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

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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

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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 multi-locus 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.

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References

Adams, A. & Angas, G.F. (1864) Descriptions of new species of shells, chiefly from Australia, in the collection of Mr. Angas. *Proceedings of the Zoological Society of London*, 32, 35–40.

Bartsch, P. (1915) Report on the Turton collection of South African marine mollusks, with additional notes on other South Africa shells contained in the United States National Museum. *Smithsonian Institution - United States National Museum Bulletin*, 91, 1–1305.

Brenzinger, B., Padula, V. & Schrödl, M. (2013) Insemination by a kiss? Interactive 3D-microanatomy, biology and systematics of the mesopsammic cephalaspidean sea slug *Pluscula cuica* Marcus, 1953 from Brazil (Gastropoda: Euopisthobranchia: Philinoglossidae) *Organisms, Diversity and Evolution*, 13, 33–54.
http://dx.doi.org/10.1007/s13127-012-0093-3

Christa, G, Gould, S.B., Franken, J., Vleugels, M., Karmeinsky, D., Händeler, K., Martin, W.F. & Wägele, H. (2014) Functional kleptoplasty in a limpontioid genus: Phylogeny, food preferences and photosynthesis in *Costasiella* with focus on *C. ocellifera* (Gastropoda, Sacoglossa). *Journal of Molluscan Studies*.
http://dx.doi.org/10.1093/mollus/eyu026

Cimino, G. & Ghiselin, M.T. (1998) Chemical defense and evolution in Sacoglossa (Mollusca: Gastropoda: Opisthobranchia). *Chemoecology,* 8, 51–60.
http://dx.doi.org/10.1007/PL00001804

Dayrat, B., Conrad, M., Balayan, S., White, T.R., Albrecht, C., Golding, R., Gomes, S., Harasewych, M.G. & deFrias Martins,

A.M. (2011) Phylogenetic relationships and evolution of pulmonate gastropods (Mollusca): new insights from increased taxon sampling. *Molecular Phylogenetics and Evolution*, 59, 425–437.
http://dx.doi.org/10.1016/j.ympev.2011.02.014

Dinapoli, A. & Klussmann-Kolb, A. (2010) The long way to diversity—phylogeny and evolution of the Heterobranchia (Mollusca: Gastropoda). *Molecular Phylogenetics and Evolution,* 55, 60–76.
http://dx.doi.org/10.1016/j.ympev.2009.09.019

Evertsen, J., Burghardt, I., Johnsen, G. & Wägele, H. (2007) Retention of functional chloroplasts in some sacoglossans from the Indo-Pacific and Mediterranean. *Marine Biology,* 151, 2159–2166.
http://dx.doi.org/10.1007/s00227-007-0648-6

Famà, P., Wysor, B., Kooistra, W.H. & Zuccarello, G.C. (2002) Molecular phylogeny of the genus *Caulerpa* (Caulerpales, Chlorophyta) inferred from chloroplast *tufA* gene. *Journal of Phycology,* 38, 1040–1050.
http://dx.doi.org/10.1046/j.1529-8817.2002.t01-1-01237.x

Fischer, M. (1857) Description d'espèces nouvelles. *Journal de Conchyliologie*, 5, 2me sér 1, 273–277.

Gascoigne, T., Sartory, P.K. & Lemche, H. (1974) The teeth of three bivalved gastropods and three other species of the order Sacoglossa. *Journal of Molluscan Studies,* 41, 109–126.

Göbbeler, K. & Klussmann-Kolb, A. (2011) Molecular phylogeny of the Euthyneura (Mollusca, Gastropoda) with special focus on Opisthobranchia as a framework for reconstruction of evolution of diet. *Thalassas,* 27, 121–153.

Gosliner, T.M. (1987) Biogeography of the opisthobranch gastropod fauna of southern Africa. *American Malacological Bulletin,* 5, 243–258.

Guangyu, L. (1978) A new species of *Cylindrobulla* (Opisthobranchia) of the Xisha Islands, Guangdong Province, China. *Oceanologia et Limnologia Sinica*, 9, 95–98.

Händeler, K. & Wägele, H. (2007) Preliminary study on molecular phylogeny of Sacoglossa and a compilation of their food organisms. *Bonner Zoologische Beiträge,* 55, 231–254.

Händeler, K., Grzymbowski, Y., Krug, J.P. & Wägele, H. (2009) Functional chloroplasts in metazoan cells - a unique evolutionary strategy in animal life. *Frontiers in Zoology,* 6, 1–28.
http://dx.doi.org/10.1186/1742-9994-6-28

Hamatani, I. (1969) A new species of the rare shelled sacoglossan genus *Cylindrobulla* from middle Japan (Opisthobranchiata). *Publication of the Seto Marine Biological Laboratory,* 17, 171–174.

Hamatani, I. (1971) A new species of *Cylindrobulla*, sacoglossan opisthobranch, from California; with a comparison with *C. japonica* Hamatani, 1969. *Publication of the Seto Marine Biological Laboratory,* 19, 111–117.

Huson, D.H. & Bryant, D. (2006) Application of phylogenetic networks in evolutionary studies. *Molecular Biology and Evolution*, 23, 254–267.
http://dx.doi.org/10.1093/molbev/msj030

Jeffreys, J.G. (1856) Contribution to the Conchology of France. *Annals and Magazine of Natural History,* series 2, 18, 471–472.

Jensen, K.R. (1989) A new species of *Cylindrobulla* from Phuket, Thailand, with a discussion of the systematic affiliation of the genus. *Phuket Marine Biological Center Research Bulletin,* 52, 1–11.

Jensen, K. (1996a) Phylogenetic systematics and classification of the Sacoglossa (Mollusca, Gastropoda, Opisthobranchia). *Philosophical Transactions of the Royal Society of London B Biological Sciences,* 351, 91–122.
http://dx.doi.org/10.1098/rstb.1996.0006

Jensen, K.R. (1996b) The Diaphanidae as a possible sister group of the Sacoglossa (Gastropoda , Opisthobranchia). *In:* Taylor, J. (Ed.), *Origin and evolutionary radiation of the Mollusca* (pp. 247). Oxford University Press, Oxford, pp. 231–247.

Jensen, K.R. (2007) Biogeography of the Sacoglossa (Mollusca, Opisthobranchia). *Bonner zoologische Beiträge*, 55, 255–281.

Jensen, K.R. (2011) Comparative morphology of the mantle cavitiy organs of shelled sacoglossa, with a discussion of relationships with other Heterobranchia. *Thalassas*, 27, 169–192.

Jensen, K.R. & Wells, F.E. (1990) Sacoglossa (=Ascoglossa) (Mollusca, Opisthobranchia) from southern Western Australia. In: *R. Proceedings of the Third International Marine Biological Workshop: The Marine Flora and Fauna of Albany; Western Australia*. Perth: Western Australian Museum, pp. 297–331.

Jörger, K.M., Stöger, I., Kano, Y., Fukuda, H., Knebelsberger, T. & Schrödl, M. (2010) On the origin of Acochlidia and other enigmatic euthyneuran gastropods and implications for the systematics of Heterobranchia. *BMC Evolutionary Biology,* 10, 1–323.
http://dx.doi.org/10.1186/1471-2148-10-323

Katoh, K., Misawa, K., Kuma, K.-I. & Miyata, T. (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research,* 30, 3059–3066.
http://dx.doi.org/10.1093/nar/gkf436

Kobelt, W. (1896) Die Familie Bullidae. *Systematisches Conchylien-Cabinet von Martini und Chemnitz,* 1, 1–190.

Kocot, K.M., Halynych, K.M. & Krug, P.J. (2013) Phylogenomics supports Panpulmonata: Opisthobranch paraphyly and key evolutionary steps in a major radiation of gastropod molluscs. *Molecular Phylogenetics and Evolution*, 69, 764–771.
http://dx.doi.org/10.1016/j.ympev.2013.07.001

Kohnert, P., Brenzinger, B., Jensen, K.R. & Schrödl, M. (2013) 3D-microanatomy of the semiterrestrial slug *Gascoignella aprica* Jensen, 1985—a basal plakobranchacean sacoglossan (Gastropoda, Panpulmonata). *Organisms, Diversity and Evolution*, 13, 583–603.
http://dx.doi.org/10.1007/s13127-013-0142-6

Lin, G. (1978) A new species of *Cylindrobulla* (Opisthobranchia) of the Xisha Islands, Guangdong Province, China.

Oceanologia et Limnologia Sinica, 9, 95–98.

Maeda, T., Kajita, T., Maruyama, T. & Hirano, Y. (2010) Molecular phylogeny of the Sacoglossa, with a discussion of gain and loss of kleptoplasty in the evolution of the group. *Biological Bulletin,* 219, 17–26.

Marcus, E. (1972) On some opisthobranchs from Florida. *Bulletin of Marine Science,* 22, 284–308.

Marcus, E. & du Bois-Reymond Marcus, E. (1956) The tectibranch gastropod *Cylindrobulla. Dos Anais da Academia Brasileira de Ciencias*, 28, 118–130.

Marcus, E. & du Bois-Reymond Marcus, E. (1970) Opisthobranchs from Curaçao and faunistically related regions. *Studies on the Fauna of Curaçao and other Caribbean Islands,* 122, 1–129.

Marcus du Bois-Reymond, E. (1970) Opisthobranchs from northern Brazil. *Bulletin of Marine Science,* 20, 922–951.

Marín, A. & Ros, J. (2004) Chemical defenses in sacoglossan opisthobranchs: taxonomic trends and evolutive implications. *Scientia Marina,* 68, 227–241.

Melvill, J.C. (1918) Descriptions of thirty-four species of marine Mollusca from the Persian Gulf, Gulf of Oman, and Arabian Sea, collected by Mr. F.W. Townsend. *Annals and Magazine of Natural History*, 1, 137–158.
http://dx.doi.org/10.1080/00222931808562296

Mikkelsen, P.M. (1998) *Cylindrobulla* and *Ascobulla* in the western Atlantic (Gastropoda, Opisthobranchia, Sacoglossa): Systematic review, description of a new species, and phylogenetic reanalysis. *Zoologica Scripta,* 27, 49–71.
http://dx.doi.org/10.1111/j.1463-6409.1998.tb00428.x

Mikkelsen, P.M. (2002) Shelled Opisthobranchs. *Advances in Marine Biology*, 42, 67–136.
http://dx.doi.org/10.1016/S0065-2881(02)42013-5

Neusser, T.P., Fukuda, H., Jörger, K.M., Kano, Y. & Schrödl, M. (2011) Sacoglossa or Acochlidia? 3D reconstruction, molecular phylogeny and evolution of the Aitengidae (Gastropoda: Heterobranchia). *Journal of Molluscan Studies*, 77, 332–350.
http://dx.doi.org/10.1093/mollus/eyr033

Nevill, G. & Nevill, H. (1869) On Some New Marine Gastropoda from the Southern Province of Ceylon. *Journal of the Asiatic Society of Bengal,* 32, 65–69.

Pierce, S.K., Curtis, N.E., Massey, S.E., Bass, A.L., Karl, S.A. & Finney, C.M. (2006) A morphological and molecular comparison between *Elysia crispata* and a new species of kleptoplastic sacoglossan sea slug (Gastropoda: Opisthobranchia) from the Florida Keys, USA. *Molluscan Research*, 26, 23–38.

Rumpho, M.E., Pelletreau, K.N., Moustafa, A. & Bhattacharya, D. (2011) The making of a photosynthetic animal. *Journal of Experimental Biology,* 214, 303–311.
http://dx.doi.org/10.1242/jeb.046540

Rumpho, M.E., Summer, E.J. & Manhart, J.R. (2000) Solar-Powered Sea Slugs. Mollusc/Algal Chloroplast Symbiosis. *Plant Physiology,* 123, 29–38.
http://dx.doi.org/10.1104/pp.123.1.29

Schrödl, M., Jörger, K., Klussmann-Kolb, A. & Wilson, N.G. (2011) Bye bye “Opisthobranchia”! A review on the contribution of mesopsammic sea slugs to euthyneuran systematics. *Thalassas*, 27, 101–112.

Souverbie, M. & Montrouzier, R.P. (1874) Descriptions d'espèces nouvelles del'Archipel Calédonien. *Journal de Conchyliologie*, 22, 187–201.

Sowerby, G.B. (1894) Marine shells of South Africa. *Journal of Conchology*, 7, 368–378.

Stamatakis, A., Hoover, P. & Rougemont, J. (2008) A Rapid Bootstrap Algorithm for the RAxML Web-Servers, *Systematic Biology*, 75, 758–771.
http://dx.doi.org/10.1080/10635150802429642

Swofford, D.L. (1998) PAUP*. Phylogenetic Analysis Using Parsimony (* and other methods). Version 4*. Sunderland: Sinauer Associates.

Thompson, T.E. (1985) Aposematic colour patterns of two Mediterranean species of opisthobranch molluscs: a proposal for co-operative investigation. *Journal of Molluscan Studies,* 51, 222–226.

Verbruggen, H., Tyberghein, L., Pauly, K., Vlaeminck, C., Van Nieuwenhuyze, K., Kooistra, W.H.C.F., Leliaert, F. & De Clerck, O. (2009) Macroecology meets macroevolution: Evolutionary niche dynamics in the seaweed *Halimeda. Global Ecolology and Biogeography*, 18, 393–405.
http://dx.doi.org/10.1111/j.1466-8238.2009.00463.x

Wägele, H. & Klussmann-Kolb, A. (2005) Opisthobranchia (Mollusca, Gastropoda) - more than just slimy slugs. Shell reduction and its implications on defence and foraging. *Frontiers in Zoology,* 2, 1–18.
http://dx.doi.org/10.1186/1742-9994-2-3

Wägele, H. & Martin, W.F. (2013) Endosymbioses in sacoglossan seaslugs: Plastid-bearing animals that keep photosynthetic organelles without borrowing genes. *In*: Löffelhardt, W. (Ed.), *Symbiosis,* Springer, pp. 291–324.

Wägele, H., Klussmann-Kolb, A., Verbeek, E. & Schrödl, M. (2014) Flashback and foreshadowing—a review on the taxon Opisthobranchia. *Organisms, Diversity & Evolution,* 14, 133–149.
http://dx.doi.org/10.1007/s13127-013-0151-5

Zapata, F., Wilson, N.G., Howison, M., Andrade, S.C.S., Jörger, K.M., Schrödl, M., Goetz, F.E., Giribet, G. & Dunn, C.W. (2014) Phylogenomic analyses of deep gastropod relationships reject Orthogastropoda. *Proceedings of the Royal Society B: Biological Sciences*, 281 (1739), 1471–2954.
http://dx.doi.org/10.1098/rspb.2014.1739