *P. natalense*–*devriesii* clade were shown to possess compressed axes with depressed apices and plastids in both utricles and medulla. The discovery of *P. mucronatum* reinforces the conclusions of De Clerck et al. (2008) because this species is clearly associated with the *P. natalense*–*devriesii* clade in the phylogenetic tree and is characterized by compressed axes, depressed apices, and plastids in the utricles and medullar siphons.

Our molecular clock analysis is the first attempt to calibrate the phylogenetic history of *Pseudocodium* in absolute time. The initial, justified critique of the molecular clock has led to the development of more realistic models that relax the assumption of the molecular clock by allowing rate heterogeneity across lineages (Lepage et al. 2007). We used a model that assumes that rates of molecular evolution are autocorrelated, meaning that rates on parent and daughter branches are correlated. This correlation is achieved by optimizing branch rates with a Brownian motion model (Thorne et al. 1998). Molecular clock analyses are most commonly calibrated in geological time using information from the fossil record. *Pseudocodium* has been reported as a fossil once, as *P. convolvens* from the Jurassic (Praturlon 1964). However, the spirally twisted peripheral siphon and extensive medulla of *P. convolvens* precludes it from being a member of

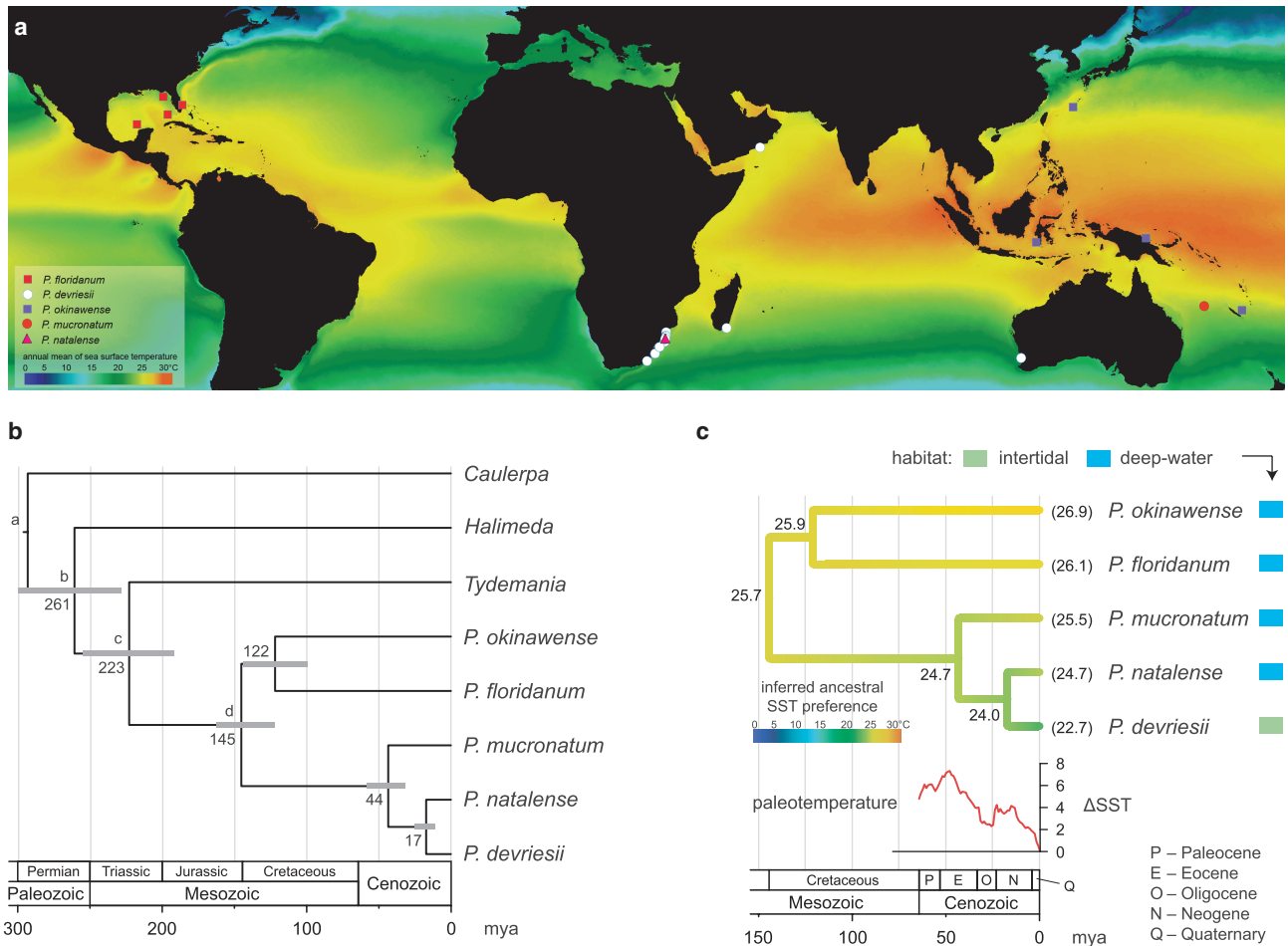


FIG. 6. Evolution of climatic preferences in *Pseudocodium*. (a) Map of the world showing annual mean sea surface temperature (SST) and collecting localities of five *Pseudocodium* species. (b) Chronometric phylogram of *Pseudocodium* and selected outgroups obtained using relaxed molecular clock methods. Numbers at nodes are mean node ages; the bars represent the variation around the mean (95% confidence interval). Letters at nodes represent calibration points (see text). (c) Evolution of climatic preferences. Inferred ancestral SST are traced along the tree in colors and indicated at nodes (in °C). The habitat is indicated behind the species name. The graph below the tree represents the evolution of global temperatures through the Cenozoic. The y-axis represents the difference in tropical SST from the present value. Data for this graph were taken from Zachos et al. (2001). The  $\delta^{18}\text{O}$  data were converted assuming that each part per thousand change in  $\delta^{18}\text{O}$  corresponds to  $\sim 1.75$  SST units (Veizer et al. 2000).

*Pseudocodium*. As Pratulon (1964) described this species as “*Pseudocodium convolvens* n. gen. n. sp.,” it is obvious that he was unaware of the existence of the extant genus and inadvertently introduced this mistake. As an alternative to using fossil information to calibrate our phylogenetic tree, we used information derived from a relaxed molecular clock analysis of the siphonous algae (Bryopsidales and Dasycladales). For four nodes common between our phylogeny and that of Verbruggen et al. (2009), the previously inferred 95% node age confidence intervals were used as minimum and maximum constraints in our calibration.

Our results allow for some conclusions regarding the evolution of ecological preferences in *Pseudocodium*. Our results reinforce the hypothesis that climate is an important determinant of macroalgal distributions and a crucial driving force behind their evolution and

biogeography. The genus originated in the tropics and subsequently one of its lineages adapted to colder water (the lineage leading to *P. natalense* and *P. devriesii*). Our results suggest that the adaptation to colder waters may have taken place gradually. Whereas *P. mucronatum* can be regarded as a tropical species, *P. natalense* shows intermediate climatic affinities and occurs on the southernmost reefs of the East African coast (northern KwaZulu Natal). The only species that has fully adapted to colder water is *P. devriesii*, which occurs in eastern South Africa, southern Madagascar, the Arabian Sea (Oman), and SW Australia. The observed range of annual mean SST values for this species is 20°C–26°C, and the upper limit of this range is most likely an overestimate because it represents the records from Oman, where the species has only been found during the SW monsoon season, when upwelling makes for considerably colder water. *P. devriesii* is

also the only species that has adapted to shallow-water and intertidal habitats, a feature that is also associated with changes in tolerance limits.

In some key publications about macroalgal biogeography, the hypothesis that part of the subtropical and warm-temperate seaweed floras was derived from tropical lineages was proposed (van den Hoek 1984, Lüning 1990). Our results confirm this hypothesis for *Pseudocodium*. As anticipated by van den Hoek and Lüning, the tropical lineages invaded colder-water habitats during the past 50 million years (Cenozoic), a period characterized by gradually decreasing global temperatures and narrowing of the tropical belt (Fig. 6c) (Zachos et al. 2001). A similar pattern of invasion of the subtropics by a genus of tropical origin was shown for *Halimeda* (Verbruggen et al. 2009). In this genus, several lineages adapted to colder water independently during the same historical epoch.

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### Supplementary Material

The following supplementary material is available for this article:

**Table S1.** Geographical information and derived sea temperature data for *Pseudocodium* collecting localities, with voucher numbers (Ghent University Herbarium and NHN Leiden).

**Table S2.** Specimen table with geographic origins and GenBank accession numbers. Newly generated sequences are indicated with an asterisk.

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