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RESOLVING THE 150 YEAR DEBATE OVER THE ECOLOGICAL HISTORY OF THE COMMON PERIWINKLE SNAIL, *Littorina littorea*, IN NORTHEAST NORTH AMERICA.

BY

APRIL M.H. BLAKESLEE

B.A., Boston University, 1998

M.A., Boston University, 2001

DISSERTATION

Submitted to the University of New Hampshire

in Partial Fulfillment of

the Requirements for the Degree of

Doctor of Philosophy

in

Zoology

May, 2007

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DEDICATION

I wish to dedicate this dissertation to my parents, Dr. Jan Laws Houghton and Dr. Raymond C. Houghton, Jr., for their continued encouragement and support over the years in helping me work towards the kind of education that would make me happiest. I am grateful for the opportunities they have provided for me, and I only hope I can do the same someday for my son, Westley.

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program has been extremely rewarding—I have made some lasting friendships, collaborations, and have gone on some great overseas trips (twice to Denmark!) all thanks to this program and to Andy Rosenberg who selected me to be part of it. In addition, various UNH departments and programs have also been very generous: I have received travel funding to attend conferences for the past 5 years from the graduate school, the Zoology department, and the Marine Program. In addition, the Marine Program provided a couple small research grants, which helped fund my molecular work and travel to collection sites. The Zoology department also awarded me a Hatch Grant summer 2006. Finally, the UNH Writing Center provided me two years of a Graduate Fellowship, and the Zoology Department gave me three Teaching Assistant positions. I have also received small grants from several sources outside the University, including Sigma Xi, which provided me research grants two years in a row; Lerner-Gray (American Museum of Natural History); and the American Malacological Society. Jeb Byers also received an NSF SGER grant (OCE 05-03932), which was used to fund many aspects of my research, especially the expensive molecular parts. Finally, the Shoals REU program has provided me with a lot of support in the form of research help and in my experimental and mentoring education.

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ABSTRACT

RESOLVING THE 150 YEAR DEBATE OVER THE ECOLOGICAL HISTORY OF THE COMMON PERIWINKLE SNAIL, *Littorina littorea*, IN NORTHEAST NORTH AMERICA.

by

April Monica Houghton Blakeslee

University of New Hampshire, May, 2007

Littorina littorea (common periwinkle snail) is highly abundant in both Europe and North America. A known native of Europe, its presence in North America has been the subject of a one-hundred year debate. Prior published work attempting to resolve its cryptogenic (=uncertain origin) status with historical, archaeological, ecological and/or genetic data were not successful. I therefore included novel parasite and molecular evidence to definitively resolve *L. littorea's* North American cryptogenic status.

First, I explored trematode species richness patterns in European versus North American *L. littorea* and two co-occurring native congeners, *L. saxatilis* and *L. obtusata.* Through extensive field and literature surveys, I found only *L. littorea* to possess significantly fewer trematode species in North America, while all *Littorina sp.* North American trematodes were a nested subset of Europe. Overall, these results suggest a recent invasion to North America for *L. littorea* and an older, natural expansion to North America for *L. saxatilis* and *L. obtusata.*

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Second, I explored genetic founder effect signatures in North American *L. littorea*. I sequenced a ~1200 base-pair region of mitochondrial DNA in nearly 400 Europe and North American snails. My results demonstrate a significant reduction in overall genetic diversity in North America versus Europe, nested and common haplotype frequencies in North America, and a divergence estimate of no greater than 450 years ago.

Third, I explored genetic founder effect signatures in *L. littorea's* most common trematode parasite, *Cryptocotyle lingua*. I sequenced a 1043 base-pair region of mitochondrial DNA and found *Cr. lingua* to show several signatures of a recent introduction to North America, including a significant reduction in haplotype diversity in North America, nested and common haplotype frequencies in North America, and a range of divergence estimates between 240-480 years ago.

Altogether, these three corroborative pieces of evidence suggest a recent, likely human-mediated introduction of North American *L. littorea* from Europe. This work represents the first time ecological studies of parasites and genetics have been used in concert to resolve the cryptogenic status of an important marine species. The successful application of these techniques can therefore be useful in cryptogenic investigations worldwide.

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INTRODUCTION

In recent years, species invasions have become recognized as a major component of human-mediated impacts on natural systems. Specifically, species invasions in marine environments have been shown to cause considerable harm to native communities—as exemplified by San Francisco Bay on the United States (U.S.) west coast. From 1961 to 1995, one new species invaded the bay about every 14 weeks, and these marine exotics made up 40-100 percent of the common species within the bay, up to 97 percent of its species abundance, and up to 99 percent of the species biomass (Cohen and Carlton 1998). Due to this high rate of invasion, San Francisco Bay is presently considered one of the most heavily invaded aquatic ecosystems in the world, and these invasions greatly contribute to the bay's high level of degradation (Cohen and Carlton 1998). Thus, marine invasions can have profound effects on ecosystems, resulting in significant implications for ecological and management work.

One major dilemma in the study of invasion biology, however, is that not all species observed in a particular ecosystem can be demonstrably classified as native or non-native. These species are referred to as cryptogenic and can comprise a considerable number of the species observed in a particular region (Carlton 1996). For example, Carlton (1996) found that *possible* invasions – i.e., cryptogenic species – would increase by as much as one-third the number of known invasions in San Francisco Bay if these proved to be introduced. The number of cryptogenic species that are actually non-

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indigenous on the east coast of the U.S. is predicted to be even higher than on the west coast because the east coast was settled by Europeans much earlier than the west coast and before rigorous inventories of native biota were consistently undertaken (Ruiz et al. 2000). In Chesapeake Bay, for example, close to 30% of the total species found in the Bay may actually be cryptogenic (Ruiz et al. 2000). For resource managers and conservation biologists attempting to restore and maintain native species, biological diversity, and ecosystem function and services, knowledge of the native members of the community is a fundamental starting point—especially as little can be done (or is unlikely to be done) to address potential impacts of these species when it is uncertain whether they are non-native. Especially in the case of conspicuous, abundant species, resolution of the ambiguous ecological history of cryptogenic species is a critical conservation goal (even if removal of a given species may now be impossible, other conservation and management goals may then defendably take priority, such as protecting truly native or endangered species).

When the evidence for invasions is not obvious (typically because they have occurred prior to human exploration of the oceans and their coastlines) (Carlton 2003), methodologies for resolving their native or non-native status have been carried out using certain 'criteria', or criteria-based evidence for introductions (e.g., Chapman and Carlton 1991). These criteria include evidence from geographical sources, the biology and ecology of the species, historical data, and genetics (Chapman et al. 2007a, Chapman et al. 2007b). For example, close association with human mechanisms of transport or as a food source are often correlated with anthropogenic introductions (Chapman et al.

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2007a). Furthermore, the absence of a fossil record for the purported founding population and the lack of a natural dispersal mechanism (such as stepping stone invasion) are also potential signatures for an introduction. Finally, strong genetic bottlenecks can result from recent founding events (Grosberg and Cunningham 2000). Because these criteria represent clues for a species' status as native or non-native, they can therefore be used as signatures for resolving cryptogenic histories.

One North American cryptogenic species, whose status as native or non-native has been debated for the past 150 years (e.g., Ganong 1886, Clarke and Erskine 1961, Clarke 1963, Berger 1977, Wares et al. 2002, Chapman et al. 2007a, Chapman et al. 2007b, Wares and Blakeslee, in press) is the marine snail, *Littorina littorea* (common periwinkle). Not only has this species been used as a model species for marine introductions, but this snail has profoundly impacted marine communities, making the resolution of its cryptogenic status even more important. Furthermore, because a species' cryptogenic status often stems from incomplete or unknown historical knowledge of its presence in a region (Ruiz et al. 2000), the lingering ambiguity surrounding *L. littorea* is particularly surprising given the vast amount of research over the past several decades that has been conducted on this species, which has included historical, archaeological, ecological, and genetic data (e.g., Ganong 1886, Clarke and Erskine 1961, Clarke 1963, Bird 1968, Berger 1977, Carlton 1982, Wares et al. 2002, Chapman et al. 2007, Wares and Blakeslee, in press). The following sections provide detail on this research, including ecological information on *L. littorea's* range, spread, and impacts on North American

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populations, as well as evidence that has thus far been gathered attempting to resolve the snail's North American cryptogenic history.

Littorina littorea **– ecology and current ranges**

Littorina littorea is a marine gastropod with planktotrophic larvae that exists in widely varying environmental conditions, including the rocky intertidal zone, estuarine, , and, at times commonly, both sandy and muddy environments (Moore 1937, Brenchley and Carlton 1983, Reid 1996). Snails can attain relatively large shell lengths; e.g., the largest individual ever recorded was 52.8 mm (Reid 1996); 33.71 mm was the largest individual personally observed in North America. Furthermore, populations of *L. littorea* can be extremely abundant, reaching numbers upwards of 150 individuals per 0.05 m^2 (pers. obs.), which converts to about 3000 individuals per m^2.

Littorina littorea is currently found in two large North Atlantic populations, Europe and North America, but is absent from North Atlantic islands (including the Faroe Islands, Iceland and Greenland) where congeners, *L. saxatilis* and *L. obtusata*, have historically been and are presently found. In the European population, *L. littorea* is found from the White Sea, Russia to Portugal (Reid 1996), and in North America, established populations are found from Red Bay, Labrador to Cape May, NJ (Reid 1996; pers. obs.), though ephemeral populations have been found as far south as Virginia (Reid 1996). *Littorina littorea* is known to be native to Europe, but its presence in North America is less understood.

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Spread and impacts

What is known about *Littorina littorea's* presence in North America is that in the 1850s, *L. littorea* spread rapidly and sequentially southwards from Halifax, Nova Scotia (with oral reports placing it about 15 years earlier in Pictou, Nova Scotia) into the United States, reaching Cape May, New Jersey only 30 years later. This invasion was well documented in the literature and secondary sources, and the pattern of this invasion was pieced together by Carlton (1982) and by Steneck and Carlton (2001).

The influence of this spread on intertidal communities is also well known. *Littorina littorea* is the dominant herbivore in the U.S. rocky intertidal zone and has been shown to significantly impact algal communities (Lubchenco and Menge 1978, Lubchenco 1983, Bertness 1984). It is also believed to have competitively displaced native snails, including *L. saxatilis* and *Ilyanassa obsoleta*, in areas where it is abundant (Yamada and Mansour 1987, Brenchley and Carlton 1982). Thus, *L. littorea's* presence in the U.S. within the last 150 years has had major impacts on both marine flora and fauna in the intertidal zone.

Littorina littorea's cryptogenic status

Although *Littorina littorea's* invasion into the U.S. was well documented, uncertainty regarding its status as native or non-native in North America has persisted to this day. What remains uncertain is whether the maritime Canada population from which the spread into the U.S. originated was: 1) native and confined to maritime Canada until the mid-1800s, or 2) an anthropogenically introduced population from Europe that was

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first established in maritime Canada and then invaded southward in the mid-1800s (Reid 1996). Due to this uncertainty, *L. littorea* is presently considered cryptogenic in North America.

Evidence for *L. littorea's* ecological history in North America

The puzzle over *Littorina littorea's* ecological history in northeast North America has been assessed by several researchers since 1886 using a number of different sources of evidence, including historical, archaeological, ecological and genetic data (Table 1). Within each section, I have summarized the evidence to date and then critically analyzed the conclusions.

Historical Evidence. *Littorina littorea's* first recorded sighting was at Pictou, Nova Scotia, sometime around 1840. This date is based upon a word-of-mouth account from Dr. J.W. Dawson (reported by Verrill 1874) that *L. littorea* had been collected by Dr. Dawson approximately thirty years prior to Verrill's (1874) account. Thus, conservatively, the date of the original sighting has been set at ~1840. Upon this 1840s 'discovery' of *L. littorea* in North America, many scientists believed that the snail had been recently and accidentally introduced from Europe; yet others (including Dr. Dawson) believed it was native to North America and had been overlooked by North American conchologists (Verrill 1874). Willis (1863) stated that he had oral testimony from several older Nova Scotian inhabitants that they had collected periwinkles as 'school boys,' which he suggested implied that the periwinkle was indigenous to North

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America; however, Ganong (1886) disputed this claim as unscientific and suggested *L. littorea* might have been confused with native periwinkles.

While the oldest recorded dates for North American *L. littorea* were based on sightings and oral testimonies, the first live specimens collected and then preserved were taken from Halifax, Nova Scotia in 1854 and are now found at the Smithsonian Institution (Willis 1863, Ganong 1886, Chapman et al. 2007a). Willis (1863) stated that at the time of these first collected specimens, several eminent British conchologists were consulted, and these scientists believed it nearly impossible that the common periwinkle snail existed in North America.

Until about 1870, *L. littorea* was unknown to American conchologists (Bequaert 1943). Prior to this time, dozens of shell catalogues from the early-mid 1800s recorded the presence of other rocky intertidal and estuarine snails, including *L. saxatilis*, *L. obtusata*, and *Ilyanassa obsoleta*, throughout northeast North America (e.g., Binney 1863, Gould 1851), but *L. littorea* was never listed in any of these catalogues. Steneck and Carlton (2001) doubted that such a conspicuous species, especially one commonly utilized as a food source in Europe (Packard 1870, Reid 1996), could have been overlooked by so many nineteenth century naturalists.

Those scientists that have proposed a European introduction for *L. littorea* have offered two historical alternative hypotheses for potential vectors of the snail to North America: intentional introduction as a food source and accidental introduction with ballast rocks (Reid 1996, Steneck and Carlton 2001). Several authors have suggested an intentional introduction, including Packard (1870), who hinted that *L. littorea* may have

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been introduced as a food source in his statement: "this species is identical with the common periwinkle of the English coast and its increase may be hoped for, as it will introduce a new article of food to our poorer classes." Others have also proposed an intentional introduction, including Steneck and Carlton (2001), who advocated that an intentional introduction as a food source was a likely explanation for *L. littorea's* presence in North America, and Spjeldnaes and Henningsmoen (1963a, 1963b), who suggested that Vikings may have deliberately carried *L. littorea* snails across the North Atlantic on their vessels as a food source. As an alternative, others have suggested ballast rocks may have resulted in the introduction of *L. littorea* (and potentially other species as well). Intact ballast rocks were collected directly from the intertidal zone at sites nearby European ports and then deposited at arrival ports in North America; these rocks were likely covered in European flora and fauna, and a few species may have been introduced to North America via this vector (Lindroth 1957, Carlton 1982, Chapman et al. 2007a), such as algal seaweeds like *Fucus serratus* (Coyer et al. 2006). Spjeldnaes and Henningsmoen (1963a, 1963b) have also proposed that Vikings may have carried ballast across the North Atlantic on their vessels. Finally, it remains possible that these two hypotheses are not mutually exclusive.

Archaeological evidence. No verified fossil record exists for *Littorina littorea* in the United States (Steneck and Carlton 2001); however, 19 specimens have been found in archaeological sites in maritime Canada (Chapman et al. 2007a). A single specimen from Nova Scotia was dated at 40,000 years before present (Wagner 1977); however, this

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dating was based on stratification and not radiocarbon dating; thus its accuracy has been questioned (Chapman et al. 2007a). The majority of the remaining archaeological specimens have been found at Nova Scotian sites and have been dated at ~1000 to ~1500 A.D. (~500 to ~1000 ybp) using radiocarbon dating (Clarke and Erskine 1961, Clarke 1963). Bird (1968) found two additional "ancient" specimens at a Newfoundland site, but these specimens were not radiocarbon dated. In all, the majority of the shells found in southeast Canada have not predated Norse visits to the maritime region. The two "ancient" specimens found in Nova Scotia and Newfoundland were not dated using radiocarbon techniques (Chapman et al. 2007a). Additionally, Reid (1996) noted that, on the whole, the number of archaeological *L. littorea* specimens found in North America is vastly lower than the number that have been found in Europe.

Ecological/natural history evidence. For those arguing against a native origin for *Littorina littorea* in North America, the question why the snail would suddenly expand southwards in the mid-1800s (but not much sooner) has been proposed (Reid 1996). Clarke and Erskine (1961) suggested that unfavorable currents may have prevented *L. littorea* from dispersing from the Northumberland Strait to the outer coast of Nova Scotia and that it only spread southwards when commercial shipping became more prevalent. However, trans-Atlantic shipping during this period was also more prevalent; thus the explanation of enhanced commercial shipping does not limit the snail population that invaded the U.S. to an exclusively Canadian origin. Furthermore, other marine rocky intertidal species, *Semibalanus balanoides* (acorn barnacle) and *Mytilus edulis* (blue

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mussel), with similar dispersal mechanisms to *L. littorea* and believed to have existed in glacial refugia in southeast Canada (Wares and Cunningham 2001) were not confined to maritime Canada following glacial retreat (i.e., unfavorable currents did not prevent their spread southwards). Finally, suggestions that ecological changes may have occurred which allowed the snail to be released from its southern Canadian confinement (Wares et al. 2002) are also not corroborated by any historical/natural history evidence (Reid 1996, Chapman et al. 2007a), nor did any other species show similar range expansions, which would be expected if ecological shifts led to a range expansion for North American *L. littorea* (Carlton, pers. comm.).

Furthermore, a direct crossing from Europe to North America by larval *Littorina littorea* is thought to be highly unlikely (Reid 1996) if not 'impossible' (Kraeuter 1976) based on the survivability of the larvae and the long crossing distance (~3000 km) (Reid 1996). On the other hand, a stepping stone invasion (across the North Atlantic via Iceland and Greenland) has been suggested for *L. saxatilis* and *L. obtusata*; however, this method of dispersal for *L. littorea* has been rejected as *L. littorea* is not found on any of the aforementioned North Atlantic islands that were likely stepping stones for the natural invasions of *L. obtusata* and *L. saxatilis* (Ganong 1886, Johannesson 1988, Reid 1996). Although *L. littorea* has pelagic larvae (unlike its direct developing congeners), such broadcast spawning species often have trouble retaining and establishing populations in small areas, especially islands (Johannesson 1988, Byers and Pringle 2006), which may be the reason for *L. littorea's* absence on these islands. Finally, rafting on driftwood has been suggested as a means of natural movement from Europe to North America (Bird

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1968) (especially for the aforementioned stepping stone invasion that is effective for direct developing species); however, any rafting *L. littorea* adults would again have difficulty being retained on stepping stone islands (Johannesson 1988), and a direct crossing of rafting adults from Europe to North America would likely end up south of most of *L. littorea's* present-day North American range and far south of *L. littorea's* first reported sightings in southern Canada (Kraeuter 1976).

Molecular evidence. A variety of techniques have been used to attempt to resolve *Littorina littorea's* introduction question in North America using molecular methods, beginning with allozyme data in the mid-1970s through newer methods like DNA sequencing and Amplified Fragment Length Polymorphisms (AFLPs) in recent times. The first of these molecular studies was performed in 1977. Berger (1977) used protein electrophoresis to compare allozyme variability in two *L. littorea* populations in Cape Cod, U.S. and Roscoff, France. He determined that the allozyme diversity in the Roscoff population was much greater than the Cape Cod population and that the number of alleles shared between the two populations was small. He suggested that fixed differences he found at seven of 12 allozyme loci suggested an ancient population divergence and thus a native origin for *L. littorea* in North America. However, Reid (1996) questioned Berger's (1977) conclusions due to a similar investigation (Morris 1979) that found the opposite result. Morris (1979) compared the allozyme variability in *L. littorea* populations in Maine versus Wales and found that allelic composition was not significantly different between the two populations, which supported a recent

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introduction of *L. littorea* to North America. Reviewing these opposing studies, Johannesson (1992) believed that the large differences in the allozyme patterns between the Cape Cod and Roscoff samples presented by Berger (1977) were surprising. Johannesson (1992) noted that several other allozyme studies from various European sites found much lower variability in alleles overall, and she concluded that the Roscoff population was probably one of a few areas that locally possessed an unusually high number of alleles when compared to most European populations. Furthermore, Chapman et al. (2007a) noted that Berger's (1977) European geographic sample (which was restricted to Roscoff, France) was too limited to reveal the range of genetic diversity throughout the European population. Berger's (1977) conclusions have also been discredited by two other recent sources. The first (Cunningham 2007) concluded that similar allele frequencies found at the North American site sampled by both Berger (1977) and Morris (1979) and the Welsh population sampled by Morris (1979) suggested that "something was amiss in Berger's (1977) allozyme study from France." The second (Wares and Blakeslee, in press) compared the Berger (1977) data set to their AFLP data set (which explores numerous loci throughout the entire genome) and found zero fixed differences between the North American and European populations out of 120 scored AFLP loci; this result was 'in stark contrast' with the smaller and less representative data set of Berger (1977).

In a recent investigation, Wares et al. (2002) used mitochondrial and nuclear DNA sequencing and reported 'unique' American haplotypes that were not observed in their European samples and were at least 8000 years old, using Nei and Li's (1979)

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measure of net pairwise divergence and the MDIV program (Nielsen and Wakeley 2001). Wares et al. (2002) concluded that this was evidence that *L. littorea* was indigenous to North America. However, the study's total snail (NA: n=57; Europe: n=60) and site replication (NA: n=5; Europe: n=4) were small and only included specimens from a portion of the entire extant range of *L. littorea*. In addition, their own mitochondrial sequence data is suggestive of a severe genetic bottleneck in North America due to the significantly lower number of North American haplotypes compared to European haplotypes (10 vs. 32). North American diversity made up only 24% of the total diversity of the two populations. Furthermore, native populations are expected to contain high allelic diversity and many rare alleles (and a few common ones) due to the long evolutionary history that has resulted in a high amount of genetic structure. On the other hand, as the result of a genetic bottleneck, introduced populations are expected to show lower genetic diversity and few rare alleles and many common alleles (because common founding alleles will attain high frequency as the population expands while rare alleles are often lost due to genetic drift) (e.g., Ledig et al. 1999). Wares et al. (2002, Appendix) had significantly fewer rare haplotypes in North America compared to Europe, a pattern that is expected in founding populations (Chapman et al. 2007a).

Wares et al. (2002) also had a small number of sequences (n=18; 8 North American and 10 European individuals) from the nuclear ribosomal internal transcribed region (ITS) and found no shared alleles between European and North American ITS sequences, which they stated also suggested a native origin for *L. littorea* in North America. Because confidence intervals for divergence estimates using the ITS data did

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not exclude zero, this evidence is not conclusive. Additionally, Wares and Blakeslee (in press) suggested that the Wares et al. (2002) ITS data may have been inappropriate for use in the introduction question of *L. littorea* because ITS data may harbor significant diversity for a number of reasons (including high copy number, high substitution rate, low selective constraint); and thus the small sample size used in the Wares et al. (2002) analysis would not be able to discern high diversity between the two populations due to these reasons versus an actual ancient population divergence.

One of Wares et al. (2002)'s main contentions for a native origin in North America was that they found unique haplotypes in North America not observed in Europe, which in itself should be suggestive of a population divergence in the distant past. This is because in a recent introduction all the alleles found at a locus in the founding population should be derived from the source population (Grosberg and Cunningham 2000). However, two recent investigations have shown that the Wares et al. (2002) study's low sample size (especially in Europe) likely precluded the discovery of the corresponding European haplotypes to the unique North American ones. First, Wares and Blakeslee (in press) demonstrated this point graphically (Figure 1). Visibly asymptoting accumulation curves are suggestive of the total diversity in a population (Gotelli and Colwell 2001). Figure 1 clearly shows that the North American curve is asymptoting, while the European curve is linear ($R^2 = 0.994$). This result suggests that continued sampling in Europe would reveal more haplotypes, while the discovery of haplotypes in North America appears to be leveling off. Overall, this evidence demonstrates the difficulty in satisfying the second requirement for an introduction – all the alleles found at a locus in the recipient biota

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should be derived from the source population – when the source population has so much genetic diversity compared to the recipient population (i.e., it is easy to "miss" alleles in a population with substantial genetic diversity). The second investigation (Chapman et al. 2007a) used a probability analysis to reveal that the number of "unique" haplotypes Wares et al. (2002) witnessed in North America falls within the number of unshared haplotypes that would be expected based on the number of unique haplotypes found in Europe. This strongly suggests that these "unique" haplotypes may in fact exist in Europe but due to insufficient sampling were not found. In fact, based on the number of rare (=1 occurrence) haplotypes observed in Europe in the Wares et al. (2002) data set, the genetic diversity in Europe is expected to be quite high, much higher than in North America (Figure 2). This again strongly suggests a high likelihood of missing haplotypes in Europe that are shared with those 'unique' ones observed in North America. As an example, Muira et al. (2006) found that a trematode species infecting the snail, *Batillaria attramentaria*, a known non-native on the U.S. Pacific coast, possessed a handful of haplotypes in the non-native population that were not also found in its native population in Asia. If this species were not already known to be non-indigenous, it might be suggested that the unshared haplotypes found in North America were endemic to North America. New mutations resulting in novel haplotypes would not be expected to arise in the mitochondrial gene used in this study within the short time-span since the snail was introduced to the U.S. Pacific coast; the authors suggested that insufficient sampling was likely the explanation for the unshared haplotypes found in North America. Thus, the observation of unique haplotypes does not necessarily imply endemism *a priori* (Chapman et al. 2007b).

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Finally, Wares et al. (2002) used the MDIV program (Nielsen and Wakeley 2001) to estimate the divergence time between the European and North American populations based on their sequence data. Based on this analysis, they found the maximum likelihood estimate of divergence to be 23.2 kya with a low-bounded 95 percent confidence interval of 16.1 kya. However, a complication with the Wares et al. (2002)'s divergence estimate is that the MDIV analysis assumes equal effective population sizes (N_e) for the two populations being compared. Wares et al.'s (2002) mtDNA sequence data violate this assumption (Chapman et al. 2007a). When Chapman et al. (2007a) calculated N_e using MDIV for each of the two populations separately (Europe: 60 sequences; North America: 57 sequences), the maximum likelihood N_e was found to be approximately five times larger for Europe than for North America. Because N_e is part of the calculation for the divergence estimate between the two populations ($t = T*2N_e$, where t = total divergence time in generations, T = a scaled divergence time determined from the sequence data using a maximum likelihood plot, and N_e = effective population size; Nielsen and Wakeley 2001), a great disparity in N_e's between populations will result in an inflated divergence estimate between the two populations. Europe clearly has greater diversity than North America, which suggests that the MDIV analysis was inappropriate for the Wares et al.'s (2002) data set (Chapman et al. 2007a, Cunningham 2007). Therefore Chapman et al. (2007a,b) employed a different analysis, Isolation with Migration (IM) (Hey and Nielsen 2004), to calculate the divergence between the two populations for the Wares et al. (2002) data set. IM directly estimates the divergence time ($t = t/\mu$, where t = total years of divergence time, t = the time parameter determined from the sequence data,

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and μ = the gene substitution rate; Hey and Nielsen 2004) from the sequence data and thus does not require the assumption for equal N_e's between the two populations. Using IM, Chapman et al. (2007b) found divergence estimates that were much lower than the Wares et al. (2002) divergence estimates using MDIV (the MDIV estimates were as much as 24 times greater than the IM estimates), likely due to the inappropriate assumptions in the MDIV analysis. Furthermore, Chapman et al. (2007a,b) argued that the unique North American haplotypes Wares et al. (2002) assumed as endemic would impact the divergence estimates calculated by both MDIV and IM. Thus, original divergence estimates based on the Wares et al. (2002) mtDNA data set are likely inflated.

Recently, Wares and Blakeslee (in press) used a molecular technique, Amplified Fragment Length Polymorphisms (AFLPs), to compare European and North American L. littorea. This newer molecular method explores numerous loci rather than just one or two. Although this technique showed some evidence supporting a recent introduction—of 120 loci, none were fixed in either population—it also included unexpected artifacts that hindered clear conclusive evidence.

Altogether, the molecular evidence that has been gathered to date has been debatable or unclear—with two similar investigations (Berger 1977, Morris 1979) coming to opposite conclusions, a mtDNA dataset (Wares et al. 2002) whose conclusions have been questioned due to its small sample size (Chapman et al. 2007), and recent AFLP data which has been hampered by artifacts impacting any clear conclusions (Wares and Blakeslee, in press). On the whole, the molecular data are inconclusive.

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Using parasites and genetics to resolve *L. littorea's* cryptogenic status

As a result of the ambiguous nature of all the evidence to date, additional and new lines of evidence are needed to resolve these questions. Therefore, I not only explored genetic data for the snail itself, but I also collected new evidence using parasites, which included both ecological and genetic analyses. I will describe this novel parasite evidence in two chapters: the first chapter investigates parasite release in *L. littorea's* cryptogenic North American range compared to two of its congeners, *L. obtusata* and *L. saxatilis*, which are both considered native throughout the North Atlantic (including northeast North America); the second chapter explores whether *L. littorea's* most common trematode parasite, *Cryptocotyle lingua* (see Figure I.1 for this trematode's life cycle), exhibited genetic signatures indicative of a founding event in North America compared to Europe. In this chapter, I also include a much smaller comparative dataset for another trematode species, *Cercaria parvicaudata*. The third chapter focuses on *L. littorea* itself through the exploration of a large molecular dataset, which investigated whether the snail exhibited signatures of an introduction to North America. Prior to these three chapters, I have assembled the detailed methodology I used for my dissertation. Within the chapters themselves, the methodology is described briefly.

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Table 1: Debated Evidence over *Littorina littorea*'s North American Origin.
The following is all evidence (historical, archaeological, ecological and molecular) to date for (a) a native origin for *L. littorea* in North America, or (b) a recent introduction of *L. littorea* to North America from Europe.

Native to North America		
Type of Data	**Evidence For:**	**Citation(s)**
Historical evidence	Word-of-mouth accounts that the snails had existed in maritime Canada many years prior to its first recorded sighting in Pictou, Nova Scotia in 1840.	Vernell 1874, Willis 1863
Archaeological evidence	Pre-Columbian shells found at Nova Scotian and Newfoundland sites in maritime Canada.	Bird 1968, Clarke & Erskine 1961, Clarke 1963, Wagner 1971
Ecological/Natural History evidence	Unfavorable currents preventing dispersal southwards but enhanced shipping between Canada and US in mid 1800s allowed spread into US.	Clarke & Erskine 1961
	Ecological shifts allowing for spread southwards.	Wares et al. 2002
Molecular evidence	Fixed differences in allozyme data.	Berger 1977
	Unique haplotypes in mtDNA data.	Wares et al. 2002

Introduced to North America		
Type of Data	**Evidence For:**	**Citation(s)**
Historical evidence	Rapid and sequential spread from a single point of origin.	Steneck and Carlton 2001
	Early American conchologists had no record of *Littorina littorea* prior to 1870.	Bequaert 1943
	European conchologists expressed surprise at *Littorina littorea's* presence in North America.	Vernell 1874, Willis 1863
	Suggestion that intentional introduction as a food source would have been welcomed.	Packard et al. 1870
	Evidence of rock ballast mediated introductions in other species.	Coyer et al. 2006
Archaeological evidence	No fossil record in the U.S. Only a handful of specimens found in maritime Canada; those that were radiocarbon dated do not predate Norse visits to maritime Canada.	Chapman et al. 2007
Ecological/Natural History evidence	*Littorina littorea* is not found on Iceland or Greenland, believed to be stepping stones for many North American rocky intertidal species.	Ganong 1886, Johannesson 1988, Ingolfsson 1992, Reid 1996
	Littorina littorea larvae cannot survive direct crossing from Europe to North America.	Kraeuter 1976
Molecular evidence	No fixed differences in allozyme data.	Morris 1979
	No fixed differences in AFLP data.	Wares and Blakeslee, in press

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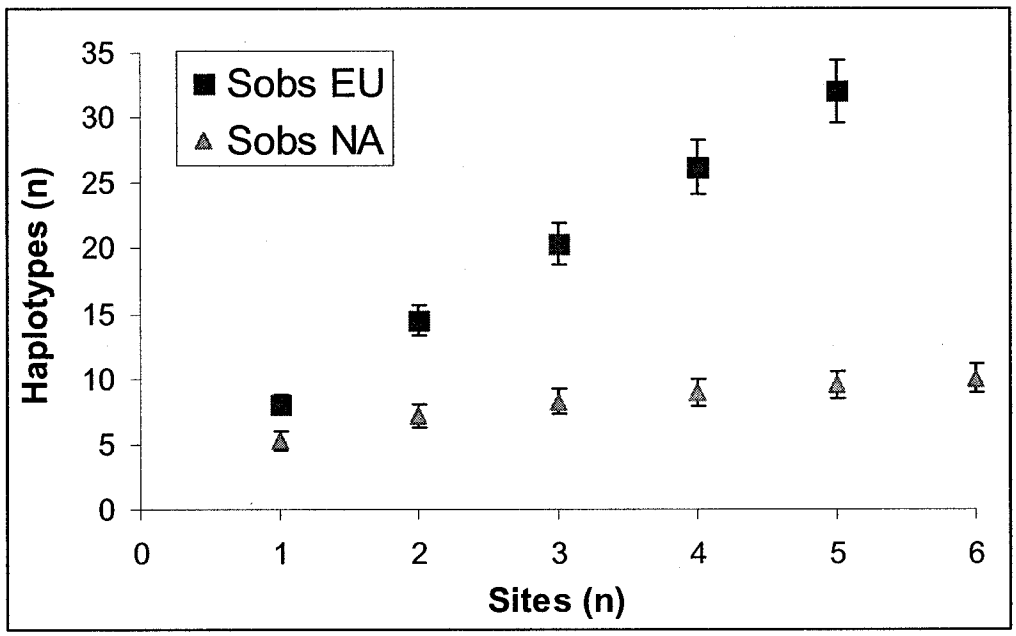

Figure 1: Haplotype Rarefaction Curves for *Littorina littorea*. These sample-based rarefaction curves for North American (▲) and European (■) haplotype diversity from the Wares et al. (2002) appendix demonstrate a clear asymptote in North America and a linear relationship of haplotype number per sampling effort. Analysis performed using ESTIMATES 8.0 (Colwell 2006).

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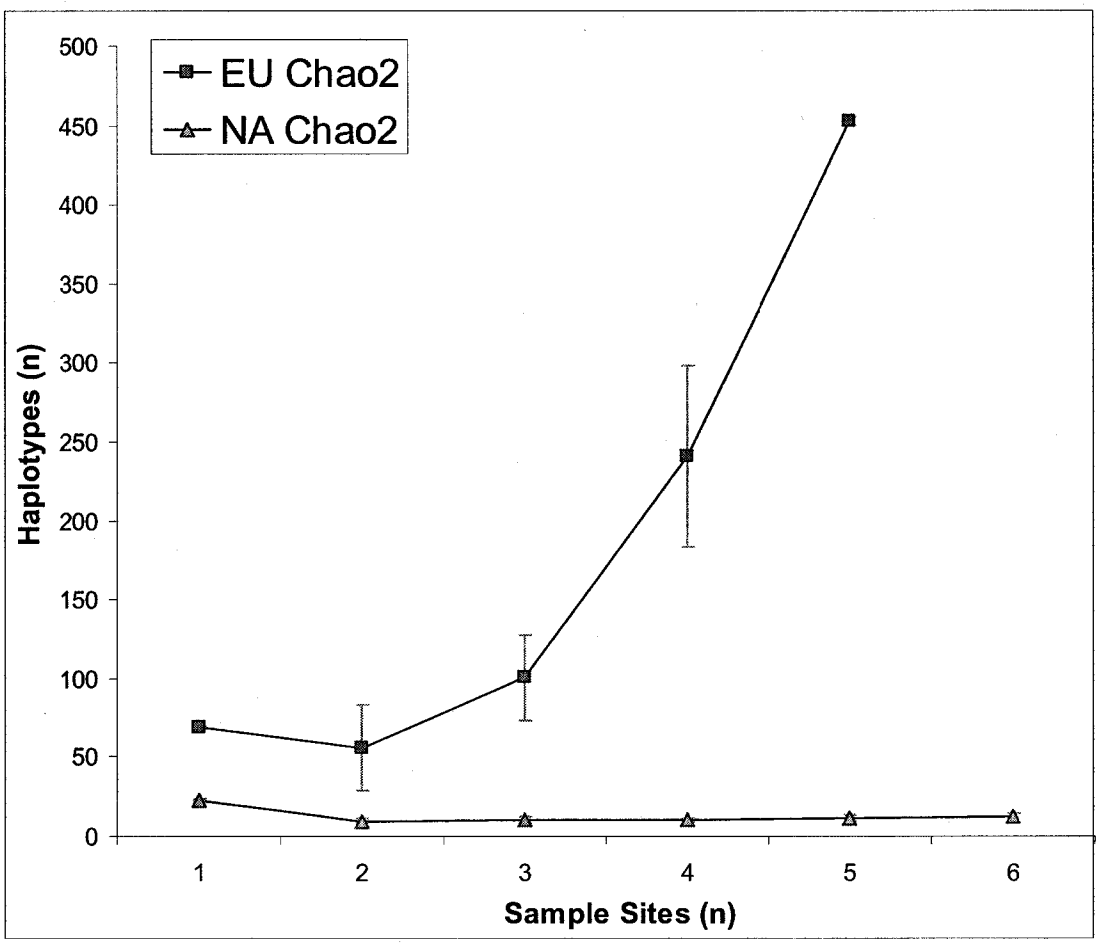

Figure 2: Expected Haplotype Diversity for *Littorina littorea*. At the maximum sampling effort, the Chao2 estimator predicts a total of 450 haplotypes in Europe (■) versus 12 in North America (▲). Analysis performed using ESTIMATES 8.0 (Colwell 2006).

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CHAPTER I

USING PARASITES TO INFORM ECOLOGICAL HISTORY: COMPARISONS AMONG THREE CONGENERIC MARINE SNAILS IN NORTH AMERICA AND EUROPE

ABSTRACT

Species introduced to novel regions often leave behind many parasite species. Signatures of parasite release could thus be used to resolve cryptogenic (uncertain) origins like that of *Littorina littorea*, a European marine snail whose history in North America has been debated for over 150 years. Through extensive field and literature surveys, I examined species richness of parasitic trematodes infecting this snail and two co-occurring congeners, *L. saxatilis* and *L. obtusata*, both considered native throughout the North Atlantic. I found only *L. littorea* to possess significantly fewer trematode species in North America, and all three *Littorina sp.* North American trematodes were a nested subset of Europe. Surprisingly, several of *L. littorea's* missing trematodes in North America were found infecting the other *Littorina* congeners. Most likely, long separation of these trematodes from their former host resulted in divergence of the parasites' recognition of *L. littorea*. Overall, my results suggest a recent invasion from Europe to North America

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for *L. littorea* and an older, natural expansion from Europe to North America for *L. saxatilis* and *L. obtusata*.

INTRODUCTION

As global human transportation continues to homogenize the world's biota, we are often dependent on historical records and baseline biological surveys to determine what species are truly native to a region. However, these records can be incomplete, resulting in uncertainties regarding some species' status as native or non-native in a region (i.e., cryptogenic) (Carlton 1996). Usually doubt over a species' origin is prompted by observations of a species with a disjunct biogeographical distribution, an odd ecological role within the community, or closely associating/co-occurring species that are known to have been introduced (Chapman & Carlton 1991, Ruiz et al. 2000). Here, I demonstrate that parasites may be useful tools to help resolve the ecological histories of such cryptogenic species.

According to the hypothesis of enemy release, introduced species often leave behind predators and parasites in their native habitats (Torchin & Mitchell 2004). Because only a small number of individuals are typically exported in an invasion event, an introduced host will likely carry with it just a subset of its native parasite fauna, resulting in a reduction in parasite species richness in introduced populations compared to native populations (e.g., Dove 2000, Torchin et al. 2002, Tsutsui et al. 2003, Prenter et al. 2004, Torchin et al. 2005). Thus, parasites may inform invasion histories through

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comparisons of patterns in their abundance and diversity in hosts from native and non-native ranges.

In an extensive review of parasitism in non-native versus native hosts across many taxa, Torchin et al. (2003) found that non-native populations possessed about half the parasite species richness and prevalence of infection of native host populations. Though many of these studies were terrestrial or freshwater, a few marine studies have also strongly supported the predictions of enemy release. For example, in northeastern North America, non-native populations of the European green crab (*Carcinus maenas*) were found to possess roughly half the number of parasites compared to native European populations. Furthermore, non-native green crabs were larger and exhibited a greater biomass than native conspecifics, consistent with predictions of the physiological benefits non-native hosts confer from escaping parasites (Torchin et al. 2001). Additionally, on the west coast of the United States, a non-native Asian snail, *Batillaria cumingi*, is infected by only a single parasitic trematode species, compared to at least 8 trematodes that infect it in its native range (Torchin et al. 2005).

Although invaders typically exhibit reduced parasite richness in an introduced population compared to their native range, this differential may decrease over time due to the probability of subsequent invasions of infected hosts or arrival of parasites through natural vectors/other hosts (Prenter et al. 2004). This difference in parasite composition between native and introduced regions depends on the time since the invasion, the amount of propagules transported between the regions, and the specificity of the host-parasite relationship (Torchin & Mitchell 2004). Highly specialized parasites obligate to

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specific hosts, such as trematode parasites, are a useful guild of parasites to explore enemy release signatures because introduced hosts rarely acquire new species of trematodes from distantly related native hosts, which would dilute parasite release signatures.

Because of extensive, consistent support for decreased parasite richness in introduced populations (Torchin et al. 2003), I propose using patterns of enemy release in reverse, i.e., to use parasite signatures to inform the ecological origin of a given host. Specifically, the predictions for parasite release can be tested among three marine congeneric snails found in the North Atlantic, whose invasion/colonization histories are hypothesized to differ greatly. All three snail species are infected by digenean trematode (flatworm) parasites, which are obligate to specific snail (first-intermediate) hosts. While the enemy release hypothesis has been used to explain heightened invasion success and ecological impact, to my knowledge, this study represents the first endeavor to use its predictions to distinguish older, natural range expansions from a recent, and purportedly human-mediated, introduction.

Study system

Littorine natural histories in the North Atlantic. *Littorina saxatilis* (rough periwinkle) and *L. obtusata* (smooth periwinkle) are gastropod mollusks found in similar ranges and habitats throughout the North Atlantic, including western Europe and northeast North America, as well as Greenland and Iceland (Reid 1996). Both snails are considered native throughout the North Atlantic—their origins in the western Atlantic are

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generally believed to have been the result of a natural invasion from Europe many thousands of years ago (Ganong 1886, Ingolfsson 1992, Reid 1996, Wares & Cunningham 2001) as is suspected for many northwest Atlantic hard-bottom species (Vermeij 1991, Ingolfsson 1992, Wares & Cunningham 2001). *Littorina obtusata*, in particular, was suggested to have colonized North America from Europe shortly after the last glacial maximum, which occurred approximately 20,000 years ago (Wares & Cunningham 2001). *L. saxatilis* and *L. obtusata* are both direct developers: *L. saxatilis* broods its young, while *L. obtusata* lays its eggs casings on nearby rock and algae.

Littorina littorea (common periwinkle) is also found in the North Atlantic rocky intertidal zone; however, both its biogeography and larval dispersal of young are different than congeners *L. saxatilis* and *L. obtusata*. *Littorina littorea* is presently found in western Europe and northeastern North America but is absent from Iceland and Greenland (Reid 1996), and it has pelagically dispersed larvae. *Littorina littorea* is a known native of Europe based upon extensive paleontological evidence (Reid 1996), but the history of its presence in North America remains less clear. What is known is that in the 1850s, *L. littorea* was witnessed to spread rapidly and sequentially southwards from Halifax, Nova Scotia into the U.S, reaching Delaware Bay only 30 years later (Steneck and Carlton 2001). What remains uncertain is whether Canadian populations were native and confined to Canada until the mid-1800's or were anthropogenically introduced from Europe. This ambiguity has been debated for over 150 years with evidence supporting both hypotheses (e.g., Ganong 1886, Clarke 1961, Berger 1977, Wares et al. 2002), but

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there has been no definitive resolution as of yet (Chapman et al. 2007, Wares & Blakeslee, in press).

Trematode parasites and trematode richness expectations in littorine snails. Recent evidence has shown that under some circumstances parasites can provide better information for identifying source populations of host species than the host itself (Criscione et al. 2006). Thus, parasites may be useful and essential tools for understanding many ecological questions, especially when other sources of information are equivocal or lacking. Digenean trematodes could provide such novel information. Trematodes infect multiple hosts within their complex life-cycles and typically use gastropods as a first-intermediate host (Figure I.1; APPENDIX I). The relationship between trematode and snail host is highly specific to a particular species of snail or a group of closely related snail species, including the three *Littorina* congeners. Larval trematodes in snail hosts are typically parasitic castrators (Kuris 1990) and do not kill their hosts; thus infections are maintained throughout the duration of a snail's life. Within the littorine snail host, trematodes asexually reproduce, producing a free-swimming cercarial larval stage that is continually shed from the snail and must encyst within a second-intermediate host, which can include many species of fish, crabs, bivalves, or other mollusks. Second-intermediate hosts must then be ingested by a definitive host, typically a gull (*Larus* spp.), where the trematode sexually reproduces (Lauckner 1980).

I predicted *L. saxatilis* and *L. obtusata* would show some reduction (likely non-significant) in trematode species richness in North America, representing a subset of the

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snails' European source trematode richness because I expected that older, natural invasions should have allowed sufficient time for the hosts to acquire trematode richness through subsequent host and trematode invasions. In contrast, if *L. littorea* is a recent invader from Europe, I expected a significant reduction in North American trematode species richness based on the predictions for enemy release. Otherwise, I expected similar richness patterns to those exhibited by the two long-established congeners, *L. saxatilis* and *L. obtusata.*

MATERIALS AND METHODS

Literature review

To look for evidence of parasite release in *Littorina* sp., I first performed an extensive literature review of trematode species richness in European and North American populations of *L. littorea*, *L. saxatilis*, and *L. obtusata.* I accepted studies that provided either trematode species richness (the total number of trematode species at a site), prevalence of infection (the proportion of snails infected by trematodes at a site), or both. I searched for every available study with these data. In all, I was able to use a total of 60 different European studies and 13 North American ones (APPENDIX A). Based on this review, I determined that while all three snail species have been well studied in Europe, they had been comparatively under-sampled in North America and had not encompassed the snails' full geographic ranges in North America compared to Europe.

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Snail collections and dissections

Observed species richness increases with sampling area and effort. Thus, any comparisons made using the much better sampled European literature data versus the North American literature data would likely suffer from sampling bias. To remedy the apparent undersampling in North America, I collected ~100 *Littorina* sp. snails of each *Littorina* species from numerous sites throughout their North American ranges (*L. littorea* n=49; *L. saxatilis* n =19; *L. obtusata* n =24; Figure I.2; APPENDIX B). I focused collections on *L. littorea* because it is more abundant and found at more sites than the other two littorines, and because this was the species of the three I suspected would exhibit the signature of parasite release; thus I wanted to ensure that sampling had been exhaustive to reveal all trematode species including potentially rare ones. Furthermore, I extensively sampled Canada, especially north and east of Pictou, NS (where *L. littorea* was first noted) because the alternative hypothesis to an introduction of *L. littorea* to North America is that it was present in Canada historically. If true, these Canadian populations would be older and could harbor a richer parasite fauna that may not have completely advanced with the snail as it invaded the U.S.

Although the European literature was quite extensive, I also collected *L. littorea* in Europe for corroboration with the literature, especially since many of the studies were from several decades ago. Also, I wanted to expand on the geographic range of samples reported from Europe, which prior to my investigation had centered on sites in the British Isles and the North Sea (APPENDIX A). Furthermore, as another potential signature of parasite release, I used this data to compare prevalence of infection for a standardized

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size class of snails between the two regions for *L. littorea* (average length $\pm$ s.d. = NA: 18.78 $\pm$ 4.41 and EU: 18.82 $\pm$ 4.42) to determine whether this species showed lower prevalence of infection in North America compared to Europe. Standardization is important for prevalence comparisons because *L. littorea* size correlates with its age and thus the length of exposure to contract trematodes from its environment (Byers et al., in revision). Therefore, I collected and dissected approximately 100 *L. littorea* snails per site from 20 different European sites ranging from Moss, Norway to Vigo, Spain (Figure I.2; APPENDIX B), recording trematode species richness and prevalence for each site. Field surveys were not performed in Europe for the other two *Littorina* species because the data in the literature was extensive, encompassing the majority of their ranges, and thus did not need to be further enhanced.

At each site, adult snails were collected haphazardly from the intertidal zone during low tide over the summer months of the years 2002-2005. Because both the snails and their trematode infections are long-lived, richness patterns at my sites were unlikely to change appreciably over the time period of my investigation—a fact I quantitatively confirmed for *L. littorea* at seven North American sites that were sampled in two study years. After snails were collected, they were each measured from the apex to the anterior tip of the aperture. Snails were dissected under a stereomicroscope and the gonadal and digestive tissues examined for presence of trematode infection. Trematode species were identified under a compound microscope using multiple published keys and descriptions of trematodes infecting *Littorina sp.* (e.g., Werding 1966, James 1968a, 1968b, and Stunkard 1983). Further detail on collection methods are found in APPENDIX G.

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Statistical analyses and species richness estimators

To resolve whether my North American sampling was complete and to assess the total expected species richness and thus compare both populations using a standard metric, I employed ESTIMATES 8.0 (Colwell 2006) to construct species accumulation and species richness estimator curves from my field and literature data. ESTIMATES uses Monte Carlo re-sampling (through randomization of sample order over a number of replicates (e.g., 500)) to determine the mean accumulation of species (S_{obs}) as samples are added over the full data set (Gotelli & Colwell 2001), while also providing standard deviations and 95% confidence intervals for each data point (Colwell 2006). Although my data was sample-based, I re-scaled my species accumulation curves to accumulated individuals in order to compare species richness across my data sets in a standardized manner (Gotelli & Colwell 2001).

Sample-based rarefaction curves may not capture the total species richness within a population for a particular sampling effort, especially if these curves have not reached a stable asymptote. Thus, non-parametric estimators, such as Chao2, can be useful in predicting the eventual asymptote in species richness for a particular population (Gotelli & Colwell 2001), and do so by including the effects of rare species on the total species richness (Chao 2004, Witman et al. 2004). Chao2 has been found to be one of the most robust estimators (see Colwell 2006 for Chao2 equation) when compared to empirical data from a variety of systems for revealing the missing species in a population and thus predicting the total expected species richness for the system (e.g., Walther & Morand 1998, Foggo et al. 2003). In fact, Walther & Morand (1998) advocated the use of Chao2

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specifically for parasite species richness. In addition, Chao2 has been shown to remain precise even under changes in sampling effort (Walther & Morand 1998), and since I included sites from both literature and field data of varying sample sizes, use of the Chao2 estimator was highly appropriate for my study.

Because an asymptoting accumulation curve indicates that the total species richness for a population has been captured (Gotelli & Colwell 2001), estimator curves and species accumulation curves that converge on the same asymptote reflect adequate sampling (Walther & Morand 1998). Therefore, I used this technique (with Chao2 as my estimator) to determine whether I had adequately sampled trematodes in North America since the snail hosts had been severely undersampled in the literature. Although the Chao2 method standardizes for variable sample sizes and thus accurately predicts the maximum expected species richness in each population, I performed an additional standardizing technique at the site level (standardized for sampling effort) to determine whether average site level richness corroborated results of the Chao2 technique and the observed richness (S_{obs}) in each population. To do this, I performed Monte-Carlo resampling (using ESTIMATES 8.0) on each site, standardizing sites at a sampling effort of 75 individuals. Following standardization, the adjusted site level species richness value (average $\pm$ standard error) was recorded for each field site and literature study (Appendices A & B). These standardized values were then used in a single-factor ANOVA to determine whether there were significant differences in average site level richness in North American versus European populations. Those few sites/studies with

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less than 75 individuals were excluded from this analysis. Further detail on statistical analyses can be found in APPENDIX G.

RESULTS

My sampling dramatically increased the total number of snails and sites investigated in North America for trematodes of all three littorines (Table I.1, APPENDIX A). In total, for *Littorina littorea*, I found 11 trematode species in Europe versus 5 in North America—a 55% reduction in trematode richness in North America. For *L. saxatilis*, there were a total of 16 European versus 11 North American trematode species, a reduction of 32% in North America. Finally, for *L. obtusata*, the difference was 16 to 12 species in Europe versus North America, a reduction of 25% in North America (Figure I.3). For all three snail species, the trematode species richness of North America was a subset of the European trematode richness (Table I.2).

Species accumulation (S_{obs}) and Chao2 species richness estimator analyses all asymptoted at a trematode species richness of 5 species for North American *L. littorea* (Figure I.4a) and at 11 species for European *L. littorea* (Figure I.4b), indicating that no further trematode species are expected to be found infecting *L. littorea* in either population. Confidence intervals in North America and Europe for both S_{obs} and Chao2 were zero or nearly zero. For *L. saxatilis*, the North American S_{obs} and Chao2 curves asymptoted at a trematode species richness value of 11 species (confidence intervals = S_{obs}: 9-14, Chao2: 11-19; Figure I.4c), while the European S_{obs} achieved a value of 15

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species and the Chao2 curve culminated at 16 species (confidence intervals = S_{obs}: 13-19, Chao2: 15-31; Figure I.4d). For North American *L. obtusata*, the S_{obs} curve achieved a value of 12 species, while the Chao2 curve culminated at ~12 species (confidence intervals = S_{obs}: 9-15, Chao2: 12-20; Figure I.4e). For European *L. obtusata,* the S_{obs} curve culminated at a trematode species richness of 15 species, while the Chao2 curve achieved a value of 16 species (confidence intervals = S_{obs}: 13-19, Chao2: 15-26; Figure I.4f). The difference in the total trematode richness in European *L. saxatilis* and *L. obtusata* recorded in Figure I.3 (=16 trematode species for each snail) and the maximum S_{obs} values shown in Figure I.4 d&f (=15 trematode species for each snail) are due to the omission of one trematode species (*Himasthla littorinae*) from the S_{obs} analyses. This omission is the result of missing prevalence data for *H. littorinae* in European *L. saxatilis* and *L. obtusata* (Table I.1), which is necessary for inclusion in the S_{obs} analysis.

After standardizing for sampling effort at each site (Appendices A & B) and statistically comparing the adjusted site level trematode richness in each region, I found European *L. littorea* to possess a significantly greater average ($\pm$ standard error) site-level trematode species richness compared to North America ($F=28.27$; d.f.=1; $p<0.001$), and this decline for North America (1.28 ± 0.11) was nearly 50% compared to Europe (2.43 ± 0.23) (Figure I.5). Both *L. saxatilis* and *L. obtusata* showed lower average trematode richness in North America compared to Europe, but neither reduction was significant (LS: $F=0.13$; d.f.=1; $p=0.71$ and LO: $F=1.37$; d.f.=1; $p=0.25$; Figure I.5). For *L. saxatilis*, the decline in North American (2.49 ± 0.26) average richness was only about 8%

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compared to Europe (2.69 ± 0.31), while for *L. obtusata*, the decline in North America (2.20 ± 0.23) was about 22% compared to Europe (2.81 ± 0.35).

Finally, I compared site level average trematode prevalence of infection (± SE) in European versus North American *L. littorea* (Table I.1) with a single-factor ANOVA analysis. Differences in prevalence between the two populations were not significant (EU: 10.2% ± 1.9%; NA: 9.7% ± 1.5%; p=0.86), nor were they significantly different when I compared the average prevalence (± SE) of just the five trematode species common between North America and Europe (EU: 8.9% ± 1.8%; NA: 9.7% ± 1.5%; p=0.74).

DISCUSSION

Both my extensive literature review and supplemental field sampling identified significantly lower total trematode species richness for *Littorina littorea* in North America versus Europe, which was a decline of ~55% in North America (Figure I.3). Average site level richness was also significantly lower in North America compared to Europe (Figure I.5) and the decline (~50%) was nearly equivalent to the decline based on the total species richness (~55%). Moreover, the Chao2 results confirmed that this difference in trematode species richness was not the result of undersampling in North America, but was the accurate total species richness for this region. Furthermore, all Chao2 curves for *L. littorea* asymptoted at the same value as the observed trematode richness (Figure I.4 a&b). In contrast, for *L. saxatilis* and *L. obtusata* only small, non-

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significant reductions in trematode species richness in North America versus Europe were demonstrated, and based on the total species richness in each region, these were declines of only 32% and 25%, respectively (Figure I.3). Average site level richness for *L. saxatilis* and *L. obtusata* also showed much lower declines in North America versus Europe compared to *L. littorea* (Figure I.5)—the decline based on average site level richness in North American *L. obtusata* (22%) was essentially equivalent to that observed in the declines based on the total richness (25%) in each region, while for *L. saxatilis*, the decline based on average site level richness was much lower (8%) than that observed in the declines based on the total richness (32%) in each region. In both *L. saxatilis* and *L. obtusata*, the maximum expected trematode richness in each population calculated by the Chao2 estimator essentially equaled the observed species richness in each region (Figs. 1.4 c-f). This result demonstrates that my field and literature sampling captured essentially all trematode species expected in the two populations; and thus, I can be confidant that the much lower differences in total richness and average site level richness observed in *L. saxatilis* and *L. obtusata* compared to *L. littorea* were accurate and not impacted by undersampling. On the whole, these analyses demonstrate a substantial distinction between *L. littorea* and its two congeners, in that the differences in richness between the regions for *L. saxatilis* and *L. obtusata* are essentially half that of the differences for *L. littorea*.

In European snails, taxonomic inflation of trematode species may exist as the result of a longer history of trematode exploration, which has produced accounts of indistinguishable subspecies and congeners. I partially controlled for this by lumping

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some of the more indistinguishable subspecies (refer to APPENDIX C for details). Despite the potential for inflated richness in the native region, trematode species richness still was not significantly depressed in *L. obtusata* nor *L. saxatilis* in North America relative to Europe. In contrast, *Littorina littorea* had almost no taxonomically challenging species to inflate its European trematode richness; yet it was the only snail to demonstrate a significant decrease in its North American trematode species richness. The reduction in trematode richness in *L. littorea* cannot be due to different environmental conditions that have resulted in across the board reductions of littorine trematodes in North America because *L. obtusata* and *L. saxatilis* are infected by the same species of trematodes and show no significant decline in trematode richness. This suggests that the significantly lower trematode richness in North American versus European *L. littorea* requires additional and alternative explanations. Thus, my results strongly support a distinctly different ecological history for *L. littorea* compared to its two congeners.

I suggest that the non-significant reduction in trematode richness in North American compared to European *L. saxatilis* and *L. obtusata* (Figure I.5) supports an older invasion for these two snails because a longer time interval should allow for subsequent invasions to enhance the number of parasite species in the invaded population (Prenter et al. 2004). Furthermore, all trematode species infecting the snails in North America were a subset of the European trematode population (Table I.2). My data is therefore consistent with other evidence that has suggested that *L. saxatilis* and *L. obtusata* naturally invaded North America from Europe, probably as recolonization events following the last glacial maximum (e.g., Ganong 1886, Ingolfsson 1992, Reid

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1996), which could have been as long as 20,000 years ago (Wares & Cunningham 2001). This natural crossing from Europe to North America was likely through dispersal to shallow water and intertidal habitats of islands in the North Atlantic, where both *L. saxatilis* and *L. obtusata* (but not *L. littorea*) are presently found, including populations in the Faeroe Islands, Iceland, and Greenland (Ganong 1886, Reid 1996). Such a scenario has been suggested for much of the western North Atlantic hard-bottom fauna, as evidenced by the Iceland fauna, which is almost entirely a subset of European fauna, and northeastern North America, which is a further reduced subset of the European and Icelandic fauna (Ingolfsson 1992). From my European literature data set, it in fact appears that the Iceland populations match Europe more closely in trematode diversity than northeastern North America (Sannia & James 1977, Galaktionov & Skirnisson 2000, Skirnisson & Galaktionov 2002), further corroborating historical, natural movement of the two littorines from the British Isles to Iceland and suggesting a filtering out of trematode species with increasing distance from the source.

Littorina littorea, on the other hand, is not found to exist on any of the aforementioned North Atlantic islands that were likely stepping stones for the natural invasions of *L. obtusata* and *L. saxatilis* (Johannesson 1988, Reid 1996). Although *L. littorea* has pelagic larvae (unlike its direct developing congeners), such broadcast spawning species often have trouble retaining and establishing populations in small areas, especially islands (Johannesson 1988, Byers & Pringle 2006). Furthermore, Kraeuter (1976) suggests that current patterns from the British Isles across the North Atlantic make a direct crossing by *L. littorea* larvae "impossible," and that any rafting adults would

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likely end up south of most of *L. littorea's* present-day North American range and far south of its first reported sightings in southern Canada. Thus, the patterns in trematode richness I observed for this snail coupled with its natural history suggest a recent introduction to North America from Europe.

One of the striking results of my dataset is that almost all of the trematode species that infect *L. littorea* in Europe are found in North America infecting *L. saxatilis* and *L. obtusata* (Table I.1); yet four of these species do not infect *L. littorea* in North America (*Ce. lebouri*, *Himasthla elongata*, *H. littorinae* and *Podocotyle atomon*). The lack of occurrence of these four trematodes in North American *L. littorea* is surprising given that they all use *L. littorea* as a host in Europe, and two of the four (*H. elongata* and *Ce. lebouri*) are believed to use *L. littorea* as their preferred primary host (James 1968b, Matthews et al. 1985, Williams & Ellis 1976). The absence of these four trematode species in North American *L. littorea* is not the result of a sampling issue because the sample size from my North American *L. littorea* field surveys is four times higher than the other littorines and my species accumulation curves suggest complete capture of all North American trematodes for *L. littorea* (Figure I.4a). The absence is also not due to lack of infection opportunities or ecological proximity because *L. littorea* was present at all sites where I observed these four species infecting *L. saxatilis* and *L. obtusata* in North America. Moreover, two species in particular, *H. elongata* and *H. littorinae*, have miracidia that directly penetrate their snail hosts and do not require ingestion for infection to occur (Stunkard 1966, Matthews et al. 1985). In addition, Matthews et al. (1985) in a study performed in Ireland suggested that *H. elongata's* free-swimming miracidia

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actively host-selected for *L. littorea* and not the other two littorines. For all these reasons, it is highly likely that the four former *L. littorea* parasites have had many opportunities to infect *L. littorea* in North America and their absence must be due to some physiological constraint between these trematodes and *L. littorea* snails—a pattern consistent with a genetic divergence between these four trematode species and their former host.

The most parsimonious explanation for this pattern is that upon a recent introduction of *L. littorea* to North America, these four former *L. littorea* parasites (being present in historical North American populations of *L. saxatilis* and *L. obtusata*) no longer recognized *L. littorea* as a suitable host due to the divergence that had occurred over their long separation. *Littorina saxatilis* and *L. obtusata* are believed to have naturally invaded North America following the last glacial maximum, ~20kya (e.g., Ganong 1886, Ingolfsson 1992, Reid 1996, Wares & Cunningham 2001), and they likely carried some of these former *L. littorea* trematode species with them upon invasion (*L. littorea's* trematodes are a nested subset of the other two littorine species, see Table I.1). Definitive seabird hosts, such as *Larus argentatus* (Herring Gull) and *Larus marinus* (Black-backed Gull) (Stunkard 1966) may have also brought trematode species to North America; however, trans-Atlantic flights by gulls are believed uncommon (J. Ellis, T. Good, pers. comm.). Because these former *L. littorea* trematodes have low prevalences in European *L. saxatilis* and *L. obtusata* (e.g., *H. elongata* prevalence = 0.6% and 0.3%, respectively), trematode colonizations in North America would have likely included extremely small founding populations. Small populations are highly susceptible to genetic drift, where genotypes allowing for physiological compatibility between parasites

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and their primary host could have been lost, leading to a divergence between these trematode populations on either side of the Atlantic, and thus a situation where these four trematode species can no longer infect their former host.

A loss of infectivity of hosts for certain parasite genotypes has been empirically and even experimentally demonstrated. For example, Little et al. (2006) experimentally showed that after several generations a particular genotype of a bacterial parasite, *Pasteuria ramosa*, lost the ability to infect a host genotype of its crustacean host, *Daphnia magna,* while other *P. ramosa* genotypes did not. Similarly, Richards (1976) found that certain strains of the trematode, *Schistosoma mansoni*, were less infective to the freshwater snail, *Biomphalaria glabrata*, than other strains and that changes in its infectivity may have been the result of shifts in gene frequencies. Finally, trematode species previously thought to represent one species have been found to be genetically distinct cryptic taxa. For example, Huspeni (2000) showed that the trematode 'species' *Parorchis acanthus* actually represents four genetically distinct species and for one of these distinct species, there were also two divergent clades representing genetic differences within this species complex. Thus, due to isolating events, morphologically similar members of a species may actually become genetically distinct cryptic taxa (Huspeni 2000). A loss of infectivity due to trematode genotype shifts or losses is a likely explanation for the absence of these four trematode species in North American *L. littorea.* Ultimately, given a small, natural and historical inoculation of the former *L. littorea* trematodes to North America, the separation of *L. littorea* from its parasites necessary for

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divergence in the loss of infection capability was most likely driven by an absence of *L. littorea* in North America over historical time.

The nested subset of *L. littorea* trematodes also helps eliminate alternative explanations for the absence of several of its European trematode species in North America. First, the absence cannot be due to the lack of appropriate second-intermediate and definitive hosts in the trematodes' complex life cycle. Not only are appropriate second-intermediate and definitive hosts present in North America (e.g., Pohley 1976, Stunkard 1983), but also their ecological functioning as hosts is assured by the successful completion and persistence of all of *L. littorea's* trematodes using the other two *Littorina* sp. snails. Second, although glaciation is believed to have been more severe in the western than the eastern Atlantic (Ingolfsson 1992), any explanation that invokes a pre-Ice Age North American history for *L. littorea* would have to explain how glaciers wiped out trematode species just from *L. littorea* that were not subsequently restored with the North American colonization of *L. obtusata* and *L. saxatilis* and their shared trematode species shortly after the last glacial maximum.

Finally, I found the prevalence of trematode infection in *L. littorea* to be similar in North America and Europe. Although prevalence has been shown to be significantly lower in founder versus source populations in other systems, Torchin et al. (2001) also showed that when only species common between populations were compared, the prevalence between the populations was not different. Presumably, this was because parasite species carried with their hosts were able to achieve equally high prevalence in the introduced range as in their source population. Because the five species common

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between my two populations of *L. littorea* account for 86% of the occurrence of all trematode species in Europe (Table I.1), it is perhaps not surprising that I did not find higher prevalence of infection in Europe versus North America.

In conclusion, the results of my trematode species richness analyses corroborate prior historical, molecular and ecological evidence supporting an older, natural invasion of North America for both *L. saxatilis* and *L. obtusata*, and meets expectations of enemy release for North American *L. littorea*, thus supporting a recent invasion for this snail. An interesting facet of parasite release uncovered here is that although *L. littorea* has escaped some trematodes in North America, it has not escaped those parasites physically, but physiologically due to an incompatibility that has apparently developed over the long separation between these trematodes and their former host. My work represents the first endeavor to use parasites to inform invasion histories. Because parasite release is an easily recognizable signature, it may prove useful for resolving the cryptogenic status of species in many systems.

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Table I.1: Prevalence of Trematode Species Infecting *Littorina littorea*, *Littorina saxatilis* and *Littorina obtusata* in Europe and North America. Data stem from my extensive literature and field surveys. The percent infection of a trematode species among all snails investigated (i.e. prevalence) is listed for each survey. Metadata are presented at the bottom of the table. N.d. = no data and refers to literature studies that recorded the presence but not the prevalence of a trematode species. When using the data presented in Table I.1 (especially for European *L. saxatilis* and *L. obtusata* where all of my data were extracted from the literature), I caution that for a few species, taxonomic issues could affect reported data and may not precisely reflect their natural prevalences. Furthermore, as a conservative approach to avoid taxonomic over-inflation of total trematode species richness, I have collapsed several subspecies into one category (*Cercaria littorinae saxatilis* sp.) and combined four species of morphologically similar microphallid species into one category (*Microphallus* sp.) in both Europe and North America (refer to APPENDIX C for details). For European *L. saxatilis*, prevalence of *Cercaria brevicauda* was 4.5×10^{-5}.

Trematode Species	Percent Infection (Prevalence) Among *Littorina sp.*									
	L. littorea				*L. saxatilis*			*L. obtusata*		
	Europe		North America		Europe	North America		Europe	North America	
	Lit	Field	Lit	Field	Lit	Lit	Field	Lit	Lit	Field
Cryptocotyle lingua	14.38%	5.23%	9.66%	9.37%	1.53%	6.60%	2.62%	0.33%	6.32%	1.53%
Cercaria parvicaudata	n.d.	2.23%	0.62%	1.13%	0.04%	1.82%	0.53%	0.15%	n.d.	0.42%
Renicola roscovita	4.75%	2.04%	0.09%	0.27%	0.47%	0.68%	1.33%	0.13%	1.09%	1.18%
Microphallus similis	0.01%	0.15%	n.d.	0.02%	11.61%	0.73%	4.14%	8.92%	1.82%	2.96%
Microphallus sp. (other than *M. similis*)	1.06%	0.23%	0.02%	0.02%	7.69%	23.38%	0.49%	7.39%	5.21%	0.28%
Cercaria lebouri	0.23%	0.15%			0.06%		0.53%	0.06%	0.55%	0.14%
Himasthla elongata	5.73%	0.92%			0.01%		0.04%	0.01%		0.10%
Himasthla littorinae	n.d.	0.27%			n.d.	1.35%	0.04%	n.d.	0.30%	0.17%
Podocotyle atomon	0.18%	1.00%			0.54%	0.05%	0.13%	0.35%	0.18%	0.28%
Cercaria emasculans	0.28%	0.04%			0.01%					
Cercaria littorinae	0.04%							0.01%		
Cercaria littorinae saxatilis sp.					0.06%		0.04%	0.04%		0.14%
Maritrema arenaria					0.11%		0.27%			
Parvatrema homeotecnum					0.09%			0.09%		0.10%
Cercaria littorinae obtusatae								0.18%		0.07%
Parapronocephalum symmetricum					0.11%			0.02%		
Maritrema linguilla					0.32%					
Cercaria brevicauda					<0.01%					
Notocotyloides petasatum								0.01%		
Cercaria islandica I								0.03%		
Trematode Species Richness	11	10	5	5	16	7	11	16	8	12
Study Sites (n)	16	20	7	49	16	2	19	13	1	24
Total Infected Snails	18787	319	670	888	5029	666	229	2811	304	212
Total Snails Investigated	70460	2600	6447	8210	22196	1925	2248	15867	1645	2875

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Table I.2: Percent Occurrence of Trematode Species Infecting *Littorina littorea*, *Littorina saxatilis* and *Littorina obtusata* in Europe and North America. Data stem from my extensive literature and field surveys. The percent occurrence of a trematode species among infected snails is listed for each survey. Metadata are presented at the bottom of the table. N.d. = no data and refers to literature studies that recorded the presence but not the prevalence of a trematode species.

Trematode Species	Percent Occurrence Among Infected *Littorina sp.*									
	L. littorea				*L. saxatilis*			*L. obtusata*		
	Europe		North America		Europe	North America		Europe	North America	
	Lit	Field	Lit	Field	Lit	Lit	Field	Lit	Lit	Field
Cryptocotyle lingua	53.91%	42.63%	92.99%	84.60%	6.74%	19.07%	25.76%	1.85%	34.21%	20.75%
Cercaria parvicaudata	n.d.	18.18%	5.97%	10.47%	0.18%	5.26%	4.95%	0.85%	n.d.	5.66%
Renicola roscovita	17.83%	16.61%	0.90%	2.48%	2.07%	1.95%	13.10%	0.71%	5.92%	16.04%
Microphallus similis	0.03%	1.25%	n.d.	0.23%	51.26%	2.10%	40.61%	50.37%	9.87%	40.09%
Microphallus sp. (other than *M. similis*)	3.97%	1.88%	0.15%	0.23%	33.94%	67.57%	4.80%	41.73%	44.41%	3.77%
Cercaria lebouri	0.87%	1.25%			0.26%		6.24%	0.36%	2.96%	1.89%
Himasthla elongata	21.50%	7.52%			0.04%		0.44%	0.04%		1.42%
Himasthla littorinae	n.d.	2.19%			n.d	3.90%	0.44%	n.d.	1.64%	2.36%
Podocotyle atomon	0.69%	8.15%			2.37%	0.15%	1.31%	1.99%	0.99%	3.77%
Cercaria emasculans	1.04%	0.31%			0.06%					
Cercaria littorinae	0.15%							0.04%		
Cercaria littorinae saxatilis sp.					0.26%		0.44%	0.25%		1.89%
Maritrema arenaria					0.48%		2.62%			
Parvatrema homeotecnum					0.42%			0.50%		1.42%
Cercaria littorinae obtusatae								1.00%		0.94%
Parapronocephalum symmetricum					0.48%			0.11%		
Maritrema linguilla					1.43%					
Cercaria brevicauda					0.02%					
Notocotyloides petasatum								0.04%		
Cercaria islandica I								0.18%		
Trematode Species Richness	11	10	5	5	16	7	11	16	8	12
Study Sites (n)	16	20	7	49	16	2	19	13	1	24
Total Infected Snails	18787	319	670	888	5029	666	219	2811	304	212
Total Snails Investigated	70460	2600	6447	8210	22196	1925	2248	15867	1645	2875

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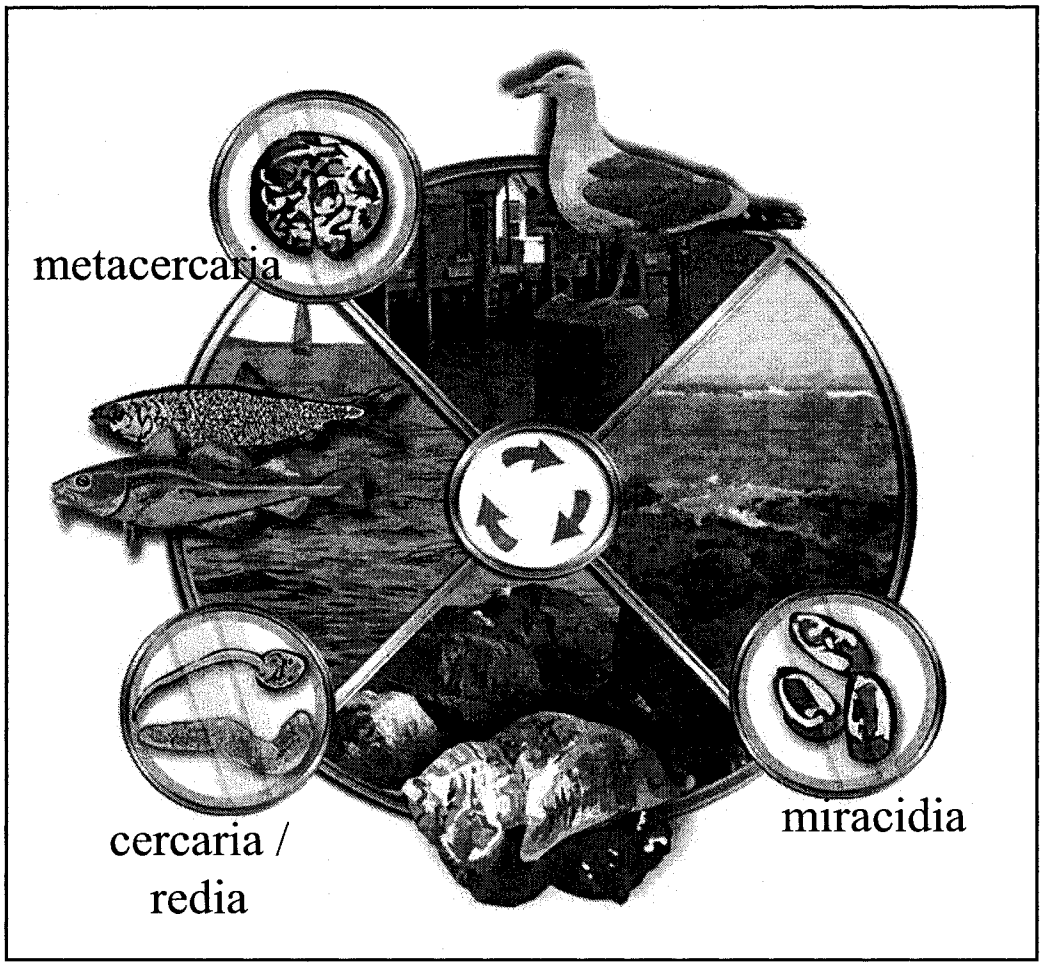

Figure I.1: Three-host Infection Cycle for *Cryptocotyle lingua*. This transmission cycle includes the larval cercarial stage, found within the snail host, where asexual reproduction takes place. The cercariae are continually shed from their snail host and proceed to seek out a second-intermediate host, which includes many species of fish. The second-intermediate host must then be ingested by the definitive host, typically a shorebird, where the trematode sexually reproduces.

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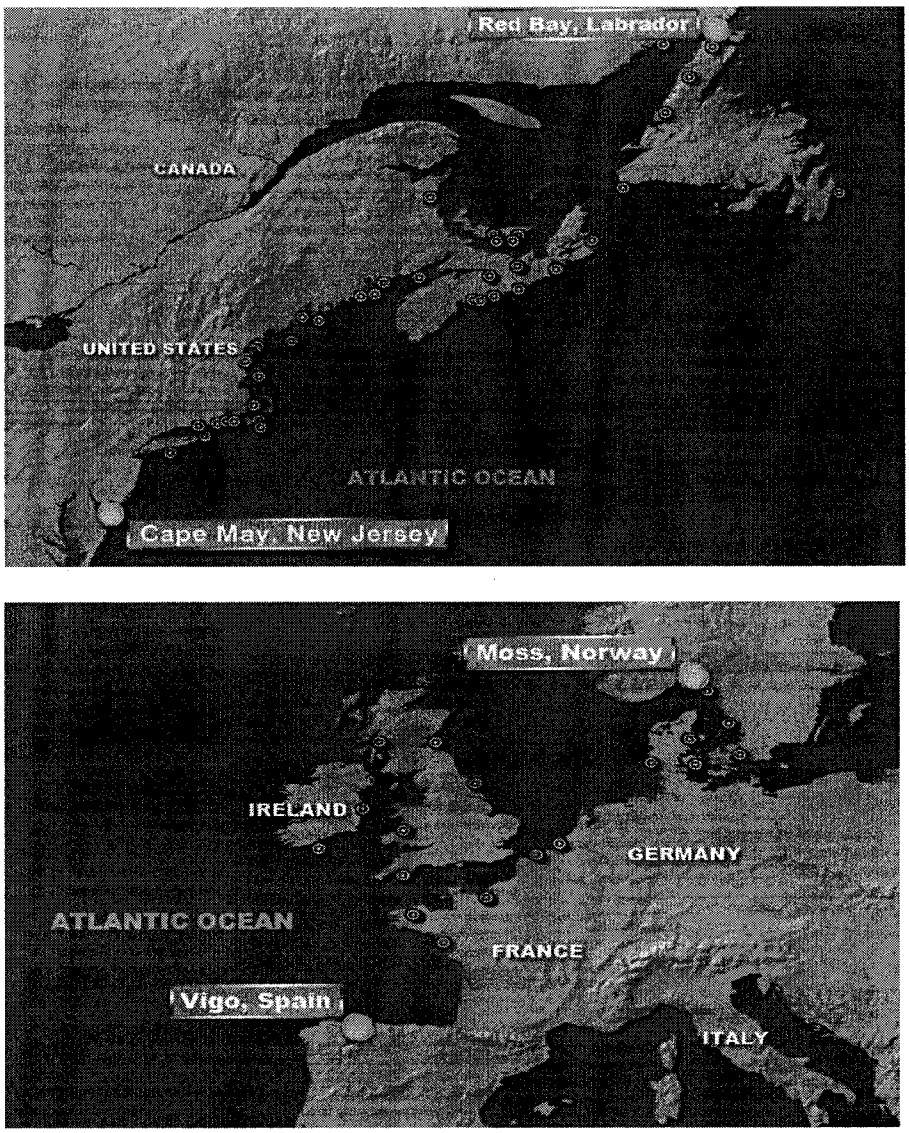

Figure I.2: North American and European Collection Sites for *Littorina littorea, Littorina saxatilis* and *Littorina obtusata*. Altogether I collected from 62 North American sites, ranging from Red Bay, Labrador to Cape May, NJ, and 20 European sites, ranging from Moss, Norway to Vigo, Spain (see APPENDIX B for detailed information on these collection sites).

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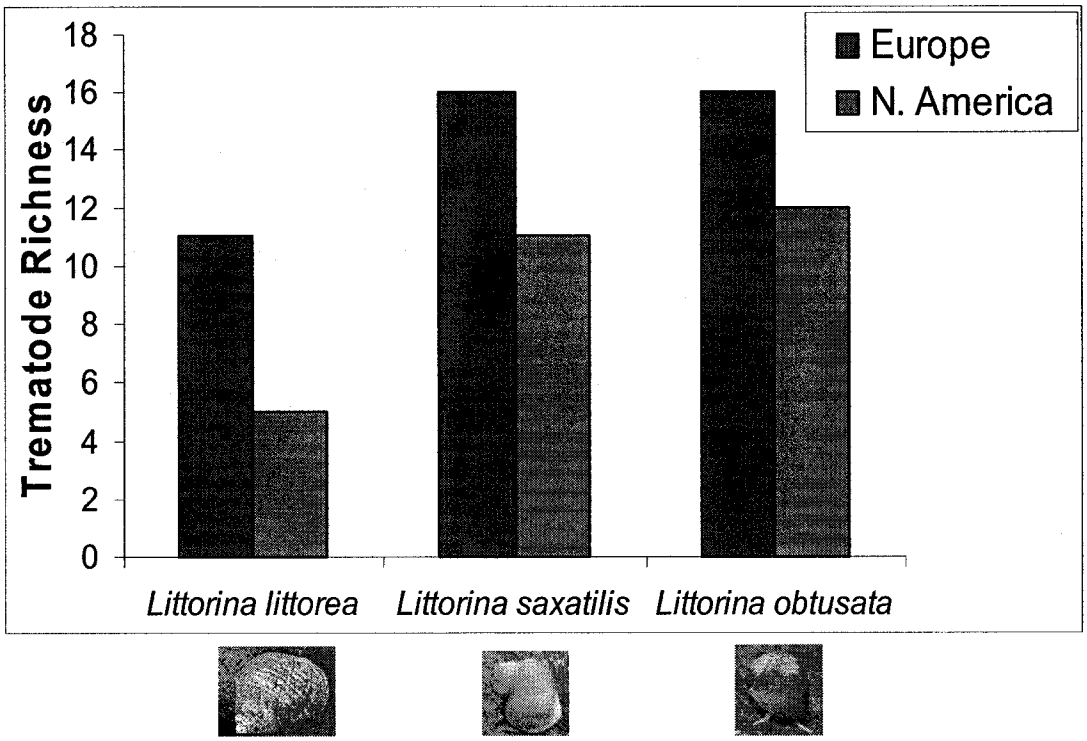

Figure I.3: Total Trematode Species Richness for *Littorina sp.* Snails in Europe versus North America. *L. littorea* shows a reduction in trematode richness that is almost twice that of congeners, *L. saxatilis* and *L. obtusata*. Total trematode species richness for *L. littorea* was 11 European versus 5 North American trematodes (55% reduction), 16 versus 11 (32% reduction) in *L. saxatilis*, and 16 versus 12 (25% reduction) in *L. obtusata*.

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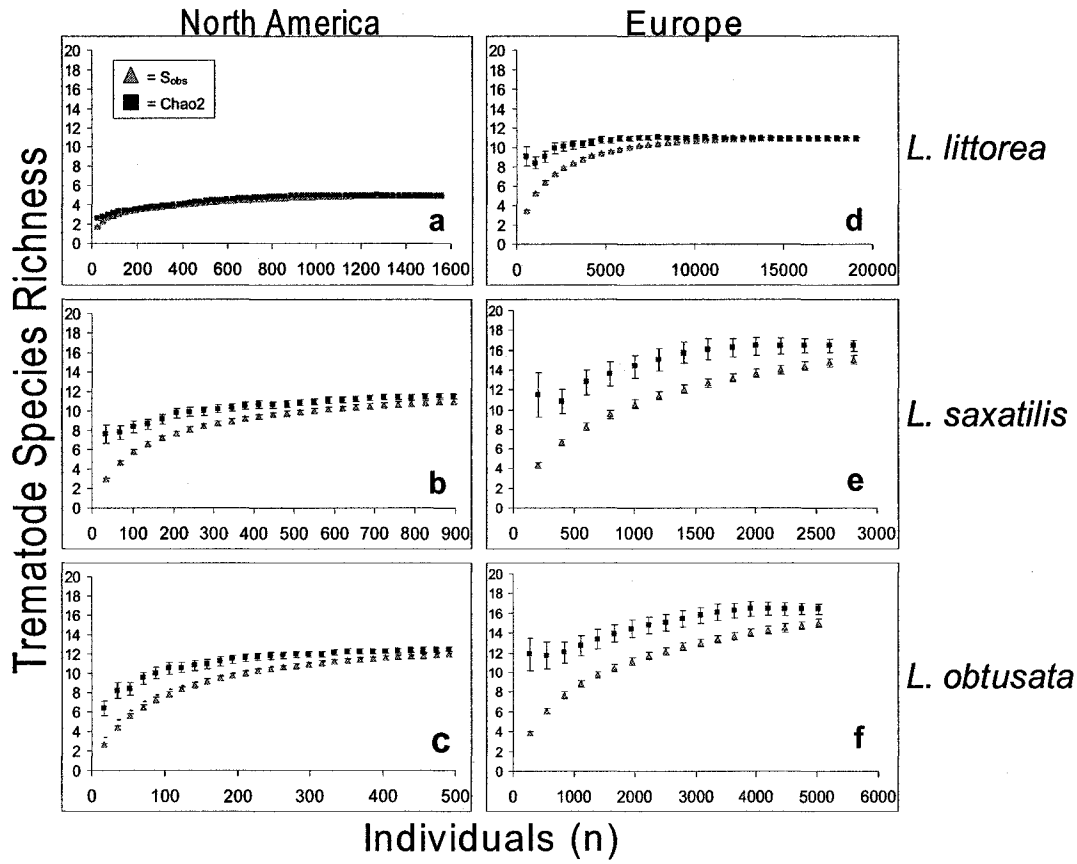

Figure I.4: Trematode Species Richness as a Function of Infected *Littorina* sp. Snails from Literature and Field Data. Richness of trematodes infecting *Littorina sp.* in both North America and Europe was estimated using species accumulation and species estimator curves (Colwell 2006). Each panel shows S_{obs} (▲) and the Chao2 (■) species richness estimate for *L. littorea* (a&b), *L. saxatilis* (c&d) and *L. obtusata* (e&f). The left and right columns depict richness in North America and Europe, respectively. For *L. littorea*, S_{obs} and Chao2 asymptote at a trematode species richness value of 5 in North America (a) and a value of 11 in Europe (b). For *L. saxatilis*, the S_{obs} and the Chao2 curves asymptote at 11 trematode species in North America (c) and in Europe, the S_{obs} culminates at 15 species and the Chao2 achieves a value of 16 species (d). For *L. obtusata*, S_{obs} culminates at ~12 species for Chao2 in North America (e), and in Europe, S_{obs} culminates at 15 species and Chao2 achieves a value of 16 species (f).

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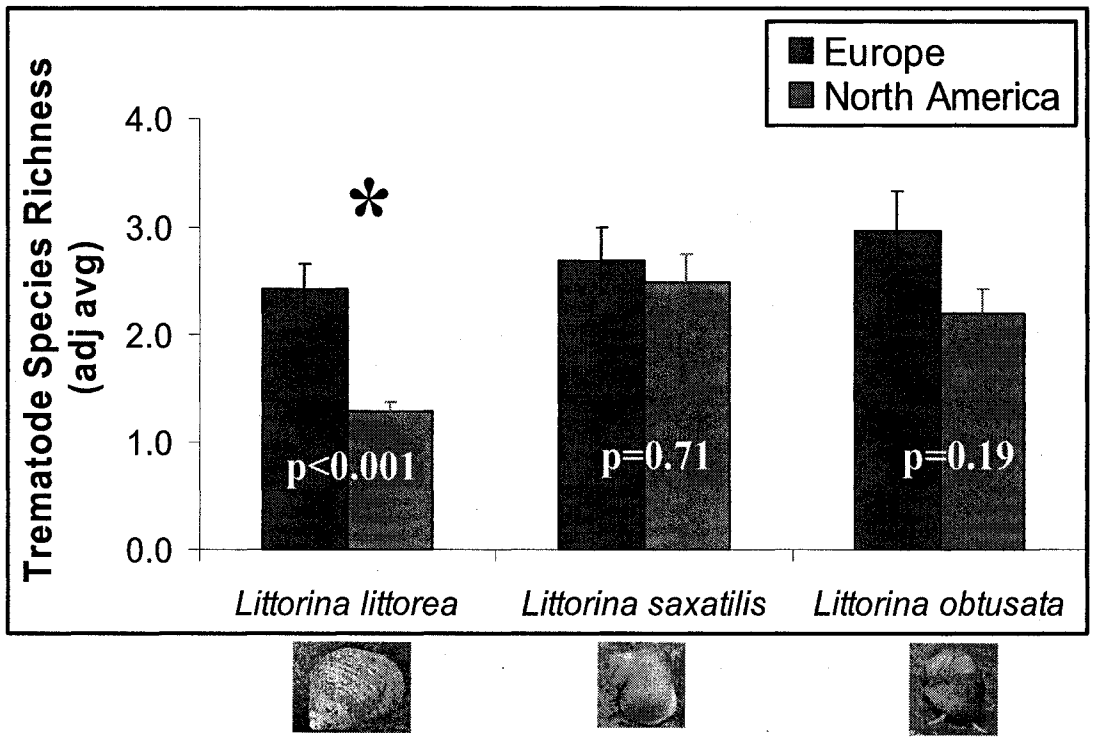

Figure I.5: Standardized Site Level Average (± Standard Error) Trematode Species Richness for *Littorina sp.* Snails in Europe versus North America. *L. littorea* shows a significantly ($p<0.001$) greater average site level trematode richness in Europe (2.43 ± 0.23) compared to North America (1.28 ± 0.11). *L. saxatilis* and *L. obtusata* both show lower trematode richness in North America (LS: 2.49 ± 0.26; LO: 2.204 ± 0.23) compared to Europe (LS: 2.69 ± 0.31; LO: 2.81 ± 0.35), but these differences were not significant ($p=0.71$ and $p=0.25$, respectively).

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CHAPTER II

RESOLVING THE 150-YEAR DEBATE OVER THE INTRODUCTION OF *LITTORINA LITTOREA* TO NORTH AMERICA

ABSTRACT

The marine snail *Littorina littorea* is an abundant intertidal snail on both North Atlantic coasts. Although definitively native to Europe, its ecological history in North America has been extensively investigated and debated for over 150 years. To resolve its cryptogenic status, I sequenced mtDNA of nearly 400 *L. littorea* snails in Europe and North America to explore molecular signatures of a potential introduction to North America. My results demonstrate a significant reduction in overall genetic diversity in North America versus Europe, nested and common haplotype frequencies in North America, and a divergence estimate of ~450 years ago from the European source. My data indicate a recent, human-mediated introduction of *L. littorea* to North America from Europe.

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INTRODUCTION

In recent years, invasive species have become recognized as a major component of human-mediated impacts on natural systems. However, there remain a considerable number of species that cannot be demonstrably classified as native or non-native in many regions, and these species are referred to as cryptogenic (Carlton 1996). For example, Carlton (1996) found that *possible* invasions – i.e., cryptogenic species – would increase by as much as one-third the number of known invasions in San Francisco Bay if these proved to be introduced. Moreover, the number of cryptogenic species that are actually non-indigenous on the US east coast is suspected to be even higher than the west coast because the east coast was settled by Europeans much earlier and before rigorous inventories of native biota were consistently undertaken (Ruiz et al. 2000). In Chesapeake Bay, for example, as much as 30% of the total species found in the Bay are cryptogenic and could potentially be non-indigenous (Ruiz et al. 2000). Knowledge of the native members of the community is a fundamental starting point for resource managers and conservation biologists attempting to restore and maintain native species, biological diversity, and ecosystem function. Especially in the case of conspicuous, abundant, or high impact species, resolution of the ambiguous ecological history of cryptogenic species is a critical conservation goal.

One North American cryptogenic species, whose status as native or non-native has been debated for the past 150 years (e.g., Ganong 1886, Clarke and Erskine 1961,

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Berger 1977, Wares et al. 2002, Chapman et al. 2007a,b, Cunningham 2007, Wares and Blakeslee, in press), is the marine snail, *Littorina littorea* (common periwinkle). Cryptogenic status stems from incomplete or unknown historical knowledge of a species' presence in a certain location. The lingering ambiguity surrounding *L. littorea's* status is particularly surprising given the vast amount of research over the past several decades that has been conducted on this species. Considering the species' conspicuousness and dominance within intertidal regions in northeast North America, and its past use as a textbook case of invasion (e.g., Steneck and Carlton 2001), the resolution of its present-day cryptogenic status is imperative. Evidence for understanding the ecological history of *L. littorea* in North America has been provided by historical, archaeological, ecological, and genetic data (e.g., Ganong 1886, Clarke and Erskine 1961, Clarke 1963, Bird 1968, Berger 1977, Carlton 1982, Wares et al. 2002, Chapman et al. 2007, Wares and Blakeslee, in press). However, its cryptogenic status remains unresolved because conclusions from these sources have been conflicting or equivocal (Johannesson 1992, Reid 1996, Wares and Blakeslee, in press). Recent ecological evidence (Blakeslee and Byers, in revision) has revived the debate by analyzing patterns in parasite species richness in *L. littorea* on both sides of the Atlantic. Using the well-supported theory of enemy escape (e.g., Torchin et al. 2003), the authors found significantly lower parasite richness in snails from North America compared to Europe, which was suggestive of a recent invasion to North America. What remains missing in the pursuit to finally resolve the 150+ year debate over *L. littorea's* presence in North America is a definitive molecular genetic analysis that includes a large and diverse sampling of snails in Europe

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and North America. Prior molecular genetic studies on the *L. littorea* introduction question (Berger 1977, Wares et al. 2001) have met with criticism due to small sample sizes (Chapman et al. 2007).

Therefore, in this study I have assembled a large molecular dataset using mitochondrial DNA to look for signatures expected in a strong genetic bottleneck, including lower genetic diversity in North America versus Europe and patterns in haplotype frequencies where the most frequent haplotypes are shared between the regions. These patterns would be expected in a strong genetic bottleneck because any substantial decline in population size would result in a significant reduction in genetic diversity in the bottlenecked population, which would only possess a subset of the genetic diversity of its original population (Grosberg and Cunningham 2000). This reduction in diversity would also result in changes in haplotype frequencies in the bottlenecked population where those few haplotypes that remained would increase in frequency leading to a few common haplotypes in the bottlenecked population compared to many, rare haplotypes in the original population under random sampling (e.g., Ledig et al. 1999). In the case of a recent introduction for *L. littorea*, not only would I expect these bottleneck signatures, but I also would expect short divergence time estimates between the North American and European populations because coalescent theory would predict a recent split (divergence time) between the two regions. On the other hand, if the alternative hypothesis were true—that *L. littorea* is native to North America having existed in pre-glacial refugia in maritime Canada until its spread into the US in the mid-1800s—I would expect a divergence estimate between the European and North American

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populations that would be prior to human contact with North America from Europe. To examine these hypotheses, I explored two regions of mitochondrial (mt) DNA within snails from both European and North American populations. mtDNA is appropriate for explorations of genetic bottlenecks due to its lack of recombination and lower effective population size (since a single copy is passed from mother to offspring) (Avise 2000). I analyzed my data using phylogenetic approaches, analysis of molecular variance (AMOVA), rarefaction curves, and the Isolation with Migration model of divergence.

Study system

Along most rocky shorelines of the North Atlantic, *Littorina littorea* is a highly abundant snail with large, well-documented influences on the community through grazing activities on micro- and macroalgae (Lubchenco 1983), competitive displacement of native snails (Brenchley and Carlton 1983, Yamada and Mansour 1987), indirect impacts on community interactions (Bertness 1984), and serving as first intermediate host to trematode parasites with complex, multi-host lifecycles (Lauckner 1980). *Littorina littorea* is presently found in western Europe and northeastern North America but is absent from Iceland and Greenland (Reid 1996); it is known to be native to Europe (Linnaeus 1758), but its status in North America remains less clear. What is known is that in the mid 1850s, *L. littorea* was observed to spread rapidly and sequentially southwards from Halifax, Nova Scotia into the U.S, reaching Delaware Bay only 30 years after its first recorded sighting in Halifax (Steneck and Carlton 2001). What remains uncertain is whether this maritime Canada population was native and confined to Canada until the

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mid-1800's or was an anthropogenically introduced population from Europe (Reid 1996, Chapman et al. 2007, Wares & Blakeslee, in press).

MATERIALS AND METHODS

Snail collections

During the summers of 2002-2005, approximately 10-15 adult snails were collected haphazardly from the intertidal zone at each of 29 North American (13 U.S. and 16 Canadian) and 22 European sites (Figure II.1). Each snail was dissected and the snail foot tissue removed and stored at –80 °C until processing. Because *Littorina littorea* snails can sometimes be infected by trematode parasites, I only used uninfected snails in my analyses to avoid contamination issues.

DNA extractions, PCR and sequencing

DNA was extracted using a standard CTAB protocol (France et al. 1996), and DNA quality and quantity were determined using a spectrophotometer. Two sets of primers were used to amplify sections of the Cytochrome b (cyt b) and Cytochrome Oxidase I (COI) mitochondrial genes: Cyt b (625 bp): *Primer1-F*, CCTTCCCGCACCTTCAAATC, and *Primer4-R*, ATGAGAAATTTTCAGGGTC (Reid et al. 1996); COI (572 bp): *LLCOIAB-F*, CTCTCCTGGGAGATGACCAG, and *LLCOIAB-R*: TTCTGGGTGACCGAAGAATC designed using COI sequence data from Williams and Reid (2004). All samples were amplified using a PCR protocol based on

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Kyle and Boulding (1997): 0.6 μL template DNA (~100 $ng^{-\mu L}$) was added to a PCR mix (3 μL Taq buffer, 0.75 μL of 3.4 mM dNTPs, 0.225 μL of each 20 μM primer, and 0.15 μL Taq (5 $U^{-\mu L}$) and molecular grade water for a final volume of 30 μL. Reaction mixes were subjected to 32 Cycles of 95 °C for 30 s, 44 °C for 30 s, and 72 °C for 30 s in an automated thermocycler. DNA was eluted from PCR products using a QIAquick Gel Extraction kit (Qiagen, Inc, Valencia, CA) and sequenced using ABI 377 DNA Automated Sequencers at the UNH Sequencing Facility. Sequences were analyzed using DNASTAR Programs (EditSeq, Seqman, and Megalign; DNASTAR, Inc., Madison, WI).

Statistical analyses

Phylogenetic relationships were analyzed using PAUP 4.0 (Swofford 2003). Phylogenetic trees were not only constructed using the full data set, but also using a truncated data set, where I excluded all third position sites (resulting in 798 total bp), which are the most variable sites in coding DNA because substitutions at these sites are often silent (i.e., they do not alter amino acid composition). This latter approach gave us a conservative estimate of haplotype diversity in Europe versus North America. Finally, I constructed a phylogenetic tree of just North American individuals so I could compare Canadian versus U.S. sites. I performed this last analysis as a way to determine whether Canadian sites showed more diversity than U.S. sites, which might be expected if *L. littorea* had existed in the Canadian maritimes for thousands or hundreds of thousands of years before spreading into the U.S. ~150 years ago (i.e., the U.S. subpopulation should show a subset of the diversity of the Canadian subpopulation if *L. littorea* had pre-glacial

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populations in Canada). For each of these phylogenetic analyses, the maximum likelihood root haplotype for each tree (designated by an asterisk) was determined using the program, TCS 1.21 (Clement et al. 2000).

Because haplotype diversity was high in my sampled populations, I used haplotype accumulation and haplotype estimation curves to estimate haplotype diversity in each population and to quantify the effects of sampling effort on haplotype diversity. Specifically, I used ESTIMATES 8.0 (Colwell 2006) to calculate haplotype accumulation and haplotype estimation curves. ESTIMATES uses Monte Carlo re-sampling (through randomization of sample order over a number of replicates (e.g., 500)) to determine the mean accumulation of haplotypes (S_{obs}) as samples are added over the full data set (Gotelli & Colwell 2001), while also providing standard deviations and 95% confidence intervals for each data point (Colwell 2006). However, sample-based rarefaction curves may not capture the entire haplotype diversity within a population for a particular sampling effort, especially if these curves have not reached a stable asymptote. Thus, non-parametric estimators, such as Chao2, can be useful in predicting the eventual asymptote in haplotype diversity for a particular population (Gotelli & Colwell 2001), and do so by including the effects of rare haplotypes on the total haplotype diversity (Chao 2004, Witman et al. 2004). The Chao2 estimator has been found to be one of the most robust estimators (see Colwell 2006 for Chao2 equation) when compared to empirical data from a variety of systems (e.g., Walther & Morand 1998, Foggo et al. 2003).

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Finally, population divergence estimates were performed using the Isolation with Migration (IM) program (Version: July 2006; Hey and Nielsen 2006). IM uses Markov Chain Monte Carlo sampling and applies the Isolation with Migration model to genetic data taken from closely related species or populations of the same species. The program provides maximum likelihood estimates of the time since two populations split (t) in terms of mutations, which can be converted to an estimate of the number of years since the populations diverged using the specific neutral mutation rate for the gene in question. I performed ten different runs/replicates of IM using the following input parameters and ten different random seeds: q1=1000, m1=m2=7, t=2, b=100,000, L=18.0. Divergence estimates were calculated using the following equation: $t = t/\mu$, where t = total years of divergence time, t = the time parameter determined by IM from the sequence data, and μ = the gene substitution rate (Hey and Nielsen 2004). The substitution rate (3% per MY; $\sim 1.8 \times 10^{-5}$ for 1197 bp) I used in calculating divergence estimates was determined by Wares and Cunningham (2001) from fossil record evidence of *Littorina* sp. provided by Reid et al. (1996) and later employed specifically for *L. littorea* in investigations by Wares et al. (2002) and Cunningham (2007).

RESULTS

A total of 370 sequences were analyzed (187 European and 183 North American) with an average ($\pm$ s.d.) of 7.25 ($\pm$ 3.06) snail sequences per site (APPENDIX D). Each sequence was 1197 bp in length when the two mtDNA regions (cyt b: 625 bp; COI: 572

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bp) were combined. Altogether, I observed a total of 175 different haplotypes. Fifty-seven haplotypes were North American and 144 were European (when including haplotypes that were shared between the regions). Of the 57 North American haplotypes, 26 were shared between the North American and European populations, 23 were unique to North America, and 8 appeared unique but were basal to European haplotypes so must be shared with a European haplotype not detected in my sampling (Figure II.2). Thus, 60% of the North American haplotypes were shared and 40% were not shared. In addition, 42% (24/57) of the North American haplotypes were found more than once (i.e., common), while 58% were observed just once (i.e., rare). Of the 144 European haplotypes, 26 were shared (18%) between the regions and 118 (82%) were unique to Europe (Figure II.2). In addition, 94% (135/144) of the European haplotypes were rare and only 6% (9/144) were common. In all, North America exhibited a significant reduction in genetic diversity compared to Europe (χ^2=37.7, d.f.=1, $p<0.001$) and possessed a significant number of common haplotypes compared to Europe (χ^2=6.8, d.f.=1, p=0.009). On the whole, no clades were completely monophyletic for North American individuals (i.e., all clades containing North American individuals also included European individuals) (Figure II.2). Furthermore, patterns observed in the order of haplotype frequencies from high to low also met expectations for a recent introduction—all the common haplotypes were either shared or in North America (except one high frequency European haplotype), while the majority of rare haplotypes were found in Europe (Figure II.3). Finally, the haplotype estimation (Chao2) curves calculated the expected, maximum number of haplotypes (mean of 500 replicate runs) in

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Europe to be 2456 (95% CI=918; 4115) versus 140 haplotypes (95% CI=89; 273) in North America (Figure II.4); thus Europe is expected to possess 17.5 times more haplotypes in Europe compared to North America.

When I analyzed the phylogenetic relationship between North America and Europe using a conservative approach (where all 3rd position sites were removed), I found nearly all haplotypes in North America to be included among the European sites. I detected only two unique North American haplotypes compared to 38 unique European haplotypes and 5 shared ones (total haplotypes n=45; Figure II.5). In total, Europe possessed nearly 96% of the total diversity, and North American diversity was significantly lower than European diversity (χ^2=25.9, d.f.=1, p<0.001). Additionally, the European haplotype estimator (Chao2) curve (mean estimate of 500 replicate runs) predicted 193 haplotypes (95% CI=120; 339), while in North America the haplotype estimator did not predict any more haplotypes than the 7 total haplotypes (95% CI=7; 7.1) observed in North America. Thus this approach vastly reduced the number of unique North American haplotypes, which made up less than 5% of the total number of haplotypes. Furthermore, the predicted number of European haplotypes (193) is 27.5 times greater than the number predicted in North America (7).

Phylogenetic comparisons within the North American region did not reveal more diversity in Canada compared to US (Figure II.6), as might be expected if *L. littorea* were confined to Canada for potentially tens or hundreds of thousands of years before moving into the US in the mid-1800s. In fact, the diversity between the two populations was essentially equal (Canada: 29 total haplotypes from 95 individuals and US: 28 total

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haplotypes from 88 individuals) and not significantly different (χ^2=0.2, d.f.=1, p=0.895). Additionally, the number of unique Canadian haplotypes was nearly identical to the number of unique U.S. haplotypes (19 to 18, respectively), and 10 haplotypes were shared between the two regions. Chao2 estimates of haplotype diversity (mean of 500 replicate runs) were 82 (95% CI=58; 139) in Canada versus 70 (95% CI=60; 117) in the U.S., suggesting consistent maximum estimates between the two regions.

Finally, I used the Isolation with Migration (IM) program (Hey and Nielsen 2006) to calculate divergence estimates based on the sequence data for Europe and North America. Over my ten replicate runs I found the average divergence estimate (± SE) to be 444 (± 88) years with 95% confidence intervals between 344 (± 73) and 644 (± 137) years ago (Table II.1).

DISCUSSION

I have demonstrated several genetic signatures that strongly support a founder effect in North American *Littorina littorea*. First, the snail showed a significant reduction in overall genetic diversity in North America versus Europe for both my complete data set and my conservative data set (i.e., the exclusion of all third position sites) (Figs. 2.2 & 2.5). In the complete data set the inclusion of the third position sites, which are the most variable sites in coding DNA, increased the likelihood that I would observe enhanced diversity in both regions. Alternatively, the latter analysis tested whether a decreased likelihood for diversity would result in all North American haplotypes nested within

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European haplotypes, which is one of the primary expectations of a founder effect (Grosberg and Cunningham 2000). In this analysis, I found all but two North American haplotypes to be nested within European haplotypes. Given the significant diversity in Europe, the fact that these two haplotypes are not found in Europe is likely a product of incomplete sampling in Europe and not because the haplotypes are endemic to North America. Had North America and Europe been independent populations over many thousands of years, the likelihood of producing a new haplotype in these mitochondrial genes is essentially equal for each population (since I used a molecular marker that should not be under any significantly different selection pressure in the two regions) and thus North America should have had many more unshared haplotypes than I observed. Furthermore, no clades were completely monophyletic for North American individuals (Figure II.2). Had North America existed independently from Europe for a long period of time (potentially hundreds of thousands of years), the expectation would be for divergence of individuals into distinct clades. Instead, all clades containing North American individuals also included European individuals. This demonstrates that not a single North American individual sequence or haplotype was completely independent from Europe, suggesting that further sampling should reveal shared status and thus nestedness of all North American genetic diversity within European diversity.

Furthermore, I found a significant difference in the number of high frequency (common) haplotypes in North America versus Europe (Figure II.3). Only one common European haplotype was also unique, and this haplotype was predominantly found at Scandinavian sites. Consistent with my conclusions, this either signifies that my North

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American sampling did not detect this frequent European haplotype or it may suggest that *L. littorea* individuals have not arrived to North America from that region of Europe. In contrast, there was a significant number of common haplotypes in North America, a pattern expected in recent founding events, where those few haplotypes carried with the founding population would increase in frequency (compared to frequencies in the source population) as the size of the population grew.

One pattern I observed that is not expected in recent founding events was the detection of 23 unique haplotypes in North America not also found in Europe (i.e., the blue sections in Figs. 2.2 & 2.3). Because a recently introduced population would not be expected to accumulate new mutations in this particular marker within the relatively short amount of time since an anthropogenic introduction could have occurred, these unique haplotypes signify two alternative scenarios. Either, 1) the haplotypes are truly endemic to North America, suggesting a long-term existence for the snail in North America, or 2) they are not endemic to North America and instead represent a sampling issue due to the vast genetic diversity of the snail in Europe (i.e., I failed to find all European haplotypes corresponding to the handful of unique ones observed in North America).

My haplotype estimation curves support the latter of the two hypotheses because they predicted European diversity to be over 1.5 orders of magnitude greater than North American diversity, requiring significantly more sampling in Europe to reveal all predicted haplotypes (Figure II.4). In fact, in Europe at the present proportion of haplotypes to snails—0.77 (which could change with more sampling)—I would need to sequence 3189 snails in order to capture the 2456 haplotypes predicted by the Chao2

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estimator. In contrast, at the proportion of haplotypes to snails (0.31) in North America, I would need to sequence only 478 snails to find the 149 predicted haplotypes. Thus, those 23 haplotypes that are currently unshared in North America are likely among the 2000+ haplotypes that have yet to be discovered in Europe. The strong likelihood for these haplotypes being shared with yet undiscovered corresponding haplotypes in Europe is further supported by the lack of unique North American clades.

Moreover, Wares and Blakeslee (in press) have shown that a prior dataset significantly undersampled *L. littorea* mtDNA (Wares et al. 2002) exhibited a high probability of undersampling, preventing the investigators from discerning whether all North America haplotypes were nested within European haplotypes. In fact, the smaller sample size of this prior investigation (Wares et al. 2002) demonstrates the profound effect sample size can have on conclusions gleaned from genetic data. The Wares et al. (2002) data set was less than one-third the size of my dataset and resulted in entirely opposite conclusions, particularly relating to a handful of unique North American haplotypes that were proposed to be endemic. However, as is the case for my own dataset, European diversity in the Wares et al. (2002) study was too great to preclude sampling as an alternative and more likely scenario for the presence of these unshared haplotypes. Furthermore, other investigations of known introductions have found unique haplotypes in the founding populations not witnessed in samples from the source populations. For example, Fonseca et al. (2001) found several unique haplotypes of a Japanese mosquito in its non-native US population and suggested that its high genetic diversity in Japan precluded the detection of the shared haplotypes in Europe. Similarly,

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Muira et al. (2006) detected a few unique U.S. haplotypes in a Japanese trematode parasite, *Cercaria batillariae*. The authors suggested that these novel haplotypes were the result of undersampling and not due to new mutations, which would be impossible within the short time period since the trematode's invasion (<100 years) and the inherent neutrality in the COI marker. Finally, Roman (2006) in an investigation of *Carcinus maenas* (European green crab) discovered a few haplotypes in eastern North America not found in Europe, which were assumed as shared between the regions (i.e., the corresponding European haplotype had gone undetected). Thus, based on the likelihood for finding unique haplotypes in founding populations when the source population is very diverse, the 23 unshared haplotypes I observed in North America do not appear to represent endemism but rather undersampling in Europe.

Divergence estimates using the IM Program (Hey and Nielsen 2006) also support a recent founding event for North American *L. littorea* (Hey and Nielsen 2006). I found these estimates (mean and 95% confidence intervals) to be within the time frame for human colonization of North America from Europe, ranging from ~350-650 years ago (Table II.1). *L. littorea's* first reported sighting was in Pictou, Nova Scotia, which was settled by Europeans in the mid-1600s (Ron Wallis, "The History of Pictou"); however, Vikings are also believed to have visited maritime Canada as far back as ~1000 years ago (Spjeldnaes and Henningsmoen 1963). Thus, these divergence estimates are within the time frame that Europeans were colonizing or visiting maritime Canada, and consistent with the mechanism of human-mediated transport for the recent introduction of *L. littorea*. In addition, because these divergence estimates are impacted by the unshared

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North American haplotypes that I have demonstrated are unlikely to be unique, the mean divergence estimate of ~450 years ago (Table II.1) is a conservative estimate and true divergence estimates are likely much earlier than 450 years ago, especially since there is no evidence of living *L. littorea* in America prior to the 1840s, and no clear indication where populations of *L. littorea* would have been and remain undetected from the 1500s to the 1800s.

Although glaciation could also result in genetic bottleneck signatures in North America, my evidence argues against a pre-glacial existence for *L. littorea* in North America. Patterns for expansion following glacial refugia typically show low genetic diversity in the latitudes furthest from the source of the population expansion (Marko 2004). My phylogenetic analysis of North American populations, which treated maritime Canada as a possible glacial refugial region (as proposed in Wares et al. 2002), and compared it against U.S. populations, found no difference in the amount of genetic diversity at either the regional level (29 Canadian versus 28 US total; Figure II.6) or at the site level (the proportion of the number of haplotypes / number of snails at each site: Canada=0.87; US=0.91). In fact, when I compared all of maritime Canada with just the southern-most portion of the US population (US sites south of Cape Cod), there was little change in site-by-site Canadian versus US diversity (proportion of haplotypes / snails: US south of Cape Cod=0.89; Canada=0.87). If *L. littorea* had existed for tens or hundreds of thousands of years in maritime Canada before suddenly expanding its range in the mid-1800s, it would have retained some level of diversity in Canada that would not have migrated with the snail when it expanded into the US. In contrast to this prediction, my

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data show an essentially equal amount of diversity between Canada and the US, and this pattern cannot simply be explained as the result of a sampling issue in Canada (as I have shown is the case in Europe) because the Chao2 estimator predicts only a handful more haplotypes (~12) in Canada compared to the US. In contrast to my results, Marko (2004) found evidence for a northern latitude glacial refugia for a Pacific North American marine snail, *Nucella lamellosa*; in particular, AMOVA tests revealed a significant amount of subdivision between northern and southern latitudes, evidence that the snail had existed in glacial refugia in northern latitudes before expanding southwards. Using the same AMOVA test and comparing my Canadian versus U.S. sites did not reveal significant F_{ST} or F_{CT} values (F_{ST}: 0.0145; $p = 0.20$; F_{CT}: -0.00038; $p = 0.37$), nor were the comparisons significant when just exploring US sites south of Cape Cod versus maritime Canada sites (F_{ST}: 0.03991; $p = 0.06256$; F_{CT}: -0.00222; $p = 0.40958$). Furthermore, my IM divergence estimates between the European and North American populations are many thousands of years later than the time period for glacial refugia for *L. littorea* (the last glacial maximum was ~20,000 years ago; Wares and Cunningham (2001)). Finally, other marine rocky intertidal species, *Semibalanus balanoides* (barnacle) and *Mytilus edulis* (blue mussel), with similar dispersing mechanisms to *L. littorea* and believed to have existed in glacial refugia in southeast Canada (Wares and Cunningham 2001) were not confined to maritime Canada following glacial retreat. Taken on the whole, these results argue strongly against a glacial refugia theory in maritime Canada for North American *L. littorea.*

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Therefore, the most parsimonious conclusion based on my results is a recent introduction of *L. littorea* to North America from Europe, which was likely human-mediated due to *L. littorea's* close association with human means of transport (e.g., through rock ballast) or intentional introduction as a food source (Carlton 1982). Furthermore, the snail's absence from North Atlantic islands, such as Iceland and Greenland, which are believed to have aided in the natural, stepping-stone invasions of several marine intertidal species following the last glaciation (Ingolfsson 1992, Johannesson 1988), is further proof that the snail did not move naturally across the North Atlantic but was more likely anthropogenically carried over. Additionally, my molecular genetic data is consistent with recent ecological patterns observed by Blakeslee and Byers (in review), which showed a significant reduction in parasite species richness in North America compared to Europe for *L. littorea* but not for native congeners, *L. saxatilis* and *L. obtusata*. Altogether, my study of *L. littorea's* invasion history in North America resolves not only a specific conundrum, but also demonstrates the success of the approach applied here to resolve cryptogenic histories even of older invasions of broadcast spawning species.

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Table II.1: IM Divergence Estimates for *Littorina littorea* in North America versus Europe. Divergence estimates were calculated using a mutation rate of 3%/MY (Reid et al. 1996, Wares and Cunningham 2001). Mean estimates (± SE for ten different runs) are listed for each mutation rate followed by low and high 95% confidence intervals. All resulting divergence estimates are within the time frame of European settlement of North America from Europe.

Divergence Estimates in Years (Average ± SE)	
Mean 't'	**444 (±88)**
95% CI Low 't'	344 (±73)
95% CI Upper 't'	644 (±137)

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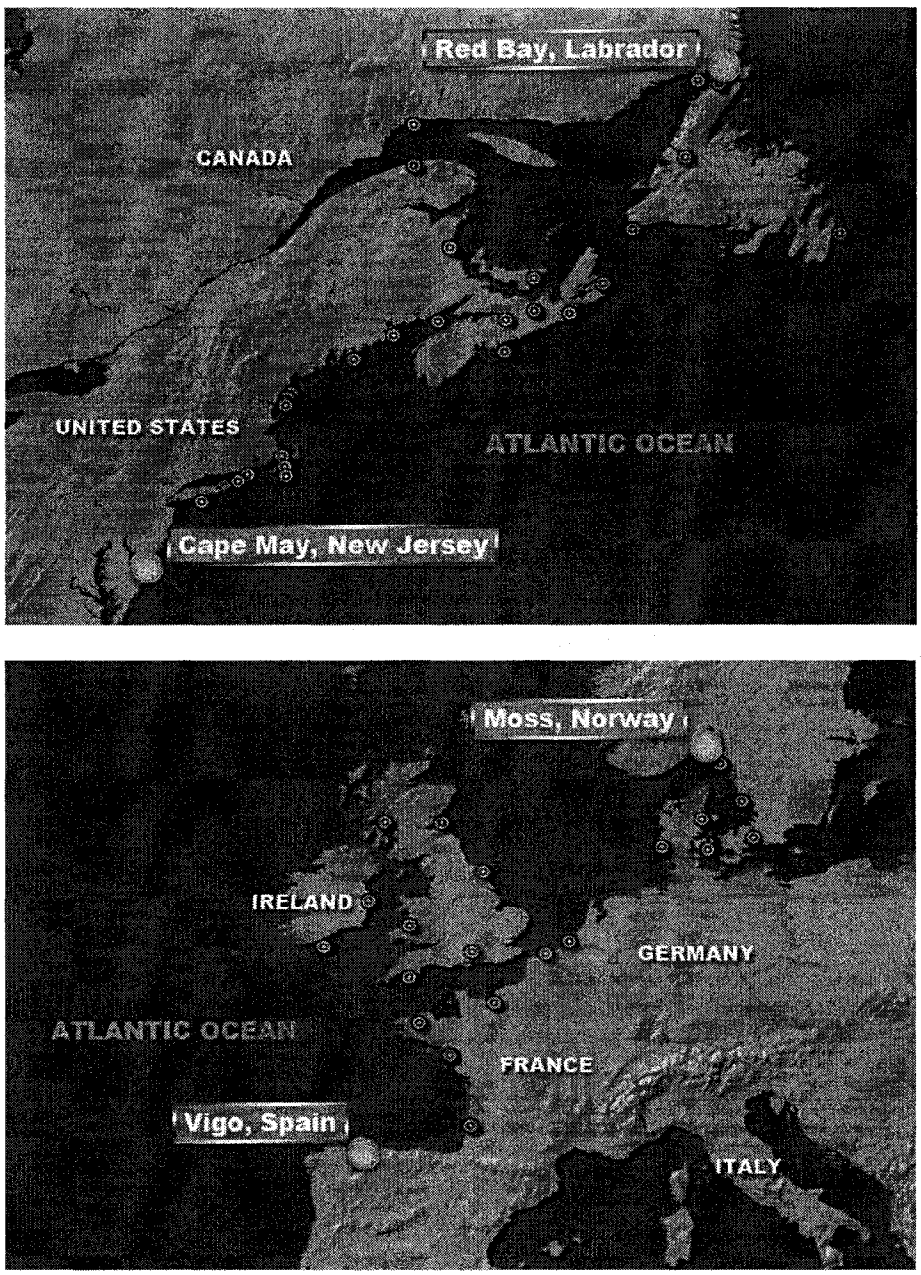

Figure II.1: North American and European Collection Sites for *Littorina littorea* mtDNA Analyses. I collected from 29 North American sites, ranging from Red Bay, Labrador to Cape May, NJ. In Europe, I collected from 22 sites, ranging from Moss, Norway to Vigo, Spain.

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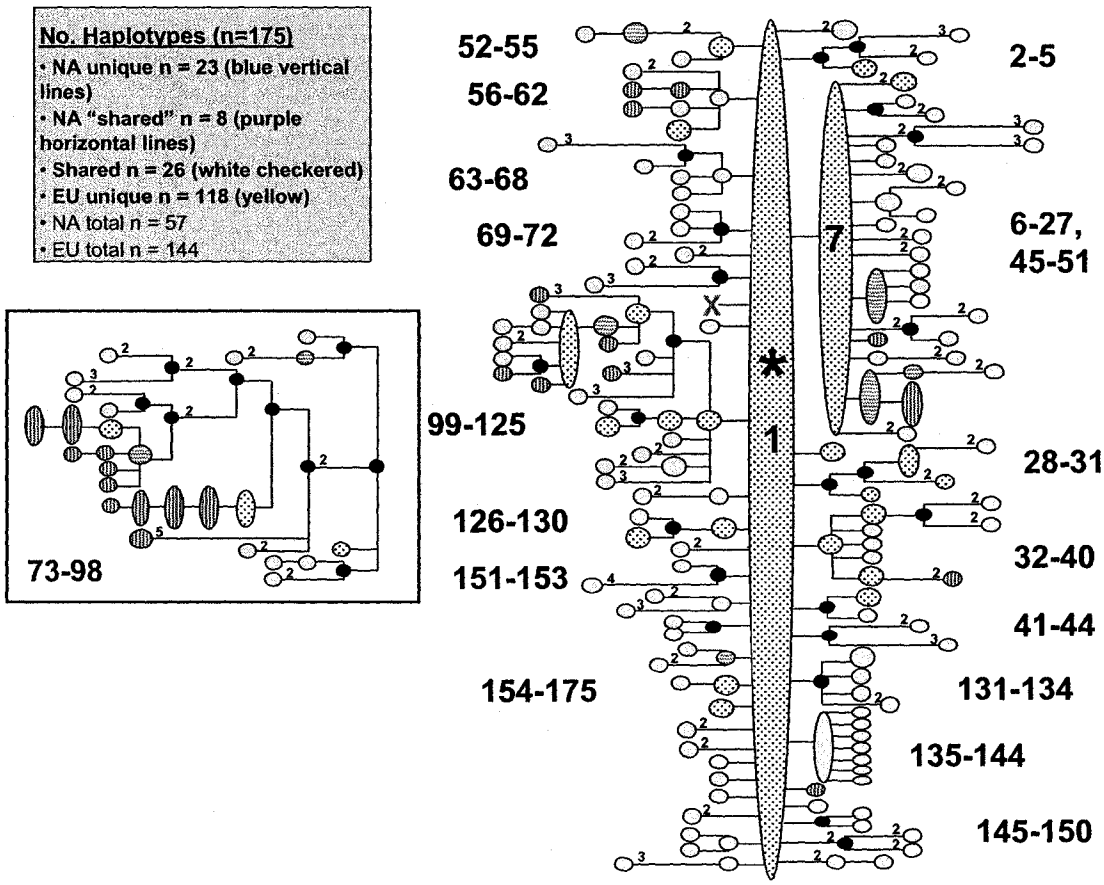

Figure II.2: Haplotype Tree for *L. littorea* mtDNA (~1200 bp). The numbers on the sides of the tree represent haplotype identities within clades/areas on the tree (see APPENDIX D). Haplotype bubbles are relatively sized based on haplotype frequencies (e.g., higher frequencies have larger-sized bubbles) and are colored according to the following categories: unique to Europe (yellow), unique to North America (blue vertical lines), and shared between populations (white checkered). A fourth category (purple horizontal lines) represents haplotypes that appeared unique to North America but which are basal to European haplotypes and are considered shared. The inset represents a clade that was too large for the scale of this diagram (see the 'X' for position on the overall tree).

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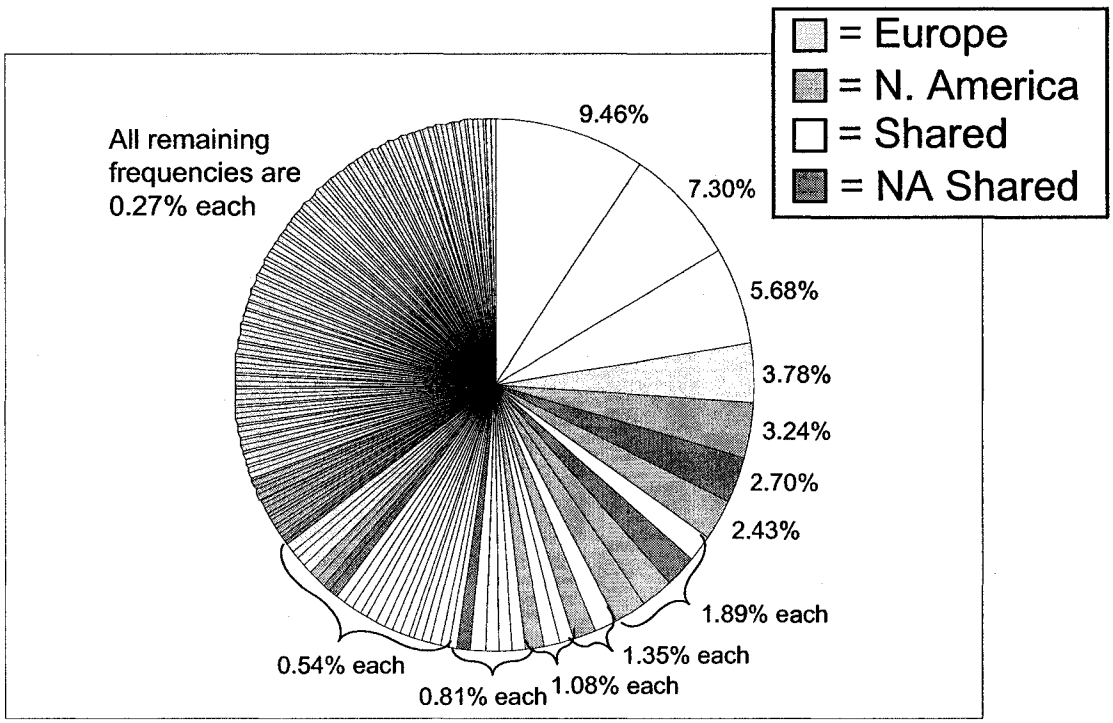

Figure II.3: Haplotype Frequencies for North American and European *Littorina littorea*. Frequencies have been sorted from highest to lowest occurrence and colored according to their status as unique to Europe (yellow), unique to North America (blue), shared between the populations (white), and North American haplotypes that are basal to European haplotypes so must be shared (purple). The majority of North American and shared haplotypes are found within the upper 50% of the frequencies while the majority of the European haplotypes are found within the lower 50%.

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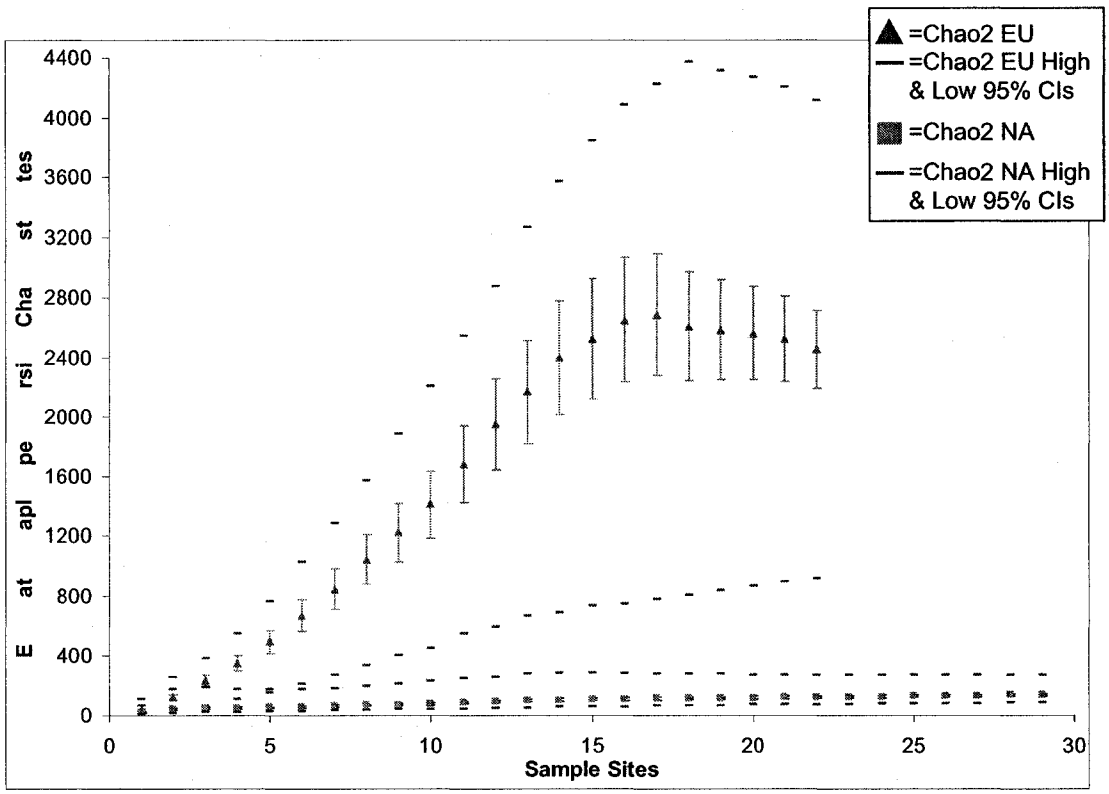

Figure II.4: Haplotype Estimation Curves for European versus North American *Littorina littorea*. The European Chao2 estimator curve suggests a maximum, expected number of haplotypes of ~2500 (with 95% confidence intervals between ~920 and ~4110 haplotypes), while the North American Chao2 estimator curve suggests a maximum, expected number of haplotypes of ~140 (with 95% confidence intervals between ~90 and ~270 haplotypes). These data graphically demonstrate the much greater genetic diversity in Europe compared to North America and additionally that the 23 unique haplotypes found in North America have a very high probability of being present in Europe, but due to Europe's massive diversity I did not detect them in my sampling.

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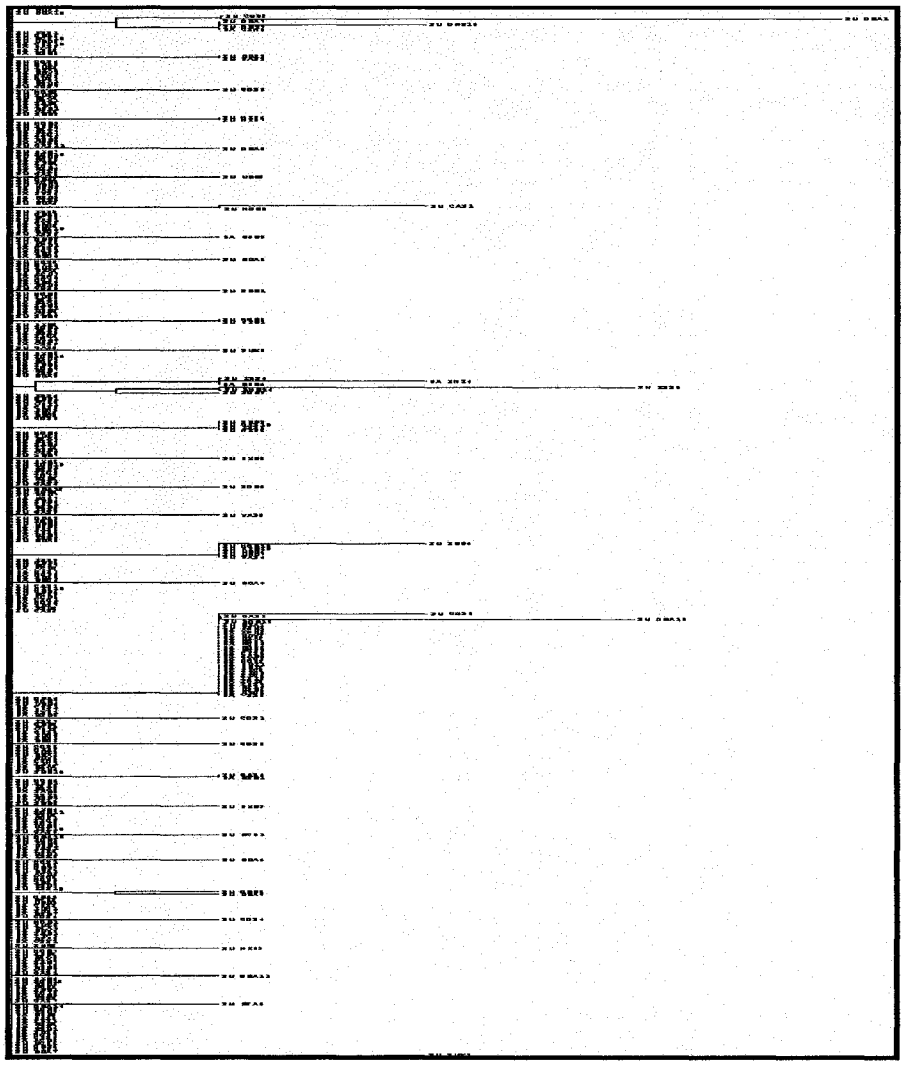

Figure II.5: Haplotype Tree for *Littorina littorea* mtDNA Excluding Third Position Sites (~798 bp). The following is a PAUP 4.0 produced phylogenetic tree showing the 45 total haplotypes. After the most variable regions were removed, the majority of individuals shared one haplotype. Altogether there were five shared haplotypes, 38 haplotypes unique to Europe and 2 haplotypes unique to North America.

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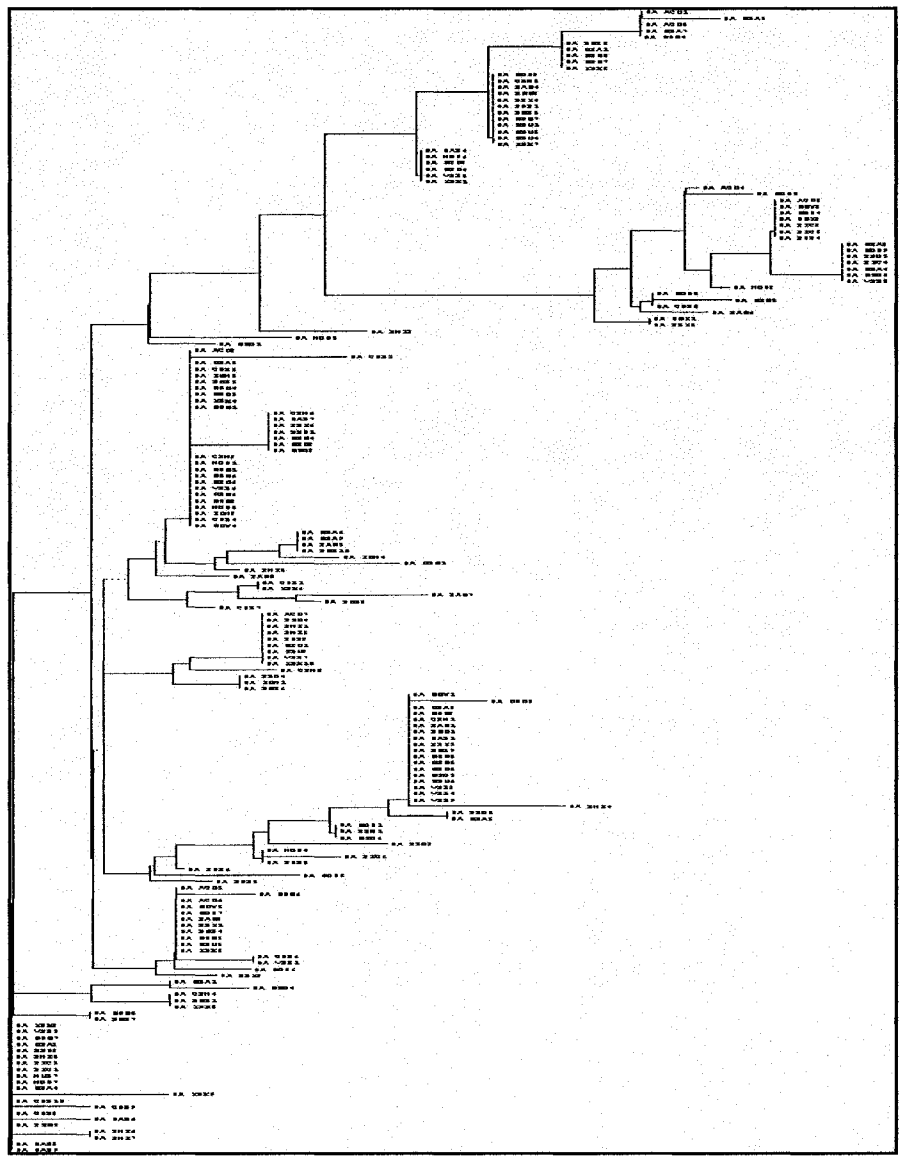

Figure II.6: Haplotype Tree for North American *Littorina littorea* mtDNA. The following is a PAUP 4.0 produced phylogenetic tree showing the North American population only. I found no significant differences between Canadian and US diversity.

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CHAPTER III

PARASITES AND INVASIONS: WHAT CAN PARASITE GENETICS TELL US ABOUT QUESTIONABLE INVASION HISTORIES?

ABSTRACT

Recently, parasites have been shown to add new and important evidence to the understanding of marine invasions, sometimes conveying more information than studies of the invading host itself. In particular, exploring the molecular genetics of invading host parasites has been shown to reveal strong genetic bottlenecks in the parasite (often stronger than in the host itself) and may also reveal the specific population from which the host originated. Such information can be especially helpful for cryptogenic species (species not demonstrably native or non-native), where current evidence regarding a species' status as native or non-native may be unclear or even unavailable. The invasion status of one highly abundant European marine snail, *Littorina littorea* (common periwinkle), has been debated for over 150 years and it is presently considered cryptogenic in northeast North America. To help resolve its cryptogenic status, I explored the molecular genetics of a prominent group of snail parasites, trematodes, to provide novel evidence. Using mitochondrial DNA sequencing, I found *L. littorea's* most common trematode parasite, *Cryptocotyle lingua*, to show a significant reduction in

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haplotype diversity in North America compared to Europe, consistent with signatures of a recent founder effect. This genetic evidence is also consistent with recent ecological evidence that has shown a significant reduction in trematode species richness in North American versus European *L. littorea* snails, suggestive of parasite release in North America. Altogether, this corroborative parasite evidence is suggestive of a recent introduction for *L. littorea* in North America.

INTRODUCTION

Recently, parasites have become recognized as important tools/indicators in the understanding of marine invasions. For example, a study by Criscione et al. (2006) found parasite genotypes to be more accurate at resolving host origins than the host genotypes themselves. Therefore, parasite genetics can be an important yet understudied tool in understanding the ecological histories of known invasions. However, there are numerous ecological histories that are not known; i.e., there exist many species in a region that cannot be demonstrably classified as native or non-native (termed "cryptogenic," Carlton 1996). These cryptogenic species may have profound effects on the communities in which they reside; yet it is unclear whether those effects are produced by a native community member or one that is non-indigenous. Because little can be (or will) be done regarding a species while it has this nebulous classification, it is important to resolve cryptogenic histories, especially for those species which exert pressure directly and/or indirectly on many different species within a community. Because cryptogenic status

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typically comes about as the result of equivocal, incomplete, or missing historical information regarding a species' presence in a region, novel information is often required to aid in the resolution of cryptogenic histories. As parasites have been shown to provide novel evidence in studies of known invasions (e.g., Criscione et al. 2006, Muira et al. 2006), they could also be useful in resolving cryptogenic histories.

One cryptogenic species whose history in northeast North America has been debated for over 150 years (e.g., Ganong 1886, Clarke and Erskine 1961, Berger 1977, Wares et al. 2002, Chapman et al. 2007a,b, Cunningham 2007, Wares and Blakeslee, in press), is the marine snail, *Littorina littorea* (common periwinkle). Evidence for and against a non-native origin for the snail in North America have been published using several different sources of data, including historical, archaeological, ecological and genetic (e.g., Ganong 1886, Clarke and Erskine 1961, Clarke 1963, Bird 1968, Berger 1977, Carlton 1982, Wares et al. 2002, Chapman et al. 2007a, Wares and Blakeslee, in press); however, *L. littorea* is presently considered cryptogenic in North America. Therefore, novel evidence, such as parasite analyses, may help in the definitive resolution of this snail's cryptogenic status.

I therefore explored the molecular genetics of an associated, host-specific trematode parasite, *Cryptocotyle lingua*. Trematodes have complex life cycles, in which they use a myriad of hosts to complete their life cycles (Figure I.1). Within their first-intermediate gastropod hosts, trematodes asexually reproduce, producing countless rediae and cercariae, which can be identified to species level (e.g., Lauckner 1980) and easily

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extracted for molecular studies. Thus, I focused on this stage in the life cycle for genetic analyses.

Cryptocotyle lingua is *L. littorea's* most dominant trematode parasite in both Europe and North America (Table I.1). Sindermann and Farrin (1962) suggested that *Cr. lingua* was introduced to North America with its host *Littorina littorea*, a hypothesis that has never been confirmed. Therefore, *Cr. lingua* is an ideal trematode species in which to explore genetic signatures of an introduction because this trematode species would be the most likely candidate for an associated introduction with the snail. Additionally, I included a second trematode species of *L. littorea* for comparison purposes—*Cercaria parvicaudata*. This species is typically *L. littorea's* second-most common trematode species (Table I.1), and its inclusion was meant for comparison with *Cr. lingua* such that parasite analyses were to some extent replicated.

In both trematode species, I looked for evident signatures of an introduction, which included significantly lower genetic diversity, patterns in haplotype frequencies where the most frequent haplotypes are shared between the regions, and nested genetic diversity within the source diversity; and in *Cr. lingua*, I also calculated divergence estimates between the source population, Europe, and the purported founding population, North America. Clear conformation to these genetic signatures would support a recent introduction for the trematode and additionally be suggestive of a recent introduction for the snail.

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MATERIALS AND METHODS

Trematode collections

In order to extract sufficient numbers of *Cryptocotyle lingua* trematodes for genetic analyses, I collected numerous *Littorina littorea* snails from sites throughout their North American and European ranges. Altogether, I were able to find and extract 182 total *Cr. lingua* individuals from 20 North American sites (n=96), which ranged from Red Bay, Labrador to Pt Judith, RI (Figure III.1). I collected snails southwards of this Point Judith site (including two sites in Connecticut, two on Long Island, and Cape May, NJ) but did not find *Cr. lingua* southwards of Point Judith, Rhode Island. In Europe, I collected snails from Moss, Norway to Vigo, Spain as well as several sites on the British Isles; altogether, I found 86 *Cr. lingua* individuals from 15 sites ranging from Moss, Norway to Mindin, France and a few sites in the British Isles (Figure III.1).

Because of its lower overall prevalence and occurrence in both North American and European *L. littorea* (Table I.1, I.2), *Cercaria parvicaudata* was much more difficult to collect; thus there are many fewer overall samples from fewer sites. Overall, I collected a total of 63 *Ce. parvicaudata* specimens: 37 were from 10 European sites ranging from Moss, Norway to Vigo, Spain and 26 were from 8 North American sites ranging from Halifax, Nova Scotia to Cape May, NJ.

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DNA extractions, PCR and sequencing

All trematode samples were stored in a -80°C freezer until they were ready for processing. DNA was extracted using a standard CTAB protocol (France et al. 1996), and DNA quality and quantity were determined using a spectrophotometer. Two sets of primers were used to amplify a 1043 bp section of the Cytochrome Oxidase I (COI) mitochondrial gene: COIa: *COI2575F*: TTTTTTGGGCATCCTGAGGTTTAT, and *COI3021R*: TAAAGAAAGAACATAATGAAAATG (Morgan and Blair 1998); COIb: *ABCOICLF*: TCTTTAGGATCATAAGCG, and *ABCOICLR*: TAAACCCCCGTATCCAAACC designed using COI sequence data from Kane et al. (2003). For *Ce. parvicaudata*, a 398 bp fraction of the COI mitochondrial gene was amplified using one set of primers: *COI2575F*: TTTTTTGGGCATCCTGAGGTTTAT, and *COI3021R*: TAAAGAAAGAACATAATGAAAATG (Morgan and Blair 1998). All trematode samples (*Cr. lingua* and *Ce. parvicaudata*) were amplified using a PCR protocol based on Huspeni (2000). For each reaction, 0.6 µl template DNA (~100 ng μl^{-1}) was added to a PCR mix (3 µl Taq buffer, 0.6 µl of 10 mM dNTPs, 1.5 µL of each 10 µM primer, and 0.18 µl Taq (5 U μl^{-1}) and molecular grade water for a final volume of 30 µl. Reaction mixes were subjected to 35 Cycles of 94 °C for 30 s, 50.9 °C for 30 s, and 72 °C for 30 s in an automated thermocycler. DNA was eluted from PCR products using a QIAquick Gel Extraction kit (Qiagen, Inc, Valenica, CA) and sequenced using ABI 377 DNA Automated Sequencers at the UNH Sequencing Facility. Sequences were analyzed using DNASTAR Programs (EditSeq, Seqman, and Megalign; DNASTAR, Inc.,

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Madison, WI). Because I was only able to amplify a 398 bp region for *Ce. parvicaudata*, analyses based on this trematode are for comparison purposes only.

Statistical analyses

Phylogenetic relationships using the full dataset were assembled with PAUP 4.0 (Swofford 2003). As a conservative estimate of haplotype diversity, I also constructed a phylogenetic tree with a truncated data set, where I excluded all third position sites (resulting in 695 total bp), which are typically the most variable sites in coding DNA since substitutions at these sites are mostly silent. In both the full and truncated data sets, the maximum likelihood root haplotype (designated by an asterisk) was determined using the program, TCS 1.21 (Clement et al. 2000).

Because haplotype diversity was high in my sampled populations, I used haplotype accumulation and haplotype estimation curves for *Cr. lingua* only to estimate haplotype diversity in each population and to quantify the effects of sampling effort on haplotype diversity. Specifically, I used ESTIMATES 8.0 (Colwell 2006) to calculate haplotype accumulation and haplotype estimation curves. ESTIMATES uses Monte Carlo re-sampling to determine the mean accumulation of haplotypes (S_{obs}) as samples are added over the full data set (Gotelli & Colwell 2001). However, sample-based rarefaction curves may not capture the entire haplotype diversity within a population for a particular sampling effort, especially if these curves have not reached a stable asymptote. Thus, non-parametric estimators, such as the well-used and robust estimator Chao2 (Walther & Morand 1998, Foggo et al. 2003), can be useful in predicting the eventual

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asymptote in haplotype diversity for a particular population (Gotelli & Colwell 2001), and do so by including the effects of rare haplotypes on the total haplotype diversity (Chao 2004, Witman et al. 2004).

Finally, population divergence estimates for *Cr. lingua* only were performed using the Isolation with Migration (IM) program (Version: July 2006; Hey and Nielsen 2006). The program provides maximum likelihood estimates of the time since two populations split (t) in terms of mutations, which can be converted to an estimate of the number of years since the populations diverged using substitution rates for the gene in question. I performed ten different runs/replicates of IM using the following input parameters and ten different random seeds: q1=1000, m1=m2=7, t=2, b=100,000, L=10.0. Divergence estimates were calculated using the following equation: $t = t/\mu$, where t = total years of divergence time, t = the time parameter determined by IM from the sequence data, and μ = the gene substitution rate (Hey and Nielsen 2004). Because trematodes do not preserve well in the fossil record, the best estimates for COI substitution rates in *Cr. lingua* is a range between 2-4% per MY (J. Morgan, pers. comm.): for 1043 bp, the rates used in calculating divergence estimates were 1.04×10^{-5} for 2% per MY, 1.56×10^{-5} for 3% per MY, and 2.09×10^{-5} for 4% per MY.

RESULTS

For *Cryptocotyle lingua*, a total of 182 sequences were analyzed (86 European and 92 North American) with an average ($\pm$ s.d.) of 5.20 ($\pm$ 2.90) sequences per site

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(APPENDIX E). Each sequence was 1043 bp in length when the two contiguous COI regions were combined. Altogether, I observed a total of 86 different haplotypes (Figure III.2). Thirty-four haplotypes were North American and 67 were European (when including haplotypes that were shared between the regions). Of the 34 North American haplotypes, 15 were shared between the North American and European populations, 16 were unique to North America, and 3 appeared unique but were basal to European haplotypes so must be shared with a European haplotype not detected in my sampling (Figure III.2; APPENDIX D). Thus, 53% of the North American haplotypes were shared and 47% were not shared. In addition, 47% (16/34) of the North American haplotypes were found more than once (i.e., common), while 53% were observed just once (i.e., rare). Of the 67 European haplotypes, 15 were shared (22%) between the regions and 52 (78%) were unique to Europe (Figure III.2). In addition, 87% (58/67) of the European haplotypes were rare and only 13% (9/67) were common. In all, North America exhibited a significant reduction in genetic diversity compared to Europe (χ^2=10.78, d.f.=1, $p<0.001$). Furthermore, Europe possessed significantly more rare haplotypes compared to North America (χ^2=21.05, d.f.=1, $p<0.001$). On the whole, no clades were completely monophyletic for North American individuals (i.e., all clades containing North American individuals also included European individuals) (Figure III.2). Furthermore, patterns observed in the order of haplotype frequencies from high to low also met expectations for a recent introduction—all the common haplotypes were either shared or in North America, while the majority of rare haplotypes were found in Europe. In fact, the top 35% of frequencies were all shared haplotypes (Figure III.3). Finally, the haplotype

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estimation (Chao2) curves calculated the expected, maximum number of haplotypes (mean of 500 replicate runs) in Europe to be 427 (95% CI=205; 1005) versus 74 haplotypes (95% CI=47; 162) in North America (Figure III.4); thus Europe is expected to possess 6 times more haplotypes in Europe compared to North America.

In the phylogenetic analysis of my truncated dataset (i.e., exclusion of 3rd position sites) for *Cr. lingua*, I found all but four haplotypes in North America included among the European diversity. Fourteen haplotypes were unique to Europe and 5 haplotypes were shared between the two regions (total haplotypes n=23; Figure III.5). In total, Europe possessed nearly 61% of the total diversity, and North American diversity was significantly lower than European diversity (χ^2=5.56, d.f.=1, p=0.018).

Finally, I used the Isolation with Migration (IM) program (Hey and Nielsen 2006) to calculate divergence estimates between the European and North American populations for *Cr. lingua*. Over my ten replicate runs I found the mean (± SE) divergence estimates to be: 479 (± 162) years with 95% confidence intervals between 192 (± 65) and 1582 (± 865) years ago for the 2%/MY mutation rate; 320 (± 108) years with 95% confidence intervals between 128 (± 43) and 1055 (± 577) years ago for the 3%/MY mutation rate; 240 (± 81) years with 95% confidence intervals between 96 (± 32) and 791 (± 432) years ago for the 4%/MY mutation rate (Table III.1).

The resulting phylogenetic data for *Ce. parvicaudata* was less clear than for Cr. lingua because *Ce. parvicaudata* had so little genetic structure overall and because my sample size was small (Figure III.6, APPENDIX E). Altogether, there were 12 haplotypes, one of which was shared and made up over 75% of the total haplotype

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frequencies (Figure III.7); 8 were unique to Europe and 3 were unique to North America. Thus, there were 4 total North American haplotypes and 9 European haplotypes. European haplotypes made up as much as 70% of the total diversity.

DISCUSSION

My results suggest a recent founding event for *Cryptocotyle lingua*, and is complimented by results of the second trematode, *Cercaria parvicaudata*, which also showed a decline in genetic diversity in North America compared to Europe. However, because I observed so little genetic structure for *Ce. parvicaudata* and because the sample size for this species was small, evidence related to this trematode species is simply corroborative, and henceforth, I will devote all future discussion to analyses involving *Cr. lingua*.

Evidence for a recent introduction of *Cr. lingua* to North America was supported by several genetic signatures. First, European diversity was significantly greater than North American diversity; e.g., European diversity made up to 78% of the total diversity. In my truncated dataset, there was a similar pattern: the majority of haplotype diversity was European (61%) and North American diversity was significantly lower than the European diversity. Second, patterns in haplotype frequencies were suggestive of a recent founding event, in that there were significantly more European haplotypes that were rare (=1 occurrence) than North America, where 47% of the haplotypes were common (>1 occurrence). If North America and Europe had existed independently of one another for a

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long period of time, it is probable that I would have witnessed many more rare haplotypes in North America, similar to what was observed in Europe. Third, mean divergence estimates between the European and North American populations calculated by IM were recent, ranging from approximately 240-480 years ago, which is well within the time frame for European settlement of North America from Europe. Altogether, these three pieces of evidence are suggestive of a recent founding event for *Cr. lingua* parasites.

However, one of my expectations for a recent founding event was not met—that all North American diversity would be a nested subset of the European diversity. I found 16 unique North American haplotypes, and these unshared haplotypes do not appear to conform to signatures associated with a recent introduction. Nonetheless, my haplotype estimation curves suggest that it is probable that these unshared haplotypes are not endemic to North America but instead represent a sampling issue, in that the diversity in Europe was so high that I missed the corresponding European haplotype to an unshared North American one. In Europe, the Chao2 haplotype estimator predicted 427 haplotypes compared to 67 that were actually observed, while for North America, the Chao2 predicted only 74 haplotypes compared to the 34 that were observed. Thus, it is highly likely that continued sampling in Europe will reveal those 16 unshared North American haplotypes among the approximately 350 haplotypes that are yet to be found in Europe. In fact, at the present proportion of European haplotypes to snails (0.77), the predicted value of 427 haplotypes would require sequencing of 556 individuals to capture 427 haplotypes at this current proportion of haplotypes to snails (which would be likely to change with more sampling). Thus, it is highly likely that those 16 unshared haplotypes

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are not endemic to North America but instead shared with yet-to-be-found European haplotypes.

On the whole, my results are suggestive of a recent introduction for *Cr. lingua* in North America. Additionally, because of the strong bottleneck I observed in North American *Cr. lingua*, my data argues against frequent, multiple introductions of the trematode. *Cr. lingua* has several different hosts in its life cycle (Figure I.1), many of which are highly mobile, including pelagic fish and several species of shorebird (Lauckner 1980). However, trans-Atlantic migrations of *Cr. lingua's* primary definitive host, *Larus* sp. gulls are believed to be rare (J. Ellis, T. Good, pers. comm.). It is also unclear whether the species of pelagic fish that *Cr. lingua* infects would be able to make such long journeys. Nonetheless, it remains possible that occasional trans-Atlantic crossings could occur for these host species in *Cr. lingua's* life cycle—yet my data appear to suggest that this is not a very strong impact on North American diversity since it remains substantially reduced even after one-hundred or more years since the first potential founding event.

Finally, my trematode genetic results are suggestive of a recent introduction for North American *L. littorea* because of the tight association between parasite and host. Although native congeners, *L. saxatilis* and *L. obtusata*, can also serve as first-intermediate hosts to *Cr. lingua*, these snails are believed to be secondary hosts for *Cr. lingua*; *L. littorea* is *Cr. lingua's* preferred host (Lauckner 1980). Thus, current infection in *L. saxatilis* and *L. obtusata* are likely recent events following *Cr. lingua's* introduction with *L. littorea*. Moreover, if *Cr. lingua* were actually native to North America, its

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genetic phylogeny should have shown strong divergence signatures between Europe and North America; e.g., distinct monophyletic clades, similar levels of diversity in the two regions, and high divergence time estimates. Instead, it showed strong associations between Europe and North America, suggestive of a recent introduction. Furthermore, this genetic evidence is corroborated by ecological evidence of trematode parasitism in *L. littorea* hosts (Chapter 1), where I found North American *L. littorea* to have significantly lower trematode species richness than European snails, supporting expectations for parasite release in the North American snail population. Altogether, my data not only finds that a dominant trematode in North American communities, *Cr. lingua*, which infects numerous native hosts in its life cycle, is an introduced species, it also corroborates ecological trematode data (as well as genetic data in the snail, described in the next chapter) that is suggestive of a recent introduction for *L. littorea* snails. Thus, I show here that parasite data can be instrumental in the resolution of cryptogenic histories, even when hundreds of years have passed since the initial founding event. Parasites are therefore useful tools in cryptogenic and invasion studies alike.

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Table III.1: IM Divergence Estimates for *Cryptocotyle lingua*. Because trematodes do not preserve in the fossil record, the best estimates for mutation rates of COI mitochondrial genes are between 2-4%/MY. Mean estimates (± SE for ten different runs) are listed for each mutation rate followed by low and high 95% confidence intervals. Mean divergence estimates for each mutation rate are within the time frame of European settlement of North America from Europe.

Mutation Rates	Divergence Estimates in Years (Average ± SE)		
	Mean 't'	95% CI Low 't'	95% CI Upper 't'
2% / MY	**479 (±162)**	192 (±65)	1582 (±865)
3% / MY	**320 (±108)**	128 (±43)	1055 (±577)
4% / MY	**240 (±81)**	96 (±32)	791 (±432)

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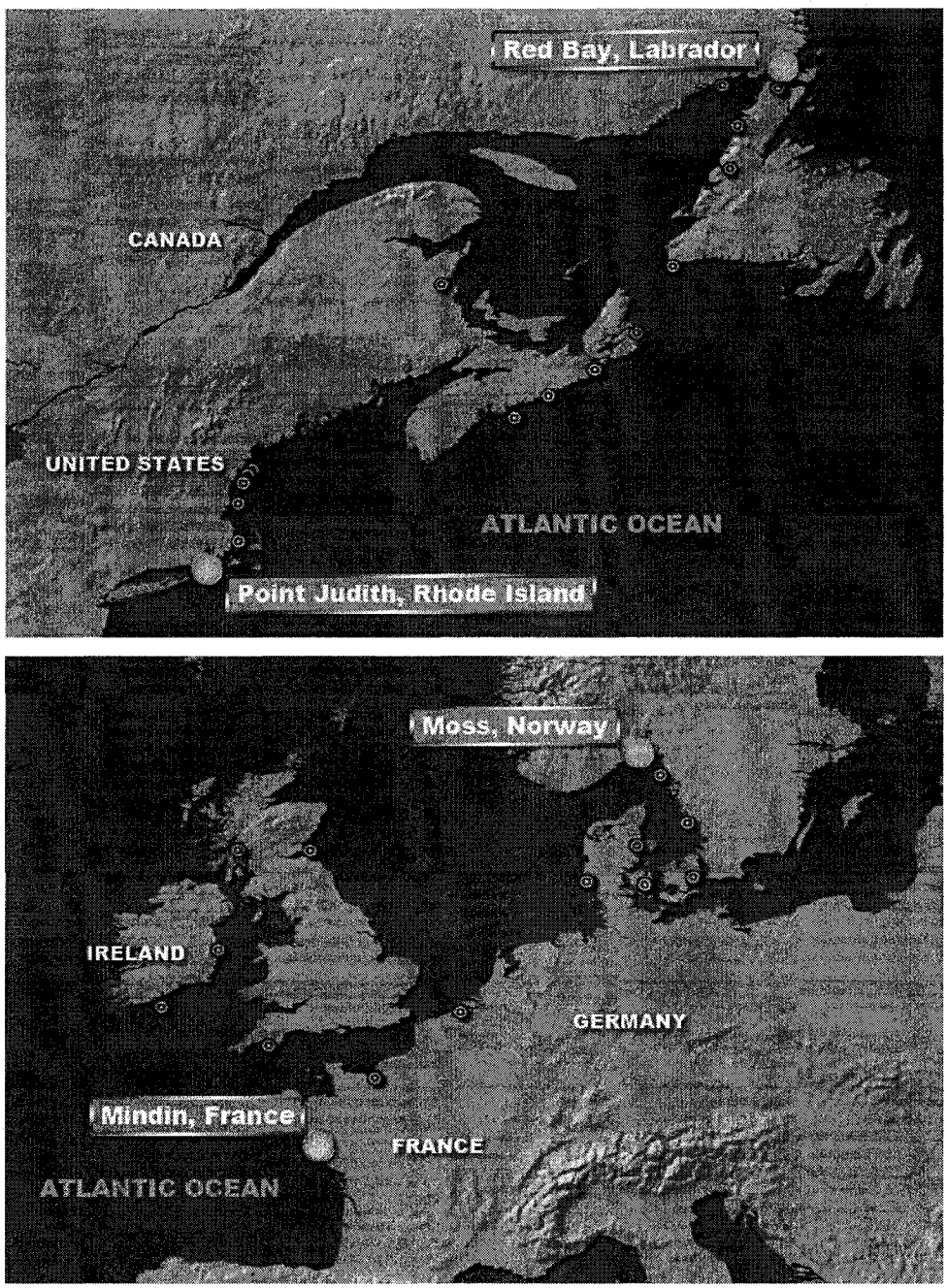

Figure III.1: North American and European Collection Sites for *Cryptocotyle lingua*. I collected from 20 North American sites, ranging from Red Bay, Labrador to Point Judith, RI. In Europe, I collected from 15 sites, ranging from Moss, Norway to Mindin, France.

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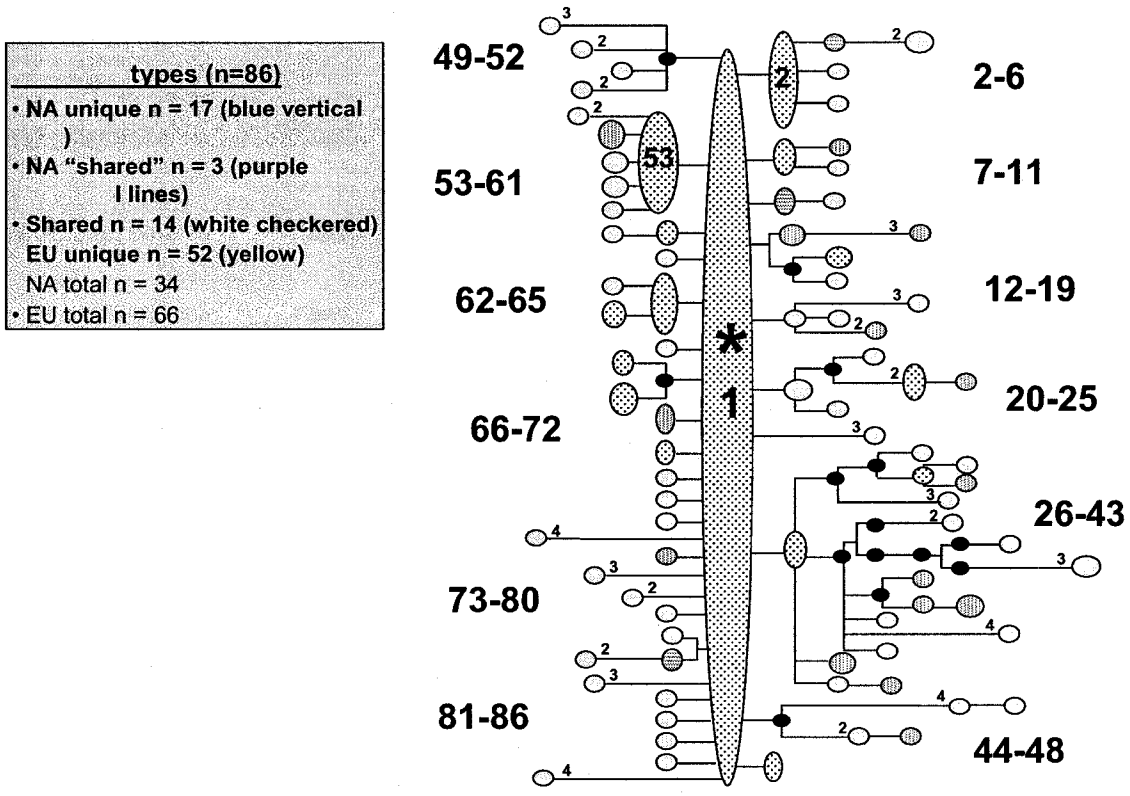

Figure III.2: Haplotype Tree for *Cryptocotyle lingua* mtDNA (1043 bp). The numbers on the sides of the tree represent the particular haplotype identities within clades/areas on the tree (see APPENDIX E). Haplotype bubbles are relatively sized based on haplotype frequencies (e.g., higher frequencies have larger-sized bubbles) and are colored according to the following categories: unique to Europe (yellow), unique to North America (blue vertical lines), and shared between populations (white checkered). A fourth category (purple horizontal lines) represents haplotypes that appeared unique to North America but which are basal to European haplotypes and are considered shared.

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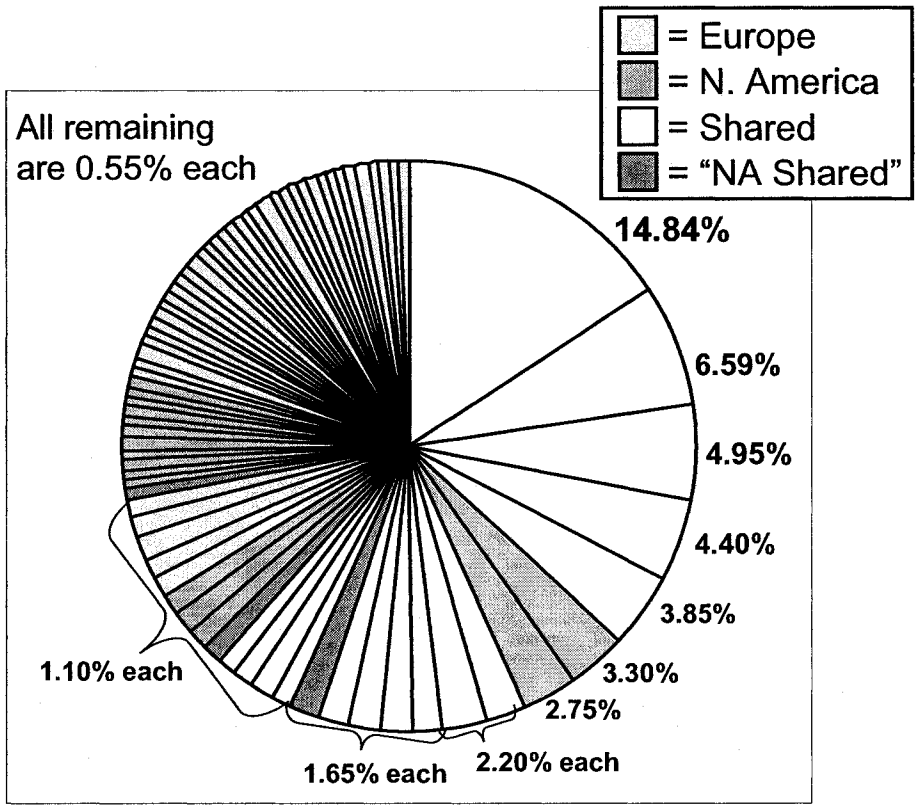

Figure III.3: Haplotype Frequencies for European and North American *Cryptocotyle lingua*. Frequencies have been sorted from highest to lowest occurrence and colored according to their status as unique to Europe (yellow), unique to North America (blue), shared between the populations (white), and North American haplotypes that are basal to European haplotypes so must be shared (purple). The majority of North American and shared haplotypes are found within the upper 50% of the frequencies while the majority of the European haplotypes are found within the lower 50%.

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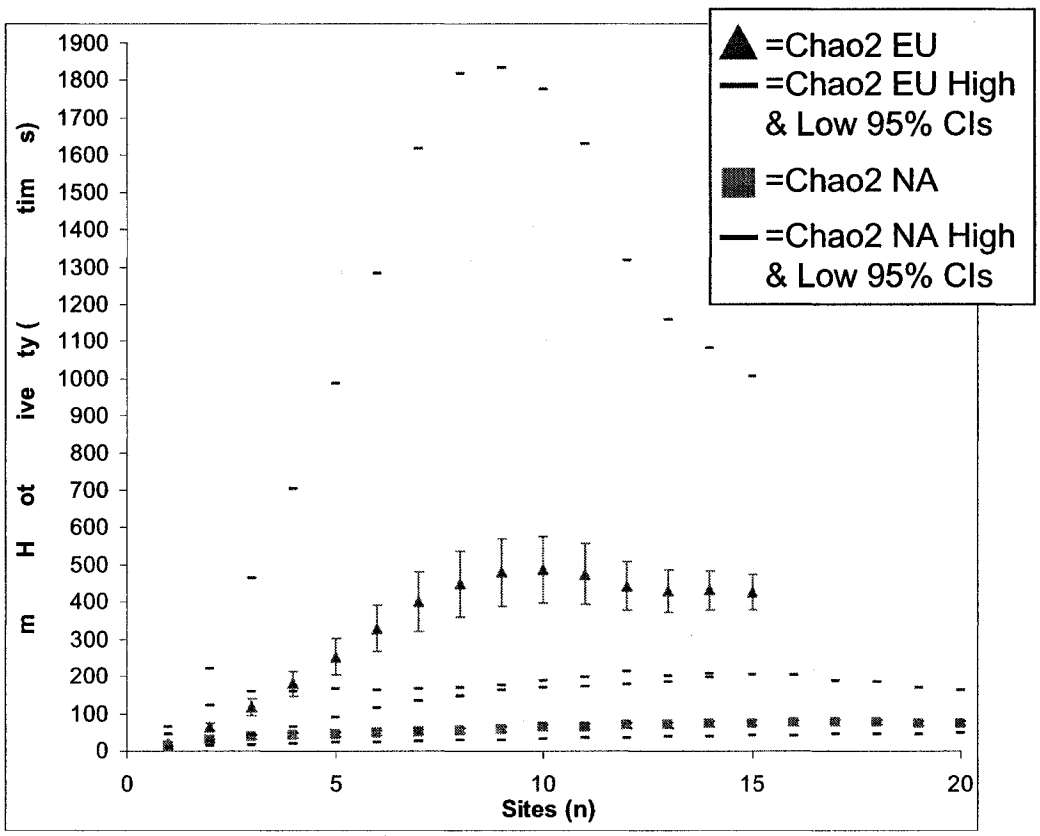

Figure III.4: Haplotype Estimation Curves for *Cryptocotyle lingua*. The European Chao2 estimator curve suggests a maximum, expected number of haplotypes of 427 (with 95% confidence intervals between 205 and 1005 haplotypes), while the North American Chao2 estimator curve suggests a maximum, expected number of haplotypes of 74 (with 95% confidence intervals between 46 and 162 haplotypes). These data graphically demonstrate the much greater genetic diversity in Europe compared to North America and additionally that the 17 unique haplotypes found in North America have a very high probability of being present in Europe, but due to Europe's massive diversity I did not detect them in my sampling.

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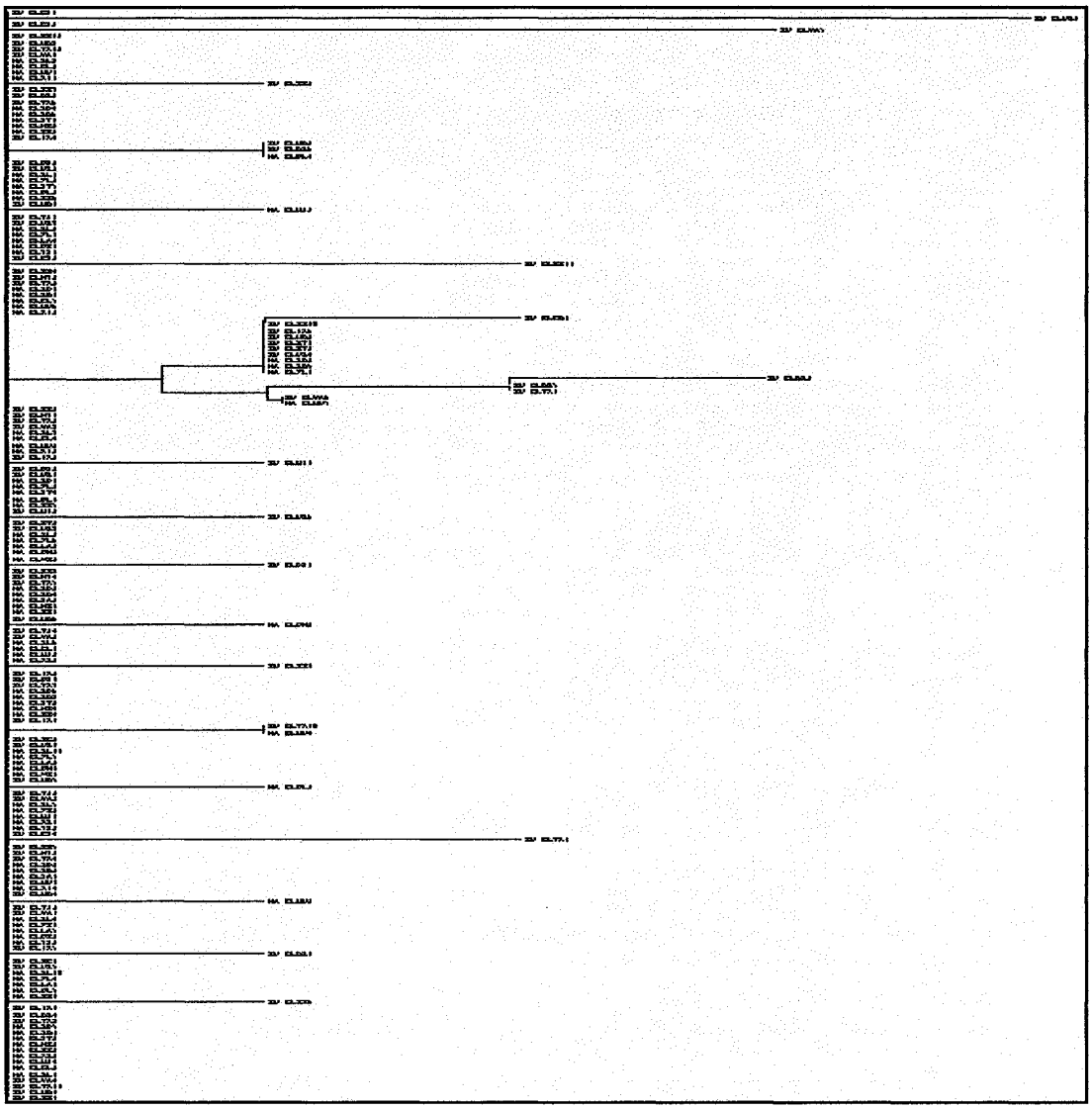

Figure III.5: Haplotype Tree for *Cryptocotyle lingua* mtDNA with Excluded Third Position Sites (695 bp). The following is a PAUP 4.0 produced phylogenetic tree showing the 23 total haplotypes and the individuals which share them. After the most variable regions were removed, the majority of individuals shared one haplotype. Altogether there were 5 shared haplotypes, 14 unique to Europe and 4 unique to North America.

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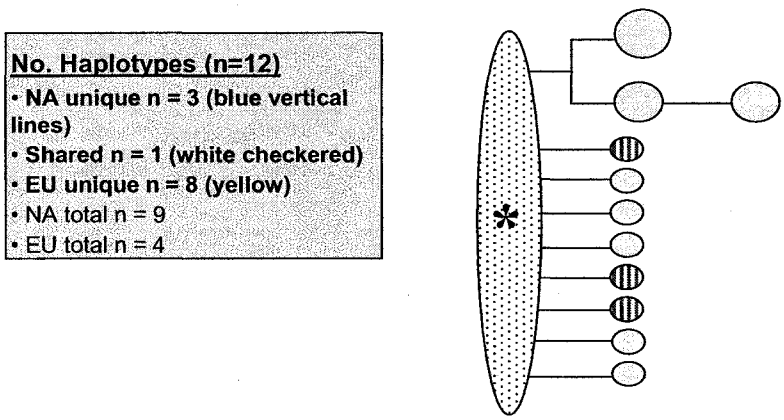

Figure III.6: Haplotype Tree for *Cercaria parvicaudata* mtDNA (398 bp). Haplotype bubbles are relatively sized based on haplotype frequencies (e.g., higher frequencies have larger-sized bubbles) and are colored according to the following categories: unique to Europe (yellow), unique to North America (blue vertical lines), and shared between populations (white checkered). See APPENDIX F for information on haplotype identities, such as total numbers within sample sites, frequencies, etc.

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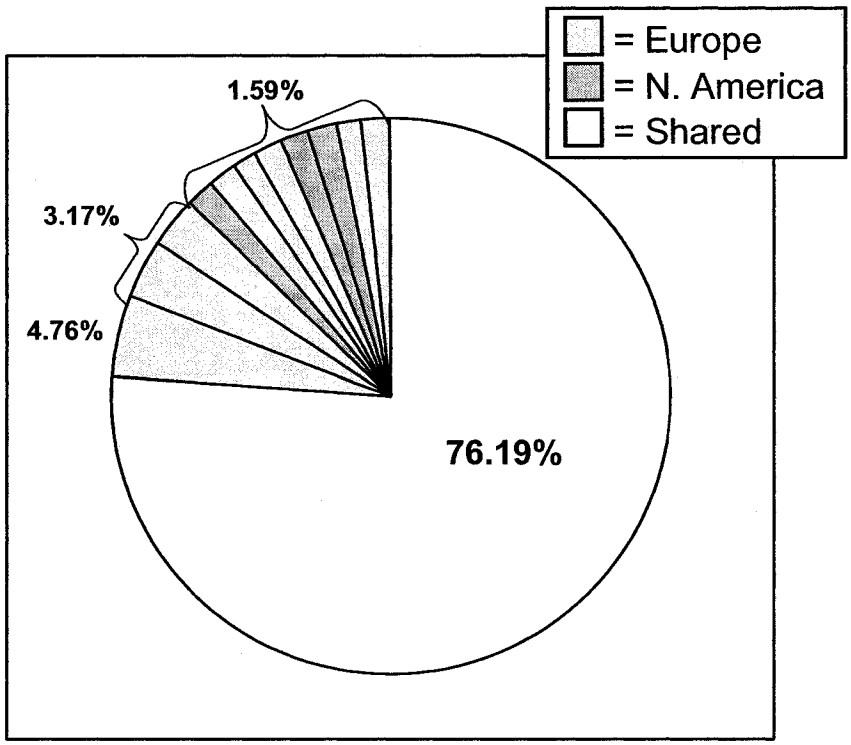

Figure III.7: Haplotype Frequencies for *Cercaria parvicaudata*. Frequencies have been sorted from highest to lowest occurrence and colored according to their status as unique to Europe (yellow), unique to North America (blue), and shared between the populations (white). The majority of individuals shared one haplotype, which made up over 75% of the total haplotype frequencies.

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CONCLUSIONS AND SIGNIFICANCE

On the whole, my corroborative and novel evidence—lower trematode species richness in North American *Littorina littorea*; founder effect signatures in *L. littorea*; and founder effect signatures in an associated trematode parasite—when taken in concert with past historical and ecological evidence, are all suggestive of a recent introduction of *L. littorea* to North America. Moreover, this introduction is likely human-mediated for several reasons: first, *L. littorea* is absent from North Atlantic islands like Iceland and Greenland, which are believed to have served as stepping-stones for several intertidal species, such as *L. saxatilis* and *L. obtusata* (Johanesson 1988, Ingolfsson 1992); thus, a natural crossing for *L. littorea* is unlikely, enhancing the probability that it was carried over by humans; second, *L. littorea* has been associated with several potential human mechanisms of introduction, including ballast rock transport and as a human food source (Reid 1996, Steneck and Carlton 2001); third, *L. littorea's* rapid, sequential southwards movement is a typical observation for a recently introduced and rapidly expanding non-native species (Steneck and Carlton 2001). Overall, these various lines of evidence make a human-mediated introduction the most likely explanation for *L. littorea's* recent introduction to North America.

This study is the first to incorporate corroborative ecological and genetic evidence of parasites in resolving the cryptogenic status of an integral marine species. The resolution of this 150+ year debate is significant for many reasons, including its utility in the understanding of model invasions (e.g., Steneck and Carlton 2001); in understanding

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the impacts of non-indigenous species on natives; and in providing new tools for the resolution of cryptogenic histories. In addition, this work demonstrates that species invasions are not always singular in nature but can result in the introduction of associated organisms, such as parasites, like *Cryptocotyle lingua*. Thus, the understanding of cryptogenic histories is important not only at the species level but may also reveal community-wide impacts. Further work resolving cryptogenic histories is therefore necessary for complete understanding of interactions among species (including species with questionable invasion histories) in communities around the world.

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APPENDICES

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APPENDIX A LITERATURE REVIEW FOR *LITTORINA* SP. DOCUMENTING SAMPLE SIZES AND TREMATODE SPECIES RICHNESS BY SITE

Literature review for *Littorina littorea* (LL), *L. saxatilis* (LS) and *L. obtusata* (LO). For North America, sites are listed north to south. For Europe, sites are listed NW to SE (with the exceptions of Iceland sites, which are placed before Scandinavian sites, and United Kingdom sites, which are binned by country and then listed north to south). Following this are the number of snails sampled for each study, the number of infected snails for each study, the total species richness in the study, and the adjusted species richness (average $\pm$ standard deviation) per site. Adjusted species richness is based upon Monte Carlo resampling at a standardized snail number of 75 individuals (sites with less than 75 individuals are represented by a "*" symbol). N.d. indicates "no data". Citations are presented below.

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Study Site(s)	Snail Species	Total Snails Sampled (LL/LS/LO)	Total Infected Snails (LL/LS/LO)	Total Sp. Richness (LL/LS/LO)	Adj. Sp. Richness (avg±stdev) (LL/LS/LO)	Citation
North America Sites						
Newfoundland, Canada	LS	780	184	2	1.17 (±0.84) / 1.97 (±1.26)	Threlfall and Goudie 1977
Eastport and Roque Bluffs, ME, US	LL, LS, LO	2040 / 1145 / 1645	96 / 482 / 304	2 / 6 / 7	1.17 (±0.84) / 4.10 (±1.38) / 4.00 (±1.31)	Pohley 1976
Roque Bluffs, ME, US	LL	651	1	1	0.12 (±0.10)	Pohley & Brown 1975
Schoodic Peninsula, ME, US	LL	109	13	1	1.17 (±0.84)	Gorman & Moring 1982
Isles of Shoals, ME/NH, US	LL	817	75	1	1.00 (±0.89)	Hoff 1941
Nahant, MA and Wickford, RI, US	LL	84	12	1	1.00 (±0.89)	Pechenik et al. 2001
Woods Hole, MA, US	LS, LO	n.d. / n.d.	n.d. / n.d.	2 / 3	n.d. / n.d.	Stunkard 1970
Woods Hole, MA, US	LL, LS, LO	n.d./ n.d. /n.d.	n.d./ n.d. /n.d.	2 / 4 / 4	n.d./ n.d. /n.d.	Stunkard 1983
Woods Hole, MA, US	LL	632	64	3	1.00 (±0.89)	Willey & Gross 1957
Saunderstown, RI, US	LL	n.d.	n.d.	1	n.d.	Muller et al. 1999
Long Island Sound, CT	LL	2114	409	2	1.77 (±0.98)	Zavras & James 1979
Europe Sites						
White Sea, Russia	LS	n.d.	n.d.	1	n.d.	Arakelova et al. 2004
Kandalaksha Bay, White Sea, Russia	LS, LO	n.d. / n.d.	n.d. / n.d.	1 / 1	n.d. / n.d.	Gorbushin & Levakin 1999
White Sea, Russia	LS, LO	2658 / 4032	449 / 713	4 / 5	3.23 (±1.44) / 3.07 (±1.35)	Granovitch et al. 2000
Kandalaksha Bay, White Sea, Russia	LS	n.d.	n.d.	3	n.d.	Kaliberdina & Granovich 2003
White Sea, Russia	LO	n.d.	n.d.	5	n.d.	Sergievsky 1985
White Sea, Russia	LS, LO	n.d. / n.d.	n.d. / n.d.	4 / 4	n.d. / n.d.	Segievsky et al. 1997
White Sea, Russia	LL	n.d.	n.d.	3	n.d.	Tschubrik 1966
White Sea, Russia	LL	n.d.	n.d.	1	n.d.	Zelikman 1951
Kandalaksha Bay, White Sea, Russia	LL	n.d.	n.d.	4	n.d.	Zelikman 1966
North Iceland	LS, LO	43 / 110	17 / 29	3 / 1	* / 1.00 (±0.97)	Sannia & James 1977
SW Iceland	LS, LO	899 / 622	149 / 118	8 / 10	4.71 (±1.57) / 6.12 (±1.66)	Galaktionov & Skimisson 2000
SW Iceland	LO	622	78	9	4.48 (±1.46)	Skimisson & Galaktionov 2002
Barents Sea Coast, Norway	LS, LO	6307 / 5475	1543 / 821	4 / 3	2.79 (±1.45) / 1.86 (±1.24)	Bustnes & Galaktionov 1999
Tromso, Norway	LS, LO	n.d. / 685 / 360	n.d. / 139 / 41	1 / 4 / 4	n.d. / 3.58 (±1.59) / 2.09 (±1.08)	Galaktionov & Bustnes 1995
Swedish west coast	LL, LS, LO	53 / 1682 / 261	19 / 106 / 31	5 / 8 / 3	* / 2.60 (±1.07) / 2.62 (±1.37)	Granovitch & Johannesson 2000
Northern Denmark coast	LL	n.d.	n.d.	3	n.d.	Mouritsen et al. 1999
Kattegat, Baltic Sea	LL	1382	158	5	2.30 (±1.21)	Lauckner 1984a
List Tidal Basin, Wadden Sea, Germany/Denmark	LL	1090	155	6	3.56 (±1.40)	Thieltges et al. 2006
German & Danish coasts, Baltic Sea; German Bay, North Sea	LL	46,569	14,221	6	3.50 (±1.35)	Lauckner 1980
German Bay	LL	n.d.	n.d.	6	n.d.	Werding 1969
Konigshafen, German North Sea	LL	n.d.	n.d.	6	n.d.	Lauckner 1984b
Shetland Islands, Scotland, UK	LL, LS, LO	470 / 287 / 262	80 / 151 / 76	4 / 8 / 6	2.28 (±1.16) / 5.29 (±1.50) / 3.24 (±1.29)	Williams & Ellis 1976
Ythan Estuary, Aberdeen, Scotland, UK	LL	800	135	5	2.35 (±1.26)	Huxham et al. 1993
Muck Island, Scotland, UK	LS	160	56	1	0.47 (±0.45)	McCarthy et al. 2000
Edinburgh, Scotland, UK	LL	2000	88	3	1.87 (±0.99)	Huxham et al. 2001
Millport, Scotland, UK	LL, LS, LO	190 / n.d. / n.d.	1 / n.d. / n.d.	3 / 1 / 1	0.39 (±0.38) / n.d. / n.d.	Lebour 1911
Millport, Scotland, UK	LS	n.d.	n.d.	3	n.d.	Lebour 1914
Northumberland coast, England, UK	LL	n.d.	n.d.	1	n.d.	Lebour 1906
Northumberland coast, England, UK	LS, LO	n.d. / n.d.	n.d. / n.d.	1 / 1	n.d. / n.d.	Lebour 1907
British coast, UK	LS	n.d.	1	7	3.23 (±1.44)	James 1969
Yorkshire, England, UK	LL	5878	2200	4	3.21 (±1.45)	Robson & Williams 1970
Yorkshire, England, UK	LL	637	367	4	3.17 (±1.43)	Robson & Williams 1971a
Yorkshire, England, UK	LL	n.d.	n.d.	4	n.d.	Robson & Williams 1971b
Yorkshire, England, UK	LL	n.d.	n.d.	2	n.d.	Williams & Ellis 1975
Whitstable, England, UK	LS	n.d.	n.d.	1	n.d.	Berry 1961
Kent, England, UK	LS	2571	184	1	1.00 (±0.96)	Berry 1962
Southampton, England, UK	LL	800	301	3	3.00 (±1.48)	Watts 1971
Isles of Scilly, England, UK	LS	403	127	6	5.03 (±1.68)	Newell 1986
Coastal regions, Wales, UK	LL	n.d.	n.d.	6	n.d.	Rees 1935
Anglesey, Wales, UK	LS	226	42	1	1.00 (±0.97)	Elner and Raffaelli 1980
Bangor, Wales, UK	LL	1255	492	4	3.12 (±1.46)	Hughes & Answer 1982
West Wales, UK	LO	200	31	6	3.41 (±1.36)	Williams & Brailsford 1990
Cardigan Bay, Wales, UK	LL, LS, LO	6165 / n.d. / 3094	297 / n.d. / 423	6 / 1 / 3	2.48 (±0.88) / n.d. / 1.07 (±0.97)	James 1968b
Aberyswyth, Wales, UK	LS, LO	4009 / 523	1093 / 383	2 / 2	1.00 (±0.97) / 1.00 (±0.97)	James 1965
Aberystwyth, Wales, UK	LS	n.d.	n.d.	n.d.	n.d.	Popiel 1976
Aberystwyth, Wales, UK	LL, LS, LO	770 / n.d. / n.d.	32 / n.d. / n.d.	2 / 6 / 1	1.06 (±0.91) / n.d. / n.d.	Rees 1936a
Aberystwyth, Wales, UK	LL	2000	2	1	0.07 (±0.07)	Rees 1936b
Aberystwyth, Wales, UK	LL	n.d.	n.d.	2	n.d.	Thomas 1974
Pembrokeshire, Wales, UK	LL, LS, LO	n.d. / n.d. / n.d.	n.d. / n.d. / n.d.	3 / 9 / 6	n.d. / n.d. / n.d.	James 1968a
Isle of Man	LO	200	39	7	5.59 (±1.62)	Williams & Brailsford 1998
North Irish coast	LS	n.d.	n.d.	3	n.d.	McCarthy et al. 2002
Strangford Lough, Ireland	LL	n.d.	n.d.	3	n.d.	Moore & Halton 1977
Portavogie, Ireland	LL	n.d.	n.d.	3	n.d.	Pan et al. 1994
Belfast Lough, Ireland	LS	350	203	4	3.37 (±1.55)	Irwin 1983
Belfast, Ireland	LL, LS, LO	401 / 106 / 106	239 / 54 / 28	2 / 4 / 1	1.92 (±1.23) / 3.41 (±1.52) / 1.00 (±0.97)	Matthews et al. 1985
Roscoff, France	LS	1760	714	8	2.79 (±1.25)	Combescot-Lang 1976
Roscoff, France	LL, LS, LO	n.d. / n.d. / n.d.	n.d. / n.d. / n.d.	3 / 2 / 1	n.d. / n.d. / n.d.	Stunkard 1931
French coast	LS	n.d.	n.d.	1	n.d.	Richard 1976
Bassin d'Arcachon, France	LL	n.d.	n.d.	2	n.d.	Lespes 1857

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APPENDIX B FIELD COLLECTION SITES IN NORTH AMERICA AND EUROPE FOR *LITTORINA SP.*

Site collections for *Littorina littorea* (LL), *Littorina saxatilis* (LS), and *Littorina obtusata* (LO). Sites are listed north to south using GPS Latitude coordinates. Following this are the number of snails sampled at each site, the number of infected snails at each site, the total species richness at a site, and the adjusted species richness (average + standard deviation) per site. Adjusted species richness is based upon Monte Carlo resampling at a standardized snail number of 75 individuals (sites with less than 75 individuals are represented by a "*" symbol).

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Field Site	GPS Coordinates	Snail	Snails Sampled (LL/LS/LO)	Infected Snails (LL/LS/LO)	Total Sp. Richness (LL/LS/LO)	Adj. Sp. Richness (avg ± stdev) (LL/LS/LO)
North America Sites						
Red Bay, Labrador, NL, CAN	51°43.788 N 56°25.537 W	LL	95	3	1	0.99 (±0.89)
Blanc Sablon, QC, CAN	51°24.755 N 57°9.580 W	LL	88	29	2	1.85 (±1.02)
Flower's Cove, NL, CAN	51°18.02 N 56°43.48 W	LS, LO	104 / 107	19 / 6	4 / 3	3.63 (±1.48) / 2.39 (±1.24)
Port Saunders, NL, CAN	50°38.39 N 57°15.52 W	LS, LO	95 / 118	3 / 7	1 / 2	1.78 (±1.15) / 1.94 (±1.25)
Bonne Bay, NL, CAN	49°31.078 N 57°52.501 W	LL, LS, LO	105 / 108 / 227	2 / 2 / 5	2 / 3 / 3	1.43 (±0.82) / 2.69 (±1.35) / 2.87 (±1.22)
Searston, NL, CAN	47°50.089 N 59°20.187 W	LL	107	14	1	1.00 (±0.89)
Portugal Cove, NL, CAN	47°35.557 N 52°53.138 W	LL, LO	79 / 108	0 / 5	0 / 2	0 / 1.89 (±1.11)
Bay du Vin, NB, CAN	47°3.377 N 65°8.206 W	LL	63	7	1	0.65 (±0.58)
North Rustico, PEI, CAN	46°27.14 N 63°17.34 W	LS	95	0	0	0
St. Peter's Harbor, PEI, CAN	46°25.582 N 62°40.539 W	LL	106	2	1	0.92 (±0.82)
North Sydney, NS, CAN	46°12.424 N 60°14.912 W	LL	109	9	2	1.90 (±1.05)
Waterside, PEI, CAN	46°11.418 N 62°57.405 W	LL	112	2	1	0.89 (±0.80)
Rice Point, PEI, CAN	46°10.54 N 63°07.57 W	LO	101	1	1	0.74 (±0.71)
Caribou, NS, CAN	45°44.777 N 62°43.407 W	LL, LO	126 / 78	1 / 1	1 / 1	0.97 (±0.87) / 0.96 (±0.58)
Venus Cove, Mulgrave, NS, CAN	45°36.888 N 61°23.541 W	LL	111	20	2	1.68 (±0.95)
Cobequid Bay, NS, CAN	45°19.322 N 63°29.917 W	LL	77	1	1	0.65 (±0.58)
Black River, NB, CAN	45°15.407 N 65°45.683 W	LL, LO	110 / 107	0 / 1	0	0 / 0.70 (±0.67)
Back Bay, NB, CAN	45°02.377 N 66°53.423 W	LS	110	6	4	3.23 (±1.37)
Marie Joseph Park, NS, CAN	44°54.33 N 62°20.17 W	LS, LO	93 / 103	9 / 7	3 / 3	2.81 (±1.39) / 2.69 (±1.36)
Eastport, ME, U.S.	44°54.145 N 66°59.074 W	LL	111	1	1	0.68 (±0.60)
Lubec, ME, U.S.	44°50.07 N 67°02.47 W	LS, LO	109 / 106	0 / 2	0 / 2	0 / 1.42 (±0.92)
Halifax, NS, CAN	44°37.479 N 63°33.850 W	LL	131	64	3	2.81 (±1.03)
Peggy's Cove, NS, CAN	44°28.05 N 63°32.28 W	LS, LO	116 / 101	1 / 5	1 / 3	0.65 (±0.62) / 2.47 (±1.27)
Prospect Harbor, ME, U.S.	44°24.26 N 63°01.29 W	LO	99	6	2	1.76 (±1.15)
Camden Hills, ME, U.S.	44°12.35 N 69°03.22 W	LL, LS, LO	115 / 127 / 125	1 / 13 / 0	1 / 3 / 0	0.65 (±0.58) / 2.56 (±1.30) / 0
Vinyl Haven, ME, U.S.	44°03.18 N 68°52.28 W	LO	69	1	1 / 2 / 1	*
Boothbay Harbor, ME, U.S.	43°50.55 N 69°37.55 W	LL, LS, LO	76 / 56 / 87	7 / 5 / 0	2 / 2 / 0	2.00 (±1.10) / * / 0
Prouts Neck, ME, U.S.	43°31.652 N 70°19.234 W	LL	185	9	1	0.99 (±0.89)
Kennebunk, ME, U.S.	43°20.23 N 70°31.34 W	LS	124	11	4	3.38 (±1.39)
Wells, ME, U.S.	43°20.087 N 70°32.554 W	LL	265	38	2	1.81 (±1.00)
York Cliffs, ME, U.S.	43°12.255 N 70°35.407 W	LL	164	5	1	0.98 (±0.87)
York, ME, U.S.	43°08.953 N 70°37.396 W	LL, LS, LO	425 / 335 / 190	20 / 65 / 33	3 / 8 / 5	1.94 (±0.83) / 5.12 (±1.40) / 3.69 (±1.20)
Scammel Bridge, Durham, NH, U.S.	43°07.771 N 70°51.117 W	LL	163	7	3	1.88 (±0.87)
Hilton Park, Durham, NH, U.S.	43°07.205 N 70°49.626 W	LL	157	3	1	0.86 (±0.77)
Adams Point, NH, U.S.	43°05.383 N 70°51.944 W	LL	151	1	1	0.50 (±0.44)
Kittery, ME, U.S.	43°05.028 N 70°42.700 W	LL	169	10	1	1.00 (±0.89)
Fort Stark, Newcastle, NH, U.S.	43°03.553 N 70°42.769 W	LL, LO	167 / 56	5 / 2	2 / 1	1.36 (±0.81) / *
Odiorne Point, Rye, NH, U.S.	43°02.586 N 70°42.654 W	LL, LS, LO	317 / 145 / 198	42 / 38 / 16	2 / 4 / 4	1.42 (±0.88) / 3.03 (±1.33) / 2.98 (±1.16)
Duck Island, Isles of Shoals, U.S.	43°00.376 N 70°36.159 W	LL	203	59	3	2.31 (±0.98)
Rye, NH, U.S.	43°00.215 N 70°44.986 W	LL, LS, LO	169 / 121 / 125	10 / 6 / 8	2 / 3 / 4	1.68 (±0.95) / 2.22 (±1.15) / 2.79 (±1.24)
Larus Ledge, Appledore Island, Isles of Shoals, U.S.	42°59.469 N 70°37.089 W	LL, LS, LO	190 / 110 / 98	90 / 13 / 12	2 / 5 / 7	1.63 (±0.93) / 4.85 (±1.50) / 5.95 (±1.71)
Broad Cove, Appledore Island, Isles of Shoals, U.S.	42°59.377 N 70°36.751 W	LL	194	14	2	1.39 (±0.87)
Transect 5, Appledore Island, Isles of Shoals, U.S.	42°59.345 N 70°37.056 W	LL	250	60	2	1.30 (±0.87)
Ledges, Appledore Island, Isles of Shoals, U.S.	42°59.092 N 70°36.785 W	LL	279	81	5	2.08 (±0.95)
Smuttynose Island, Isles of Shoals, U.S.	42°58.851 N 70°36.462 W	LL	205	63	1	1.00 (±0.89)
Star Island, Isles of Shoals, U.S.	42°58.703 N 70°36.792 W	LL	214	38	2	1.58 (±0.92)
White Island, Isles of Shoals, U.S.	42°58.123 N 70°37.618 W	LL	198	21	1	1.00 (±0.89)
Seabrook, NH, U.S.	42°55.320 N 70°49.395 W	LL	164	2	2	0.91 (±0.53)
Newbury, MA, U.S.	42°47.45 N 70°49.06 W	LL	167	1	1	0.45 (±0.40)
Gloucester, MA, U.S.	42°36.491 N 70°40.582 W	LL, LO	281 / 147	32 / 40	2 / 5	1.71 (±0.96) / 4.16 (±1.50)
Plymouth, MA, U.S.	41°57.848 N 70°40.123 W	LL, LO	276 / 107	45 / 3	2 / 1	1.89 (±0.86) / 0.98 (±0.93)
Monument Beach, Buzzard's Bay, MA, U.S.	41°42.202 N 70°37.022 W	LL	186	4	2	1.29 (±0.76)
Vineyard Haven Bridge, Martha's Vineyard, MA, U.S.	41°27.520 N 70°35.164 W	LL	148	8	2	1.75 (±0.97)
Sengekontacket Pond, Martha's Vineyard, MA, U.S.	41°25.937 N 70°33.430 W	LL, LS	285 / 83	12 / 2	3 / 1	1.86 (±0.82) / 0.99 (±0.95)
Felix Neck, Edgartown, Martha's Vineyard, MA, U.S.	41°24.13 N 70°32.48 W	LS	68	1	1	*
Point Judith, RI, U.S.	41°21.767 N 71°28.828 W	LL	178	13	2	1.94 (±1.07)
Stonington, CT, U.S.	41°19.663 N 71°54.368 W	LL, LS, LO	216 / 128 / 101	27 / 6 / 1	2 / 3 / 1	0.69 (±0.40) / 2.14 (±1.12) / 0.74 (±0.71)
Weekapaug Point, RI, U.S.	41°19.30 N 71°45.08 W	LS, LO	121 / 124	4 / 2	3 / 2	2.10 (±1.08) / 1.19 (±0.78)
Rocky Neck, CT, U.S.	41°17.878 N 72°14.757 W	LL	101	0	0	0
Montauk Point, Long Island, NY, U.S.	41°04.309 N 71°51.501 W	LL, LO	217 / 193	18 / 41	3 / 5	2.16 (±0.92) / 3.75 (±1.47)
Crane's Neck, Long Island, NY, U.S.	40°57.952 N 73°09.051 W	LL	111	2	2	1.35 (±0.78)
Cape May, NJ, U.S.	38°57.349 N 74°52.568 W	LL	114	9	2	1.98 (±1.09)
Europe Sites						
Moss, Norway	59°25.861 N 10°39.148 E	LL	133	37	5	4.37 (±1.52)
Tjamo, Sweden	58°53.107 N 11°07.117 E	LL	213	20	6	4.09 (±1.24)
Varberg, Sweden	57°06.466 N 12°14.409 E	LL	108	17	2	1.99 (±1.27)
Udbyhoj, Denmark	56°36.565 N 10°17.986 E	LL	114	30	5	4.54 (±1.55)
Oban, Scotland, UK	56°24.41 N 05°28.38 W	LL	221	16	6	3.77 (±1.13)
St. Andrews, Scotland, UK	56°22.10 N 02°48.21 W	LL	100	7	2	1.98 (±1.27)
Esbjerg, Denmark	55°28.859 N 08°24.625 E	LL	109	22	4	2.66 (±1.33)
Copenhagen, Denmark	55°41.34 N 12°35.57 E	LL	75	1	1	0.96 (±0.92)
Nyborg, Denmark	55°18.559 N 10°47.411 E	LL	43	4	1	*
Robin Hood's Bay, North Yorkshire, England, UK	54°33.18 N 00°49.58 W	LL	76	1	1	0.99 (±0.94)
Dublin, Ireland	53°19.10 N 06°06.58 W	LL	256	28	6	2.80 (±1.20)
Cardigan Bay, Wales, UK	52°23.15 N 04°06.26 W	LL	85	3	1	1.00 (±0.95)
Cork, Ireland	51°41.12 N 08°27.04 W	LL	151	4	1	0.94 (±0.89)
Scheldt Estuary, Netherlands	51°26.396 N 03°34.336 E	LL	107	2	1	0.90 (±0.86)
Ostende, Belgium	51°13.593 N 02°56.596 E	LL	376	90	7	5.12 (±1.37)
Plymouth, England, UK	50°21.46 N 04°09.10 W	LL	75	5	3	3.00 (±1.50)
Trouville, France	49°21.851 N 00°04.871 E	LL	85	20	5	5.49 (±1.81)
Roscoff, France	48°43.768 N 03°59.320 W	LL	103	1	1	0.73 (±0.69)
Mindin, France	47°16.112 N 02°10.262 W	LL	64	7	2	*
Vigo, Spain	43°34.040 N 06°37.58 W	LL	106	1	1	0.71 (±0.67)

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APPENDIX C TREMATODE TAXONOMY IN TABLE I.1 PREVALENCE DATA

When assessing the overall data presented in Table I.1, there are some taxonomic issues that might affect my reported prevalences for certain trematode species. First, two trematode species, *Cercaria parvicaudata* and *Renicola roscovita,* are typically distinguished based on the color of their sporocysts, which are "orange" for *Ce. parvicaudata* and "cream" for *R. roscovita* (James 1968a, Stunkard 1971); this can obviously be highly subjective (Galaktionov and Skirnisson 2000). Furthermore, these two species have been debated in the literature as to their status as separate species (e.g., Stunkard 1950, Galaktionov & Skinisson 2000), and some authors have lumped them as *Renicola* sp. (e.g., Granovitch et al. 2000), referred to *Cercaria parvicaudata* as *Renicola parvicaudata* (Lauckner 1980), or described *Ce. parvicaudata* as a synonym of *R. roscovita* (Pohley 1976). For my study, I have used James's *Littorina* sp. trematode taxonomic key (1968a) in order to distinguish the two species. Second, the two *Himasthla* species, *H. elongata* and *H. littorinae*, can also be difficult to distinguish morphologically (Galaktionov & Skirnisson 2000) and have sometimes been lumped in the literature as *Himasthla sp.* (e.g., Matthews et al. 1985, Galaktionov & Bustnes 1995, Mouritsen et al. 1999). For my study, I have distinguished these species using James's *Littorina* sp. trematode taxonomic key (1968a) and descriptions by Stunkard (1966, 1983). Due to the taxonomic issues for these four species, their prevalences as reported in the Literature columns of Table I.1 may not accurately reflect their true prevalence in nature because species identifications were not standard across all studies.

In addition, I have lumped two different groups of trematode species in both Europe and North America as a conservative approach to avoid taxonomic over-inflation of total trematode species richness (see Table I.1). First, *Cercaria littorinae saxatilis* sp. is represented by a group of 6 subspecies, *Cercaria littorinae saxatilis I, II, III, IV, VI, VII*, that are morphologically difficult to distinguish and are extremely rare; i.e., some of which have only been observed in the studies that originally described them (e.g., James 1968a, James 1969, and Sannia & James 1977). There has been no confirmation as to their validity as separate subspecies. Thus to be conservative, I have lumped them into one group in both populations. Second, with the exception of *Microphallus similis*, *Microphallus* sp. are a group of four microphallid species (*M. pirifomis*, *M. pygmaeus*, *M. pseudopygmaeus*, and *M. triangulatus*) that are morphologically difficult to distinguish to the species level. This is primarily due to their infection life cycle, which uses the snail as both a first and second-intermediate host. When the microphallid species develop into metacercarial cysts within their snail hosts, they become essentially indistinguishable to species level (pers. obs). As a result, these four microphallid species have often been referred to as *Microphallus pygmaeus* (the initially described microphallid) or grouped as *Microphallus* sp. in the literature (Granovitch 1992, Galaktionov & Bustnes 1995, and Saville et al. 1997), as I have done here. In sum, the highly similar morphological details of these trematode species make it very unlikely that authors throughout the years would

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have applied a consistent standard to differentiate these species correctly. See APPENDIX I for more detailed information on the life histories of these *Littorina* sp. trematodes.

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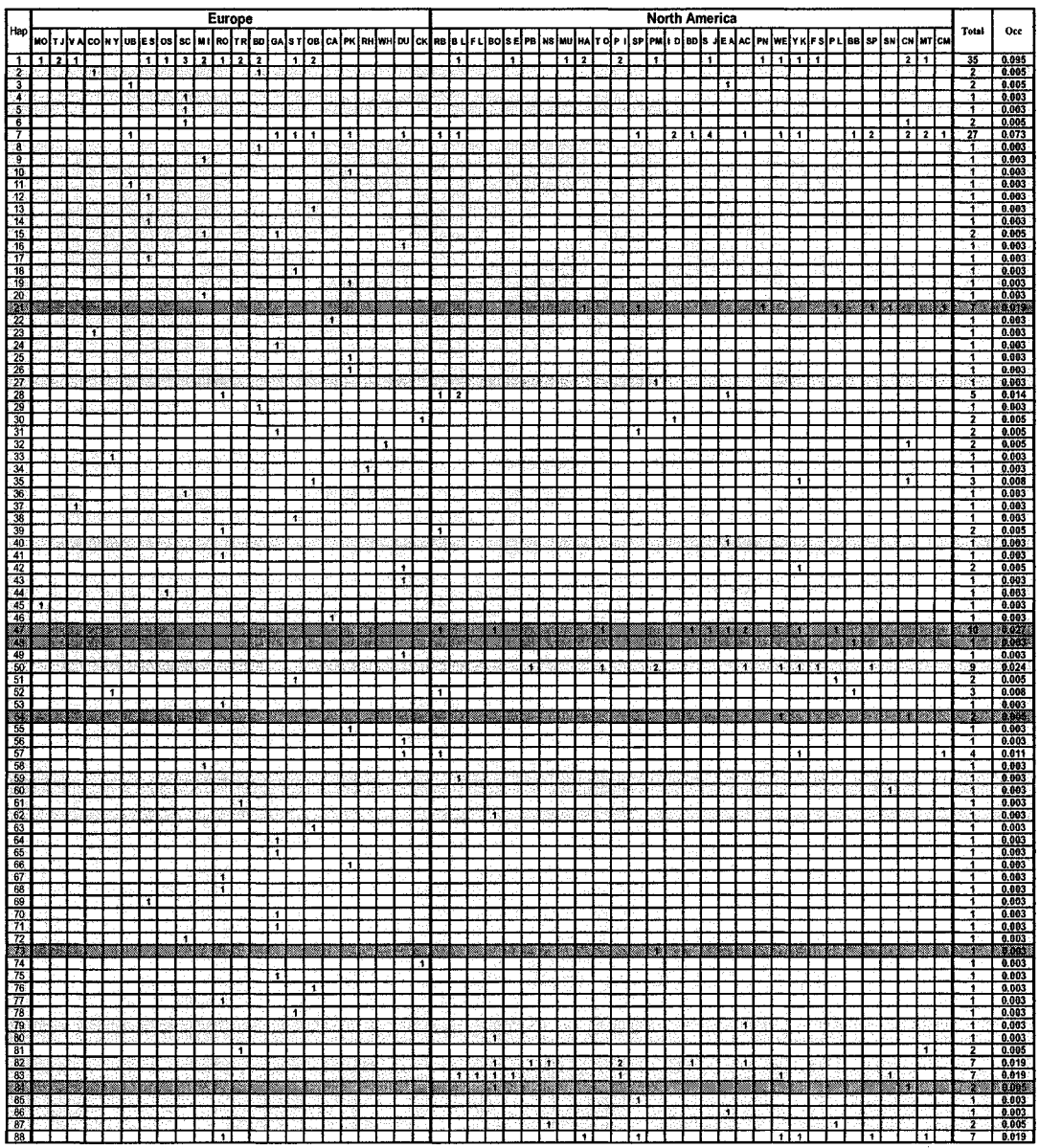
APPENDIX D HAPLOTYPE OCCURRENCE DATA FOR *LITTORINA LITTOREA* BY REGION AND SAMPLE SITE

Haplotype identities are color coded according to their status as unique to Europe (yellow), unique to North America (blue), and shared between populations (white). A fourth category (purple) represents haplotypes that appeared unique to North America but which are basal to European haplotypes and thus are considered shared.



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APPENDIX D (CONTINUED)

Hap	Europe																						North America																													Total	Occ
	MO	TJ	VA	CO	NY	UB	ES	OS	SC	MI	RO	TR	BD	GA	ST	OB	CA	PK	RH	WH	DU	CK	RB	BL	FL	BO	SE	PB	NS	MU	HA	TO	PI	SP	PM	ID	BD	SJ	EA	AC	PN	WE	YK	FS	PL	BB	SP	SN	CN	MT	CM		
89																							1			1		1				3		1					1				1	1	1						1	12	0.032
90																							1				1							1									1				1					5	0.014
91																								1																2						1						4	0.011
92																								1																												1	0.003
93			1																																																	1	0.003
94																						1																														1	0.003
95																1																																				1	0.003
96						1																																														1	0.003
97								1																																												1	0.003
98								1																																										1		2	0.005
99							1																																													1	0.003
100																			1																																	1	0.003
101												1																1																								2	0.005
102																						1																														1	0.003
103											1															1																										2	0.005
104											1																																									1	0.003
105									1																																											1	0.003
106						1																						1																						1		3	0.008
107																																	1																			1	0.003
108																									1																											1	0.003
109																									1	1																						1				3	0.008
110													1																																							1	0.003
111				1										1					1		1		1	1							1	1		1			1	1	1			3		1	1	1	1	1			1	21	0.057
112		1																																																		1	0.003
113													1																																							1	0.003
114											1																																									1	0.003
115																					1																															1	0.003
116																																			1																	1	0.003
117																																														1						1	0.003
118																									1		1																									2	0.005
119												1																1																								2	0.005
120	1																				1																															2	0.005
121																1																																				1	0.003
122		1																																																		1	0.003
123												1																																								1	0.003
124																1																																				1	0.003
125																						1																														1	0.003
126																						1																														1	0.003
127																1																																				1	0.003
128		1																					1		1											1																4	0.011
129								1																																											1	2	0.005
130								1																																												1	0.003
131						1																																														1	0.003
132								1									1																																			2	0.005
133					1																																															1	0.003
134																		1																																		1	0.003
135																																																1				1	0.003
136	1																																																			1	0.003
137	4	2		3	3	1						1																																								14	0.038
138				1																																																1	0.003
139							1																																													1	0.003
140					1																																															1	0.003
141		1																																																		1	0.003
142	1																																																			1	0.003
143				1																																																1	0.003
144			1																																																	1	0.003
145							1																																													1	0.003
146						1																																														1	0.003
147	1																																																			1	0.003
148											1																																									1	0.003
149									1																																											1	0.003
150					1																																															1	0.003
151												1																																								1	0.003
152					1																																															1	0.003
153										1																																										1	0.003
154																		1													1																					2	0.005
155								1																																												1	0.003
156											1																																									1	0.003
157							1																																													1	0.003
158								1																																												1	0.003
159																1																																				1	0.003
160								1																																												1	0.003
161													1																																							1	0.003
162										1																																										1	0.003
163		1																																																		1	0.003
164																1																																				1	0.003
165													1																																							1	0.003
166										1																									2																	3	0.008
167											1																																									1	0.003
168														1																																						1	0.003
169											1																																									1	0.003
170										1																																										1	0.003
171					1																																															1	0.003
172																				1																																1	0.003
173																																																	1			1	0.003
174																1																																				1	0.003
175													1																																							1	0.003
N	10	9	4	8	10	8	9	10	10	10	16	9	10	11	6	14	3	9	3	2	10	6	10	9	5	9	4	6	2	1	6	6	6	8	8	4	4	7	7	8	2	9	10	4	6	6	7	6	10	7	6	370	

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APPENDIX E HAPLOTYPE OCCURRENCE DATA FOR *CRYPTOCOTYLE LINGUA* BY REGION AND SAMPLE SITE

Haplotype identities are color coded according to their status as unique to Europe (yellow), unique to North America (blue), and shared between populations (white). A fourth category (purple) represents haplotypes that appeared unique to North America but which are basal to European haplotypes and thus are considered shared.

	Europe															EU	North America															NA		
Haplotype	CLMOS	CLTJA	CLVAR	CLNYB	CLUSB	CLESB	CLCOP	CLSCH	CLTRU	CLMIN	CLSTA	CLOBA	CLPUK	CLCRK	CLDIR	Totals	CLDB	CLBLA	CLRLO	CLPTS	CLBOW	CLSEA	CLNSY	CLMUL	CLMUS	CLNAL	CLBDY	CLPRR	CLWEL	CLYRK	CLWT	CLFTS	CLLAR	CLGLG	CLPLY	CLPR	Totals	Totals	Occurrence
1	1	2	1	0	1	0	0	1	1	0	0	0	0	0	1	8	0	2	1	0	2	2	1	0	1	0	1	0	0	0	2	1	3	1	2	0	19	27	0.14833846
2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	2	0	1	0	0	0	1	0	0	1	0	0	0	0	0	1	0	0	8	9	0.04945049
3	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
4	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
5	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0.010989011
6	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
7	0	0	1	0	2	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	4	0.021978022
8	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
10	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
11	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	2	2	0.010989011
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	0.00549505
14	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0.010989011
15	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
16	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
17	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
18	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0.00549505
20	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0.010989011
21	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
22	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
23	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	2	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	7	0.038461538
24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0.00549505
25	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
26	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	2	3	0.016483516
27	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
28	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	3	0.016483516
29	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0.00549505
31	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
32	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
33	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
34	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0.00549505
37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	2	2	0.010989011
38	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
39	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
40	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	2	2	0.010989011
42	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
43	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
44	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
45	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
46	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
47	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
48	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3	0.016483516
49	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
50	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
51	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
52	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
53	0	0	0	0	0	3	0	0	0	1	0	0	0	0	1	5	6	2	1	0	1	0	0	0	0	0	2	0	0	1	0	0	0	0	0	7	12	0.065934066
54	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0.00549505
55	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	2	0	0	5	5	0.027472527
56	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
57	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0.010989011
58	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0.010989011
59	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0.010989011
60	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
61	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
62	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	1	1	1	0	0	0	0	0	0	0	1	1	0	0	0	0	1	7	8	0.043956044
63	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
64	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	2	0.010989011
65	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0.00549505
66	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	2	0.010989011
67	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0	0	0	0	3	4	0.021978022
68	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	1	0	0	4	6	0.032967033
69	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	2	3	0.016483516
70	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
71	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
72	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
73	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
74	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
75	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
76	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0.00549505
77	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
78	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
79	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	0.010989011
80	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
81	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
82	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
83	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
84	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
85	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505
86	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00549505

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APPENDIX F HAPLOTYPE OCCURRENCE DATA FOR *CERCARIA PARVICAUDATA* BY REGION AND SAMPLE SITE

Haplotype identities are color coded according to their status as unique to Europe (yellow), unique to North America (blue), and shared between populations (white).

	Europe											North America										
Haplotypes	CPMOS	CPTJA	CPVAR	CPUBD	CPESB	CPOST	CPTRU	CPMIN	CPGAL	CPDIR	EU Totals	CPHAL	CPWEL	CPYRK	CPODI	CPSPD	CPPRI	CPMON	CPCMY	NA Totals	Total	Occurrence
1	6	3	1	4	1	1	2	4	1	2	25	3	3	2	1	4	2	4	4	23	48	0.7619048
2	0	1	0	0	1	0	1	0	0	0	3	0	0	0	0	0	0	0	0	0	3	0.047619
3	1	0	0	0	0	0	0	1	0	0	2	0	0	0	0	0	0	0	0	0	2	0.031746
4	0	0	0	0	0	1	0	0	0	1	2	0	0	0	0	0	0	0	0	0	2	0.031746
5	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0.015873
6	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0.015873
7	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0.015873
8	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0.015873
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0.015873
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0.015873
11	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0.015873
12	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0.015873

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APPENDIX G DETAILED METHODS FOR CHAPTER I

Snail collections and dissections

Approximately 100-150 per site (total avg ± stdev: 142 ± 70) adult *Littorina littorea* (LL avg ± stdev: 157 ± 77), *L. saxatilis* (LS avg ± stdev: 118 ± 57), and *L. obtusata* (LO avg ± stdev: 120 ± 42) snails were each collected haphazardly from the intertidal zone during low tide over the summer months of the years 2002-2006 at numerous sites in both Europe and North America (Figure I.2 and APPENDIX B). Altogether, a total of 15,933 snails (LL NA&EU: 10,810, LS NA: 2248, LO NA: 2875) were collected from 82 sites in North America (n=62) and Europe (n=20) and subsequently dissected for each study site. Snails were dissected under dissecting microscopes to assess infection trematode presence or absence. If a snail were infected, the species was determined by examination under a compound microscope and keyed out using trematode keys and diagrams (Werding 1969, Lauckner 1980, James 1968a, James 1986b, James 1969, Stunkard 1983). Following any infection, all dissection instruments were wiped down carefully with 95% ethanol to prevent any cross contamination.

Statistical analyses

To assess expected species richness in each population and for each site, I employed ESTIMATES 8.0 (Colwell 2006) to construct species accumulation and species richness estimator curves from my trematode data. ESTIMATES uses Monte Carlo re-sampling (through randomization of sample order over a number of replicates (e.g., 500)) to determine the mean accumulation of species (S_{obs}) as samples are added over the full data set (Gotelli & Colwell 2001), while also providing standard deviations and 95% confidence intervals for each data point (Colwell 2006). Although my data was sample-based, I re-scaled my species accumulation curves to accumulated individuals in order to compare species richness across my data sets in a standardized manner (Gotelli & Colwell 2001).

Sample-based rarefaction curves may not capture the total species richness within a population for a particular sampling effort, especially if these curves have not reached a stable asymptote. Thus, non-parametric estimators, such as Chao2, can be useful in predicting the eventual asymptote in species richness for a particular population (Gotelli & Colwell 2001), and do so by including the effects of rare species on the total species richness (Chao 2004, Witman et al. 2004). Chao2 has been found to be one of the most robust estimators (see Colwell 2006 for Chao2 equation) when compared to empirical data from a variety of systems for revealing the missing species in a population and thus predicting the total expected species richness for the system (e.g., Walther & Morand 1998, Foggo et al. 2003). In fact, Walther & Morand (1998) advocated the use of Chao2 specifically for parasite species richness. In addition, Chao2 has been shown to remain precise even under changes in sampling effort (Walther & Morand 1998), and since my

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data included samples of varying sizes, use of the Chao2 estimator was highly appropriate for my study.

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APPENDIX H DETAILED METHODS FOR CHAPTERS II AND III

Snail molecular methods

Snail collections. During the summers of 2002-2005, approximately 10-15 adult snails were collected haphazardly from the intertidal zone at each of 29 North American (13 U.S. and 16 Canadian) and 22 European sites (Figure II.1). Each snail was dissected and the snail foot tissue removed and stored at –80 °C until processing. Because *Littorina littorea* snails can sometimes be infected by trematode parasites, I only used uninfected snails in my analyses to avoid contamination issues.

DNA extractions. *Littorina littorea* DNA was extracted using a standard CTAB protocol (France et al. 1996): samples were homogenized in a microcentrifuge tube with 600 µl 1 x CTAB extraction buffer and 5 µl Proteinase K (20 mg ml^{-1}) and incubated at 65 °C for 2-3 hours. Samples were extracted with 600 µl of chloroform and precipitated with 1 ml of cold 100% ethyl alcohol. Following two washes with cold 70% ethanol, DNA pellets were dried and resuspended in 50 µl of molecular grade water. DNA quality and quantity were determined using a spectrophotometer at two wavelengths, 260 and 280. Quality was determined from a ratio of DNA to protein 260/280; purities between 1.8 to 2.0 were considered high quality. Quantity was determined by multiplying the 260 absorbance value by a dilution factor and a DNA specific value (equal to 5000), resulting in a DNA concentration of ng μl^{-1}.

PCR amplifications and sequencing. A 625 bp fraction of the Cytochrome b (Cyt b) mitochondrial gene and a 572 bp fraction of the Cytochrome Oxidase I (COI) mitochondrial gene were amplified using two sets of primers: Cyt b: *Primer1-F*, CCTTCCCGCACCTTCAAATC, and *Primer4-R*, ATGAGAAATTTTCAGGGTC (Reid et al. 1996); COI: *LLCOIAB-F*, CTCTCCTGGGAGATGACCAG, and *LLCOIAB-R*: TTCTGGGTGACCGAAGAATC designed using COI sequence data from Williams and Reid (2004). All samples were amplified using a PCR protocol based on Kyle and Boulding (1997). For each reaction, 0.6 µl template DNA (~100 ng μl^{-1}) was added to a PCR mix (3 µl Taq buffer, 0.75 µL of 3.4 mM dNTPs, 0.225 µL of each 20 µM primer, and 0.15 µl Taq (5 U μl^{-1}) and molecular grade water for a final volume of 30 µl. Reaction mixes were subjected to 32 Cycles of 95 °C for 30 s, 44 °C for 30 s, and 72 °C for 30 s in an automated thermocycler. Thirty µl of each reaction mix were run with a ladder and negative control on a 1% agarose gel exposed to ethidium bromide. DNA was eluted from PCR products in spin columns using a QIAquick Gel Extraction kit (Qiagen, Inc, Valencia, CA). Eluted DNA was then sequenced using ABI 377 DNA Automated Sequencers at the UNH Sequencing Facility, and sequences were analyzed using DNASTAR Programs (EditSeq, Seqman, and Megalign; DNASTAR, Inc., Madison, WI).

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Trematode molecular methods

Trematode collections. During the summers of 2002-2006, numerous adult snails were collected haphazardly from the intertidal zone at each of 20 North American (9 U.S. and 11 Canadian) and 15 European sites (Figure III.1). Each snail was dissected and assessed for presence/absence of *Cr. lingua* infection. Any mature *Cr. lingua* infections (rediae and cercariae) were collected in 1.5 mL tubes (carefully trying to avoid snail tissue as much as possible) and either placed on ethanol or stored at –80 °C until ready for processing.

DNA extractions. Prior to extractions, tubes containing *Cr. lingua* were spun in a microcentrifuge, and the ethanol (if applicable) was removed from the tubes. *Cr. lingua* samples were then transferred to a new tube with distilled water and spun a second time in the microcentrifuge to help clean the samples. The water was then removed and the samples were prepped for DNA extraction. *Cr. lingua* DNA was extracted using a standard CTAB protocol (France et al. 1996): samples were homogenized in a microcentrifuge tube with 600 µl 1 x CTAB extraction buffer and 5 µl Proteinase K (20 mg ml^{-1}) and incubated at 65 °C for 2-3 hours. Samples were extracted with 600 µl of chloroform and precipitated with 1 ml of cold 100% ethyl alcohol. Following two washes with cold 70% ethanol, DNA pellets were dried and resuspended in 50 µl of molecular grade water. DNA quality and quantity were determined using a spectrophotometer at two wavelengths, 260 and 280. Quality was determined from a ratio of DNA to protein 260/280; purities between 1.8 to 2.0 were considered high quality. Quantity was determined by multiplying the 260 absorbance value by a dilution factor and a DNA specific value (equal to 5000), resulting in a DNA concentration of ng μl^{-1}.

PCR amplifications and sequencing. For *Cr. lingua*, a 400 bp fraction of the COI mitochondrial gene and an adjacent 643 bp fraction of the COI mitochondrial gene were amplified using two sets of primers: COIa: *COI2575F*: TTTTTTGGGCATCCTGAGGTTTAT, and *COI3021R*: TAAAGAAAGAACATAATGAAAATG (Morgan and Blair 1998); COIb: *ABCOICLF*: TCTTTAGGATCATAAGCG, and *ABCOICLR*: TAAACCCCCGTATCCAAACC designed using COI sequence data from Kane et al. (2003). For *Ce. parvicaudata*, a 398 bp fraction of the COI mitochondrial gene was amplified using one set of primers: *COI2575F*: TTTTTTGGGCATCCTGAGGTTTAT, and *COI3021R*: TAAAGAAAGAACATAATGAAAATG (Morgan and Blair 1998). All trematode samples (*Cr. lingua* and *Ce. parvicaudata)* were amplified using a PCR protocol based on Huspeni (2000). For each reaction, 0.6 µl template DNA (~100 ng μl^{-1}) was added to a PCR mix (3 µl Taq buffer, 0.6 µl of 10 mM dNTPs, 1.5 µl of each 10 µM primer, and 0.18 µL Taq (5 U μl^{-1}) and molecular grade water for a final volume of 30 µl. Reaction mixes were subjected to 35 Cycles of 94 °C for 30 s, 50.9 °C for 30 s, and 72 °C for 30 s in an automated thermocycler. Thirty µl of each reaction mix were run with a ladder and negative control on a 1% agarose gel exposed to ethidium bromide. DNA was eluted from PCR products in spin columns using a QIAquick Gel Extraction kit (Qiagen, Inc,

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Valencia, CA). Eluted DNA was then sequenced using ABI 377 DNA Automated Sequencers at the UNH Sequencing Facility, and sequences were analyzed using DNASTAR Programs (EditSeq, Seqman, and Megalign; DNASTAR, Inc., Madison, WI).

Statistical analyses of snail and trematode sequence data. Phylogenetic relationships were analyzed using PAUP 4.0 (Swofford 2003) for snails and parasites. For *L. littorea*, phylogenetic trees were not only constructed using the full data set, but also using a truncated data set, where I excluded all third position sites (resulting in 798 total bp), which are the most variable sites in coding DNA because substitutions at these sites are often silent (i.e., they do not alter amino acid composition). This latter approach gave us a conservative estimate of haplotype diversity in Europe versus North America. Finally, I constructed a phylogenetic tree of just North American individuals so I could compare Canadian versus U.S. sites. I performed this last analysis as a way to determine whether Canadian sites showed more diversity than U.S. sites, which might be expected if *L. littorea* had existed in the Canadian maritimes for thousands or hundreds of thousands of years before spreading into the U.S. ~150 years ago (i.e., the U.S. subpopulation should show a subset of the diversity of the Canadian subpopulation if *L. littorea* had pre-glacial populations in Canada). For each of these phylogenetic analyses, the maximum likelihood root haplotype for each tree (designated by an asterisk) was determined using the program, TCS 1.21 (Clement et al. 2000). For *Cr. lingua*, a phylogenetic tree for the total 1043 COI region was constructed, and the maximum likelihood root haplotype was found using TCS 1.21. For *Ce. parvicaudata*, a phylogenetic tree for the 398 COI region was constructed, and the maximum likelihood root haplotype was found using TCS 1.21.

Because haplotype diversity was high in my sampled populations, I used haplotype accumulation and haplotype estimation curves to estimate haplotype diversity in each population and to quantify the effects of sampling effort on haplotype diversity. Specifically, I used ESTIMATES 8.0 (Colwell 2006) to calculate haplotype accumulation and haplotype estimation curves. Sample-based rarefaction curves may not capture the entire haplotype diversity within a population for a particular sampling effort, especially if these curves have not reached a stable asymptote. Thus, non-parametric estimators, such as the well-accepted and robust estimator Chao2 (e.g., Walther & Morand 1998, Foggo et al. 2003), can be useful in predicting the eventual asymptote in haplotype diversity for a particular population (Gotelli & Colwell 2001), and do so by including the effects of rare haplotypes on the total haplotype diversity (Chao 2004, Witman et al. 2004).

Finally, population divergence estimates for *L. littorea* and *Cr. lingua* (*Ce. parvicaudata* had too few haplotypes to run this analysis) were performed using the Isolation with Migration (IM) program (Version: July 2006; Hey and Nielsen 2006). IM uses Markov Chain Monte Carlo sampling and applies the Isolation with Migration model to genetic data taken from closely related species or populations of the same species. The program provides maximum likelihood estimates of the time since two populations split (t) in terms of mutations, which can be converted to an estimate of the number of years since the populations diverged using the specific neutral mutation rate

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for the gene in question. For *L. littorea* and *Cr. lingua*, I performed ten different runs/replicates of IM using the following input parameters and ten different random seeds: q1=1000, m1=m2=7, t=2, b=100,000, L=18.0 (L=10.0 for *Cr. lingua*). Divergence estimates were calculated using the following equation: $t = t/\mu$, where t = total years of divergence time, t = the time parameter determined by IM from the sequence data, and μ = the gene substitution rate (Hey and Nielsen 2004). The substitution rate for *L. littorea* (3% per MY; ~1.8×10^{-5} for 1197 bp) I used in calculating divergence estimates was determined by Wares and Cunningham (2001) from fossil record evidence of *Littorina* sp. provided by Reid et al. (1996) and later employed specifically for *L. littorea* in investigations by Wares et al. (2002) and Cunningham (2007). Because trematodes do not preserve in the fossil record, the best estimates for COI substitution rates for trematodes is a range between 2-4% per MY (J. Morgan, pers. comm.), which for *Cr. lingua* at a total of 1043 bp is 1.04×10^{-5} for 2% per MY, 1.56×10^{-5} for 3% per MY, and 2.09×10^{-5} for 4% per MY.

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APPENDIX I *LITTORINA* SP. TREMATODE LIFE HISTORIES

Trematode Species	1st Intermediate Host(s)	2nd Intermediate Host(s)	Definitive Host(s)	Site(s) of Citations	Citations	Population	Notes	Active / Passive Miracidia	Redia / Sporocyst
Cercaria brevicauda	*L. saxatilis*	*a) L. saxatilis;* b) encyst within 1st intermediate host, *L. saxatilis*	a) n/a; b) n/a	a) literature survey, b) Cardigan Bay, Wales	a) Pondick 1985; b) James 1969	EU			sporocyst
C. emasculans	*L. littorea, L. saxatilis*	a) intertidal crustacea and possibly intertidal fish; b) crabs, including *Cancer pagurus* and *C. meanas*, some fish; c) *C. meanas, C. pagurus*, rocklings, *Onos mustelus* and *Blennius pholis*	a) probably marine birds; b) n/a; c) n/a	a) Cardigan Bay, Wales; b) literature survey; c) Cardigan Bay, Wales	a) James 1968b; b) Pondick 1985; c) James 1969	EU			sporocyst
C. islandica I	*L. obtusata*	a) ?	a) ?	a) SW Iceland	a) Galaktionov & Skimisson 2000	EU			
C. lebouri (=P. chabaudi)	*L. littorea* (most susceptible host), *L. saxatilis, L. obtusata*	a) encyst on weed, rock surfaces or on shells of mollusks; b) encyst on hard surfaces; c) n/a; d) does not require second-intermediate host--cercariae encyst on solid surfaces; e) cercariae settle on substratum or on external surface of marine organisms and encyst; f) no second intermediate host	a) marine birds; b) marine birds; c) gulls; d) shorebirds; e) n/a; f) seabirds	a) Cardigan Bay, Wales; b) literature survey; c) sites in Maine and RI; d) German and Danish waters; e) Cardigan Bay, Wales; f) Konigshafen	a) James 1968b; b) Pondick 1985; c) Pohley 1976; d) Laukner 1980; e) James 1969; f) Lauckner 1984	EU, NA	Previously unreported in NA in *L. saxatilis* (new record by Blakeslee)	passive	redia
C. littorinae	*L. littorea, L. obtusata*	a) n/a; b) ?	a) sublittoral fish; b) ?	a) Cardigan Bay, Wales; b) West Wales	a) James 1968b; b) Williams & Brailsford 1990	EU		active	
C. littorinae oblusatae	*L. obtusata*	a) unknown; b) ? ; c) ?	a) unknown; b) ? ; c) ?	a) Cardigan Bay, Wales; b) SW Iceland; c) Isle of Man	a) James 1968b; b) Galaktionov & Skimisson 2000; c) Williams & Brailsford 1998	EU, NA	Previously unreported in NA in *L.obtusata* (new record by Blakeslee). Life cycle unknown but probably similar to *Himasthla* sp. (James 1968b)		redia
C. littorinae saxatilis I	*L. saxatilis*	n/a	n/a	Cardigan Bay, Wales	James 1969	EU	The following *C. littorinae saxatilis I - VII* are subspecies		sporocyst
C. littorinae saxatilis II	*L. saxatilis, L. obtusata*	n/a	n/a	Cardigan Bay, Wales	James 1969	EU, NA	Previously unreported in NA in *L.obtusata* & *L. saxatilis* (new record by Blakeslee)		sporocyst
C. littorinae saxatilis III	*L. saxatilis*	n/a	Gannet, *Sula bassana?*	Cardigan Bay, Wales	James 1969	EU			sporocyst
C. littorinae saxatilis IV	*L. saxatilis, L. obtusata*	n/a	n/a	a) Cardigan Bay, Wales; b) Isle of Man	a) James 1969; b) Williams & Brailsford 1998	EU			sporocyst
C. littorinae saxatilis VI	*L. saxatilis*	do not occur in same host	n/a	North Iceland	Sannia and James 1977	EU			
C. littorinae saxatilis VII	*L. saxtatilis*	crustacean?	shorebird?	Isles of Scilly	Newell 1986	EU			
C. parvicaudata	*L. littorea, L. saxatilis, L. obtusata*	a) parapodia of annelids, various species of littorinids, polyclad turbellarians; b) turbellarians, polychaetes, some bivalves, like *M. edulis*, gastropods, including littorinids; c) ? ; d) pelecypods, sometimes same snail used as first-intermediate host	a) adults unknown, probably renal parasites of birds; b) n/a; c) ? ; d) n/a	a) Woods Hole, MA; b) literature survey; c) SW Iceland; d) Danish and German coastal waters	a) Stunkard 1983; b) Pondick 1985; c) Galaktionov & Skimisson 2000; d) Lacukner 1980	EU, NA	Questions as to whether *C. parvicaudata* is a different species from *R. roscovita*	passive	sporocyst
Cryptocotyle lingua (=C. lophocerca)	*L. littorea, L. saxatilis, L. obtusata*	a) fish; b) fish, including: cunner, c) fish, especially cunners; d) many marine fish; e) n/a; f) rock-pool and sublittoral fishes; g) *P. gunnellus;* h) fishes; i) rock gunnel, *Pholis gunnellus* ; j) Arctic charr, *Salvelinus alpinus*, plaice, *Pleuronectes platessa*, cod, *Gadus morhua*, and flatfishes; k) *Gadus morhua;* l) n/a; m) n/a; n) n/a	a) shorebirds (primarily gulls); b) fish-eating birds and mammals, including: terns (*S. hirundo*), gulls, wild rats near docks in Woods Hole, not found in domestic ducks; c) naturally in birds, gulls and terns, experimentally in dogs, cats and rats but do not persist; d) many marine birds, including *Larus* sp.; e) gulls; f) marine birds, including gulls, and some mammals; g) shorebirds; h) gulls; i) n/a; j) shorebirds; k) *Larus* birds; l) Eider ducks; m) gulls; n) *Larus* sp. (argentatus, marinus, fuscus) gulls	a) Danish and German waters; b) Woods Hole, MA; c) Woods Hole, MA; d) literature survey; e) sites in Maine and RI; f) Cardigan Bay, Wales; g) Portavogie, Ireland; h) Swedish west coast; i) Maine; j) Northern Norway; k) Danish coast; l) Newfoundland coast; m) Tromso, Norway; n) Pembrokeshire, Wales	a) Laukner 1980; b) Stunkard 1930; c) Stunkard 1983; d) Pondick 1985; e) Pohley 1976; f) James 1968b; g) Matthews et al. 1985; h) Granovitch and Johannesson 2000; i) Gorman and Moring 1982; j) Kristoffersen 1991; k) Koie 1984; l) Bishop & Threlfall 1974; m) Bustnes & Galaktionov 1995; n) Harris 1964	EU, NA		passive	redia
Himasthla elongata (=H. leptosoma-- misidentification, *C. proxima, C. himasthla secunda)*	*L. littorea, L. saxatilis, L. obtusata*	a) bivalve mollusks of *lamellibranch* sp.; b) bivalves, such as *M. edulis* and *M. arenaria*; c) bivalves, pelecypods, and sometimes same snail used as first-intermediate host; d) bivalves, *Littorina;* e) *Cerastoderma edule;* f) molluscs, e.g., *M. edulis* and *C. edule* and annelids; g) n/a	a) shorebirds; b) many Larus sp.; c) shorebirds, primarily *Larus* gulls; d) gulls; e) shorebirds; f) seabirds; g) *Larus* sp. (argentatus, fuscus) gulls	a) Cardigan Bay, Wales; b) literature survey; c) Danish and German waters; d) Swedish west coast; e) Danish Wadden Sea; f) Konigshafen, North Sea; g) Pembrokeshire, Wales	James 1968b; b) Pondick 1985; c) Lauckner 1980; d) Granovitch and Johannesson 2000; e) Wegeberg et al. 1999; f) Lauckner 1984; g) Harris 1964	EU, NA	Previously unreported in NA in *L.obtusata* & *L. saxatilis* (new record by Blakeslee)	active	redia
H. littorinae	*L. obtusata, L. saxatilis, L. littorea*	a) various mollusks, including snail from which they emerged; b) bivalve mollusks, including *M. edulis* and *M. arenaria* as well as the snails from which they emerged; c) ? ; d) n/a	a) laboratory raised gulls; b) gulls, no infection in ducks; c) ? ; d) pelecypods, sometimes same snail used as first-intermediate host	a) Woods Hole, MA; b) Woods Hole, MA; c) Dale, Pembrokeshire; d) Danish and German waters	a) Stunkard 1983; b) Stunkard 1966; c) James 1968a; d) Lauckner 1980	EU, NA		active	redia

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APPENDIX I (CONTINUED)

Trematode Species	1st Intermediate Host(s)	2nd Intermediate Host(s)	Definitive Host(s)	Site(s) of Citations	Citations	Population	Notes	Active / Passive Miracidia	Redia / Sporocyst
Maritrema arenaria (=C. littorinae saxatilis V)	*L. saxatilis, L. obtusata?*	a) barnacle, *Semibalanus balanoides;* b) n/a; c) crustacean?	a) n/a; b) gulls, Oyster Catcher, Redshank; c) n/a	a) Northern Ireland; b) Millport, Great Britain; c) Cardigan Bay, Wales	a) McCarthy et al. 2002; b) Lebour 1914; c) Popiel 1976	EU, NA	Described to be a synonym of C. litt sax V in McCarthy et al. 2002 (originally described by Popiel 1976). Previously unreported in NA (new record by Blakeslee)		sporocyst
Ma. linguilla	*L. saxatilis*	*Ligia oceanica*	shorebirds	Isles of Scilly	Newell 1986	EU			
Microphallus piriformes	*L. saxatilis, L. obtusata*	a) encysts in 1st intermediate host, *L. saxatilis;* b) encysts in 1st intermediate host; c) ? ; d) ? ; e) n/a	a) gull; b) gulls, ducks; c) ? ; d) ? ; e) Eiders	a) Northern Ireland; b) Swedish west coast; c) White Sea, Russia; d) SW Iceland; e) Tromso, Norway	a) McCarthy et al. 2002; b) Granovitch and Johannesson 2000; c) Granovitch et al. 2000; d) Galaktionov & Skimisson 2000; e) Galaktionov & Skimisson 1995	EU, NA?	Morphologically indistinguisable from other microphallids in metacercarial state	passive	sporocyst
M. pseudopygmaeus	*L. obtusata, L. saxatilis*	a) *L. obtusata, L. saxatilis* (encysts in 1st intermediate host); b) ? ; c) ?	a) marine bird; b) ? ; c) ? ; d) Eiders	a) literature survey; b) White Sea, Russia; c) SW Iceland; d) Tromso, Norway	a) Pondick 1985; b) Granovitch et al. 2000; c) Galaktionov & Skimisson 2000; d) Galaktionov & Skimisson 1995	EU, NA?	Morphologically indistinguisable from other microphallids in metacercarial state	passive	sporocyst
M. pygmaeus	*L. littorea, L. obtusata, L. saxatilis*	*a) L. littorea, L. obtusata, L. saxatilis;* b) n/a; c) encysts in first-intermediate host, which also serves as second-intermediate host; thus *L. littorea, L. obtusata, L. saxatilis;* d) encysts in 1st intermediate host; e) n/a; f) encysts in 1st intermediate host; g) n/a; h) ?	a) many marine birds, including *Larus* sp. and some mammals; b) gulls; c) shorebirds; d) gulls, ducks; e) Eider ducks, gulls; f) gulls; g) Eider ducks; h) Eiders	a) literature survey; b) sites in Maine and RI; c) Danish and German waters; d) Swedish west coast; e) Maine; f) Pembrokeshire; g) Newfoundland coast; h) Tromso, Norway	a) Pondick 1985; b) Pohley 1976; c) Laukner 1980; d) Granovitch and Johannesson 2000; e) Pohley and Brown 1975; f) James 1968a; g) Bishop & Threlfall 1974; h) Galaktionov & Skimisson 1995	EU, NA	Morphologically indistinguisable from other microphallids in metacercarial state	passive	sporocyst
M. triangulatus	*L. obtusata, L. saxatilis*	a) ? ; b) ?; c) ? [encysts in first-intermediate host like other microphallids?]	a) ? ; b) ? ; c) Eiders	a) White Sea, Russia; b) SW Iceland; c) Tromso, Norway	a) Granovitch et al. 2000; b) Galaktionov & Skimisson 2000; c) Galaktionov & Skimisson 1995	EU, NA?	Morphologically indistinguisable from other microphallids in metacercarial state	passive	sporocyst
M. similis (=C. ubiquita, C. ubiquitoides)	*L. littorea, L. obtusata, L. saxatilis (L. obtusata and L. saxatilis are most susceptible hosts)*	a) *Carcinus maenas;* b) *Carcinus maenas* and other shore crabs; c) *C. maenas*, *Gammarus* sp., *Cancer pagurus;* d) n/a; e) *Carcinus maenas;* f) *Carcinus maenas* (primarily) and *Cancer pagurus* (less likely), sometimes in *Gammarus locusta* and *Hyas araneus;* g) *Carcinus maenas;* h) shorecrabs; i) *C. meanas;* j) n/a; k) n/a	a) Herring Gull, *Larus argentatus* and tern, *S. hirundo;* b) Herring Gull, *Larus argentatus*, and some other marine birds; c) many marine birds, including Larus sp.; d) gulls; e) n/a; f) *Larus* gulls; g) Herring Gull, *Larus argentatus;* h) gulls; l) Eider ducks, gulls; j) gulls; k) *Larus* sp. (argentatus, marinus) gulls	a) Woods Hole, MA; b) Cardigan Bay, Wales; c) literature survey; d) sites in Maine and RI; e) Northern Ireland; f) Cardigan Bay, Wales; g) Angelesy, Great Britain; h) literature review; I) ; j) Tromso, Norway; k) Pembrokeshire, Wales	a) Stunkard 1983; b) James 1968b; c) Pondick 1985; d) Pohley 1976; e) McCarthy et al. 2002; f) James 1969; g) Einer and Raffaelli 1980; h) Stunkard 1957; I) ; j) Bustnes & Galaktionov 1995; k) Harris 1964	EU, NA		passive	sporocyst
Notocotyloides petasatum	*L. obtusata*	a) gastropods; b) ?	a) fish; b) ?	a) literature survey; b) Pembrokeshire	a) Pondick 1985; b) James 1968	EU			
Podocotyle atomon (=C. linearis)	*L. saxatilis, L. littorea*	a) amphipods, *Gammarus sp., Carcinogammarus mucronatus, Amphithoe longimana,* natural infections in fishes and experimentally in eels and sticklebacks; b) many species in Malacostraca, inlcuding *Gammarus* sp.; c) amphipods; d) n/a; e) Gammarus locusta, Hyale nilissoni and other amphipods; f) crustaceans; g) amphipods, Gammarus sp.; h) amphipods, Isopods, mysids; i) amphipods, Isopods, mysids; j) amphipods	a) n/a; b) rays and many species of marine fish; c) fishes (mainly pleuronectid flatfishes); d) fish, like flounder; e) n/a; f) fishes; g) *Acanthocottus scorpius, Salmo salar, Sebastes marinus, Gasterosteus aculeatus, Scomber scombrus, Phols chuss, Hemitripterus americanus, Pholis gunnellus, Anarhichas lupus, Anguilla rostrata, Apeltes quadracus*, among many other fish; h) various fish families; i) cod, flounder, dabs; j) rockpool fish	a) Woods Hole, MA; b) literature survey; c) Danish and German waters; d) Portavogie, Ireland; e) Cardigan Bay, Wales; f) Swedish west coast; g) Cape Cod; h) Danish coast; I) Danish coast; j) Cardigan Bay	a) Stunkard 1983; b) Pondick 1985; c) Laukner 1980; d) Matthews et al. 1985; e) James 1969; f) Granovitch and Johannesson 2000; g) Hunninen and Cable 1943; h) Koie 1984; i) Koie 1983; j) James 1968b	EU, NA		active	sporocyst
Parvatrema homoeotecnum	*L. saxatilis, L. obtusata*	a) littorinids; b) n/a; c) n/a; d) n/a; e) use littorinids as second and first-intermediate host	a) shorebirds; b) ? ; c) Eider ducks; d) Oystercatchers; e) n/a	a) literature survey; b) SW Iceland; c) Tromso, Norway; d) Aberystwyth, Wales; e) German and Danish coast	a) Pondick 1985; b) Galaktionov & Skimisson 2000; c) Galaktionov & Bustnes 1995; d) James 1968c; e) Lauckner 1980	EU, NA	Previously unreported in NA in *L.obtusata* (new record by Blakeslee)		sporocyst
Parapronocephalum symmetricum	*L. saxatilis, L. obtusata*	a) encysts in 1st intermediate host, *L. saxatilis;* b) ? ; c) ?	a) Sand-pipers, Turnstones; b) ? ; c) ?	a) Cardigan Bay, Wales; b) SW Iceland; c) Isles of Scilly	a) James 1969; b) Galaktionov & Skimisson 2000; c) Newell 1986	EU			redia
Renicola roscovita (=C. roscovita)	*L. littorea, L. saxatilis, L. obtusata*	a) pelecypod bivlaves and littorinids; b) mollusks and crabs, including *C. meanas*; c) bivalves, including M. edulis, gastropods, including littorinids, *C. meanas;* d) other littorinids or the same snail, sometimes *C. maenas;* e) bivalves, *Littorina;* f) mussels and cockles	a) shorebirds (primarily gulls); b) probably marine bird; c) *Larus* sp.; d) probably marine birds; e) gulls; f) marine birds	a) Danish and German waters; b) Cardigan Bay, Wales; c) literature review; d) Cardigan Bay, Wales; e) West coast of Sweden; f) Konigshafen, North Sea	a) Lauckner 1980; b) James 1968b; c) Pondick 1985; d) James 1969; e) Granovitch and Johannesson 2000; f) Lauckner 1984	EU, NA	Questions as to whether *R. roscovita* is a different species from *C. parvicaudata*	passive	sporocyst

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