

# Mollusc museum voucher and GenBank accession numbers from a global sample set (Neuston Phylogeny project)

**Website:** <https://www.bco-dmo.org/dataset/505494>

**Version:** 2014-02-21

## Project

» [Community Phylogeny and Global Phylogeography of the Neuston](#) (Neuston Phylogeny)

Contributors	Affiliation	Role
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## Dataset Description

Taxonomy, museum registration number of voucher specimen, and GenBank accession numbers for sequences for four molecular markers (mitochondrial 16S rDNA, nuclear 28S rDNA, Histone-H3, Histone-H4) from the 19 caenogastropod species included in the molecular phylogeny of Janthinidae (Fig. 1) are listed below.

These data were used in a study of Janthinidae morphology and molecular systematics.

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 drooping. Our phylogeny also reveals sequential modifications of float formation and function among janthinid lineages.

## Methods & Sampling

### Sampling

*Janthina* spp. were collected as part of a global sampling effort of neustonic taxa via neuston tow and beach collection from 2005-2010 from: National Oceanographic & Atmospheric Administration (NOAA) Southeast Area Monitoring and Assessment Project (SEAMAP), NOAA Pacific Island Fisheries Science Center, Woods Hole Oceanographic Institute SEA Semester Program, P. Colman, C.K.C.C.

### Molecular data

Whole genomic DNA was extracted from dissected foot tissue when possible, or whole body extractions for very small specimens, using the E.Z.N.A. Mollusc DNA Kit (Omega Bio-Tek). A total of 2,217 aligned nucleotides were amplified from four molecular markers using previously published primers. 536 nt of mitochondrial 16S rDNA was amplified using universal primers combination 16Sar/16Sbr (named primer pairs are in the format 5'/3') [S1] and an annealing temperature of 49C. 1,192 nt of nuclear 28S rDNA (DI-DIII) was amplified using molluscan primers D23F/D6R [S2] and an annealing temperature of 50C. 328 nt of nuclear Histone-H4 was amplified using universal primers HexAF/HexAR [S3] and an annealing temperature of 53C. 160 nt of nuclear Histone-H4 was amplified using universal primers H42FS/H4F2er [S4] and an annealing temperature of 50C. All

PCRs followed a general protocol: initial denaturation (95C, 2 min); 35 cycles of (94C, 30 sec; XC, 30 sec; 72C, 1 min); final elongation (72C, 5 min), where X = Annealing temperature. After verifying the size of amplified fragments via gel electrophoresis, PCR products were directly sequenced using an ABI 3730xl (Applied Biosystems, Inc.) automated sequencer by the University of Michigan DNA Sequencing Core. Sequences were aligned using the MUSCLE alignment method [S5] implemented in CodonCode Aligner 3.7.1.1 (CodonCode Corporation) and verified by eye. Two sequences for *Littoraria intermedia* (mt 16S rDNA, nuclear 28S rDNA) used in this study were previously published in GenBank. Museums holding voucher specimens are identified by prefixes before catalog numbers: FLMNH, Florida Museum of Natural History, University of Florida, Gainesville, USA; UMMZ, University of Michigan Museum of Zoology, Ann Arbor, USA; RMNH, Netherlands Centre for Biodiversity Naturalis, Leiden, The Netherlands; FMNH, Field Museum of Natural History, Chicago, USA. Asterisks indicate no sequence data.

### Phylogenetic analyses

For the molecular analysis, best-fit models of nucleotide substitution were selected statistically by Akaike Information Criterion in jMODELTEST 0.1.1 [S6] for each molecular marker: mt 16S rDNA (TPM2uf+Γ), nuclear 28S rDNA (TIM3+I+Γ), Histone-H3 (GTR+I+Γ), and Histone-H4 (SYM+I+Γ). Bayesian phylogenetic analysis was conducted in MrBayes 3.1.2 [S7] (4 chains, 10 million generations) with a partitioned data set; the model of nucleotide substitution chosen for each partition was the closest approximation to the AIC best-fit model available in MrBayes: mitochondrial, (GTR+Γ); nuclear, (GTR+I+Γ). Convergence was estimated by plotting the average sums of split frequencies every 1000 generations. Bayesian posterior probabilities were calculated after a burn-in of 25%. Maximum likelihood phylogenetic analysis was conducted in PAUP\* 4.0 [S8], using the AIC best-fit models of nucleotide substitution for respective markers. Likelihood bootstrap values were also calculated with PAUP\* 4.0 [S8] with 300 replicates.

### Morphological data

To confirm and add to previous studies [4, 5, 7] supporting *Recluzia* as a plesiomorphic janthinid, ethanol-preserved oviparous and ovoviviparous *Janthina* spp., and *Recluzia* cf. *jehennei*, were dissected using standard techniques [S9] Morphological data for benthic janthinoideans (=Epitoniidae) came from previously published studies [4, 7] (Table S1).

**Table S1:** Taxonomic distribution of janthinoidean characters used to support *Recluzia* cf. *jehennei* as a transformative janthinid. [Churchill et al, 2011]

Character	Benthic janthinoideans (= Epitoniidae)	<i>Recluzia</i> cf. <i>jehennei</i>	<i>Janthina</i>
Statocysts [7]	yes	yes	no
Stylets in the inner paired salivary glands [7]	yes	yes	no
Cephalic tentacle structure [7]	uniramous	uniramous	branched
Pre-female stages associated with egg mass or float [5, 6]	yes	yes	no
Unpaired labial gland*	large	large	reduced
Metapodial attachment to egg mass or float*	mucus stalk	mucus stalk	mucus sheets

### Data Processing Description

## Relevant References:

- \* Celia K.C. Churchill, Diarmaid Ó Foighil, Ellen E. Strong, and Adriaan Gittenberger. (2011) Females floated first in bubblerafting snails. *Current Biology* Vol 21 No 19, R802. doi:10.1016/j.cub.2011.08.011
- S1. Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., and Flook, P. (1994). Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a comparison of conserved polymerase chain reaction primers. *Ann. Entomol. Soc. Am.* 87, 81-144.
- S2. Park J.K., and Ó Foighil, D. (2000). Sphaeriid and corbiculid clams represent separate heterodont bivalve radiations into freshwater environments. *Mol. Phylogenet. Evol.* 14, 75-88.
- S3. Colgan, D.J., McLauchlan, A., Wilson, G.D.F., Livingston, S.P., Edgecombe, G.D., Macaranas, J., Cassis, G., and Gray, M.R. (1998). Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. *Aust. J. Zool.* 46, 419-437.
- S4. Pineau, P., Henry, M., Suspène, R., Marchio, A., Dettai, A., Debruyne, R., Petit, T., Lécu, A., Moisson, P., Dejean, A., Wain-Hobson, S., and Vartanian, J.-P. (2004). A universal primer set for PCR amplification of nuclear Histone H4 genes from all animal species. *Mol. Biol. Evol.* 22, 582-588.
- S5. Edgar, R.C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* 32, 1792-1797.
- S6. Posada, D. (2008). jModelTest: Phylogenetic Model Averaging. *Mol. Biol. Evol.* 25, 1253-1256.
- S7. Ronquist, F., and Huelsenbeck, J.P. (2003). MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19, 1572-1574.
- S8. Swofford, D.L. (2002). PAUP\*. Phylogenetic Analysis Using Parsimony (\*and Other Methods). Version 4. (Sunderland: Sinauer Associates).
- S9. Strong, E. E. (2003). Refining molluscan characters: morphology, character coding, and a phylogeny of the Caenogastropoda. *Zool. J. Linn. Soc.* 137, 447-554.

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## Data Files

File
<b>mollusc_vouch_acc_nums.csv</b> (Comma Separated Values (.csv), 1.52 KB) MD5:0ed7706a4e46f4fa1c62c617c472e5f0
Primary data file for dataset ID 505494

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## Parameters

Parameter	Description	Units
family	taxonomic family	unitless
species	species of mollusc	unitless
voucher	museum voucher number	unitless
rRNA_16S	GenBank accession number for rRNA-16S	unitless
rRNA_28S	GenBank accession number for rRNA-28S	unitless
Histone_H3	GenBank accession number for Histone H3 marker	unitless
Histone_H4	GenBank accession number for Histone H4 marker	unitless

## Instruments

<b>Dataset-specific Instrument Name</b>	Automated Sequencer
<b>Generic Instrument Name</b>	Automated DNA Sequencer
<b>Dataset-specific Description</b>	ABI 3730xl (Applied Biosystems, Inc.)
<b>Generic Instrument Description</b>	General term for a laboratory instrument used for deciphering the order of bases in a strand of DNA. Sanger sequencers detect fluorescence from different dyes that are used to identify the A, C, G, and T extension reactions. Contemporary or Pyrosequencer methods are based on detecting the activity of DNA polymerase (a DNA synthesizing enzyme) with another chemoluminescent enzyme. Essentially, the method allows sequencing of a single strand of DNA by synthesizing the complementary strand along it, one base pair at a time, and detecting which base was actually added at each step.

## Deployments

### OFoighil\_2014

<b>Website</b>	<a href="https://www.bco-dmo.org/deployment/505320">https://www.bco-dmo.org/deployment/505320</a>
<b>Platform</b>	OFoighil_2014
<b>Description</b>	Mollusc genetic analyses from globally collected samples.

## Project Information

### Community Phylogeny and Global Phylogeography of the Neuston (Neuston Phylogeny)

**Coverage:** 5 subtropical gyre systems: North Atlantic, South Atlantic, North Pacific, South Pacific, Indian Ocean.

Abstract:

This project aims to construct the first marine community phylogeny. It is inspired by the integrative perspective that lies at the core of the modern concept of biodiversity. Empirical realization of this integration requires that study systems be simple enough to be tractable, yet (ideally) contain compelling ecological and evolutionary phenomena. The target marine community, the neuston, embodies these characteristics to an exceptional degree. It consists of a relatively small number of interacting species that drift at the water/atmosphere interface of the planet's subtropical gyres (40% of oceanic surface area) and play an important role in open-ocean epipelagic food webs. The ecological base of the neuston community is an endosymbiosis involving chondrophore cnidarian hosts (Porpitidae) and their dinoflagellate photosymbionts. Chondrophores are preyed upon by a variety of predators, chief among them two lineages of highly specialized gastropods (Janthinidae and Glaucinae). This prominent open ocean community has been poorly studied, apart

from its resident insect genus Halobates. The investigator aims to complete a combined phylogeny/phylogeography of neuston taxa across three trophic levels (photosymbionts, chondrophore hosts, predatory gastropods) and all 5 subtropical gyre systems. The two main goals of the community phylogeny section are to determine the evolutionary origins of the photosymbiosis, and to establish the benthic sister lineages of both gastropod lineages in order to identify the synapomorphic changes associated with ancestral ecological transitions from benthos to neuston. The primary aims of the phylogeographic section are to establish the spatial scale of speciation for the target neustonic taxa, and to test three hypotheses of within-species genetic structuring: global panmixis; ocean basin panmixis; within gyre panmixis. The investigator has developed a multi-faceted sampling strategy that involves ichthyoplankton research colleagues in multiple gyres systems, the bi-coastal, ocean-going ships and students of the Woods Hole Semester at Sea program, national and international museum collections, and an informal network of colleagues worldwide that will sample spontaneous neuston stranding event. He has also established collaborative relationships with colleagues expert in cnidarian, nudibranch, caenogastropod and epitoniid diversity, who will work closely with him on their respective groups within the neuston and, for gastropods, also within benthic sister lineages. To-date, preliminary samples from 3 of the 5 gyres have yielded results that not only demonstrate the feasibility of the primary project goals, but also provide exciting initial insights into the generality of the photosymbiotic association, the putative benthic sister lineages of neustonic gastropods, the likely presence of cryptic species complexes, and the divergent patterns of among-gyre and among-basin genetic structuring exhibited by sister taxa.

This project has an unusually extensive student outreach component in the form of the >100 Sea Education Association (SEA) undergraduates and high school students that will participate in multiple upcoming SSV Seamans & SSV Cramer cruises and who will collect neuston taxa. The investigator will provide detailed electronic feedback to the SEA courses, in the field, so that students will be able to connect the organisms they collect with the biogeographic and evolutionary hypotheses being testing, and to determine for themselves which hypotheses the available data reject, or corroborate. One graduate and three undergraduate UM students will receive in-depth training during this project. The graduate student, Celia Churchill, has participated in offshore neuston sampling, generated much of the preliminary data, and recently presented at her first scientific meeting. She will work/train with the P.I., and also with 4 expert collaborators, directly in their laboratories for 2 of them. One UM undergraduate student has already worked directly on this project and the investigator will recruit at least two more. Substantial international outreach and collaborative activities are planned across research specialties, especially involving ichthyoplankton colleagues working in different gyre systems, as well as international museum colleagues. This study promises to significantly enhance background knowledge of the vast subtropical gyre surface ecosystem, now heavily impacted, even in mid-ocean, by the incremental accumulation of non-biodegradable, plastic flotsam. This is a major new marine conservation issue that is just now entering public discourse, e.g., see recent descriptions of the North Pacific's Giant Garbage Patch in the popular press.

## **PUBLICATIONS PRODUCED AS A RESULT OF THIS RESEARCH**

Ó Foighil, D; Lee, T; Slapcinsky, J. "Prehistoric anthropogenic introduction of partulid tree snails in Papua New Guinean archipelagos," JOURNAL OF BIOGEOGRAPHY, v.38, 2011, p. 1625. View record at Web of Science

Churchill, CKC; Ó Foighil, D; Strong, EE; Gittenberger, A. "Females floated first in bubble-rafting snails," CURRENT BIOLOGY, v.21, 2011, p. R802. View record at Web of Science

Churchill, CKC; Strong, EE; Ó Foighil, D. "Hitchhiking Juveniles in the Rare Neustonic Gastropod *Recluzia* Cf. *Jehennei* (Janthinidae)," Journal Of Molluscan Studies, v.77, 2011, p. 441. View record at Web of Science

Ó Foighil, D; Li, JC; Lee, T; Johnson, P; Evans, R; Burch, JB. "Conservation Genetics of a Critically Endangered Limpet Genus and Rediscovery of an Extinct Species," PLOS ONE, v.6, 2011. View record at Web of Science

## **Focal Publications**

Churchill, C.K., Valdés, Á. Ó Foighil, D. 2014. Afro-Eurasia and the Americas present barriers to gene flow for the cosmopolitan neustonic nudibranch *Glaucus atlanticus*. Marine Biology, In Press.

Churchill, C.K., Valdés, Á. Ó Foighil, D. 2014. Molecular and morphological systematics of neustonic nudibranchs (Mollusca, Gastropoda, Glaucidae, *Glaucus*) with descriptions of three new cryptic species. In Press, Invertebrate Systematics.

Churchill, C.K., Alejandrino, A., Valdés, Á. Ó Foighil, D. 2013. Parallel changes in genital morphology delineate cryptic diversification of planktonic nudibranchs. Proceedings of the Royal Society B. 280:20131224. <http://dx.doi.org/10.1098/rspb.2013.1224>

Exemplar media coverage: [www.earthtimes.org/nature/glaucus-twin/2394/](http://www.earthtimes.org/nature/glaucus-twin/2394/)

Churchill, C.K. and Ó Foighil, D. 2013. Bubble rafting snails. McGraw-Hill Yearbook of Science and Technology 2013, pp. 56-58.

Churchill, C.K., Ó Foighil, D., Strong, E.E. and Gittenberger, A. 2011. Females floated first in bubble-rafting snails. Current Biology. [21: R802-R803](#). Featured on the [journal cover](#), in [Science](#) and in multiple online media outlets, e.g., [http://www.msnbc.msn.com/id/44849146/ns/technology\\_and\\_science-science/#.UPMcgY5\\_dsR](http://www.msnbc.msn.com/id/44849146/ns/technology_and_science-science/#.UPMcgY5_dsR)

Churchill C.K., Strong, E.E. and Ó Foighil, D. 2011. Hitchhiking juveniles in the rare neustonic gastropod *Recluzia cf. jehennei* (Janthinidae). Journal of Molluscan Studies. [77:441-444](#).

### Non-Focal publications

Li, J., Ó Foighil, D. & Park, J.K. 2013. Triton's trident: cryptic Neogene divergences in a marine clam (*Lasaea australis*) correspond to Australia's three temperate biogeographic provinces. Molecular Ecology 22:1933-1946.

<http://onlinelibrary.wiley.com/doi/10.1111/mec.12220/full>

Exemplar media: <http://phys.org/news/2013-03-cryptic-clams-biologists-species-plain.html>

Scott, P.V., Ó Foighil, D. & Li, J. 2013. Where's Waldo? A new commensal species, *Waldo arthuri* (Mollusca: Bivalvia: Galeommatidae), from the Northeastern Pacific Ocean. Zookeys, 316:67-80. [www.pensoft.net/journals/zookeys/article/4256](http://www.pensoft.net/journals/zookeys/article/4256)

Exemplar media coverage: <http://www.sciencedaily.com/releases/2013/07/130716120022.htm>

<http://www.foxnews.com/science/2013/07/20/heres-waldo-strange-new-alien-like-clam-species-found/>

Miura, O., Köhler, F., Lee, T., Li, J. & Ó Foighil, D. 2013. Rare, divergent Korean *Semisulcospira* spp. mitochondrial haplotypes have Japanese sister lineages. Journal of Molluscan Studies, 79:86-89. <http://mollus.oxfordjournals.org/content/79/1/86.full.pdf+html>

Li, J., Ó Foighil, D. & Middelfart, P. 2012. The evolutionary ecology of biotic association in a megadiverse bivalve superfamily: sponsorship required for permanent residency in sediment. PLoS ONE, [7\(8\): e42121](#). Featured in multiple online media outlets, e.g., <http://www.sciencedaily.com/releases/2012/08/120809090308.htm>

Li, J. and Ó Foighil, D. 2012. Host-specific morphologies but no host races in the commensal bivalve *Neaeromya rugifera*. Invertebrate Biology, [131:197-203](#).

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### Funding

Funding Source	Award
<a href="#">NSF Division of Ocean Sciences (NSF OCE)</a>	<a href="#">OCE-0850625</a>

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