

Teaching Biodiversity & Evolutionary Biology in a North American Marine Coastal Environment

SEBASTIAN KVIST, SHAENA A. MONTANARI,
HONGYU YI, BELLA FUKS,
MARK E. SIDDALL

ABSTRACT

The oceans are among the most biodiverse of Earth's environments. Introducing students to this diversity in the field provides an opportunity to examine the evolution of animals. We detail how readily a field-based biodiversity and evolution class can be designed and taught in a phylogenetically diverse marine setting in North America.

Key Words: *Teaching biodiversity; marine field stations; taxonomy; molecular phylogenetics; morphology.*

An understanding of biodiversity – and, specifically, of how diversity is tied to evolution – is difficult to fully convey in a classroom setting. Taxonomy and biodiversity are often taught through rote memorization of taxonomic names in the spirit of the Linnaean hierarchy, not allowing students the opportunity to handle real, tangible specimens. Field-based courses permit students to have hands-on, practical training in these areas without relying solely on textbooks. For biology teachers, this may entail the uncertainty of finding accessible and relatively small areas in which substantial marine phyletic biodiversity can be found. Indeed, it may surprise some to hear that such localities exist in North America. In May 2009, we conducted an 11-day field course in Passamaquoddy Bay, New Brunswick, based out of Huntsman Ocean Sciences (HOS) in St. Andrews. The main objective of the course was to survey and inventory the local marine biodiversity in terms of taxonomic richness and, moreover, to use genetic material from collected specimens to create an animal “tree of life” showing the evolutionary history of the organisms collected by the students. The variety of environments in Passamaquoddy Bay and the resources provided by HOS create a rich field experience that provides firsthand experience in discovering biodiversity and in using various methods of organismal collection. This article is meant to highlight the availability of such a location for conducting a marine field course that focuses on surveying biodiversity. Furthermore, we suggest a model for conducting subsequent molecular phylogenetic analyses that can discern the evolutionary relationships of the collected specimens. Although this course was conducted at a graduate level, it is applicable to undergraduate biology students and could even be adapted to fit a high school biology curriculum. Field studies conducted in the manner of this course illustrate the

Field studies conducted in the manner of this course illustrate the interdisciplinary nature of modern organismal biology.

interdisciplinary nature of modern organismal biology and engage students in scientific inquiries outside the walls of the classroom.

○ Course Location

All around the world are rainforests, coral reefs, and islands, each extremely high in biodiversity, and such phylogenetically diverse regions can also be found in North America. St. Andrews (Fig. 1) is the base for HOS, an extensively equipped research station (costs associated with accommodations at HOS, including meals and vessel hire, can be found at <http://www.huntsmanmarine.ca>). Located on the shores of Passamaquoddy Bay at the mouth of the Bay of Fundy, HOS houses full laboratory facilities complete with stereo and compound microscopes as well as numerous holding tanks and aquaria with flowing seawater for live specimens. For the offshore collections conducted during this course, the HOS's 50-foot research vessel, the *Fundy Spray*, was used for oceanographic sampling, including bottom dredging, sediment grabbing, and midwater trawling. In highlighting the use of HOS resources, we note that we have no official affiliation with this facility, nor do we benefit from endorsing it. A recently published review of marine laboratories (Hodder, 2009) included several useful Web sites, such as the Organization of Biological Field Stations (<http://www.obfs.org>) and the National Association of Marine Laboratories (<http://www.naml.org>), which contain databases of marine labs and other field stations all around North America that

can be utilized for teaching courses like this one. Our intent here is merely to underscore the availability and utility of such field stations in teaching.

○ Setting of Passamaquoddy Bay

Passamaquoddy Bay is an inlet of the Bay of Fundy, on the Atlantic coast of North America between the Canadian provinces of Nova Scotia and New Brunswick. The Bay of Fundy has one of the largest vertical tidal ranges in the world, and Passamaquoddy Bay experiences a mean tidal range of about 6 m (~20 feet; Lacroix & McCurdy, 1996). At its highest point, the tide in the Bay of Fundy can range upward of 16 m a day (~52 feet; Dalrymple & Choi, 2007). This macrotidal environment creates numerous,

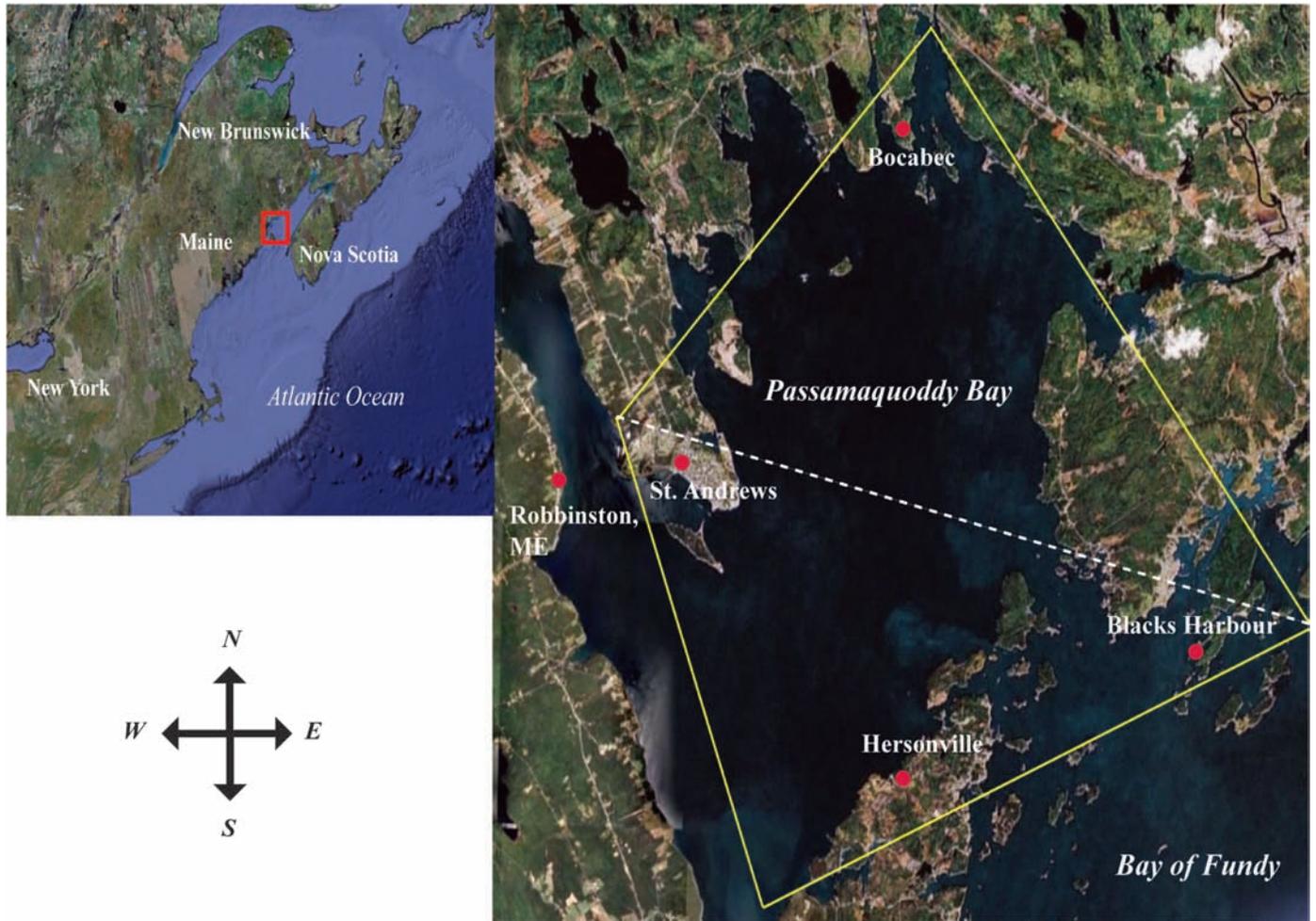


Figure 1. Map of the setting of Passamaquoddy Bay, an inlet of the Bay of Fundy. All sampling localities lay within the yellow box, and the discontinued line represents an 11-km stretch.



Figure 2. Pea Point is an example of a rocky intertidal environment located in Passamaquoddy Bay where a variety of gastropods and echinoderms are found.



Figure 3. As a contrast with Figure 2, extensive mud flats provide an ideal habitat for mud-dwelling worms and bivalves.

varied habitats in the vicinity of Passamaquoddy Bay that can be explored even without the use of a boat (Figure 2). Within a short drive (or walk) from the research station, mud flats, sandy intertidal zones, salt marshes, algal fields, mussel banks, and rocky intertidal zones are accessible (Figure 3). The numerous ecological niches of the organisms, coupled with the

many microhabitats that the changing tides create (e.g., tidal pools and rock crevasses), allows the bay to maintain a wide array of life and is ideal for a class designed to survey biodiversity. Moreover, and mostly owing to the tidal range, the bay exhibits a large variation in salinity, wave energy, and depth, which mediates the presence of numerous different species with

different habitat preferences, all within mere meters of each other. Visits to these environments, combined with oceanic excursions and sampling on the available HOS vessels, allow students to get excellent experience with various collection methods and see the immense diversity of life that can be collected in this one region. Notably, many of the phyla collected can be found at most intertidal coastal sites. This could allow schools to conduct courses under the regime detailed here without costs associated with transportation. However, we want to highlight the experiential side of learning about biodiversity, and we believe that students' experiences will be more memorable if a field trip is conducted to an unfamiliar place.

○ Equipment, Sampling & On-site Lab Work

In order to create a biodiversity inventory, it was important for the students in our course not only to learn about the taxonomic biodiversity of Passamaquoddy Bay, but also to grasp how this biodiversity can be assessed in the field – within the time constraints of an academic course. With basic field gear such as rubber boots, forceps, nets, buckets, shovels, sieves, and collection jars, the students surveyed the diversity of the various intertidal environments. Different sampling strategies, such as transects and plots, were utilized to more systematically cover the area sampled, and for this, students used a tape measure and poles to map out the sampling plot. Because the richness of the students' experiences during a biodiversity course is dependant on unveiling this diversity, finding a successful sampling strategy is pivotal. Discussions and empirical testing of different strategies may be useful for increasing the students' understanding of diversity inventories. For example, insufficient sampling effort and equipment bias may obscure the impressions of the actual diversity. Discussions of the effect of sampling strategies on apparent diversity are numerous in the literature (e.g., Gray, 2000; Stockwell & Peterson, 2002).

There are a variety of ways to fix and preserve specimens. Absolute (100%) ethanol was used here for preserving specimens meant for DNA sequencing because the high percentage of ethanol helps ensure that genetic material can be successfully extracted in the laboratory. However, it has been suggested that absolute ethanol should not be used for preserving some taxa (e.g., zooplankton; <http://www.cmarz.org/>). Instead, a lower-alcohol-content preservative (e.g., 95%) is used, and this may also be advisable when preserving other taxa (see Dawson et al., 1998). Formalin can be used to preserve specimens for collection purposes and morphological analyses, but genetic material cannot be extracted from these specimens. Formalin, ethanol, and RNAlater can be obtained from any biology supply catalogue. Parasites and various types of small organisms can be fixed, stained, and cleared on glass slide mounts for viewing internal morphology. Preservation of identified organisms is necessary for all specimens that are intended for DNA sequencing and, in addition, preserved specimens can be used for teaching purposes within the classroom. Identification of organisms collected in the field was based on detailed regional field guides for marine life that are catalogued at the research station (e.g., Smith, 1964; Gosner, 1971; Bromley & Bleakney, 1985).

Parasites, too, can be part of the biodiversity survey, and these are easily found when dissecting and examining the intestines of ray-finned fishes and skates and when performing blood smears on microscope slides. This is an example of an unexpected unit of study that can be presented to students to demonstrate that there is considerably more diversity in the marine realm than meets the eye.

The scope of morphological adaptations that can be found by the students provides a great setting in which to introduce them to the evolution of life and form. A unit describing the taxonomy, life history, and evolutionary position of each marine phylum helps the students begin to understand how organisms found in the bay can illustrate some of the evolutionary transitions that these forms of life have undergone. For example, the presence of sea squirts in Passamaquoddy Bay

permits the students to study tunicates (phylum Chordata), which are related to all later vertebrates, including humans. Constructing such lessons for all the collected phyla gives the students an evolutionary context for the organisms they are finding and help them comprehend the tree of life before they create their own hypothesis with the gathered specimens.

Allowing students to engage in hands-on sampling has numerous benefits. Beyond learning about the lifestyle strategies of various organisms, how physical properties of the ocean such as tides and water depth influence biodiversity, and how to sample these organisms most effectively, students are exposed to the pure joy of field collecting.

○ Molecular Phylogenetics

Studying biodiversity in terms of taxonomic richness is essentially an exercise in recognizing morphological traits and relating them to existing taxonomic and nomenclatural keys, the final result of which is relating the specimen to a scientific name (authoritative taxonomic information on plants, animals, fungi, and microbes of North America can be found on the Integrated Taxonomic Information System Web site at <http://www.itis.org>). Although this is no trivial exercise given the ongoing debates over the taxonomy and systematics of a multitude of organisms (e.g., because of competing categories of characters; see Bowker, 2000; Cantino & de Queiroz, 2004), studies that highlight descent from common ancestors may help students understand why certain organisms share higher-level taxonomic names. For example, lobsters and barnacles are both included in the subphylum Crustacea, something that may be hard to understand from viewing only adult specimens. Biramous limbs and nauplius larvae, apomorphic characters for crustaceans, can readily be optimized on an evolutionary tree, showing students why these taxa and other crustaceans share taxonomic identity. Thus, phylogenetics (the study of evolutionary relatedness) can be a logical continuation of taxonomic studies to further students' knowledge of the hierarchical categorization of biodiversity.

The results of phylogenetic studies are commonly illustrated in the form of a tree of life, also known as a phylogenetic tree, that depicts the evolutionary paths the organisms have taken (see <http://tolweb.org/tree/>). Rather simplified, a phylogenetic tree consists of bifurcating branches. At the tips of these branches are the sampled specimens, and the bifurcations represent the points at which two or more ancestors diverged morphologically and/or genetically. Through phylogenetic analysis one can trace evolution backwards, from the specimens under study to the root of the tree, via a series of common ancestors. As such, phylogenetics can greatly facilitate the learning of morphological and genetic evolution and the taxonomic classification of life, because the tree provides a visual representation of the evolutionary pathways. We believe that the value of adding a phylogenetic level to a study of biodiversity comes from understanding why the presence of some morphological characters (apomorphies) will place organisms in different taxonomic groups. In addition, because similarity does not equal common ancestry, a phylogeny will ensure that the students have correctly identified specimens that may be more or less nondescript or morphologically dubious.

DNA sequencing is no longer a prohibitively advanced technology. We encourage any teacher who wants to perform a field course similar to this to consider how students will benefit from being taught molecular techniques such as DNA sequencing. Students are more likely to appreciate and remember information gained by practical applications (especially those carried out by themselves) than that gained by theoretical applications alone (Zervanos & McLaughlin, 2003). The layout of this course naturally welcomes further analyses of the organisms that were collected. Having been the collectors, the students already have an affinity for the specimens, which will make any subsequent analyses increasingly interesting to them.

○ Laboratory-Based Portion

For this course, the result of the collection and genetic analyses was intended to be a phylogenetic tree encompassing a substantial part of the marine animal kingdom and based on specimens collected in Passamaquoddy Bay.

In total, 141 animal species were recorded within an 11-km radius (~7 miles), including representatives of 12 animal phyla (see Table 1). We preserved 53 specimens in absolute ethanol for DNA sequencing, and a total of 115 specimens were preserved in either ethanol, RNAlater, or formalin. At least one specimen from each of the represented phyla was chosen for the DNA sequencing to ensure that the resulting tree encompassed the broad diversity found.

Standard protocols were used for the total genomic DNA extraction, and for the amplification and sequencing of the cytochrome-*c* oxidase subunit I gene (COI) and the small nuclear subunit ribosomal gene (18S). The protocol in its entirety can be found at <http://sites.google.com/site/kvistetalab/home>. The ribosomal 18S was used for the phylogenetic analysis because it has previously been shown to evolve at a rate that is useful for reconstructing deeper (more ancient) phylogenetic relationships among animal phyla (e.g., Zrzavy et al., 1998). The COI gene, on the other hand, was used to verify field identifications through DNA barcoding

(see Hebert et al., 2003). The purpose of DNA barcoding is to allow for genetic identification of unknown, partial, or damaged specimens, so its use is ideal for nonexperts. The attention directed toward this method has greatly increased since its conception, with possibilities and limitations being widely published (e.g., Hajibabaei et al., 2006; Siddall et al., 2009). Regardless of this, the COI gene has in several cases been shown to be a good indicator of differing evolutionary histories among organisms.

This stage of the course was meant to give important insights and practical knowledge of basic DNA isolation, amplification, and sequencing, all under the umbrella of molecular systematics and evolutionary mechanisms.

○ Phylogenetic Analysis

At the phyletic level, biodiversity is greater in the marine than in the terrestrial, freshwater, or symbiotic realms, with several phyla being exclusively endemic to the oceans (Grassle et al., 1991). Biodiversity is frequently discussed at the phyletic level (e.g., Gray, 1997; Lopez et al., 1999), and therefore our phylogenetic analysis was geared toward including representatives of each phylum collected. Initially, our COI sequences were compared with the Barcoding of Life Data System (BOLD; <http://www.barcodinglife.org>), which currently manages more than 800,000 COI sequences. BOLD

Table 1. Alphabetical list of phyla found in Passamaquoddy Bay, including lower hierarchical ranks of the organisms.

Phylum	Lower Taxonomic Rank	Phylum	Lower Taxonomic Rank
Annelida	Class: Polychaeta	Cnidaria	Class: Anthozoa
	Class: Clitellata		Class: Hydrozoa
	Subclass: Oligochaeta		Class: Scyphozoa
	Subclass: Hirudinea		
		Echinodermata	Class: Echinoidea
Arthropoda	Subphylum: Crustacea		Class: Holothuroidea
	Class: Malacostraca		Class: Asteroidea
	Order: Amphipoda		Class: Ophiuroidea
	Order: Isopoda		
	Order: Decapoda	Hemichordata	Class: Enteropneusta
	Class: Maxillopoda		
	Subclass: Thecostraca	Mollusca	Class: Bivalvia
	Infraclass: Cirripedia		Class: Gastropoda
Subclass: Copepoda		Class: Polyplacophora	
Class: Ostracoda			
		Nematoda	Class: Unknown
Bryozoa	Class: Gymnolaemata		
	Order: Ctenostomata	Nemertea	Order: Hoplonemertea
			Order: Heteronemertea
Chordata	Subphylum: Urochordata		
	Class: Ascidiacea	Platyhelminthes	Class: Turbellaria
	Subphylum: Vertebrata		Order: Polycladida
	Class: Actinopterygii		Class: Cestoda
	Class: Sarcopterygii		Class: Trematoda
		Porifera	Class: Demospongiae

is open-access and rapidly reports the identity of the closest match to the query sequence. In all but four cases, the COI sequences from our identified specimens were matched with representatives of the same phylum and, in most cases, these were also matched with the same genus as determined by the students. In five cases, however, the COI sequences found no match in the database, which suggests that these particular species have not been sequenced before. Clearly, BOLD is a great instrument for establishing the identity of specimens and, for this project, identification of specimens need not necessarily go further than the phyletic level.

It is important to understand that any sequencing effort performed at this level will be more or less incomplete in terms of taxonomic and genomic inclusiveness, which could lead to skewed phylogenetic hypotheses. There are simple (albeit rather costly) ways of remedying this (e.g., more complete taxon sampling and gene sequencing). We chose to minimize the error involved in recovering phylogenetic relationships (while keeping costs low) by also including forty-two 18S sequences downloaded from GenBank (<http://www.ncbi.nlm.nih.gov/>), thus stabilizing the tree,

because phylogenetic algorithms benefit from larger sample sizes.

The phylogenetic analysis discussed here is described in its entirety at <http://sites.google.com/site/kvistetalab/home>, and the phylogenetic tree is presented in Figure 4. The tree agrees with recent phylogenetic hypotheses in many respects, including the monophyly of Deuterostomia and Chordata (colored blue in Figure 4). Although each of the phyla are recovered as monophyletic in the tree, some of the relationships between these are somewhat questionable with respect to reigning hypotheses of the tree of life. Specifically, Bryozoa groups as sister to Crustacea, which contrasts with its position among the Lophotrochozoa in other hypotheses (e.g., Halanych et al., 1995; Halanych, 2004). In addition, in our tree, Nematoda represents the sister-group of Platyhelminthes, although the former is commonly nested within Ecdysozoa, including, among other phyla, arthropods and tardigrades (Hillis, 2004 and references therein). The shortcomings of our hypothesis are likely the result of incomplete taxon and gene sampling. Nevertheless, it is notable that our tree is largely consistent with current

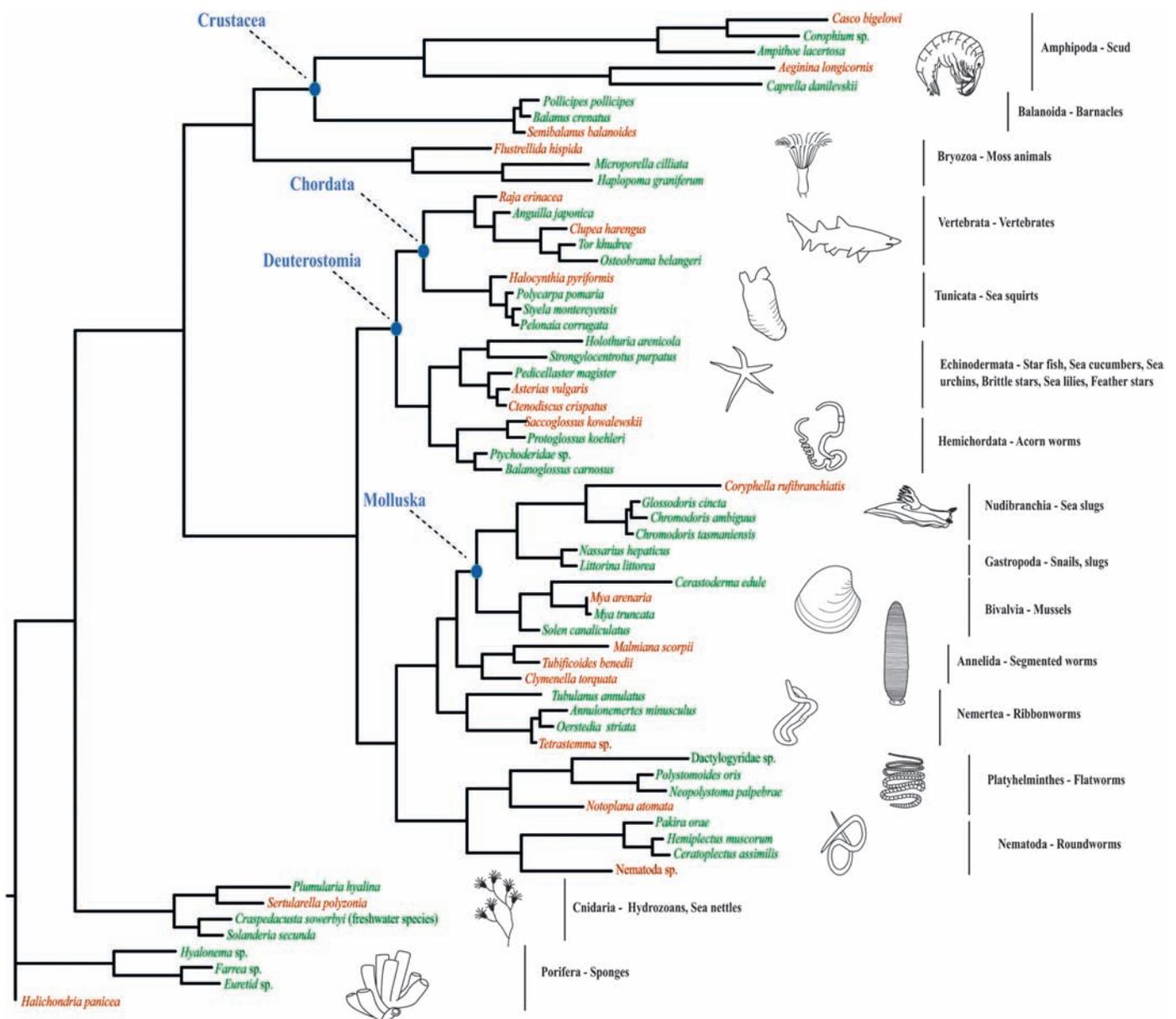


Figure 4. Phylogenetic tree of the 18S gene, constructed using parsimony. Specimens in orange were collected by the authors, and specimens in green were downloaded from GenBank. Although the tree largely agrees with previous hypotheses of phylogenetic relationships, it is shown here foremost to illustrate the large diversity found in Passamaquoddy Bay.

hypotheses. This illustrates the power of choosing a gene with an evolutionary rate that fits the taxonomic level under study, as well as the added power that comes from taking advantage of extensive databases such as GenBank and EMBL.

There are several ways to lower costs associated with DNA sequencing; prices of materials, costs of analyses, and ways to lower these costs are detailed in our Web supplement at <http://sites.google.com/site/kvistetalab/home>.

○ Conclusions

An enriching biodiversity field course for college or high school students need not be prohibitively expensive or difficult. Huntsman Ocean Sciences in St. Andrews, New Brunswick, provides a good example of easy access to a phylogenetically diverse marine system. There are numerous other facilities like HOS in North America that can be utilized for any field-based course. Moreover, DNA sequencing and phylogenetic analyses are workable, and several methods exist to increase the chances of success. Biodiversity field work coupled with the subsequent molecular phylogenetics lab work will provide students with a new look at the natural world that would be impossible solely in the classroom.

○ Acknowledgments

We thank Stephen Thurston at the AMNH for providing drawings of the organisms in Figure 4. We also thank the staff at HOS for aiding in specimen collection. An early draft of the manuscript was greatly improved by thoughtful and productive comments from three anonymous reviewers.

References

- Bowker, G.C. (2000). Biodiversity datadiversity. *Social Studies of Science*, 30, 643–683.
- Bromley, J.E.C. & Bleakney, J.S. (1985). *Keys to the Fauna and Flora of Minas Basin*. Ottawa, ON: National Research Council of Canada, Atlantic Research Laboratory.
- Cantino, P.D. & deQueiroz, K. (2004). PhyloCode: a phylogenetic code of biological nomenclature. Available online at <http://www.ohio.edu/phylocode/>.
- Dalrymple, R.W. & Choi, K. (2007). Morphologic and facies trends through the fluvial–marine transition in tide-dominated depositional systems: a schematic framework for environmental and sequence-stratigraphic interpretation. *Earth-Science Reviews*, 81 (3–4), 135–174.
- Dawson, M.N., Raskoff, K.A. & Jacobs, D.K. (1998). Field preservation of marine invertebrate tissue for DNA analyses. *Molecular Marine Biology and Biotechnology*, 7, 145–152.
- Edgar, R.C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, 32, 1792–1797.
- Gosner, K.L. (1971). *Guide to Identification of Marine and Estuarine Invertebrates: Cape Hatteras to the Bay of Fundy*. New York, NY: John Wiley & Sons.
- Grassle, J.F., Lasserre, P., McIntyre, A.D. & Ray, G.C. (1991). Marine biodiversity and ecosystem function. *Biology International Special Issue 23*. Paris, France: International Union of Biological Sciences.

- Gray, J.S. (1997). Marine biodiversity: patterns, threats and conservation needs. *Biodiversity and Conservation*, 6, 153–175.
- Gray, J.S. (2000). The measurement of marine species diversity, with an application to the benthic fauna of the Norwegian continental shelf. *Journal of Experimental Marine Biology and Ecology*, 250(1–2), 23–49.
- Hajibabaei, M., Janzen, D.H., Burns, J.M., Hallwachs, W. & Hebert, P.D.N. (2006). DNA barcodes distinguish species of tropical Lepidoptera. *Proceedings of the National Academy of Sciences*, 103, 968–971.
- Halanych, K.M. (2004). The new view on animal phylogeny. *Annual Review of Ecology, Evolution and Systematics*, 35, 229–256.
- Halanych, K.M., Bacheller, J.D., Aguinaldo, A.-M.A., Liva, S.M., Hillis, D.M. & Lake, J.A. (1995). Evidence from 18S ribosomal DNA that the Lophophorates are protostome animals. *Science*, 267, 1641–1643.
- Hebert, P.D.N., Ratnasingham, S. & deWaard, J.R. (2003). Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society of London B*, 270(Supplement), S96–S99.
- Hillis, D.M. (2004). Assembling the Tree of Life. In Cracraft, J. & Donoghue, M.J. (Eds.), *The Tree of Life and the Grand Synthesis of Biology* (pp. 545–547). New York, NY: Oxford University Press.
- Hodder, J. (2009). What are undergraduates doing at biological field stations and marine laboratories? *BioScience*, 59, 666–672.
- Lacroix, G.L. & McCurdy, P. (1996). Migratory behaviour of post-smolt Atlantic salmon during initial stages of seaward migration. *Journal of Fish Biology*, 49, 1086–1101.
- Lopez, J.V., McCarthy, P.J., Janda, K.E., Willoughby, R. & Pomponi, S.A. (1999). Molecular techniques reveal wide phyletic diversity of heterotrophic microbes associated with *Discodermia* spp. (Porifera: Demospongia). *Memoirs of the Queensland Museum*, 44, 329–341.
- Siddall, M.E., Fontanella, F.M., Watson, S.C., Kvist, S. & Erséus, C. (2009). Barcoding bamboozled by bacteria: convergence to metazoan mitochondrial primer targets by marine microbes. *Systematic Biology*, 58, 445–451.
- Smith, R.I. (1964). *Keys to Marine Invertebrates of the Woods Hole Region*. Boston, MA: Spaulding.
- Stockwell, D.R.B. & Peterson, A.T. (2002). Effects of sample size on accuracy of species distribution models. *Ecological Modelling*, 148, 1–13.
- Swofford, D. (2002). *PAUP*: Phylogenetic Analysis Using Parsimony (*and other methods), Version 4.0b*. Computer software and manual. Sunderland, MA: Sinauer Associates.
- Zervanos, S.M. & McLaughlin, J.S. (2003). Teaching biodiversity and evolution through travel course experiences. *American Biology Teacher*, 65, 683–688.
- Zrzavy, J., Mihulka, S., Kepka, P., Bezdek, A. & Tietz, D. (1998). Phylogeny of the Metazoa based on morphological and 18S ribosomal DNA evidence. *Cladistics*, 14, 249–285.

SEBASTIAN KVIST is a graduate student at the Richard Gilder Graduate School at the American Museum of Natural History, Central Park West at 79th Street, New York, NY 10024; e-mail: skvist@amnh.org. SHAENA A. MONTANARI is a graduate student at the Richard Gilder Graduate School at the American Museum of Natural History; e-mail: smontanari@amnh.org. HONGYU YI is a graduate student at Columbia University/American Museum of Natural History. BELLA FUKS is an undergraduate student at Long Island University Brooklyn Campus, 1 University Plaza, Brooklyn, NY 11201; e-mail: bella.fuks@my.liu.edu. MARK E. SIDDALL is Curator of Invertebrate Zoology at the American Museum of Natural History; e-mail: siddall@amnh.org.