

Correspondences

Females floated first in bubble-rafting snails

Celia K.C. Churchill^{1,*},
Diarmaid Ó Foighil¹, Ellen E. Strong²,
and Adriaan Gittenberger^{3,4,5}

Ever since Mivart asked Darwin to explain a bird's use for half a wing, biologists have been challenged to explain extraordinary evolutionary change mechanistically. Here, we investigate the enigmatic evolutionary origins of Janthinidae, a family of marine snails that raft passively in the neuston, a vast oceanic surface habitat, by constructing floats of mucus bubbles. We present the first molecular phylogeny including Janthinidae, which confirms that janthinids are derived from Epitoniidae (wentletraps) — benthic predators and parasites of sea anemones and corals. Our data support the hypothesis that floats and rafting evolved *via* modified epitoniid egg masses rather than by juvenile droguing. Our phylogeny also reveals sequential modifications of float formation and function among janthinid lineages. We interpret these changes as sequential adaptations to a neustonic existence, a conclusion supported by the positive association of derived janthinid traits with ecological prevalence.

The marine environment comprises three primary ecological domains: benthos (sea floor), pelagos (water column), and the neuston (water surface). The latter contains a community of approximately 100 species drifting in subtropical oceans, a vast area covering 40% of the planet [1]. These include all members of Janthinidae, a predatory snail family that achieves buoyancy by constructing bubble floats. The family comprises two genera: *Janthina* (5 species), the violet snails, and *Recluzia* (2–3 global species), the rare brown janthinas [2].

Janthinids are grouped with the large (~630 species) benthic snail family Epitoniidae in the

superfamily Janthinoidea based on morphological and ecological synapomorphies and by a single cladistic analysis [3]. Epitoniids are specialized predators and ectoparasites of benthic cnidarians [4]. Like janthinids, epitoniids develop as protandric hermaphrodites [5,6]. Smaller epitoniid males associate with larger females, which remain attached to tethered egg masses by elastic mucus threads [4]. Relationships within Janthinoidea remain ambiguous, but one morphological study supports *Recluzia* as a transitional form

between Epitoniidae and Janthinidae [3,4,7].

Here, we present the first molecular phylogeny incorporating Janthinidae (Figure 1). We included representatives of seven potential sister families of Epitoniidae, based on published molecular and morphological caenogastropod phylogenies [3]. Bayesian and maximum likelihood analyses of one mitochondrial and three nuclear molecular markers reveal robust statistical support (PP = 100; BS_{ML} = 100) for the monophyly of Janthinoidea, strongly corroborating the earlier cladistic analysis [3]. Our

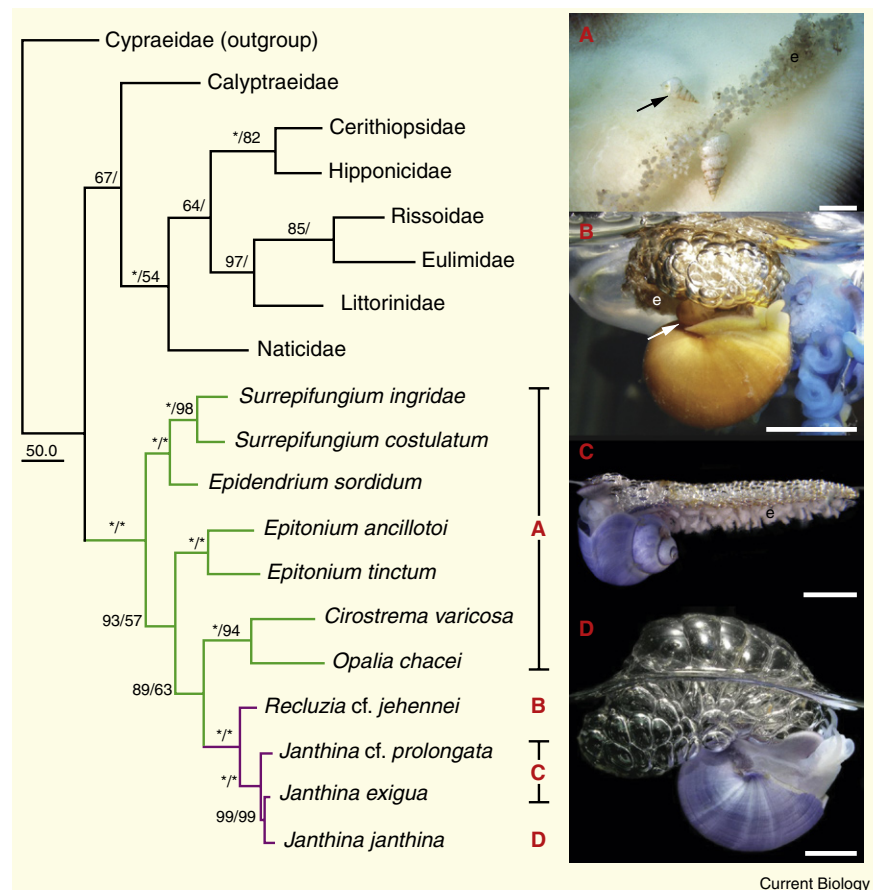


Figure 1. Proposed evolutionary steps to rafting in Janthinidae.

Left: Bayesian phylogram of Janthinoidea and seven potential sister families. DNA sequence data come from four loci: mitochondrial 16S rDNA, nuclear 28S rDNA, Histone-H3, Histone-H4. Bayesian posterior probabilities are followed by maximum likelihood bootstrap percentages above internal branches. Asterisks indicate support values of 100. Janthinidae and 'Epitoniidae' are represented in purple and green, respectively. Red letters indicate the evolution of four key rafting apomorphies, represented by photographs to the right of the topology. Right: (A) Tethered egg masses in Janthinoidea. Mature female *Epifungium nielsi*, a benthic 'epitoniid', on its coral host with egg capsules (e). An arrow indicates a small associated male. (B) Floating via modified egg masses in Janthinidae. Mature female *Recluzia jehennei* with bubble raft and husks of egg capsules (e), feeding on the cnidarian *Physalia physalis*. An arrow indicates a small associated conspecific. (C) Autonomous floating of juveniles and males in *Janthina*. Mature female *Janthina exigua* with bubble raft and egg capsules (e). (D) Loss of egg capsules from the float in ovoviparous *J. janthina*. Mature female *J. janthina* with large bubble raft. (Scale bars in A–D: 1 cm.)

results place Janthinidae within a paraphyletic 'Epitoniidae', but support levels for the nested placement of Janthinidae are not as robust (PP = 89–93; BS_{ML} = 57–63) as for other basal nodes (Figure 1). A more extensive sampling of 'epitoniid' diversity may be required to identify the closest living benthic sister lineage of Janthinidae.

Confirmation of the origins of Janthinidae allows us to ask, how did a benthic 'epitoniid' become neustonic? The fundamental janthinid adaptation to life in the neuston is the bubble float, and we propose two hypotheses of its evolutionary origin. First, the float may be derived from a modified juvenile drogue thread. Many benthic snail species are capable, as juveniles, of short-term pelagic dispersal via mucus threads that may become captured by surface tension [8]. Second, the float may represent a modified 'epitoniid' egg mass, which typically has capsules in various stages of development, from newly encased embryos to empty husks [6]. These husks could trap air in an intertidal species, providing temporary buoyancy for both the egg mass and the attached female. Both scenarios would initially produce temporary periods of passive rafting, but adding air-filled mucus bubbles to the drogue thread or the egg mass would be a prerequisite to attaining a fully neustonic existence.

To test our evolutionary hypotheses, we examined the distribution of janthinid life-history traits across the phylogeny (Figure 1A–D). *Janthina* spp. juveniles build floats [9], consistent with the juvenile drogue hypothesis, but there is no record of autonomous float formation by a juvenile *Recluzia* spp. [5]. Instead, a recent study documents larval recruitment and prolonged association of small-bodied *R. jehennei* on the float and egg capsules of larger conspecific females [5] (Figure 1B), a life history consistent with the egg mass hypothesis. Which neustonic genus (and float ontogeny) is plesiomorphic? *Recluzia* exclusively shares six morphological character states, independent of float ontogeny, with 'epitoniids' but *Janthina* has none (Supplemental information). These data support the egg mass hypothesis.

Our molecular phylogeny allows us to reconstruct how an ancestral janthinoidean lineage may have evolved a neustonic mode of life. The proto-janthinid was benthic and females formed tethered egg masses with associated males (Figure 1A, represented by an extant 'epitoniid'). The key adaptation of rafting derives from an egg mass modified for buoyancy (Figure 1B). Subsequent evolutionary change in the janthinid float involved a sequential loss of reproductive functions. In *Recluzia jehennei* (Figure 1B), the float is a female-only trait [5] and serves as a raft, a substrate for egg masses, and a platform for post-larval juveniles. The latter function is not present in the derived genus *Janthina*, in which all post-metamorphic individuals make autonomous floats. In the plesiomorphic oviparous condition, the float serves as a raft and (in females) a substrate for egg masses (Figure 1C). In the most derived condition, the ovoviviparous *J. janthina*, the float functions only as a raft during all life history stages (Figure 1D).

We interpret these sequential float modifications as adaptations to a neustonic existence. The evolution of autonomous float building by juvenile *Janthina* spp. removed the necessity of meeting a conspecific female before metamorphosis, allowing juveniles to exploit local resources earlier. The relatively compact and buoyant float of ovoviviparous *J. janthina* can be completely rebuilt [10] and is the most specialized for rafting, whereas oviparous species are tied to a single, trailing float encumbered with egg capsules (Figure 1B,C). Our inference of adaptive change is strengthened by the observation that ecological prevalence of janthinid species is correlated with the possession of derived float traits [2] (Supplemental information). Through sequential modification of float construction and function, janthinids have become increasingly successful at exploiting neustonic resources.

Supplemental Information

Supplemental Information includes experimental procedures, a supplemental figure and a supplemental table and can be found with this article online at doi:10.1016/j.cub.2011.08.011.

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References

1. Marshall, H.G., and Burchardt, L. (2005). Neuston: Its definition with a historical review regarding its concept and community structure. *Arch. Hydrobiol.* 164, 429–448.
2. Lalli, C.M., and Gilmer, R.W. (1989). *Pelagic Snails: The Biology of Holoplanktonic Gastropod Mollusks* (Stanford: Stanford University Press).
3. Ponder, W.F., Colgan, D.J., Healy, J.M., Nützel, A., Simone, L.R.L., and Strong, E.E. (2008). Caenogastropoda. In *Phylogeny and Evolution of the Mollusca*. W.F. Ponder and D.R. Lindberg, eds. (Berkeley: University of California Press).
4. Robertson, R. (1963). Wentletraps (Epitoniidae) feeding on sea anemones and corals. *Proc. Mal. Soc. (Lond.)* 35, 51–63.
5. Churchill, C.K.C., Strong, E.E., and Ó Foighil, D. Hitchhiking juveniles in the rare neustonic gastropod *Recluzia* cf. *jehennei* (Janthinidae). *J. Moll. Stud.*, (In press).
6. R. Robertson. (1980). *Epitonium millecostatum* and *Coralliophila clathrata*: Two prosobranch gastropods symbiotic with Indo-Pacific *Palythoa* (Coelenterata: Zoanthidae). *Pac. Sci.* 34, 1–17.
7. Thiele, J. (1928). Über ptenoglosse Schnecken. *Z. Wiss. Zool. Abt. A* 132, 73–94.
8. Vermeij, G. J. (1995). *A Natural History of Shells*. (Princeton: Princeton University Press).
9. Laursen, D. (1953). The genus *Janthina*: a monograph. *Dana Report* No. 38, 40 pp.
10. Wilson, D.P., and Wilson, M.A. (1956). A contribution to the biology of *Janthina janthina* (L.). *J. Mar. Biol. Assoc. U.K.* 35, 291–305.

¹Museum of Zoology and Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, Michigan, 48109-1079, USA. ²Smithsonian Institution, Washington, District of Columbia, 20013-7012, USA. ³Netherlands Centre for Biodiversity Naturalis, Leiden, 2300 RA, The Netherlands. ⁴Institute of Biology, Leiden University, Leiden, 2300 RA, The Netherlands. ⁵GiMaRIS, Leiden BioScience Park, Leiden, 2333 CA, The Netherlands.
*E-mail: celia.churchill@gmail.com