

Current Biology

Animal evolution at the ocean's water-air interface

Highlights

- We examine the evolutionary history of ocean surface-dwelling organisms
- Most surface species evolved from ancestors that attached to a substrate
- Floating animal diet and reproduction is similar to substrate-attached sister taxa
- Floating morphologies likely evolved from ancestral attachment structures

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In brief

Anthony et al. test a 66-year-old assumption that animals living on the ocean's surface evolved from seafloor ancestors. Ancestral reconstructions reveal that surface animals largely evolved from substrate-attached ancestors, although not necessarily from the seafloor. This transition was likely facilitated by modifications of attachment structures.



Report

Animal evolution at the ocean's water-air interface

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SUMMARY

Innovation is a key to evolutionary success and entrance into novel ecosystems.¹ Species that float freely at the ocean's surface, termed *obligate neuston* (also called *pleuston*, here referred to simply as *neuston*), include highly specialized taxa from distinct evolutionary lineages that evolved floating morphologies.² In 1958, Soviet scientist, A.I. Savilov,³ stated that floating animal species are derived from benthic ancestors, rather than species from the adjacent pelagic zone, and that floating morphologies are homologous to benthic attachment structures. To test Savilov's hypothesis, we constructed molecular phylogenies and ancestral states for all major floating groups for which molecular data were available. Our results reveal that four of the five clades examined arose directly from a substrate-attached ancestor, although that substrate was not necessarily the benthos, as Savilov stated, and instead included epibiotic and rafting ancestors. Despite their diverse evolutionary origins, floating animals use gas-trapping mechanisms to remain at the surface,^{4–6} and many of these gas-trapping structures appear to be homologous to substrate attachment structures. We also reconstruct the trophic habits of floating mollusks and their sister species, revealing that prey preference remains conserved upon entering the ocean's surface ecosystem. Colonization of the ocean's surface seems to have occurred through successive evolutionary steps from the seafloor. Our results suggest that these steps often included transitions through epibiotic (where species attach to other living organisms) or rafting (where species attach to floating debris) habits. The water-air interface, despite its unique properties, may, in some ways, be just another substrate.

RESULTS AND DISCUSSION

Phylogenetic hypotheses and ancestral states

Most floating animal lineages in our analysis arose from substrate-attached ancestors; these lineages include barnacles, cnidarians, nudibranchs, and snails (Figures 2 and 3).

In our results, the floating barnacle, *Dosima* Gray 1825 (Figure 1A), was nested within the rafting genus *Lepas* Linnaeus 1758 in a well-supported clade forming the family Lepadidae Darwin 1852 (Figure S1A), consistent with Chan et al.⁷ Rafting species attach to floating debris at the ocean's surface, and *Dosima* barnacles attach to small floating debris as larvae^{6,8,9} and then form an autonomous float as they outgrow their larval substrate.⁶ When we step deeper into the tree, we see that the rafting and floating lineages of Lepadidae and the epibenthic/parasitic lineages of Heteralepadidae Nilsson-Cantell 1921 form a highly supported monophyletic clade, sister to a group of epibiotic barnacles of Pociasmatidae Annandale 1909. This implies that the ancestor of the floating *Dosima* transitioned from the benthos (maximum likelihood [ML] > 99%) to epibiotic (ML = 98.05%), to rafting (ML = 98.32%), and ultimately a neustonic habitat (Figure 2A).

We next examined phylogenetic relationships among the clade containing the floating *Porpita* Lamarck 1801 (blue-

buttons) and *Velella* Lamarck 1801 (by-the-wind sailors). These two sister genera form the clade Porpitidae Goldfuss 1818 within the suborder Capitata. Porpitidae is sister to a monophyletic clade consisting of the epibiotic genera *Zanclaea* Gegenbaur 1856, *Asyncoryne* Warren 1908, *Millepora* Linnaeus 1758, and *Solanderia* Duchassaing and Michelini 1846. Our results are consistent with prior phylogenetic hypotheses.^{10–12} Ancestral capitates were almost certainly benthic (ML > 99%); however, it is not clear whether floating capitates evolved from an epibiotic ancestor (ML = 49.89%) or a benthic ancestor (ML = 25.54%). Although the substrate identity is not well resolved, the ancestor of *Porpita* and *Velella* most likely had a complex, meroplanktonic life cycle (polyp and medusa), similar to their life cycle today, with a substrate-attached polyp (ML > 81.22%) giving rise to pelagic medusae (Figure 2C).

The floating siphonophore (Cnidaria) *Physalia* Lamarck 1801 is the only neustonic lineage we examined that likely arose from a pelagic (drifting) ancestor rather than an attached ancestor (ML = 92.47%). In our phylogenetic analysis, *Physalia* is nested within the pelagic Cystonectae, as sister to Rhizophysidae (bootstrap [BS] = 1) (Figure S1B), consistent with Munro et al.¹³ Although the habitat of the last common ancestor of siphonophores is uncertain,¹¹ given their phylogenetic placement within Hydroidolina,¹⁴



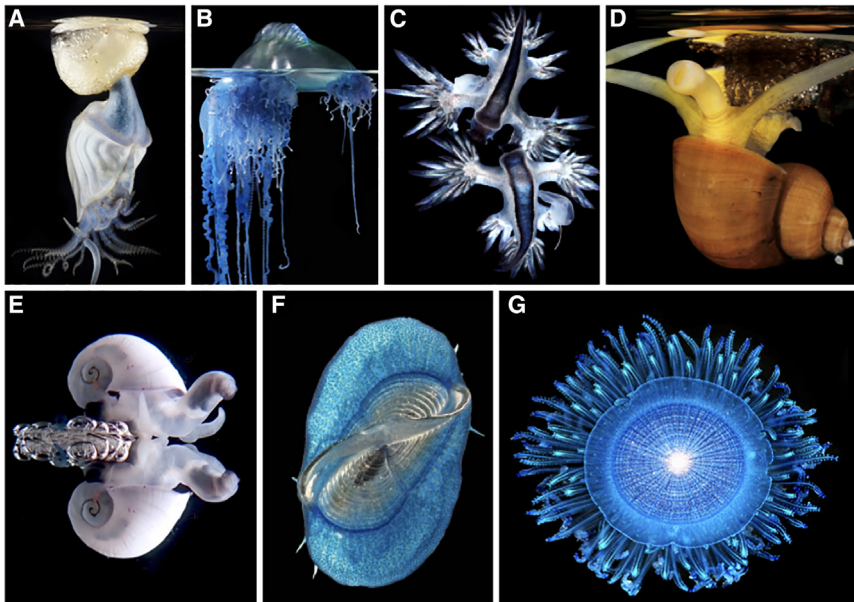


Figure 1. The neustonic groups represented in this study

This includes (A) buoy barnacle *Dosima fascicularis*, (B) Portuguese man-o-war *Physalia*, (C) blue sea dragon nudibranchs *Glaucus*, (D) floating sea snail *Recluzia*, (E) violet sea snail *Janthina*, (F) by-the-wind sailor *Velella*, and (G) blue button *Porpita*. Images by Denis Riek, modified from Helm 2021.²

Upon entering a new ecosystem, traits often shift to adhere to a new set of ecological pressures.^{1,23,24} There are a number of extreme morphological specializations that appear to have converged during colonization of the surface (Figure 1); however, broader feeding preferences and general reproductive strategies seem to remain relatively conserved.

Feeding strategies of floating barnacles and hydrozoans are unchanged, although this is not necessarily surpris-

ing. Barnacles and capitates feed on small zooplankton, similar to their substrate-attached sister clades.^{25–28} Damian-Serrano et al.²⁹ found that *Physalia* maintains its ancestral preference for fish. These barnacles and cnidarians passively collect pelagic food, which is accessible from the benthos and surface; therefore, it is not necessarily surprising that floating lineages have maintained this feeding style.

In contrast, floating mollusks are highly specific micropredators.³⁰ Both molluscan groups maintained ancestral prey preferences upon entering the surface: Hydrozoa prey for floating nudibranchs and Actiniidae prey for floating snails (Figure 3). Our ancestral state reconstruction indicates that for the nudibranch lineage Aeolidioidea, there is hydrozoan prey specificity across the backbone, with some switches in prey preference (Figure 3A). One of these switches is in the sister clade of Glaucidae, which includes *Favorinus* species that prey on eggs; however, given that egg predation appears stochastically, we hypothesize that Glaucidae maintained the prey specificity for hydrozoa evident across the phylogenetic backbone and consistent with the maximum parsimony reconstruction (Figure 3A). The floating snails *Recluzia* and *Janthina* have also retained characteristics of their likely ancestral diets. *Recluzia* maintained its ancestral preference for Actiniidae and preys on the rarely seen floating sea anemones *Actinecta*.²¹ However, *Janthina* appears to have switched prey from Actiniidae to neustonic hydrozoans (Figure 3B), and then, different *Janthina* species diverged in their preference within the neustonic hydrozoans.³⁰

Aeolioidea and Epitoniidae are some of the only Cnidaria-specific lineages of mollusks and are the only two lineages with floating members. Our results suggest ancestral specialization for cnidarian prey by benthic mollusks, a rare trait, may have facilitated the evolution of a neustonic life history.

In addition to the conservation of ancestral diet in some lineages, all floating species have complex life cycle stages that enter the pelagic zone. *Physalia* releases reproductive structures into the water column, which Totten³¹ and Munro et al.³² proposed

the pelagic siphonophores most likely arose from an ancestor with a complex life cycle that included a substrate-attaching polyp and pelagic medusan.

We examined the phylogeny of the floating blue sea dragon nudibranch family Glaucidae Gray 1827 (Figure 1C) and discovered that it is nested within a paraphyletic clade of benthic Facelinidae Bergh 1889 (Figure S1D). This clade was confidently resolved (BS = 0.87) as a sister to the rest of the superfamily Aeolidioidea Gray 1827 (Figure S1D). Although this phylogenetic hypothesis is in agreement with previously inferred relationships,^{15–17} this is the first phylogenetic analysis including Glaucidae within the broader context of Aeolidioidea.

The phylogeny of floating snails was examined within the larger clade Epitoniidae. Epitoniidae contains a monophyletic clade (BS = 1) of the floating genera *Janthina* Röding, 1798 (violet sea snails) (Figure 1E) and *Recluzia* Petit de la Saussaye 1853 (Figure 1D), albeit with low support (Figure S1E). The monophyly of this relationship, and overall phylogeny inferred herein, is consistent with prior phylogenetic studies of Epitoniidae.^{18–20} Although independent evolution of the floating *Janthina* and *Recluzia* has been suggested because of their variable shell morphology, prey preference, and fossil record,²¹ our analysis suggests that a single ancestor entered the neustonic zone as a sea anemone predator and then diversified their morphology and prey preference afterward (Figure 3B). Regardless of whether *Janthina* or *Recluzia* evolved from the same common ancestor, it is clear from our analysis that in all possible cases, each evolved from a substrate-attached ancestor.

Nudibranchs and snails moved into the neustonic zone from benthic ancestors (ML > 99%) (Figure 3); however, both neuston-containing molluscan clades are considered largely epibiotic given their tight association with cnidarian prey, a rare trait outside of these two systematic groups.²²

Ancestral trait conservation

Floating organisms form a strange, inverted community attached to a liquid-air interface instead of a solid-liquid interface (Figure 1).

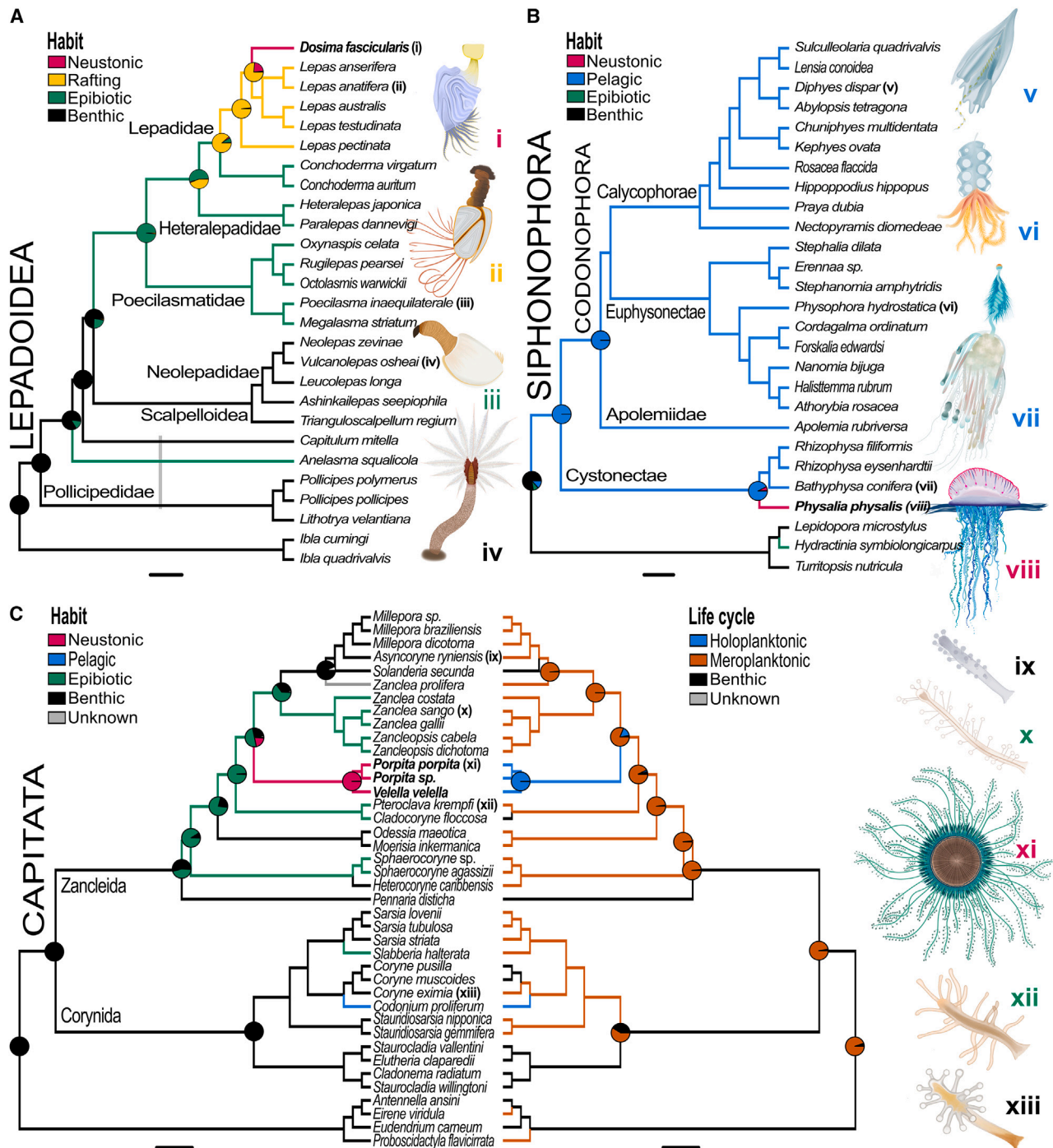


Figure 2. Ancestral state reconstructions for barnacles, siphonophores, and capitata hydrozoans

(A) Lepadoidae barnacles show a stepwise transition from benthic, to epibiotic, rafting, and ultimately neustonic habits.

(B) Among siphonophores, *Physalia* evolved a neustonic habit from a pelagic ancestor.

(C) In addition to the polyp-specific habit reconstruction (left), Capitata were also encoded as benthic (both polyp and medusa are benthic), meroplanktonic (polyp is benthic and medusan is pelagic), and holoplanktonic (both polyp and medusa are pelagic) given their unique and diverse life cycle habits. Reconstructions suggest that the neustonic capitates *Veleva* and *Porpita* most likely evolved from an epibiotic, meroplanktonic (benthic polyp and pelagic adult) habit. Maximum parsimony is represented by branch color and maximum likelihood (ML) is represented by pie charts. Ancestral trait reconstructions were mapped onto ML trees with proportionally transformed branches. Neustonic taxa are in bold. Vertical gray lines represent paraphyletic taxonomic groups.

See also Figure S1 and Table S1.

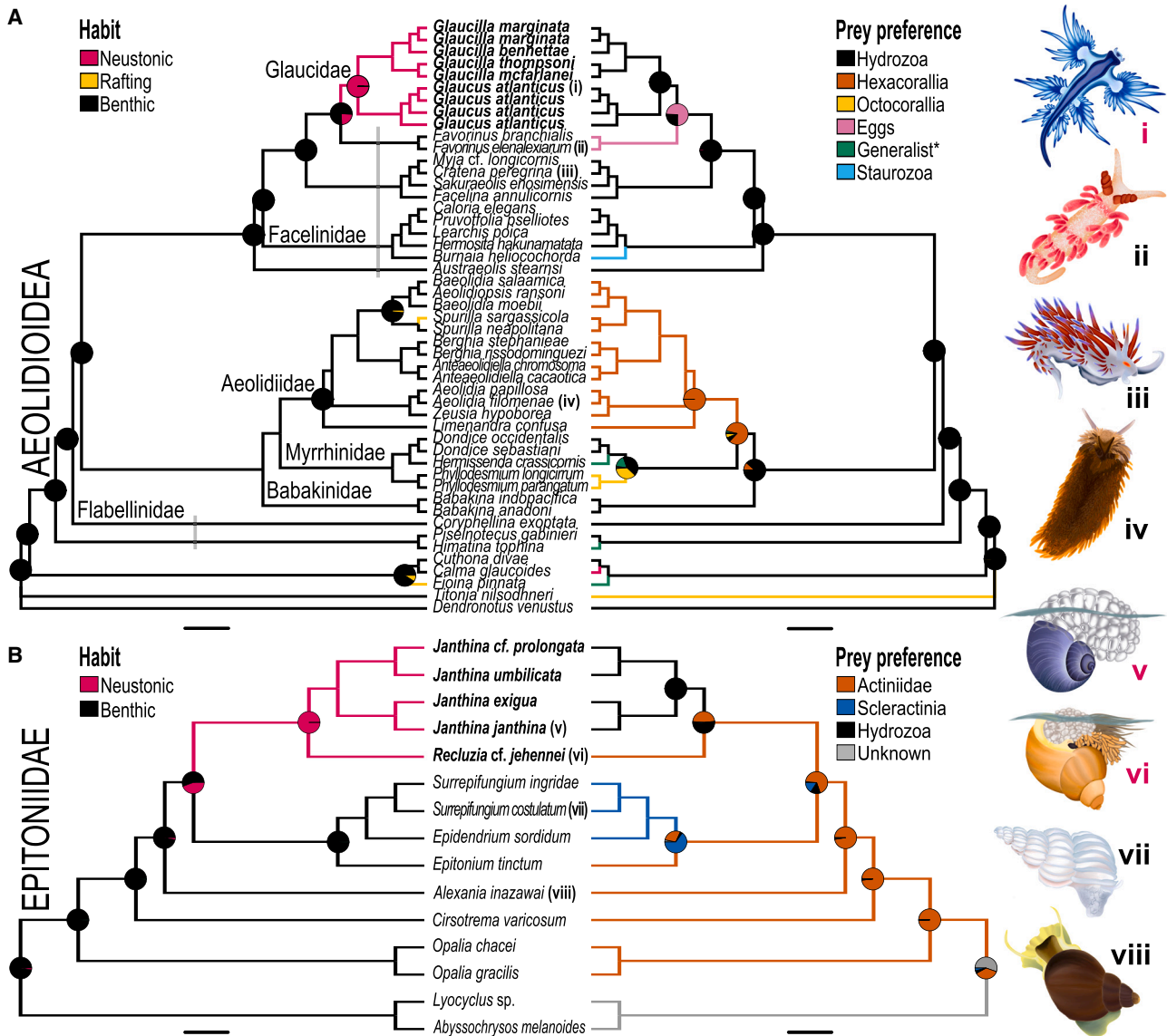


Figure 3. Ancestral state reconstructions for neuston-containing nudibranchs and snails

Both neuston-containing mollusk groups evolved from benthic ancestors with a cnidarian prey-preference; however, neustonic nudibranchs (A) maintained their ancestral prey preference, whereas neustonic sea snails diverged from their ancestral prey preference after entering the neuston (B). Maximum parsimony is represented by branch color and maximum likelihood (ML) is represented by pie charts. Ancestral trait reconstructions were mapped onto ML trees (see Figure S1) with proportionally transformed branches. Neustonic taxa are in bold. Vertical gray lines represent paraphyletic taxonomic groups. See also Figure S1 and Table S1.

may swim. Floating barnacles,³³ nudibranchs,³⁴ and snails³⁵ all have larvae that reside in the pelagic zone. Our results show that floating hydrozoan polyps *Velella* and *Porpita* have maintained their ancestral complex life cycle (Figure 2C). In hydrozoa and other cnidarians, life cycle type is generally related to dispersal ability.^{36–40} Gibbons et al. revealed³⁶ that benthic hydrozoans without a pelagic medusa stage have a narrow distribution, pelagic species without a benthic polyp stage have broad distributions, and species with both polyps and medusae in one life cycle have intermediate distributions. However, in the case of

floating hydrozoans, the polyps can disperse hundreds, possibly thousands of miles on the ocean's surface.^{2,41,42} In floating species, life cycle type and dispersal ability may be partially or fully decoupled. There are several hypotheses for why ancestral complex life cycles with “dispersal stages” persist in floating species. First, because these life cycles are ancestral, there may be little selective pressure against the persistence of pelagic stages, regardless of dispersal. Second, environmental uncertainty at the ocean's surface may promote the maintenance of these complex life cycles with stages in the comparatively stable pelagic zone. Third,

Savilov³ hypothesized that neustonic life histories exist in a kind of loop: surface stages are blown from their starting point by prevailing winds, and pelagic stages are carried back to that starting point on deeper counter-currents.^{2,3,43} However, very little is known about the longevity and depth of pelagic stages. Until we can answer basic questions about the biology and ecology of these stages, any possible selective mechanisms on pelagic stages remain obscure.

Modification of substrate attachment structures

We hypothesize that morphological floats may be modified attachment structures across floating lineages, thus indicating that the ocean's surface is just another substrate (Figure 2).

In barnacles, the homology of substrate-attaching cement and floats is well documented.^{6,33,44} Barnacle cement is packaged and secreted from gland cells at basal plates or peduncles. The floating buoy barnacle *Dosima* attaches to floating substrata as a larva using cement^{8,33} and then secretes a gas-filled cement to build a foam-like float in the adult stage.^{6,45} Lin et al.⁴⁴ described a wide array of cement proteins that diversified relatively early in barnacle evolution, presumably to attach to a variety of substrates. Zheden et al.⁴⁵ found that *Dosima* cement is similar to other barnacles, with only a few chemical variations allowing it to form its cement float.

Cnidarians have invaded the neustonic zone at least three times: (1) the capitates *Porpita* (Figure 1G) and *Velevella* (Figure 1F), (2) the siphonophore genus *Physalia* (Figure 1B), and (3) the rare floating anemone *Actinecta* (not covered in this paper due to lack of sequence data). All floating cnidarians have a gas-filled chamber at the aboral pole,⁴ the site of substrate attachment.

The enigmatic sea anemone *Actinecta* has mucus-covered bubbles in place of a substrate-attaching aboral pedal disc, which allows it to float at the ocean's surface.⁴ In the case of *Actinecta*, the location of the float is homologous to the site of attachment in benthic relatives. For floating hydrozoans, insights into the possible evolutionary homology between attachment structures and floating structures comes from embryonic development.

Floating hydrozoans all have multiple mouths, which makes the oral-aboral anatomy more complex. For hydrozoan colonies, there is a single colony aboral pole that corresponds to the larval aboral pole. In most hydrozoans that attach to a substrate, the aboral pole is the site of attachment,^{46–48} and in the case of floating hydrozoans, the colony aboral pole becomes the float.^{32,47,49–51} Although none of these species have been cultivated through full life cycles, we can infer developmental similarities from wild-collected larvae. The youngest recognizable larvae of *Velevella* and *Porpita*, known as “Conaria Larvae”, have clear aboral floats in place of attachment structures.^{52–54} In both genera, the aboral float is also chitinous.^{4,55,56} In benthic-attaching species, chitin forms the perisarc around the stolon or base of polyps.⁵⁷ Thus, both the location of the float at the aboral pole and its chitinous structure suggest that the floats of *Porpita* and *Velevella* are homologous to the attachment perisarc of substrate-attaching ancestors. In siphonophores, as in Poritidae, the colony aboral pole is the site of the float, opposite the mouth of the first zooid, known as the protozooid or first feeding zooid.^{31,32,51,58} The pelagic ancestors of *Physalia* likely already possessed a float at the aboral end of the colonies' first

zooid.^{13,32,59,60} Similar to *Porpita* and *Velevella*, this aboral pole is at the same location as benthic hydrozoans use to attach to substrates.^{46–48} According to Bentlage and Collins,¹⁴ the most probable phylogenetic hypothesis for Hydroidolina nests siphonophore within a primarily benthic dwelling clade. From this, we can assume that the ancestors of siphonophores (including *Physalia*) likely evolved from the benthos to the pelagic zone. According to Dunn et al.,⁶⁰ the float, or pneumatophore, appears ancestral. In the case of siphonophores, this float formation preceded invasion into the neustonic zone. Similar transient mechanisms of flotation are found in otherwise benthic hydrozoans. For example, the model organism *Hydra* attaches to substrates via its pedal disk but occasionally secretes a gas bubble from this disk and floats to the surface.⁴ It is easy to see how simple gas capturing mechanisms at the aboral surface could precede float evolution.

Floating mollusks attach to the surface using several methods. Flotation in *Janthina* and *Recluzia* also uses gas-trapping mucus secretions. Both species float using mucus-constructed bubble rafts.^{20,21,30,61,62} Churchill et al.²⁰ found that bubble rafts are likely modified attachment structures for anchoring egg cases to substrate. To construct the raft, snails capture a bubble from the surface with their foot, coat it in mucus, and adhere it to the raft.⁶² Both sexes make rafts in *Janthina*, but in *Recluzia*, females use their float as a raft and a substrate for egg masses, whereas males do not make rafts.²⁰ Further specialization in *Janthina* has allowed floats with egg rafts to become autonomous and repairable.^{20,30,61}

The only surface-associated group without an obvious float or raft is the nudibranch family Glaucidae. The ancestors of Glaucidae crawled along a substrate using a muscular ventral foot. Glaucidae rests with this ventral foot adhering to the water-air interface and swallows air for additional buoyancy.⁵ Thus, their crawling surface, once used to glide over benthic substrate, is now used to glide on the ocean's surface.

In addition to the extant clades examined here, there is evidence that the modified attachment structures as surface floats have a long history. The extinct floating crinoid *Scyphocrinites* used a multi-chambered, gas-filled float to drift either at the surface or in the water column. This float was at the aboral pole and was derived from the traditional crinoid substrate attachment structure, or holdfast.⁶³

A novel trait will not evolve if the underlying developmental structures and processes do not support it.^{64,65} We see that secretory cells, across all groups, appear to be key in the transition from hard substrate to the surface. Flotation and/or attachment appears to rely directly on the modification of attachment structures and their underlying secretory cells. In *Porpita* and *Velevella*, secreted chitinous scaffolding creates a flotation device at their aboral pole.^{4,55,56,66} In *Janthina* and *Recluzia*, the secreted mucous of bubble rafts is stiff in both water and air.^{20,21,30,61,62} In *Dosima* barnacles, the pedal disk secretes a cement that is airy, analogous to a marshmallow.⁶ In *Physalia*, secreted gas fills the float.^{4,67} In Glaucidae, secretory cells on the ventral surface may enhance adhesion.

Conclusions

In partial agreement with Savilov's hypothesis first set forth in 1958,³ we report that the neustonic habitat is more evolutionarily

and ecologically similar to the benthos than the pelagic zone; however, most floating lineages stepped across an intermediate substrate, including epibiotic or rafting substrates, before entering the neustonic ecosystem. We suggest this was facilitated by the continued modification of substrate attachment structures. Accompanying the modification of attachment structures are relatively conserved prey preferences and reproductive habits. From an evolutionary perspective, the ocean's surface may serve as a unique substrate type. The evolution of neustonic lineages may have been accompanied by convergent changes in underlying physiological, morphological, and developmental processes. Comparative development and genomics of these lineages, and structures related to attachment, may shed light on processes that facilitate evolution into novel habitats. Unfortunately, the open ocean's neustonic habitat is difficult to access and neustonic organisms only sporadically wash ashore, limiting research accessibility. Identifying surface-life hotspots and starting successful laboratory cultures will be critical to future work on this fascinating ecosystem.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- **KEY RESOURCES TABLE**
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 - Materials availability
 - Data and code availability
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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.cub.2023.11.013>.

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AUTHOR CONTRIBUTIONS

Conceptualization, C.J.A. and R.R.H.; data curation, C.J.A.; software C.J.A. and B.B.; formal analysis C.J.A. and B.B.; investigation C.J.A. and R.R.H.; visualization, C.J.A. and R.R.H.; writing – original draft, C.J.A. and R.R.H.; writing – review & editing, all authors.

DECLARATION OF INTERESTS

The authors declare no competing interests.

INCLUSION AND DIVERSITY

We support inclusive, diverse, and equitable conduct of research.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Character Traits	This paper	Table S1; https://github.com/AnthonyCuog/NeustonAncestralReconstruction
Phylogenetic RAxML Trees	This paper	Figure S1; https://github.com/AnthonyCuog/NeustonAncestralReconstruction
Sequence Alignments	This paper	https://github.com/AnthonyCuog/NeustonAncestralReconstruction
Molecular Data	NCBI GenBank	Table S1; https://www.ncbi.nlm.nih.gov/genbank/
Software and algorithms		
Evolutionary Analyses	This paper	https://github.com/AnthonyCuog/NeustonAncestralReconstruction
MAFFT v7.475	Katoh and Standley ⁶⁸	https://mafft.cbrc.jp/alignment/software/
Ubuntu 20.04 LTS terminal environment on Windows 10	Canonical Group Limited	https://apps.microsoft.com/store/detail/ubuntu-2004/9N6SVWS3RX71?hl=en-gu&gl=gu&rtc=1
Seaview v5.0.4 GUI	Gouy et al. ⁶⁹	https://doua.prabi.fr/software/seaview
W-IQ-TREE	Trifinopoulos et al. ⁷⁰	http://iqtree.cibiv.univie.ac.at/
RAxML v8.2.11	Stamatakis ⁷¹	https://github.com/stamatak/standard-RAxML
Mesquite v3.70 GUI	Maddison and Maddison ⁷²	https://mesquiteproject.org
R v4.2.2	R Core Team	https://r-project.org/
RStudio v1.4	Posit Software	https://posit.co/download/rstudio-desktop/
ape v5.6.2	Paradis and Schliep ⁷³	http://ape-package.ird.fr

RESOURCE AVAILABILITY

Lead contact

Further information and requests should be directed to and will be fulfilled by the lead contact, Rebecca R. Helm (rh1203@georgetown.edu).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- All data used for this research is publicly available; accession numbers, character states, and associated citations are available in [Table S1](#) and [Figure S1](#).
- This paper analyzes existing, publicly available data. These accession numbers for genetic sequences are listed in the [Figure S1](#).
- All original code and its necessary input files have been deposited on Github and are publicly available as of the date of publication (Github: <https://github.com/AnthonyCuog/NeustonAncestralReconstruction>).
- Any additional information required to reanalyze the data reported in this paper is available from the [lead contact](#) upon request.

METHOD DETAILS

Phylogenetic analyses

Phylogenies were inferred for all major neuston-containing systematic groups: Lepadoidea (Crustacea), Siphonophora (Cnidaria), Capitata (Cnidaria), Epitoniidae (Mollusca), and Aeolidioidea (Mollusca). Representative sequences of interest were extracted from NCBI GenBank (16S, 18S, 28S, COI, H3, H4). Outgroups were assigned based on results from prior broad phylogenetic

investigations.^{7,14,19,74,75} Molecular markers were independently aligned using the G-INS-i strategy implemented in MAFFT v7.475⁶⁸ within the Ubuntu 20.04 LTS terminal environment on Windows 10 with Windows Subsystem for Linux 2 (WSL2). Poorly aligned regions were excluded using Gblocks,⁷⁶ as implemented in the Seaview v5.0.4 GUI^{69,77} allowing for smaller final blocks, gaps, and less strict flanking positions. Alignments were concatenated and taxa were removed from final alignments if they were missing molecular markers, did not cluster with congeners, or had unusually long branch lengths, as determined by initial maximum likelihood (ML) phylogenies inferred with W-IQ-TREE.⁷⁰ Taxa with incomplete sequence information were retained if they were the sole representative of their genus or if their retention increased node support of relationships determined with 1000 ultrafast bootstrap replicates. Final ML trees for Lepadoidea, Siphonophora, Capitata, and Epitoniidae were inferred using RAxML v8.2.11⁷¹ under the GTR substitution model plus gamma distribution to account for rate heterogeneity, estimating model parameters separately for each alignment partition. For Aeolidioidea, relationships were unstable when inferring the phylogeny directly from the concatenated alignment, making it necessary to infer the phylogeny under a constrained backbone phylogeny. The constrained backbone was constructed within the Mesquite v3.70 GUI⁷² reflecting previously established phylogenetic relationships.⁷⁴

Ancestral state reconstructions

Ancestral state reconstructions were limited to obligate traits. Life history traits were characterized as neustonic, benthic, pelagic, epibiotic/parasitic, or rafting (Figure S1). Capitata were also encoded as benthic (both polyp and medusa are benthic), meroplanktonic (polyp is benthic and medusan is pelagic), and holoplanktonic (both polyp and medusa are pelagic), given their unique life cycle. For molluscs (Epitoniidae and Aeolidioidea), character states were encoded as neustonic, rafting, and benthic. Molluscs were also encoded for prey-preference, given their tight predator-prey associations and near-parasitic lifestyle.^{74,78–80} Ancestral states were inferred using maximum parsimony (MP) with the Mesquite v3.70 GUI⁷² and maximum likelihood (ML) with ape v5.6.2⁷³ using R v4.2.2 in RStudio v1.4. Original code and associated data for this evolutionary analysis has been deposited on Github and is publicly available (<https://github.com/AnthonyCuog/NeustonAncestralReconstruction>).