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The new species *Codium recurvatum* from Tanzania

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Codium recurvatum is described from material collected on a Tanzanian reef slope. The diminutive species is easily recognized by its unusual morphology, as its thallus consists of several dorsiventrally flattened lobes originating from a central holdfast and curving back towards the substratum. DNA barcodes (*rbc*L exon 1 and *tuf*A) confirm the distinctness of the species and the phylogenetic placement of the new species is inferred from a concatenated alignment of *rbc*L and *rps*3–*rpl*16 sequences.

Key words: Bryopsidales, Chlorophyta, *Codium recurvatum*, molecular phylogeny, morphology, Tanzania, taxonomy

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Introduction

The green seaweed genus *Codium* shows a wide variety of thallus forms. Whereas some species spread out over the substratum as tightly to loosely adhesive mats or crusts, others are spherical or have cylindrical or compressed branched thalli that can either be upright or sprawling. The genus's highest species diversity is found in warm-temperate regions such as Japan, southern Australia and South Africa (Silva & Womersley, 1956; Silva, 1959*b*), but several tropical and cold-temperate species are also known (Dellow, 1952; Silva, 1959*a*; Abbott & Huisman, 2004; Kraft, 2007). The total number of species in the genus is currently estimated at about 140 (Guiry & Guiry, 2011).

The use of molecular sequences to address species-level taxonomic questions has proven very useful in macroalgae (e.g. Zuccarello & West, 2003; Saunders & Lehmkuhl, 2005; Verbruggen *et al.,* 2005; Coyer *et al.,* 2006; Lane *et al.,* 2007; Leliaert *et al.,* 2009). In the genus *Codium, rbc*L exon 1 sequences have been used as DNA barcodes to study species boundaries (Shimada *et al.,* 2004; Verbruggen *et al.,* 2007). Verbruggen *et al.* (2007) defined 'evolutionarily significant units' (ESU) as clusters of sequences that (1) contained little intracluster sequence divergence, (2) received very high bootstrap support, and (3) were subtended by relatively long branches. In general, this approach yielded clearly circumscribed clusters that are likely to represent species. The fact that morphological differences exist between many ESU adds to the evidence that such sequence clusters indicate species boundaries.

During a sampling campaign in Tanzania, a collection of a lobed *Codium* entity was made that could not be identified using floristic treatments or monographs of the genus from the country or nearby regions (Silva, 1959*b*; Jaasund, 1976; Van den heede & Coppejans, 1996; Oliveira *et al.,* 2005). Our goal in this study is to characterize this entity morphologically and to study its molecular taxonomy and phylogenetic position.

Materials and methods

The Tanzanian entity was photographed in the field, collected and preserved in 95% ethanol. Additional pictures of the specimen were taken in the laboratory. An Olympus BX51 microscope was used to measure and draw several aspects of its anatomy, including the lengths and diameters of 47 utricles and the diameters of 13 medullar siphons from two different lobes (a small and a large one). Utricles were also measured for multiple specimens belonging to species that were morphologically similar to the Tanzanian entity or closely related to it in the phylogeny. Measurements in the description are given as (minimum−) 20 percentile–80 percentile (–maximum).

Chloroplast DNA sequences of *rbc*L exon 1 and the *rps*3–*rpl*16 region were determined following previously described protocols (Shimada *et al.,* 2004; Verbruggen *et al.,* 2007). In addition, part of the *tuf*A gene was sequenced as described in Famà *et al.* (2002) and Verbruggen *et al.* (2009). All new sequences have been submitted to Genbank (Supplementary Table S1). DNA sequences were aligned manually based on

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corresponding amino-acid sequences. The spacer between *rps*3 and *rpl*16 was excluded from analyses.

Two types of phylogenetic analyses were carried out. First, we inferred a global phylogeny of the genus *Codium* to determine the overall position of the entity from Tanzania. This analysis used concatenated sequences of *rbc*L and *rps*3–*rpl*16. Species were represented in this dataset with a single concatenated sequence. Second, we used *rbc*L sequences as DNA barcodes to investigate species boundaries in more detail between the Tanzanian entity and its closest relatives. This involved phylogenetic analyses of multiple sequences per species.

For the global phylogeny, the *rbc*L sequence of the Tanzanian entity and the *rbc*L and *rps*3–*rpl*16 regions of the related species *C. arenicola* were added to the alignments of Verbruggen *et al.* (2007). After cropping the ends of both alignments and the *rps*3–*rpl*16 intergenic spacer, the alignment counted 1062 characters (720 *rbc*L and 342 *rps*3–*rpl*16) and 73 taxa. We selected suitable partitioning strategies and models of sequence evolution for the concatenated dataset using a previously described model selection procedure based on the Bayesian Information Criterion (BIC) (Verbruggen & Theriot, 2008; Cocquyt *et al.,* 2010; Verbruggen *et al.,* 2010). Six partitioning strategies and 20 models of sequence evolution were evaluated (see Results). Phylogenetic analyses were carried out with maximum likelihood searches (ML) and Bayesian phylogenetic inference (BI). Maximum likelihood analyses were carried out with RAxML version 7.2.8 (Stamatakis, 2006). Searches were started from 200 distinct randomized maximum parsimony starting trees and branch support was assessed with the classic bootstrapping algorithm (1000 replicates). Bayesian phylogenetic inference was carried out with MrBayes version 3.1.2 (Ronquist & Huelsenbeck, 2003). Five independent runs, each consisting of four incrementally heated chains, were run for 10 million generations using default priors and other settings. Trees were sampled every thousand generations. Convergence of likelihood and parameter values was assessed with Tracer version 1.5 (Rambaut & Drummond, 2009) and a suitable burn-in value was chosen. Bayesian posterior probabilities for clades were computed from the post-burn-in sample of trees and indicated on the ML tree.

For species delimitation purposes, we carried out analyses of an *rbc*L exon 1 dataset of our Tanzanian collection and some closely related species, with multiple specimens per species included in the analysis. The alignment was cropped to 666 sites and subjected to UPGMA clustering and Bayesian phylogenetic inference. UPGMA was carried out with MEGA 5.0 (Tamura *et al.,* 2011), with 1000 bootstrap replicates and Tamura–Nei distances. Bayesian analyses were conducted in BEAST version 1.6.2 (Drummond & Rambaut, 2007), using a HKY+G_4 model of nucleotide substitution, an uncorrelated lognormal relaxed clock model, and a coalescent tree prior assuming a constant population size with a Jeffreys prior. Three independent analyses were started from a random starting tree and run for 10 million generations, sampling every thousand generations. Convergence and suitable burn-in values were determined as mentioned above, and an extended majority rule consensus tree was computed from the posterior set of trees.

Results and discussion

Phylogenetic tree

The BIC-based procedure for finding a suitable partitioning strategy and model of sequence evolution led to the selection of a global model with four partitions and a GTR+G_4 substitution model (Supplementary Table S2). The four partitions are (1) *rbc*L, cp1 + 2; (2) *rbc*L, cp 3; (3) *rps*3–*rpl*16, cp1 + 2; and (4) *rps*3–*rpl*16, cp3, where 'cp' stands for codon position.

The maximum likelihood phylogenetic tree (Fig. 1) was nearly identical to the Bayesian topology and differed from it only in poorly supported regions. Both trees obtained in this study were also highly similar to the phylogenies previously published by Verbruggen *et al.* (2007) and those of Oliveira-Carvalho *et al.* (in press), all of which were based on a similar selection of sequences. Two name changes have been applied here, relative to Verbruggen *et al.* (2007): the species previously called *C.* cf. *latum* 1 has now been described as *C. tenuifolium* (Shimada *et al.,* 2007), and the species previously called *C. geppiorum* 3 has been identified as *C. parvulum* (Israel *et al.,* 2010).

The three main clades (A, B, C) described and discussed in detail by Verbruggen *et al.* (2007) were recovered in our analyses, but the resolution within these clades was only moderate; the backbones of clades A and C in particular were poorly resolved. The entity from Tanzania, described below as *C. recurvatum,* was nested in clade C within a well-supported subclade indicated with a star in Fig. 1. The other species in this subclade were *C. arenicola, Codium* sp. 7, *C. taylorii* and *C. geppiorum* 4.

Species delimitation

To investigate the boundaries between the five species in the starred subclade in more detail, we inferred haplotype trees from an alignment with multiple sequences per species, at least for those species where more than one specimen was available (Fig. 2). The trees obtained confirm the existence of five clearly separated, species-level haplotype clusters. While species clusters generally received high support in the UPGMA bootstrap analysis, the Bayesian posterior probabilities for *C. geppiorum* 4 and *C. arenicola* were moderate to low. This is not unexpected because between-species *rbc*L sequence divergences are low in clade C (Verbruggen *et al.,* 2007) and may not

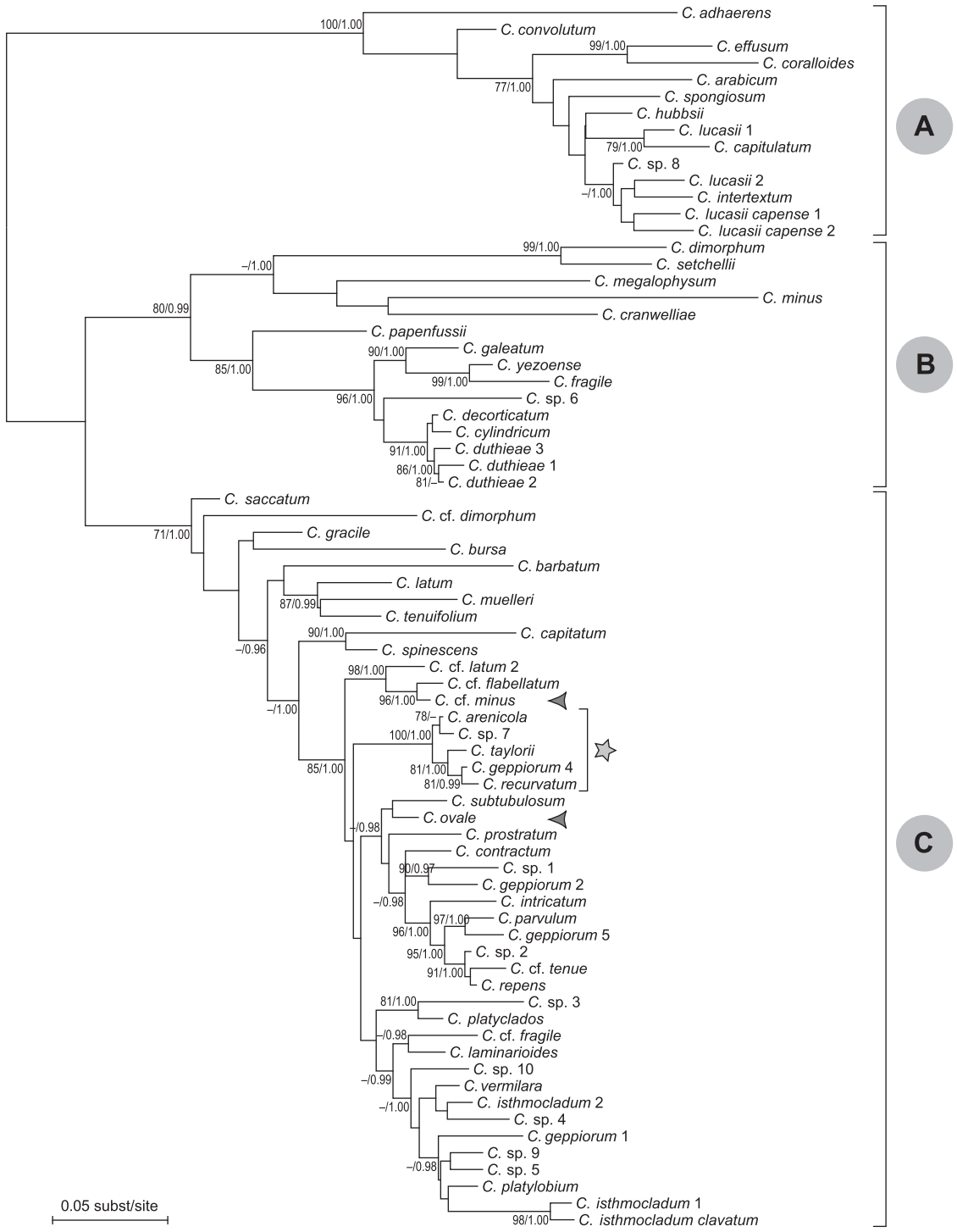

Fig. 1. Maximum likelihood phylogenetic tree of 73 *Codium* species based on the chloroplast markers *rbc*L and *rps*3–*rpl*16. Branch support is given as ML bootstrap values (before slash, when exceeding 70) and Bayesian posterior probabilities (after slash, when exceeding 0.90). The subclade containing the new species *Codium recurvatum* is marked with a star.

yield sufficient signal to separate the species in these coalescence-based analyses. Our analysis also showed a high degree of sequence variation within *C. taylorii,* which may be interpreted as an indication of hidden diversity within this species in the Lesser Antilles (specimens DML30732 and DML30928). This, however, is beyond the scope of our study.

While the *rbc*L gene has been proposed as a DNA barcoding marker for the genus *Codium* by Verbruggen *et al.* (2007) and more recently for land plants in combination with *mat*K (Hollingsworth *et al.,* 2009), a recent study indicates that *tuf*A yields better separation between closely related species of green macroalgae (Saunders & Kucera, 2010). The utility of *rbc*L to discriminate between

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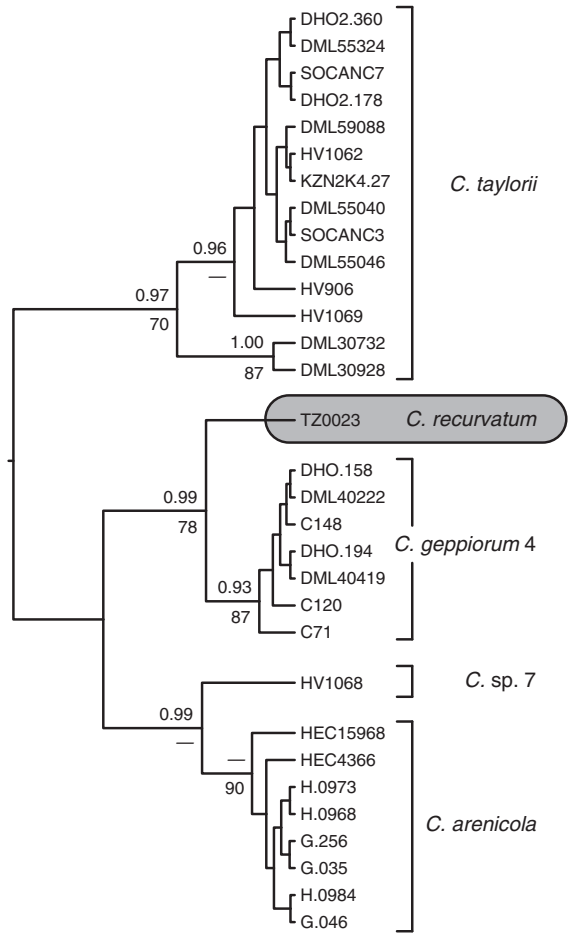

Fig. 2. Bayesian haplotype tree for the new species *Codium recurvatum* and closely related species. Branch support is given as Bayesian posterior probabilities (above branch, when exceeding 0.90) and UPGMA bootstrap values (below branch, when exceeding 70).

the most closely related of species has also been questioned for *Codium* (Verbruggen *et al.,* 2007; this study). For comparative purposes and to permit molecular identification with *tuf*A barcodes, we have also sequenced this gene of *C. recurvatum* (Genbank accession JQ706338).

Taxonomic treatment

The phylogenetic analyses reported above, as well as the distinctive morphology (see below), lead us to conclude that the entity from Tanzania should be recognized as a distinct species.

Codium recurvatum* Verbruggen, *sp. nov

Diagnosis: Differs from its congeners in having a compact thallus that consists of several lobes originating from the holdfast region. Lobes are flattened, inverse-pear-shaped, and curve back towards the substrate. Reference sequences for molecular identification are JQ706336 (*rbc*L) and JQ706338 (*tuf*A).

Etymology: The epithet *recurvatum* is Latin for 'curved back', referring to the shapes of the lobes.

Holotype: TZ0023 (GENT), collected at *c.* 20 m depth on a coral rubble slope off Mbudya Island, Tanzania (6°37.091′ S, 39°16.318′ E) on 20 January 2008.

Distribution: Presently known with certainty only from the type locality.

Description: The thallus consists of several lobes originating from the holdfast (Figs 3–5). Lobes are flattened, shaped like an upside-down pear (obpyriform), arch back towards the substratum, and are 15–28 mm long, 6–20 mm wide, and 4–8 mm thick. Mature lobes have a central cavity. The light-green thallus is surrounded by a halo of white hairs (Fig. 5). Utricles separate individually rather than in clusters (Fig. 6); they are club-shaped (clavate), and (145−) 173–279 (−375) μm broad at the apices and (560−) 720–920 (–1360) μm long. In contrast to utricle diameter, which is fairly constant, utricle length differs substantially between lobes, the larger lobes having longer utricles (Fig. 7). The utricle apices are broadly rounded and lack wall thickenings. A single hair or hair scar is present subapically on most utricles, and an occluding plug is present near the base of the utricle in one of the two medullar siphons. Medullary siphons are (32−) 42–57 (−67) μm in diameter. Gametangia could not be observed in the material available.

Comparison with similar species: The new species is very unusual among *Codium* species. However, if one were to ignore the fact that the lobes are flattened and curved back towards the undersurface, these hollow structures would resemble the thalli of *C.* cf. *minus* and *C. ovale*. The first of these taxa, which is not a formally described taxon but deserves recognition at the species level (Verbruggen *et al.,* 2007), is currently known only from the Arabian Sea (Oman), where it occurs as part of the temperate-water flora that flourishes during the annual upwelling events that take place during the summer monsoon (Schils & Coppejans, 2003, as '*C. ovale*'; Wynne, 2004, as '*C. minus*'). This entity differs from *C. recurvatum* in having subspherical thalli occurring in clusters, longer utricles (Fig. 7) and a distinct DNA signature (Fig. 1, 1st arrowhead).

Codium ovale has been reported from several places in the Indo-Pacific and the Caribbean, including the Seychelles, which lie off the East African coast where *C. recurvatum* is found (Silva *et al.,* 1996; Van den heede & Coppejans, 1996;

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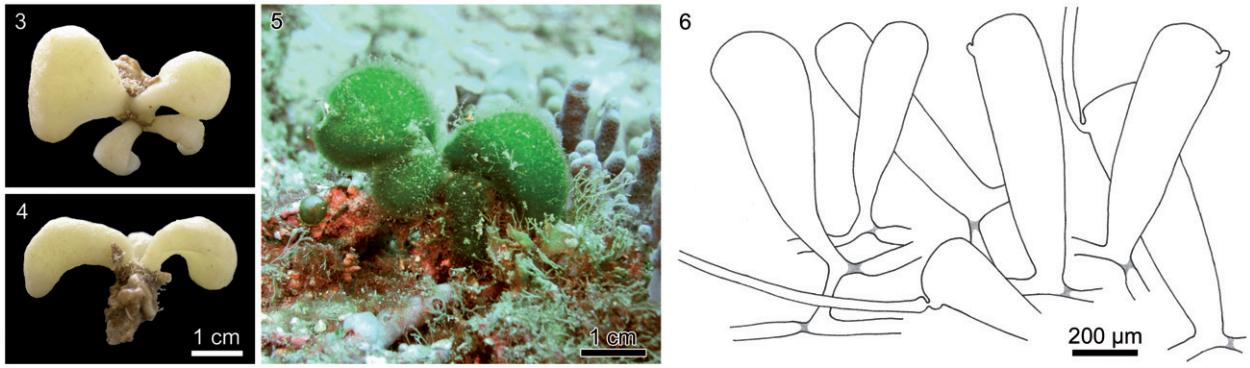

Figs 3–6. Holotype specimen of the new species *Codium recurvatum* (GENT TZ0023). **3.** Thallus viewed from above, showing the rosette-like habit composed of four inverse-pear-shaped lobes originating from a single holdfast. **4.** Side view of thallus showing the flattened and curved nature of the lobes. **5.** *In situ* photograph of the holotype specimen on a Tanzanian coral rubble slope. **6.** Clavate utricles, one of each of the two basal medullary siphons occluded by a plug.

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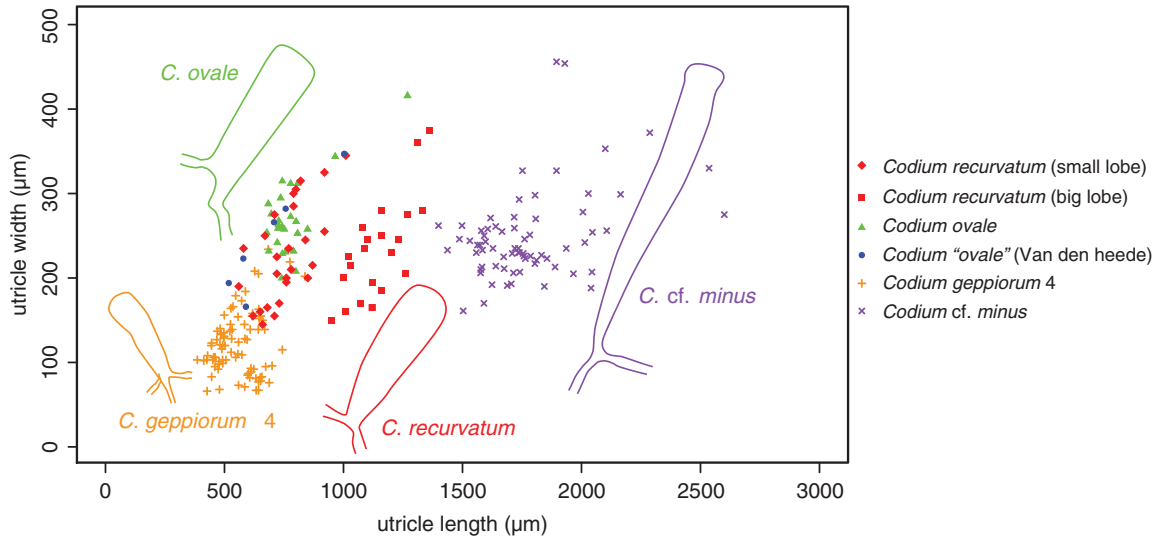

Fig. 7. Comparison of utricle dimensions in *Codium recurvatum* and some similar species. Measured specimens are TZ0023 for *C. recurvatum,* DML40050 (Fiji) and G.122 (Indonesia) for *C. ovale,* TZ0676 and HV158 for *C. geppiorum* 4, and DHO-015 and DHO2-188 for *C.* cf. *minus*. All specimens are in the Ghent University herbarium (GENT) except DML40050 in the Smithsonian Institution (US). For *Codium* '*ovale*', the measurements were made on the utricle drawings of Van den heede & Coppejans (1996).

Littler & Littler, 2000, 2003). This species differs from *C. recurvatum* in having inverse-drop-shaped thalli and very different DNA sequences (Fig. 1, 2nd arrowhead). Its utricle measurements overlap those of *C. recurvatum* (Fig. 7), but the utricle tips of *C. ovale* are usually flatter. A specimen from Raja Ampat (Indonesia), very close to the type locality of *C. ovale,* clusters with the Fijian *C. ovale* sample that we have used in our phylogenetic tree (data not shown), so this clade almost certainly represents the true *C. ovale*. The utricle measurements in Fig. 7 include the Indonesian and Fijian specimens.

It is worth noting that, in their monograph of tropical East African *Codium,* Van den heede & Coppejans (1996) report a single, minute specimen of *C. ovale* from the Seychelles, speculating that it is a young specimen with smaller utricles. However, the utricle measurements of this specimen fall completely within the range of those of the small lobe of our *C. recurvatum* specimen, so it is possible that the specimen from the Seychelles represents a second record of *C. recurvatum*. If this is the case, the description of the external morphology of the new species would have to be expanded to include minute spherical thalli. However, the measurements also overlap with those of genuine *C. ovale* (Fig. 7), so a second possibility is that the Seychelles entity belongs to this species.

In a phylogenetic context, only *C. geppiorum* 4 is closely related to *C. recurvatum*. This species,

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which is known to range from Fiji to the East African coast, including Tanzania, is one of the entities within the *C. geppiorum–repens* complex that should be recognized at the species level (Verbruggen *et al.,* 2007, and unpublished results). Morphologically, this undescribed species differs from *C. recurvatum* in having branched, sprawling thalli with relatively smaller utricles (Fig. 7).

The description of *C. recurvatum* is a step forward but our results also show that there is much more to be done before an updated global taxonomy of the genus will be available. After all, of the five species-level clusters recognized in the starred clade of Fig. 1, only two have been formally described (*C. taylorii* and now *C. recurvatum*), whereas *C. arenicola* remains a *nomen nudum, Codium* sp. 7 is an uncharacterized entity, and *C. geppiorum* 4 is one of many unrecognized entities within a complex of sprawling species known as the *C. geppiorum–repens* complex. At present we hesitate to describe the other species-level clusters in this lineage as new species because their morphology is not as distinctive as that of *C. recurvatum,* and a formal description of these other species would require comparison with relatively large numbers of morphologically similar species from other parts of the phylogenetic tree – a daunting task that requires more expansive sampling both in geographical and phylogenetic dimensions.

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Supplementary material

The following supplementary material is available for this article, accessible via the Supplementary Content tab on the article's online page at http://dx.doi.org/10.1080/09670262.2012.690106.

Supplementary Table 1. Sequences used in the phylogenetic analysis with voucher and Genbank accession numbers.

Supplementary Table 2. Results of the selection procedure for partitioning strategies and models of sequence evolution.

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