The *Cylindrobulla* / *Ascobulla* complex—unraveling problems in identification and adding to *Cylindrobulla* diversity (Gastropoda, Heterobranchia, Sacoglossa) by describing a new species

ELISE LAETZ, GREGOR CHRISTA, KATHARINA HÄNDELER & HEIKE WÄGELE

Zoologisches Forschungsmuseum Alexander Koenig, Adenauerallee 160, 53113 Bonn, Germany.
E-mail: melaetz@gmail.com, gchrista@uni-bonn.de, k.haendeler@gmx.de, h.waegele@zfmk.de

Abstract

Sacoglossa (Gastropoda: Heterobranchia) are generally considered a monophyletic group, previously associated within the now defunct “Opisthobranchia”, but now basally located within Panpulmonata. In the light of this new phylogenetic hypothesis, detailed knowledge of the most basal groups within Sacoglossa is of paramount importance. This study focuses on the genus *Cylindrobulla*, which is usually considered the most basal group within the Sacoglossa from a morphological point of view, because it does not share the typical elongate radula teeth of all other Sacoglossa. We describe a new species, *Cylindrobulla schuppi* sp. nov., and provide data on its food. We reexamined and clarify the radula of the type species *C. beauii*, review the genus with all other valid species, provide new characters to aid in the proper identification of species within this genus, compare it to the very similar genus *Ascobulla*, present a determination key using external characters to ensure proper identification of the two similar genera, and discuss phylogenetic relationships within the shelled sacoglossan, the Oxynoacea.

Key words: Food, barcoding, *rbcL*, *tufA*, Oxynoacea, character analysis, phylogeny

Introduction

The Sacoglossa comprises a group of marine heterobranchs that feed on algae using a modified radular tooth that allows them to suck out algal cell sap (Jensen 1996a). Many sacoglossan species are known to sequester chloroplasts from their algal food and maintain them for weeks to months (kleptoplasty) (e.g., Rumpho et al. 2000; Evertsen et al. 2007; Händeler et al. 2009; see also reviews of Rumpho et al. 2011 and Wägele and Martin 2013). Numerous species are capable of “stealing” secondary metabolites from their food sources (kleptochemistry) for their own chemical defense (e.g., Cimino & Ghiselin 1998; Marín & Ros 2004).

The affiliation of Sacoglossa within Heterobranchia has changed often in recent years, certainly due to taxon and gene selection (see e.g., Jensen 2011), but was recently included in the new taxon Panpulmonata (see Schrödl et al. 2011) based on earlier analyses including several genes and with good support values (e.g., Dinapoli and Klussmann-Kolb 2010; Jörger et al. 2010, Göbbeler & Klussmann-Kolb 2011, Wägele et al. 2014). Kocot et al. (2013) and Zapata et al. (2014) confirmed this affiliation by genomic analyses. Sacoglossa is generally considered monophyletic (Wägele & Klussmann-Kolb 2005; Jörger et al. 2010; Dayrat et al. 2011; Kohnert et al. 2013; see Wägele et al. 2014), although the relative positions of groups within this taxon remain disputed (Jensen1996a, b; Händeler & Wägele 2007; Händeler et al. 2009; Maeda et al.2010, Jensen 2011). The most basal group within the Sacoglossa has been debated for many years and varies according the methods used for phylogenetic analysis. The taxa with characters considered as rather basal and plesiomorphic compared to taxa within the former Opisthobranchia, (large shells, no rhinophores, but see also Brenzinger et al. 2013) are *Cylindrobulla* Fischer, 1857, and *Ascobulla* Marcus, 1972, which appear almost identical in morphology. *Cylindrobulla* has been excluded from Sacoglossa in the past (Jensen 1996a; 1996b) mainly based on the presence of a radula with a broad median tooth not adequately adapted for piercing and sucking, whereas *Ascobulla* and all other sacoglossan species have a
published by Verbruggen et al. (2009) in GenBank. According to AlgalBase, this species is not distributed in the Atlantic, or even in the Caribbean Sea. *Halimeda opuntia* sequences differ from our sequences by about 6%, indicating a different species. We are not able to sort out the taxonomy of *Halimeda* species; however, this might be necessary in future for correct food identification by barcoding.

When comparing *Cylindrobulla* and *Ascobulla* with the remaining oxynoacean taxa based on morphology alone, these two genera are still very close. This sharply contrasts the molecular data as was already depicted by Maeda et al. (2010). Unlike Händeler et al. (2009), who deliberately chose *Cylindrobulla* as outgroup for their phylogenetic analysis (based on the strong differences in the radula), Maeda and coworkers considered the genus as member of the ingroup and used a caenogastropod as the outgroup. In their results, as in ours, *Cylindrobulla* and *Ascobulla* are not closely related. *Cylindrobulla* is not the basal group in either analysis, but it is rendered the most basal taxon within the Oxynoacea in the four gene analysis. This implies that *Cylindrobulla* either has secondarily evolved a broad radula tooth that is not able to penetrate cell walls in a similar way as all other sacoglossans do, or that the piercing form has evolved twice in Sacoglossa (in Oxynoacea and Plakobranchacea). We consider the results on paraphyly of *Ascobulla* and Juliidae in the analysis based on mitochondrial genes alone as an artefact, probably due to the high substitution rate of these markers and especially the 3rd position in the CO1 gene.

Jensen (2011) recently doubted the close affinities of Siphonariidae with Sacoglossa as suggested by multilocus analyses and considered their morphological similarities as homoplasies. It is known that outgroup selection as well as taxon sampling and the selection of genes has a great effect on molecular analyses. We therefore do not consider our results as a final conclusion of oxynoacean phylogeny.

This study summarizes and adds considerably to our knowledge of two morphologically similar genera, *Cylindrobulla* and *Ascobulla*. Nevertheless, many gaps in the morphological data matrix solidify this report as a preliminary one and suggest that proper assessment, especially of the taxa *C. systremma* and *C. souverbiei*, as well as of all recent *Ascobulla* species, is still warranted.

**Acknowledgements**

We wish to thank especially Patrick Krug (Los Angeles), Annika Putz (Bonn), Conxita Avila (Barcelona) and Gabi König (Bonn) for their collecting efforts in Guam. Without Peter Schupp (Oldenburg), this expedition would have been far less successful. Karin Ulmen (Bonn) helped us in the scanning electron microscopic studies and Claudia Etzbauer (Bonn) in the molecular analyses. Dario Karmeinsky provided the *rbcL* sequence of *Cylindrobulla beauii*. We thank Paula Mikkelsen for helping us with literature. We are very grateful to two anonymous reviewers who contributed with valuable comments. This study was supported by the German Science Foundation to HW (618/12).

**References**


