Evolution and Phylogeography of New World Gastropod Faunas

by

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Abstract

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This dissertation covers a broad range of evolutionary topics, but its primary focus is on the use of molecular phylogenetics to reconstruct phyletic relationships and biogeography of gastropod molluscs, and in particular, the clade Patellogastropoda. The first chapter tests the long held view that the Gulf of California, Mexico is an isolated basin and as such behaves as an island having insular effects on gastropod evolution. Tests are performed that compare the level of endemicity in the Gulf to the surrounding eastern Pacific fauna. The remaining four chapters apply molecular phylogenetics to specific biological methodologies and questions. Chapter 2 examines the combinability of 16S and COI mitochondrial genes in a phylogenetics analysis of New World Patellogastropoda and presents a robust phylogenetic hypothesis. In chapter 3 molecular and morphological techniques are used to resolve a putative cryptic species complex, while in chapter 4, the patellogastropod phylogeny is used in conjunction with an innovative collecting methodology to clarify a long-standing confusion surrounding the identity of several northeast Pacifc patellogastropods.

This includes the discovery of a new species in the Gulf of California. Lastly, chapter 5 uses a molecular phylogeny to examine the biogeography of the New World patellogastropods and generates testable biogeographical hypotheses for the northeast Pacific, Chilean, and tropical New World faunas. These hypotheses are unified by a global hypothesis that ties the evolutionary history of these patellogastropod faunas to a western Tethyan origin.

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Introduction

This dissertation is composed of five chapters, each focused on the evolutionary history of near shore gastropods. The first chapter examines the hypothesis that the Gulf of California is an isolated body of water and as such behaves as an island having insular effect on gastropod evolution. In support of this hypothesis, there have been many claims that the Gulf of California is a repository for endemics of many taxa. Chapter one tests whether the level of endemism in the Gulf of California differs from that of the rest of the tropical eastern Pacific continental shelf. Using a “window” analysis of a new gastropod database containing the ranges of over 2,400 tropical eastern Pacific species does this. The “window” analysis examines the number of species ranges restricted to fixed lengths of coastline (the “window”). As the “window” is incrementally shifted along the eastern Pacific coastline, the percentage of ranges restricted to each window increment is tallied. This produces a coastal profile of the level of endemism for a particular “window” size. One of the “windows” is equal to the total coastal length of the Gulf of California, thereby permitting a comparison of the level of endemicity in the Gulf of California to the rest of the tropical eastern Pacific.

The remaining chapters (2-5) focus on the evolutionary history and phylogeography of the New World Patellogastropoda. The Patellogastropda are an ideal study group for phylogeography because they primarily occupy the near shore rocky intertidal of every New World coastline, which permits their

evolutionary history to be correlated with the geological history of the New World continental margins. Chapter 2 is a phylogenetic analysis of New World Patellogastropoda and an analysis of the compatibility of the two molecular data sets employed to infer Patellogastropoda phylogeny. The mitochondrial markers for the large subunit ribosomal RNA gene (16S) and Cytochrome c Oxidase subunit I (COI) were employed for the analysis. Only 31 of the OTUs were successfully sequenced for COI resulting in an uneven sampling between 16S (57 OTUs) and COI (31 OTUs) data. Many tests were performed to determine whether these two sources of molecular data were compatible in a combined phylogenetic analysis. In addition to testing for uneven sample size, the compatibility of informative phylogenetic content was also examined. The results suggested that 16S and COI are both phylogenetically informative, but at different phylogenetic levels. COI was more informative for more recent evolutionary events while 16S was better at resolving deeper events and when combined in a single analysis produced poorly supported hypotheses incompatible with biogeographical distributions.

The results from chapter 2 were used to examine specific biological topics within the evolutionary history of patellogastropods, three of which are included as chapters 3, 4, and 5. In chapter 3 variation found in COI was used to examine the validity of a morphological cryptic species complex. It had been suggested that *Notoacmea fascicularis* was a complex of two species based on difference in radular morphology. Radular morphology has been the primary diagnostic feature in the systematization of gastropods for over two centuries. However the

recent availability of techniques, which enable the sequencing of DNA has broadened our ability to accurately reconstruct the evolutionary history of life. These techniques were used to examine the possibility that *N. fascicularis* is a cryptic species complex.

Chapter 4 describes a collecting methodology that suggests that the current taxonomy of living gastropod groups is seriously flawed. The more organisms that are collected and sequenced, the more it becomes apparent that the current nomenclature for patellogastropods is incomplete and misleading. Therefore, I adopted a collecting strategy that intentional avoided all preconceived notions of taxonomic assignment. Instead, collections were made and the slightest variation in morphology and habitat were sampled. The objective of this strategy was to build a large database of molecular sequences from many different habitats and localities and be able to discover misnamed and unnamed species. In chapter 4, a molecular phylogeny based on my collecting strategy reveals several species of limpets formerly believed to be a single wide ranging species (*Lottia strigatella*), one of which was undescribed until now.

Chapter 5, the final chapter, describes six biogeographical patterns observed for New World patellogastropods and presents hypotheses for the origins of these patterns. The biogeographical patterns were discovered by mapping the localities of the specimens onto a complete 16S New World patellogastropod phylogeny. The chapter is concluded with a global hypothesis synthesizing all of the biogeographical hypotheses into a single testable biogeographical hypothesis.

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Chapter One

A New View of the Gulf of California

Introduction

Endemism is generally defined as the restriction of any taxon or community to a particular geographic area. Patterns of endemism are central to inferring a variety of evolutionary processes, including speciation, extinction, and community turnover (Hillis, 1996; Cox, 1993). Therefore, it is paramount that we scrutinize the methods that are used to identify endemism patterns. Endemism patterns in the Gulf of California have been the focus of considerable attention and have provoked a series of evolutionary and biogeographic explanations (Hubbs, 1960; Walker, 1960; Karig & Jensky, 1972; Chen, 1975; Gastil *et al*., 1975; Moore & Curray, 1982; Present, 1987). In this study, the basic pattern of anomalously high endemism of the Gulf is challenged, and several causal hypotheses are evaluated critically. Rigorous analyses of biogeographic patterns are an essential prerequisite to an understanding of evolutionary process in the gulf and other biogeographic realms.

Taxonomic “isolation” is central to theories of island biogeography and therefore laden with assumptions about processes and patterns. In general, insularity implies the ecological and/or physical isolation of faunas resulting in patterns of high endemicity and low diversity (Diamond, 1972; Wilcox, 1978; Brown & Gibson, 1983; Case & Cody, 1987; Case *et al*., 1992; Myers, 1988; to mention only a few). The mechanisms suggested to be responsible for these patterns include varying levels of speciation, extinction, competition, migration, and to some extent chance. It is difficult to quantify or even identify the contribution to patterns each of these processes makes. Endemism, as in

paleoendemism, however, may also be the result of other processes independent of insularity, such as relictual populations left behind by the extinction of surrounding populations. Here, ‘insularity’ represents the suite of processes generally believed to be responsible for patterns associated with the isolation of taxa and communities.

Biogeographic setting

The Gulf of California (Gulf) is often treated as an island and viewed as a relatively isolated basin. The geographic history, geomorphology, and latitudinal orientation of the Gulf is an ideal setting for isolation, and contributes to expectations of high endemicity of its marine biota. Terrestrial barriers surround the Gulf on the east, north, and west coasts and its latitudinal orientation sets up a potential tropical barrier at the mouth of the Gulf. The temperate head of the Gulf lies just below the 32nd parallel, while the mouth opens 9º to the south in tropical waters.

This island-like setting has been used to explain endemicity in the Gulf for a variety of lineages from crustaceans and mollusks to fishes (Skogland, 1992; (Present, 1987; Houston, 1980; Brusca, 1980; Keen, 1971; Soule, 1963; Hubbs, 1960; Garth, 1960; Walker, 1960). For example, Walker (1960) estimated that 17% of continental shelf fish species are endemic to the Gulf of California. However, the significance of such a number is unknown without comparable data from other clades and comparisons to comparable regions. Indeed, it remains to

be demonstrated that the percentage of marine faunas confined to the Gulf of California is significantly higher than similarly sized regions elsewhere.

In this study, the hypothesis of high Gulf endemism is tested by using a new database of tropical and neo-tropical gastropod distributions. Specifically, these data are employed to detect the presence or absence of an isolating barrier at the mouth of the Gulf by examining range endpoints near the mouth of the Gulf, and by tallying ranges that do and do not cross the barrier. In addition, the distribution database is used to determine whether the level of endemism in the Gulf is significantly higher than the rest of the neo-tropical and tropical eastern Pacific (TEP) by comparing the ranges of marine gastropods restricted to the Gulf of California to those of similar neo-tropical and TEP habitats using sliding window analyses. Sliding windows are used to compare the sizes of the Gulf ranges to those of the TEP. Finally, diversity in the Gulf is compared to the TEP using sliding window analyses. The goal of all of these analyses is to better understand patterns of diversity in the Gulf of California and evaluate previous assertions about processes controlling diversity in the Gulf of California.

Materials and Methods

Neo-tropical and tropical gastropod database

Gastropods are one of the most species-rich metazoan taxa and the most abundant of all benthic marine invertebrates on the continental shelf. Their abundance and the linear nature of their ranges along the eastern Pacific margin, which are associated with the continental shelf, make coastal marine gastropods ideal for comparative studies of distribution patterns. The correspondence of species ranges with the linear shoreline makes comparisons between ranges a simple matter of comparing differences in length and position.

The database compiled for this study contains distribution and depth data for 2,439 TEP marine gastropods catalogued from Keen (1971), the supplement to Keen by Skogland (1992), and locality data from the UCMP collections. The sources used to build the gastropod range database are descriptions of neo-tropical and TEP taxa, because the marine waters of the Gulf are primarily neo-tropical to tropical habitat. In general, the TEP begins near Isla Cedros (28º N. lat.)on the west coast of central Baja California. Consequently, many of the Californian taxa, which range into Baja California, have not been included here, leaving the northern temperate coast of Baja underrepresented for gastropod

species in general. However, in this analysis, Gulf taxa are being compared to those of the rest of the TEP, so temperate ranges have been ignored.

Many databases catalogue locality information only by latitude and geographic locations. However, latitudinal groupings and comparisons are inappropriate for this study because the gulf has internal and external coastlines in the same latitudinal range. For example, a range south of Baja California, which extends throughout the Gulf and around the tip of the peninsula north to California, would have three sets of identical latitudinal positions, one each for the outer peninsula, the inner peninsula, and the mainland coast of the Gulf (Fig. 1). Therefore, in this study, ranges are described more quantitatively; I have adapted the mapping system used by Keen (1971) into a Paradox 8® database. Here, localities are numbered along the eastern Pacific coastline in ascending order from north to south, following the coastline around the tip of the Baja California peninsula, through the Gulf and down the mainland side southward towards Chile (Fig. 2). This method permits a more accurate description of nearshore ranges associated with the Gulf of California.

The task of estimating Gulf diversity is further complicated by the vagueness of published range descriptions involving the Gulf of California. Fully 268 range descriptions are noted only as ‘also found in the Gulf’. Unique codes have been applied to these ranges, depending on whether the vague reference refers to the northern or southern endpoint of the distribution or if the description refers to some region between endpoints. If the description of a Gulf-spanning range was vague about localities between non-Gulf endpoints, then the extent of

a Gulf range was estimated by its most northerly locality outside of the Gulf. If a range description was vague about its southern Gulf endpoint, then the range was estimated to reside on the west side of the Gulf. If the description was vague about its northern Gulf endpoint, then the range was estimated to be on the east side of the Gulf. There are also 96 records of ranges spanning the mouth of the Gulf that mention nothing about occurrence within the Gulf. These 96 records are treated here as non-Gulf ranges.

These vagaries force an underestimation of the Gulf diversity because the 268 Gulf-vague records must be treated conservatively; surely many of the 96 non-Gulf records do range into the Gulf. However, they do not affect the overall diversity estimates for latitudes associated with the Gulf and peninsula, because their ranges are included in the estimation if their northern range includes any portion of the Baja peninsula or Gulf.

The Gulf of California

The Gulf of California is one of the few places in the world where a cool body of water is surrounded by hot arid desert. The Gulf of California is unique not only in the stark contrast between its marine and terrestrial environments, but also in its coastal geomorphology and oceanography.

The formation of the modern Gulf of California began approximately 13.5 million years ago. The waters of the Gulf fill a deep rift depression left by the oblique northwest drifting of the Baja peninsula as it was sheared away from the Mexican mainland. This deep trough lies at the Pacific and North American plate

boundary and defines the shear zone between the two plates (Ferrari, 1995; Lonsdal, 1989). This rift divides the Gulf by a deep-water trough of 650 m at the southern end of the Sal Si Puedes Basin, near the head of the Gulf, to waters as deep as 3,500 m at the mouth of the Gulf. Two parallel marine shelves have thus been produced on either side of the Gulf of California with a combined coastal length of approximately 2,000 km and a latitudinal traverse of 9º.

The appendix-shaped Baja peninsula points southward and is parallel with the mainland coast of Mexico confining the Gulf waters between these two landmasses. This coastal geography creates three parallel coastlines all roughly perpendicular to latitude (Fig 1). The southern end of the Gulf, situated in tropical waters, is the only part of the Gulf opening to the Pacific Ocean. The rest of the Gulf is neo-tropical to temperate. It is reasonable to postulate that the tropical waters at the mouth of the Gulf serve as a barrier to dispersal for many forms of life, including gastropods, because the successful settlement of planktonic larvae is very difficult, especially in temperatures outside their native range (Vermeij *et al*. 1990 and references within). Moreover, gastropods with nonplanktonic larval stages are known to have weaker dispersal capabilities than do those with planktonic stages (Hansen, 1978; Perron & Kohn, 1985), at least across latitudes (Vermeij et al. 1990).

Another notable feature of the Gulf is that over 90% of the west coast of the Gulf is rocky habitat, while more than 80% of the east coast is sandy. Many gastropods require rocky nearshore environments and are not found on sandy beaches, while others prefer sandy habitats. The different substrates found on

the east and west shores of the Gulf provide different community resources and therefore support different faunas. The west coast of the Baja peninsula has both sandy and rocky substrates and is exposed to heavy surf year round, while Gulf localities rarely experience surf of any kind. Therefore, the three parallel coastlines associated with the peninsula and the Gulf have different environmental characteristics and are hypothesized to support different gastropod communities.

Analyses

Four hypotheses can be tested based on the notion that the Gulf of California is insular. (1) Range sizes in the Gulf should be constrained by insular barriers and therefore should be shorter than those outside of the Gulf. (2) Endemism in the Gulf should be elevated relative to the rest of the TEP. (3) There should be a marine barrier near the mouth of the Gulf restricting dispersal into and out of the Gulf. (4) The inverse relationship between isolation and diversity predicts less diversity in the Gulf than in the rest of the TEP.

To test the insular hypothesis, the ranges of marine gastropod species occupying the benthic, littoral and neritic habitats were used. (1) Potential reduction of range sizes in the Gulf was examined using sliding windows. (2) The level of endemism in the Gulf was also compared to the rest of the eastern Pacific using sliding windows. (3) The presence of a barrier was investigated by examining the distribution of range endpoints and estimating the percentage of ranges that penetrate the mouth of the Gulf. (4) Comparing diversity profiles

generated by six different sliding window analyses tested the level of Gulf diversity against that of the TEP.

Two types of window analyses were used: distance and latitudinal. The distance windows were 500 km (window 1), 1,000 km (window 2), and 2,000 km (window 3) and the latitude windows were 5º (window 4), 9º (window 5) and 18º (window 6) (Tables 1-5, Fig 3). All of the windows were initially positioned on the Pacific side of the peninsula at Isla Cedros and were shifted down and around the tip of the peninsula, then up to the head of the Gulf and down the eastern shores to Chile. The latitudinal windows were shifted southward in 5º increments whereas the distance windows were shifted in 250 (window 1 & 2) and 500 km (window 3) increments. At each increment, the total number of species with any portion of their range contacting the window was tallied. All ranges fully contained within the window were considered endemic to that window and recorded. When comparing the Gulf to non-Gulf regions, the percentage of ranges restricted to each window was averaged using a weighted average. This percentage was calculated by dividing the sum of all ranges restricted to each window by the sum of all ranges in contact with each window.

Potential biases

Before presenting the tests three features that present potential problems with the window analyses must be addressed: (1) The pattern known as Rapoport's rule; (2) the latitudinal diversity gradient; and (3) the folding coastline associated with the Gulf and Baja California peninsula.

1) Rapoport's Rule

The sliding window analyses performed here were susceptible to bias if gastropod range length decreases with decreasing latitudes as suggested by Rapoport's rule (Stevens, 1989). If ranges in lower latitudes are shorter than in higher latitudes, then the shorter ranges would have a higher probability of being entirely contained within a particular window. However, Roy et al. (1994) examined the ranges of 2,838 species of marine gastropods and bivalves from the eastern Pacific coast and found no evidence of Rapoport's rule. Gaston et al. (1998) compared the relationship of range size and latitude from 25 published studies and found no evidence for Rapoport's rule as a global trend. Both studies provide convincing evidence that the eulogy for Rapoport's rule has been read and thus it will not be considered further here.

2) Latitudinal diversity gradient

Another potential bias to the sliding window analyses is the well-documented presence of a latitudinal diversity gradient. If diversity increases with decreasing latitudes, then sampling size would increase as windows were slid from higher to lower latitudes. The percent endemism for the windows should not be expected to be affected by latitudinal diversity, but because there are a relatively small number of windows, differences in sample sizes could profoundly skew the overall results. To determine whether gastropod diversity is influenced

by latitude, a sliding window analysis much like that used by Roy et al. (1994) was used to estimate the distribution of gastropod species diversity along the TEP. Total species diversity for a 5º window shifted every 5º from 32º north to 10º south was recorded and plotted against latitude (fig. 4 & 5).

3) Gulf coastline

The geographic position of the Baja California peninsula complicates the estimation of latitudinal diversity because the peninsula adds two additional coastlines to the diversity profiles between 23º N and 32º N latitude. Previous studies of latitudinal diversity for this region have revealed an elevated level of diversity (Roy et al., 1994). Calculating latitudinal diversity for latitudes across the Gulf of California, Roy et al. treated the Gulf waters separately from the waters outside the Baja peninsula "… because two very different water masses exist at the same latitude there…." While this is true, they were looking at shelled gastropods and bivalves living in waters shallower than 200m. As described earlier, the Gulf is divided by a deep trough, so there are actually two parallel shelves in the Gulf shallower than 200m. I argue here that there are actually three separate faunas existing at the same latitude (the east and west coast of the Gulf and the west coast of Baja) and should be associated with coastal shelves rather than bodies of water.

To compensate for the added diversity imposed by the Baja peninsula, the two coastlines of the Baja California peninsula are treated separately from the mainland coast of Sonora and Sinaloa in the estimation of latitudinal diversity for

Gulf latitudes (Fig. 5). Ranges simultaneously occupying both the peninsula and the mainland were excluded from the diversity estimations for the peninsula. This avoids counting the same ranges twice for particular latitudes and provides an estimation of the peninsular contribution to the overall latitudinal diversity of Baja latitudes.

Tests

1) Range size

To examine the effect of Gulf insularity on range sizes, three sliding windows were employed (windows 1, 2, & 5). Windows 2 and 5 are each approximately half the coastal length of the Gulf, while window 1 is approximately a quarter of total coastal length of the Gulf (Fig. 3). If range lengths have been reduced, then a particular window of Gulf coastline should contain, on average, more ranges restricted to that window than elsewhere along the TEP. The total number of ranges restricted to each window increment was recorded. And, the weighted averages for all increments within the Gulf and outside of the Gulf were calculated and compared.

2) Level of endemism

To compare the level of endemism in the Gulf to the rest of the tropical eastern Pacific, two windows (window 3 & 6) approximately equal to the coastal length of the Gulf were used. These estimate, for the entire TEP, the total number of taxa restricted to a window equal in size to the coastal length of the Gulf of California. This reveals whether the total number of taxa restricted to the

Gulf is elevated relative to the rest of the TEP. The windows were incrementally shifted from Isla Cedros, around the tip of the Baja peninsula, up through the head of the Gulf and south to Chile every 500 km for the 2,000 km window and every 5º for the 18º window. The percentages of ranges restricted to each window increment were compared.

3) Barrier

A key requirement for the Gulf to be insular is that a barrier exists between the Gulf waters and the Pacific Ocean. The most reasonable place to expect such a barrier is the mouth of the Gulf because the waters here are considerably warmer than inside the Gulf and is the only passage to the Pacific. If a barrier exists at the mouth of the Gulf, two more hypotheses can be made: One would expect to find an accumulation of range endpoints near the proposed barrier. And, few ranges would be expected to penetrate the proposed barrier. To detect the presence of a barrier at the mouth of the Gulf, all range endpoints were tallied from Baja California to 15º-north latitude. An elevated collection of endpoints near the proposed barrier would support a barrier hypothesis because a barrier would serve as a provincial boundary between the cooler waters in the Gulf and the warmer waters south of the Gulf. The numbers of range endpoints near the Gulf are plotted against locality numbers (Fig. 6).

However, to falsify the hypothesis, it must be demonstrated that most ranges that encounter the barrier penetrate it. The efficacy of a barrier at the mouth of the Gulf was tested by determining the percentage of ranges proximal to the

mouth of the Gulf, which breach the mouth of the Gulf. Two measures were made: (1) the percentage of western Baja ranges and (2) the percentage of ranges south of the Gulf, which enter the Gulf were calculated (Fig. 7). From the north, all ranges occupying the Pacific shores of the Baja California peninsula were considered, and from the south, all ranges crossing 20º N were considered.

4) Diversity

While there are a few exceptions to the inverse relationship between isolation and diversity, namely Drosophila of Hawaii (Williamson, 1981; Carson & Yoon, 1982) and cichlids of the great lakes of central Africa (Fryer & Iles, 1972; Dominey, 1984), there are no a priori reasons to suspect the Gulf to be an exception to this rule. Competition, extinction, and restricted immigration into the Gulf should contribute to the expected reduction in diversity if the Gulf is insular. Diversity is estimated by separately tallying all species associated with each window for each of the sliding window analyses and plotting the values against localities along the TEP (Fig. 8). Diversity is also compared to the TEP by examining a latitudinal diversity profile of the TEP (Fig. 4A & 9). These plots permit regional comparisons of total species diversity along the TEP.

Results

Biases

1) Rapoport's rule – Excluded.

2) Latitudinal diversity gradient and 3) Multiple latitudinal Gulf coastlines.

The Gulf has the highest diversity in the eastern Pacific (fig. 4A). However, if the Baja California peninsula is excluded from this estimate, the diversity profile for the mainland coast in the Gulf of California is similar to the rest of the TEP (fig. 4B & 5), with an average diversity of 986 species per 5º-latitude versus an average of 931 species per 5º-latitude for the rest of the TEP (Table 6). The additional diversity contribution for Gulf latitudes from the Baja peninsula, estimated by excluding Gulf ranges that occupy any portion of the east coast of the Gulf, is 333 species per 5º-latitude.

The latitudinal diversity profiles generated here demonstrate that the latitudinal diversity profile for the TEP is relatively flat from the head of the Gulf of California to Panama, therefore, a latitudinal diversity gradient for TEP gastropods does not exist and does not bias the sliding window analyses performed in this study.

Tests

1) Range size

There are twenty-three 500 km window positions from Isla Cedros to Chile, six of which are entirely contained within the Gulf (Table 1, Fig. 10A). For the six windows restricted to the Gulf, the weighted average of ranges restricted to each window is 5.00%. The weighted average for ranges restricted to windows outside the Gulf is 6.90%. There are twenty-two 1,000 km window positions from Isla Cedros to Chile, five of which are entirely contained within the Gulf (Table 2, Fig. 10B). For the five windows restricted to the Gulf, the weighted average of ranges restricted to each window is 7.71%. The weighted average for ranges restricted to windows outside the Gulf is 9.30%. For the 9º windows, there are 10 windows from Isla Cedros to Chile, three of which are restricted to the Gulf (Table 3, Fig. 10C). The weighted average of ranges restricted to each window in the Gulf is 8.92% while the weighted average for the rest of the east Pacific is 13.25%. None of these sliding window analyses indicates that the ranges in the Gulf are shorter than the rest of the east Pacific; in fact, the data indicate that the ranges are larger.

2) Endemism level

There are ten 2,000 km windows and eight 18º windows from Isla Cedros to Chile with a single window restricted to the Gulf for each analysis (Fig. 11). For the 2,000 km analysis, a single window occupies the gulf with 16.37% of the taxa

restricted to the Gulf, while the rest of the TEP has a weighted average of 16.11% endemism per 2,000 km window (Table 4). The 18º analysis reveals 17.81% window endemism for the Gulf compared to a weighted 21.92% endemism for the rest of the 18º windows of the TEP (Table 5). Both of these sliding window analyses demonstrate that there is no difference in endemism levels between the Gulf and the TEP.

3) Barrier

The range endpoint profile associated with the Gulf reveals six localities with a conspicuously elevated number of endpoints; Cabo San Lucas, La Paz, Puerto Peñasco, Bahia San Carlos, Guaymas, and Mazatlán (Fig. 6). Mazatlán and Cabo San Lucas have the highest and third highest collection of range endpoints associated with the Gulf and therefore supports the presence of a barrier.

A test that may falsify the barrier hypothesis is to calculate the percentage of ranges that actually breach the proposed barrier. Of 570 ranges north of the mouth, 390 (64.4%) penetrate the mouth of the Gulf. Of all ranges south of the mouth (those straddling 20º-north latitude), 80.7% penetrate the mouth of the Gulf (Fig. 7). Therefore, the majority of gastropod geographic ranges are not limited by the mouth of the Gulf, but a certain number do appear to be influenced in their range limits.

4) Diversity

Total species diversity was tallied for six sliding window analyses: (1) 500 km/250 km; (2) 1,000 km/250 km; (3) 2,000 km/500 km; (4) 9º/5º; (5) 18º/5º; (6) 5º/5º (Tables 1-5). Each of these window analyses, except the 9º analysis, shows that the greatest diversity is in the Gulf (Fig. 8). The 9º analysis shows Panama Bay to have the highest diversity with the Gulf a close second.

Discussion

The hypothesis that the Gulf of California is insular predicts relatively small range sizes, elevated endemism, and low diversity in the Gulf. It also requires that a barrier to dispersal in and out of the Gulf exists. The window analyses performed here, falsify the hypotheses that range sizes are reduced relative to ranges found outside of the Gulf, that there is an elevated level of endemism in the Gulf, and that TEP diversity is actually greatest in the Gulf. The presence of a barrier at the mouth of the Gulf is ambiguous given the tests performed here.

The confining nature of insularity can be expected to limit range sizes to suitable habitats available within the boundaries of the barriers. Sliding window analyses show that range sizes in the Gulf are comparable to range sizes outside of the Gulf. The fact that Gulf ranges are not smaller than those outside of the Gulf does not necessarily suggest that the Gulf is not isolated. The Gulf's parallel coastlines may expand tolerable habitats across the Gulf and promote the expansion of endemic ranges, or the size of the Gulf may be enough that typical TEP range size is not constrained. If range sizes were smaller than those found

in surrounding regions, then it could be said that range size is being constrained. Given these results, range sizes in the Gulf are not different from the rest of the TEP.

Endemism in the Gulf of California is not special relative to the rest of the TEP shores. The sliding window analyses reveal similar levels of endemism in and out of the Gulf. The consequences of this biogeographic pattern are significant because any statement about the level of endemism in the Gulf is meaningless unless it is accompanied by an evaluation of the level of endemism outside of the Gulf. For instance, a claim of 17% endemism for a Gulf taxon is not special if the surrounding habitat of equal size also houses 17% endemism. If, on the other hand, the Gulf had twice as many endemic taxa as similar sized portions of the TEP, then this would imply that some mechanism unique to the Gulf is influencing the Gulf fauna differently than that of neighboring faunas. Clearly, any study of endemism in the Gulf of California or any other region suspected of insularity, must be accompanied by a comparative analysis of endemism for similar taxa and habitats.

All of the sliding window analyses reveal that the highest TEP diversity is found in the Gulf (Fig 8). The same is true for the eastern Pacific diversity profiles from Roy et al. (1998) (Fig. 9). The expectation that diversity is reduced by insularity is not found in the Gulf; in fact, Gulf diversity is the highest of the entire eastern Pacific coast. Clearly, the Gulf cannot be considered isolated based on its elevated levels of diversity.

The results of the range endpoint analysis and the test of the efficacy of a barrier at the mouth of the Gulf contradict one another. The endpoint analysis indicates that many ranges do end near the mouth of the gulf, but the barrier test shows that most ranges, especially from the south, enter the Gulf.

The range endpoint profile associated with the Gulf reveals six localities with conspicuously elevated endpoints: Cabo San Lucas, La Paz, Puerto Peñasco, Bahia San Carlos, Guaymas, and Mazatlán (Fig. 6). Not coincidentally, these are the most populated and sampled localities in the Gulf. Mazatlán and Cabo San Lucas lie at the boundary of the proposed barrier and represent the highest and third highest collections of endpoints, thereby supporting the presence of a barrier. However, these numbers may be more a reflection of sampling bias rather than some natural process affecting distributions.

A falsifying test of the effect of a barrier at the mouth of the Gulf is to calculate the percentage of ranges unaffected by a barrier, i.e. those that enter the Gulf. 64.4% of the taxa from the Pacific side of the Baja peninsula penetrate the mouth of the Gulf. This is a conservative estimate because 96 of all ranges crossing the tip of the peninsula are part of the list of previously discussed vague range descriptions, which do not mention whether or not any part of the range occupies the Gulf. Consequently, the number of ranges penetrating the Gulf from the north is certainly higher than 64%. Over 80% of the ranges south of the Gulf enter the Gulf. Given the high percentage of ranges unaffected by the proposed barrier at the mouth of the Gulf of California, any barrier that may exists has little influence on the gastropod ranges from the south. More distributional data of

other taxa need to be examined before a definitive declaration regarding the presence of a barrier can be made.

The potential problems with the sliding window analyses, Rapoport's rule, latitudinal diversity, and the overlapping coastlines associated with the Gulf, do not bias the analyses herein. However, in determining the extent of latitudinal diversity and the potential affects it might have on the sliding window analyses, two interesting features emerge: First, based on center-of-origin arguments (Croizat, 1974), the spike in latitudinal diversity associated with the Gulf of California would suggest that eastern Pacific molluscs originated in the Gulf. A more reasonable argument is that elevated diversity is primarily due to the additional habitat provided by the Baja peninsula. Second, Latitudinal diversity of the eastern Pacific, which has been described as a gradient (Stevens, 1989; Roy et al. 1994), is better described as a latitudinal step.

The greatest molluscan diversity of the eastern Pacific is found at the Gulf of California (Roy *et al*., 1998). By arguments of the center-of-origin hypothesis, the Gulf should be the birthplace of eastern Pacific molluscs. However, the formation of the Gulf (13 Ma) is relatively recent relative to the molluscan fossil record of the eastern Pacific (500 Ma). Ricklefs and Schulter (1993) counter this type of evidence against center-of-origin by arguing that "Moreover, because of the dynamic nature of the earth's surface, one expects major taxa both to undergo wholesale shifts and to colonize new areas where they may proliferate. As taxa shift, they may carry their centers of origination, if not centers of origin, with them." This argument undermines the utility of the center-of-origin concept,

for if levels of diversity determine the center of origin and that diversity moves great distances, then which method do we apply to find the original center of origin? Based on diversity patterns generated by sliding window analyses in this study, the two additional coastlines of the Baja peninsula are responsible for the elevated diversity estimates for latitudes intersecting the Gulf. For, if the additional diversity contributed by the Baja peninsula is ignored, latitudinal diversity for Gulf latitudes is comparable to the rest of the TEP (fig. 5).

The estimations of latitudinal diversity for this study do not conflict with the latitudinal diversity profile for the TEP estimated by Roy et al. (1998). However, they describe their latitudinal diversity profile as a gradient, it is more accurately described as a latitudinal step; between 35º and 30º north, eastern Pacific species diversity jumps more than 350% from 200 species to over 900 species. North of these latitudes, diversity does not vary more than 25% except at the Aleutians, which is primarily parallel to latitude and therefore contains much more habitat per degree latitude than the rest of the coast. South of this step, diversity is level to Colombia, where diversity again steps down by over 40% (Fig. 9). The latitudinal diversity profile estimated by Roy et al. for the western Atlantic has an even more pronounced step-like profile. These profiles and the one here, suggest that a series of thresholds control latitudinal diversity. The concordance of latitudinal threshold positions in the Pacific and Atlantic are striking and may be a clue to significant biological processes associated with latitude. A shift in perspective from viewing latitudinal diversity as a gradient to a series of threshold

points might open the door for new questions about controls on biological diversity.

Range sizes, the level of endemism, and diversity, do not support the hypothesis that the Gulf of California is isolated. These results emphasize the need for careful use of the term “endemism.” At some level everything is endemic, but when is it special relative to its surrounding provinces? Based on the results from this study, there is no evidence that the Gulf fauna is any different from that of the rest of the tropical eastern Pacific.

Tables

500 KM window/250KM					
Window #	Window position[1]	Total	Endemic	% Endemic	Gulf
1	1 to 7	720	79	10.97%	
2	4 to 14	842	99	11.76%	
3	7 to 22	844	72	8.53%	Gulf
4	14 to 29	897	28	3.12%	Gulf
5	22 to 34	1001	50	5.00%	Gulf
6	29 to 42	1140	52	4.56%	Gulf
7	34 to 44	1077	21	1.95%	Gulf
8	42 to 47	1124	81	7.21%	Gulf
9	44 to 52	1164	104	8.93%	
10	47 to 54	1052	40	3.80%	
11	52 to 56	1006	25	2.49%	
12	54 to 61	1019	42	4.12%	
13	56 to 65	1019	40	3.93%	
14	61 to 71	1047	53	5.06%	
15	65 to 75	1084	64	5.90%	
16	71 to 81	1103	54	4.90%	
17	75 to 86	1188	98	8.25%	
18	81 to 90	1185	151	12.74%	Panama
19	86 to 94	961	35	3.64%	
20	90 to 97	817	42	5.14%	
21	94 to 104	837	55	6.57%	
22	97 to 111	858	144	16.78%	
23	104 to 119	732	139	18.99%	
	Total Weighted AVE			6.90%	
	Gulf Weighted AVE			5.00%	

Table 1. The numbers of ranges wholly and partially contained by each window in the 500 km sliding window analysis. Windows 3-8 include the Gulf. Weighted averages for the Gulf were calculated by dividing the sum of all Gulf ranges restricted to each window (Endemic) by the sum of all Gulf ranges in contact with each window (Total). The weighted average for the TEP was calculated the same way.

[1]Window position numbers refer to locality numbers in the database (see fig. 2 for an example of locality numbers associated with the gulf).

1,000K window/250K

Window #	Window position[1]	total	endemic	% endemic	Gulf
1	1 to 14	862	122	14.15%	
2	4 to 22	908	114	12.56%	
3	7 to 29	982	102	10.39%	Gulf
4	14 to 34	1022	64	6.26%	Gulf
5	22 to 42	1184	89	7.52%	Gulf
6	29 to 44	1089	57	5.23%	Gulf
7	34 to 47	1237	113	9.14%	Gulf
8	42 to 52	1183	105	8.88%	
9	44 to 54	1182	116	9.81%	
10	47 to 56	1057	42	3.97%	
11	52 to 61	1039	45	4.33%	
12	54 to 65	1045	46	4.40%	
13	56 to 71	1092	73	6.68%	
14	61 to 75	1103	75	6.80%	
15	65 to 81	1148	84	7.32%	
16	71 to 86	1238	130	10.50%	
17	75 to 90	1209	195	16.13%	
18	81 to 94	1188	154	12.96%	Panama
19	86 to 97	965	47	4.87%	
20	90 to 104	871	66	7.58%	
21	94 to 111	895	147	16.42%	
Total Weighted AVE				8.83%	
Gulf Weighted AVE				7.71%	

Table 2. The numbers of ranges wholly and partially contained by each window in the 1,000 km sliding window analysis. Windows 3-7 include the Gulf. Weighted averages for the Gulf were calculated by dividing the sum of all Gulf ranges restricted to each window (Endemic) by the sum of all Gulf ranges in contact with each window (Total). The weighted average for the TEP was calculated the same way.

[1]Window position numbers refer to locality numbers in the database (see fig. 2 for an example of locality numbers associated with the gulf).

9º window/5º

Window #	Window position[1]	total	endemic	% endemic	Gulf
1	1 to 21	921	125	13.57%	
2	7 to 29	982	102	10.39%	Gulf
3	22 to 41	1180	84	7.12%	Gulf
4	32 to 47	1247	118	9.46%	Gulf
5	43 to 54	1194	117	9.80%	
6	49 to 70	1157	81	7.00%	
7	56 to 89	1346	306	22.73%	
8	72 to 95	1252	243	19.41%	
9	91 to 109	912	127	13.93%	
10	99 to 119	874	163	18.65%	
	Total Weighted AVE			13.25%	
	Gulf Weighted AVE			8.92%	

Table 3. The numbers of ranges wholly and partially contained by each window in the 500 km sliding window analysis. Windows 2-4 include the Gulf. Weighted averages for the Gulf were calculated by dividing the sum of all Gulf ranges restricted to each window (Endemic) by the sum of all Gulf ranges in contact with each window (Total). The weighted average for the TEP was calculated the same way.
[1]Window position numbers refer to locality numbers in the database (see fig. 2 for an example of locality numbers associated with the gulf).

18º window/5º

Window #	Window position[1]	total	endemic	% endemic	Gulf
1	1 to 41	1370	323	23.58%	
2	7 to 46	1443	257	17.81%	Gulf
3	22 to 53	1412	239	16.93%	
4	32 to 63	1366	197	14.42%	
5	42 to 78	1387	227	16.37%	
6	48 to 94	1443	357	24.74%	
7	56 to 104	1420	398	28.03%	
8	71 to120	1394	465	33.36%	
	Total Weighted AVE			21.92%	
	Gulf Weighted AVE			17.81%	

Table 4. The numbers of ranges wholly and partially contained by each window in the 500 km sliding window analysis. Window 2 contains the Gulf. Weighted averages for the Gulf were calculated by dividing the sum of all Gulf ranges restricted to each window (Endemic) by the sum of all Gulf ranges in contact with each window (Total). The weighted average for the TEP was calculated the same way.
[1]Window position numbers refer to locality numbers in the database (see fig. 2 for an example of locality numbers associated with the gulf).

2,000 km window/500 km

Window #	Window position[1]	total	endemic	% endemic	Gulf
1	1 to 34	1191	219	18.39%	
2	7 to 44	1295	212	16.37%	Gulf
3	22 to 52	1390	237	17.05%	
4	34 to 56	1319	158	11.98%	
5	44 to 65	1246	150	12.04%	
6	52 to 75	1184	105	8.87%	
7	56 to 86	1338	205	15.32%	
8	65 to 94	1307	277	21.19%	
9	75 to 104	1254	256	20.41%	
10	86 to 119	1093	214	19.58%	
	Total weighted AVE			16.11%	
	Gulf Weighted AVE			16.37%	

Table 5. The numbers of ranges wholly and partially contained by each window in the 500 km sliding window analysis. Window 2 contains the Gulf. Weighted averages for the Gulf were calculated by dividing the sum of all Gulf ranges restricted to each window (Endemic) by the sum of all Gulf ranges in contact with each window (Total). The weighted average for the TEP was calculated the same way.
[1]Window position numbers refer to locality numbers in the database (see fig. 2 for an example of locality numbers associated with the gulf).

5º window/ 5º

º Latitude	Mainland	Peninsula	total
35	923		
30	994*	279	1273
25	1042*	387	1429
20	960		
15	989		
10	1174		
5	703		
0	662		
AVE	930.87vs.986*	333	1351

Table 6. Latitudinal diversity table. For latitudes intersecting the Gulf, ranges restricted to the Baja peninsula were tallied and subtracted from the total diversity at those latitudes. This permits a comparison between the mainland coast of the Gulf* and the rest of the TEP. The average diversity for the Gulf mainland (986*) and the entire mainland coast of the TEP (930) were calculated.
[1]Window position numbers refer to locality numbers in the database (see fig. 2 for an example of locality numbers associated with the gulf).

Figure 1. The orientation of the shoreline associated with the Baja peninsula and the Gulf creates three unique intersections (points 1, 2, & 3) with latitude and biases measures of latitudinal diversity.

1 Isla Cedros
2 Bahía San Bartolomé
3 Punta Abreojos
4 Bahía San Juanico
5 Isla Magdalena
6 Todos Santos
7 Cabo San Lucas
8 Punta Gorda
9 Cabo Pulmo
10 La Paz
11 Isla Espíritu Santo
12 Isla San José
13 Punta San Marcial
14 Puerto Escondido
15 Isla Carmen
16 Loreto
17 Punta Pulpito
18 Bahiá Concepción
19 Mulegé
20 Punta Chivato
21 Santa Rosalía
22 Bahía Las Animas
23 Bahía de los Angeles
24 Isla Angel de la Guarda
25 Bahía Calamajue
26 Bahía San Luis Gonzaga
27 Isla San Luis
28 Puertocitos
29 San Felipe
30 Puerto Peñasco
31 Bahía San Jorge
32 El Desemboque
33 Bahía Tepoca
34 Cabo Tepopa
35 Isla Tiburón
36 Bahía Kino
37 Isla San Pedro Martír
38 Puerto San Carlos
39 Ensanada Bocachibampo
40 Cabo Haro
41 Guaymas
42 Bahía de Yavaros
43 Bahía Topolobampo
44 Altata
45 La Cruz
46 Mazatlán

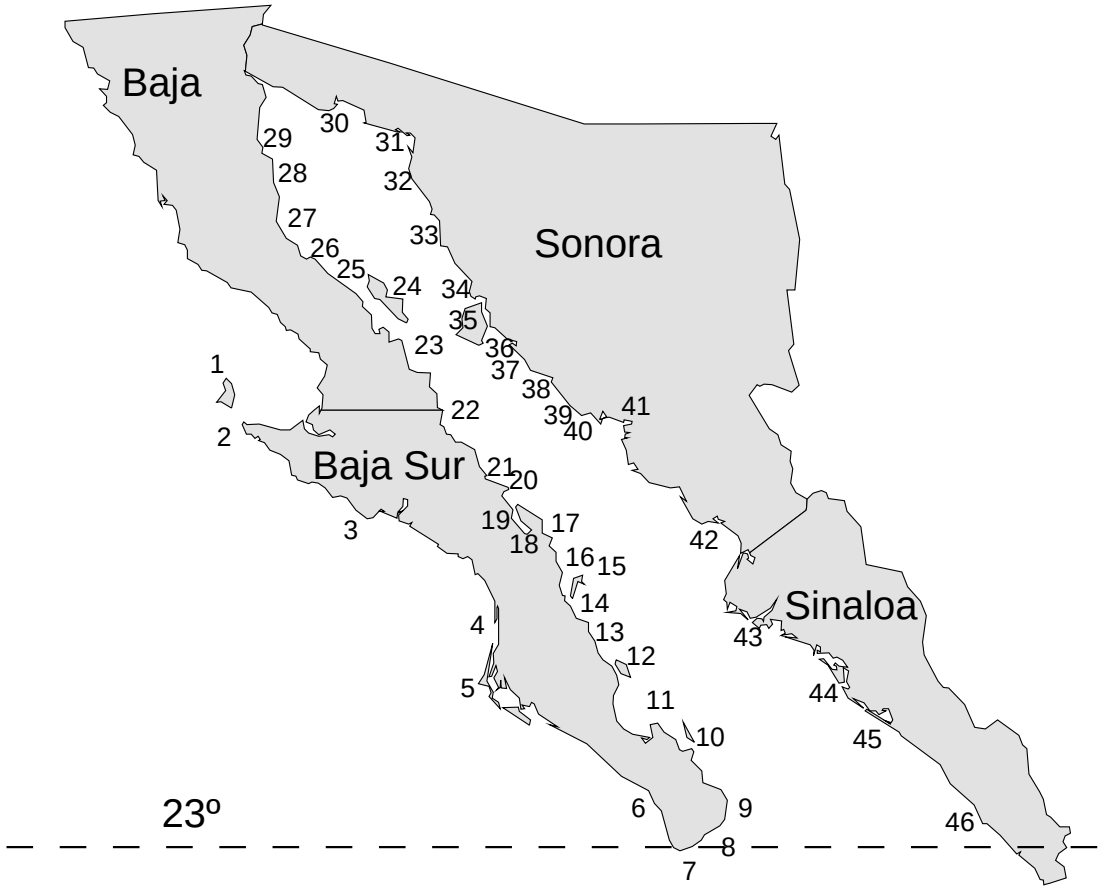

Figure 2. locality mapping system employed to better describe ranges associated with the Gulf of California. Each locality is numbered beginning at Isla Cedros (1) and ending in Mazatlán. The rest of the eastern Pacific is similarly numbered south to Chile.

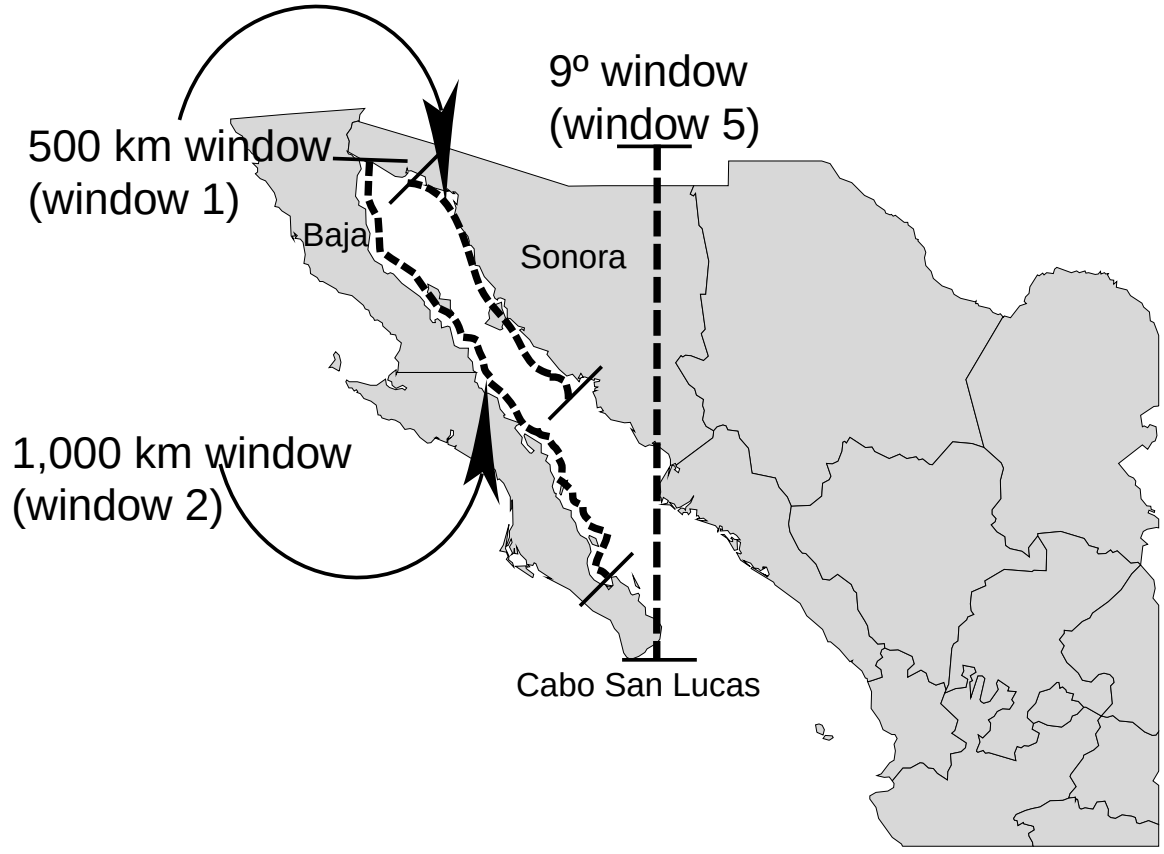

Figure 3. A graphical view of dimensions for windows 1, 2 and 5. Windows 3, 4, and 6 (not pictured) are 2,000 km, 5º, and 18º respectively. Note how the distance windows (1 & 2) conform to the coastline and the latitudinal window (5) does not.

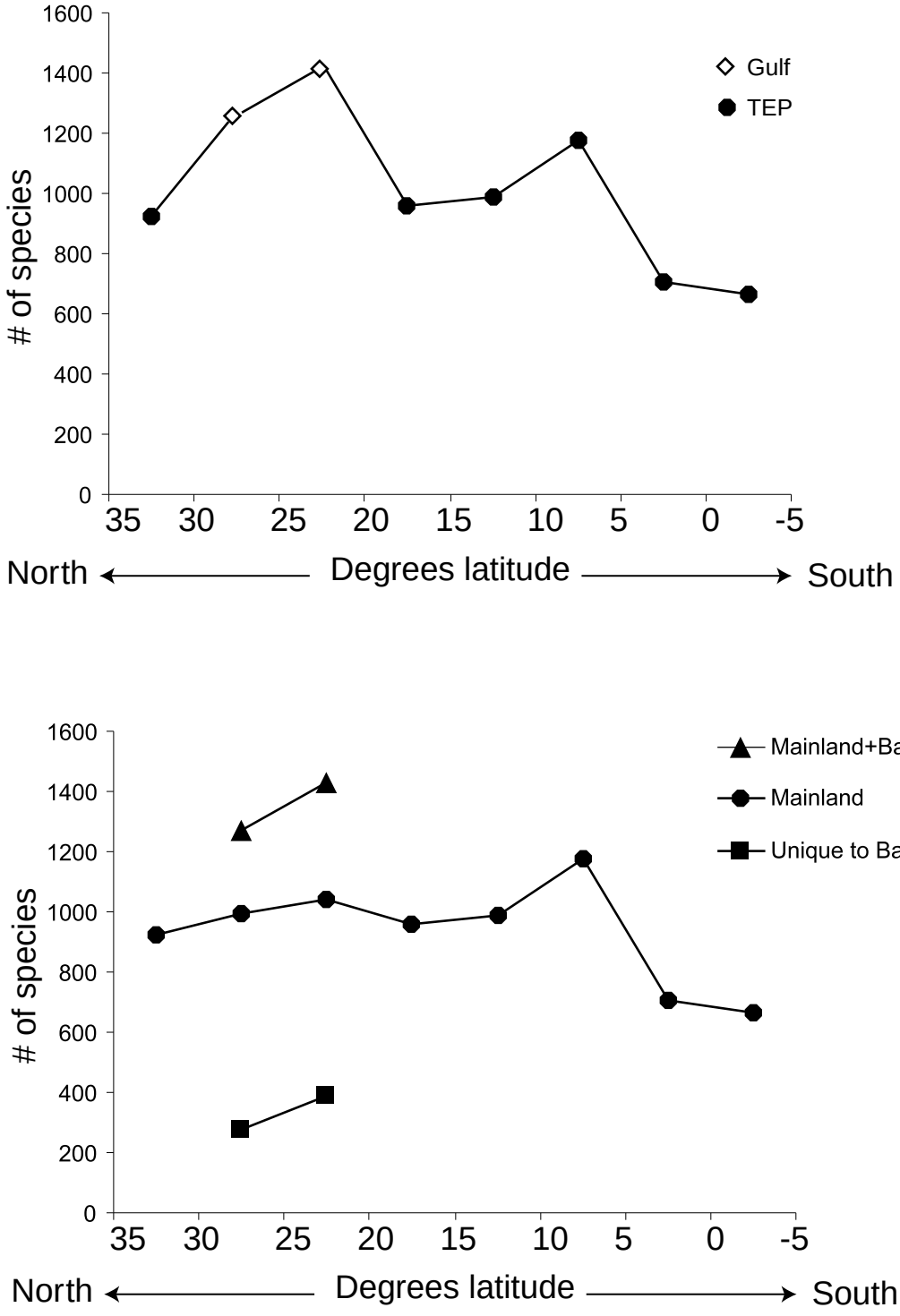

Figure 4. Latitudinal diversity plots generated using the 5º sliding window analysis. Plot A is uncorrected for the Baja peninsula. Plot B accounts for the additional diversity created by the Baja peninsula.

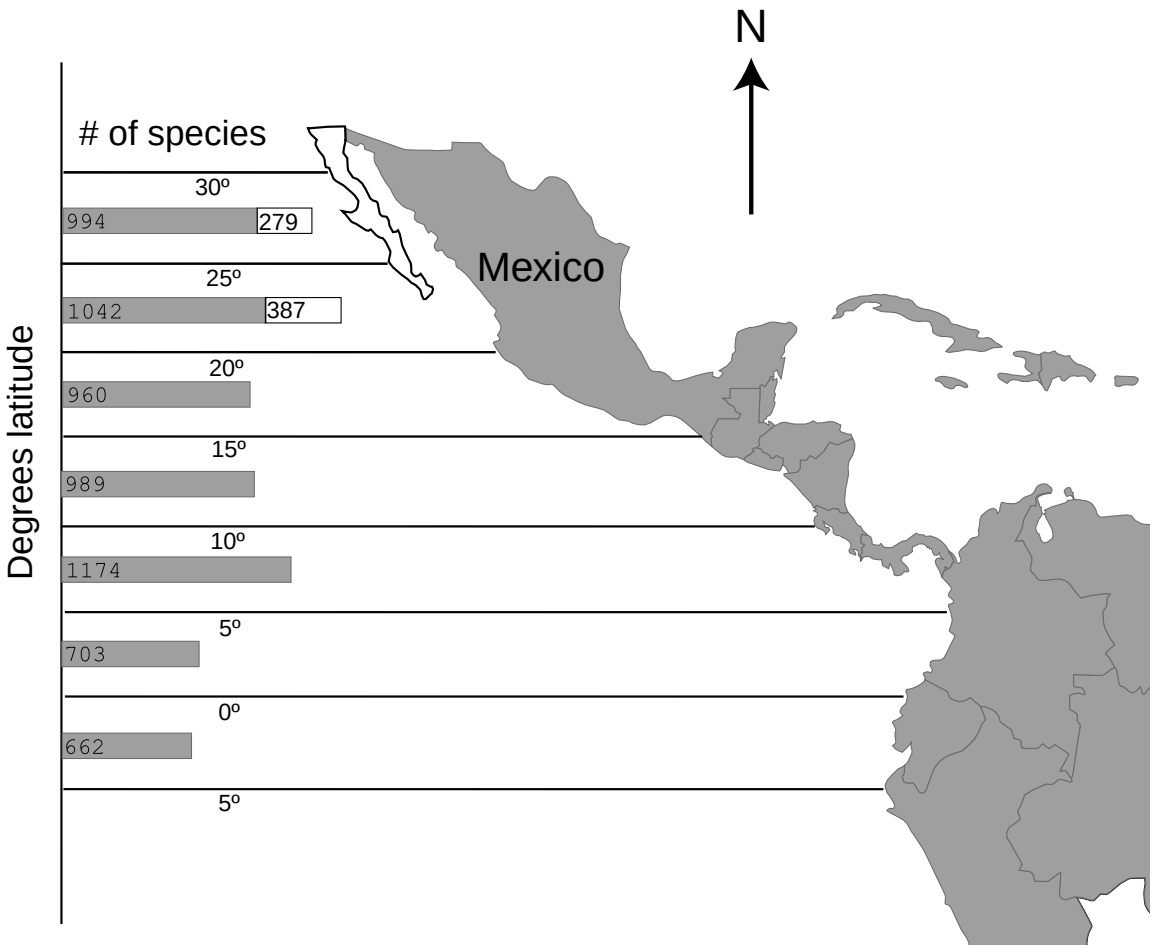

Figure 5. A graphical representation of the latitudinal diversity added to the mainland shores of the TEP. The unshaded bars represent latitudinal diversity unique to the Baja peninsula.

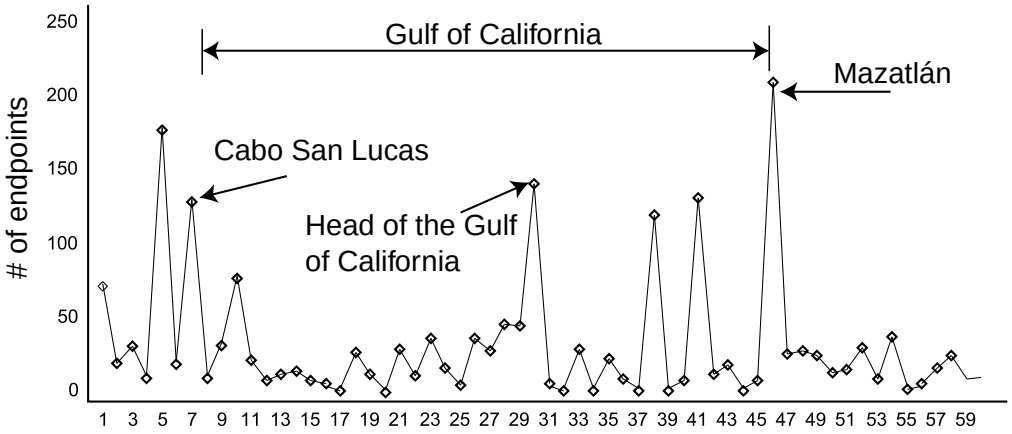

Figure 6. A plot of accumulated range endpoints associated with the Gulf of California. The x-axis represents locality numbers from figure 2.

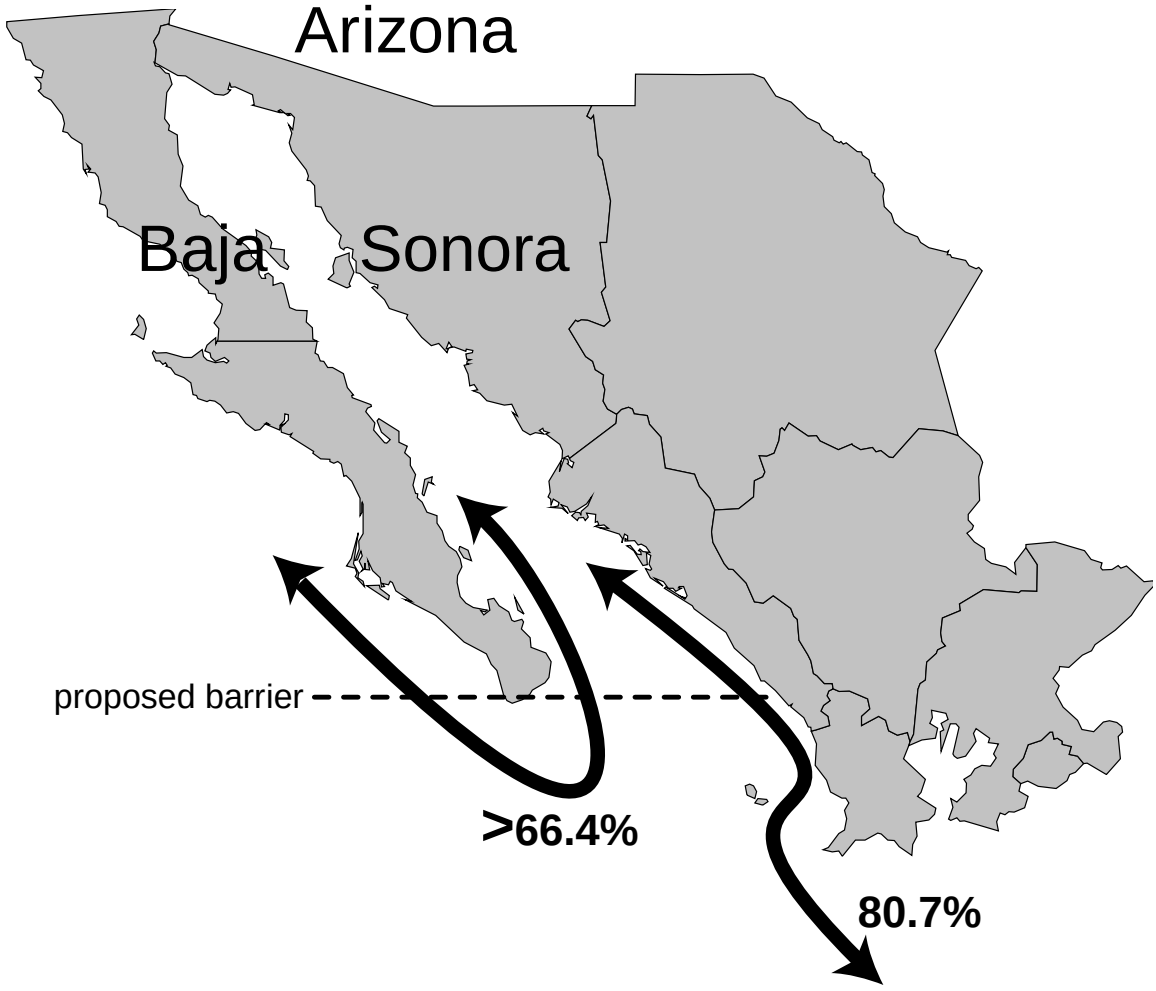

Figure 7. A graphical representation of the percentage of ranges from the north and south, which are not influenced by the mouth of the Gulf.

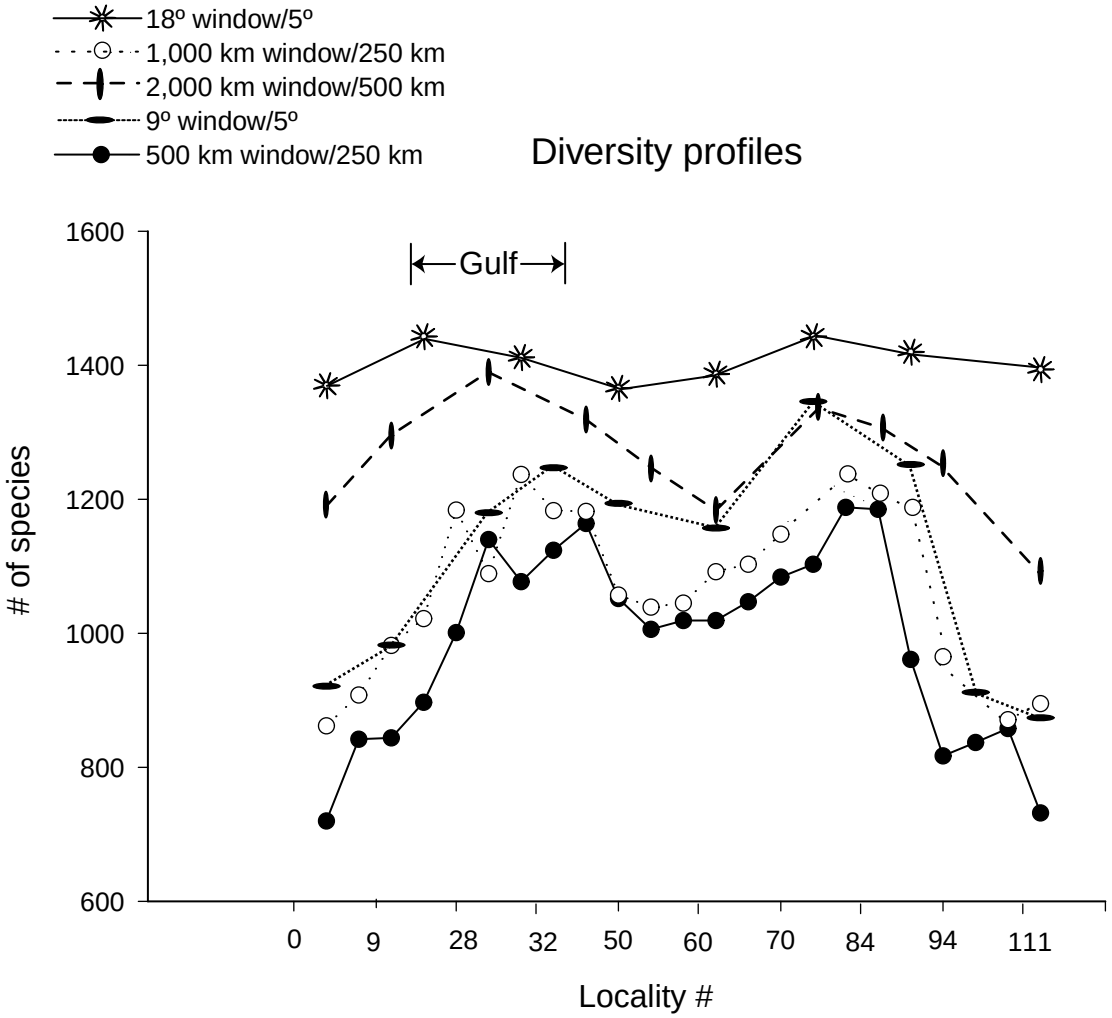

Figure 8. A plot of the diversity profiles generated by the 5 sliding window analyses. These analyses differ from the latitudinal diversity analysis in that they slide along the interior coastline of the Gulf and around the Baja peninsula, thereby counting ranges throughout the Gulf rather than counting ranges along a single latitudinal line, which intersects the three coastlines of the Gulf.

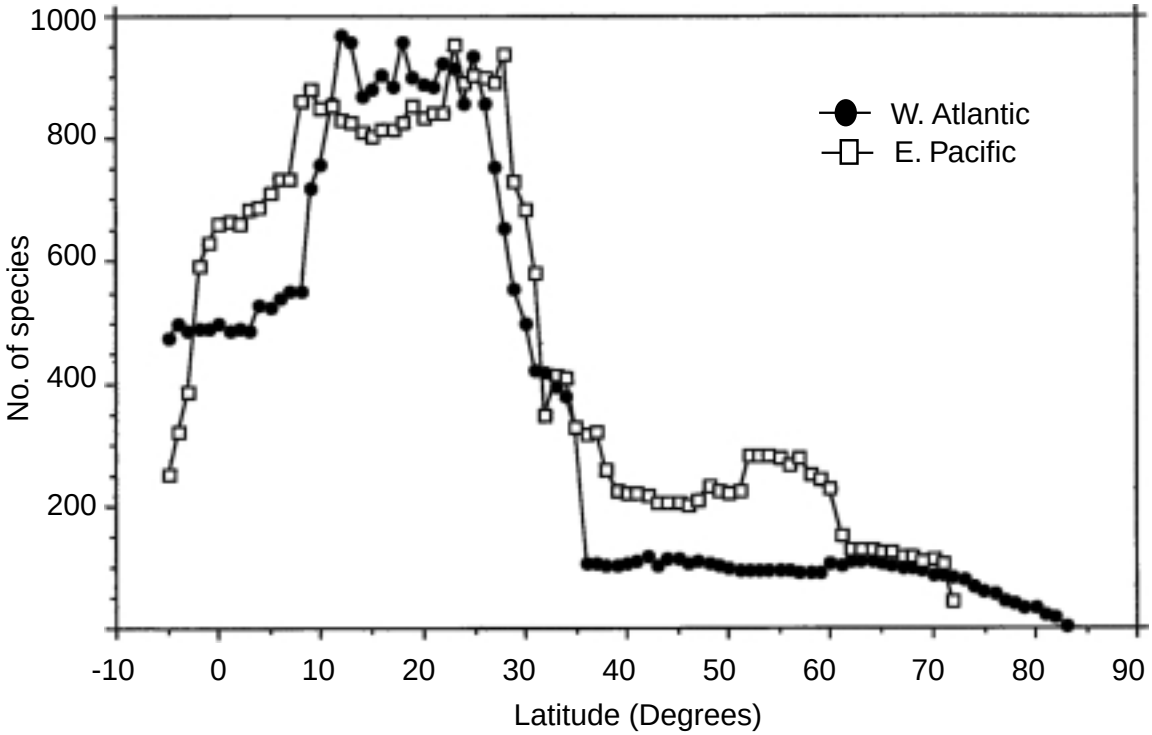

Figure 9. Latitudinal diversity profiles for the western Atlantic and eastern pacific redrawn from Roy et al. (1998). The latitudes examined in this study are between 30º N to 10º S. From 10º to 30º N. the profile is relatively flat.

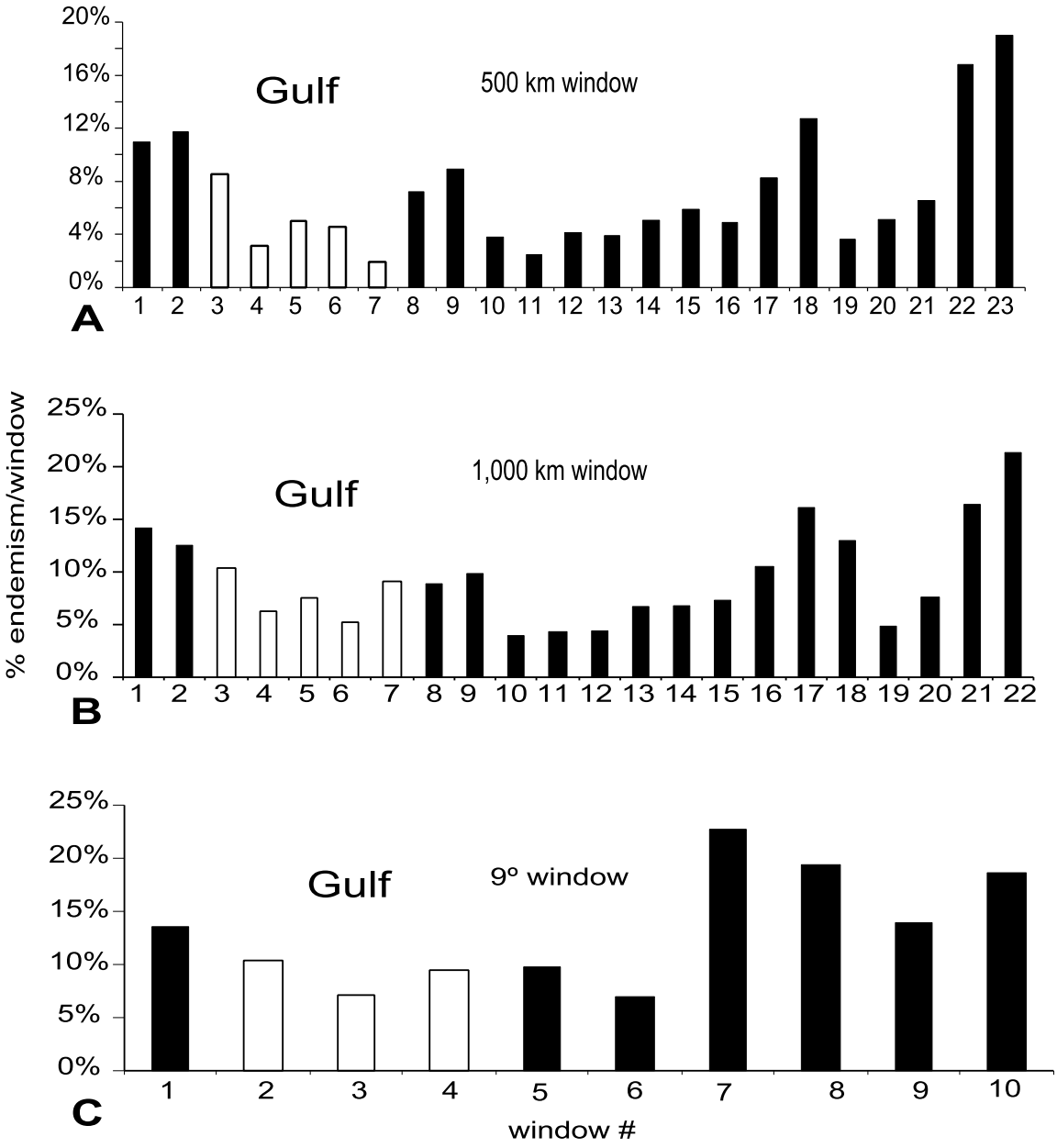

Figure 10. Plots of data from tables 1, 2, and 3. Each of these window analyses reveals the level of endemism per window for the Gulf and the rest of the TEP. If Gulf ranges were smaller than those of the TEP, then more ranges would be expected to fit into each window and we would see greater endemism per window.

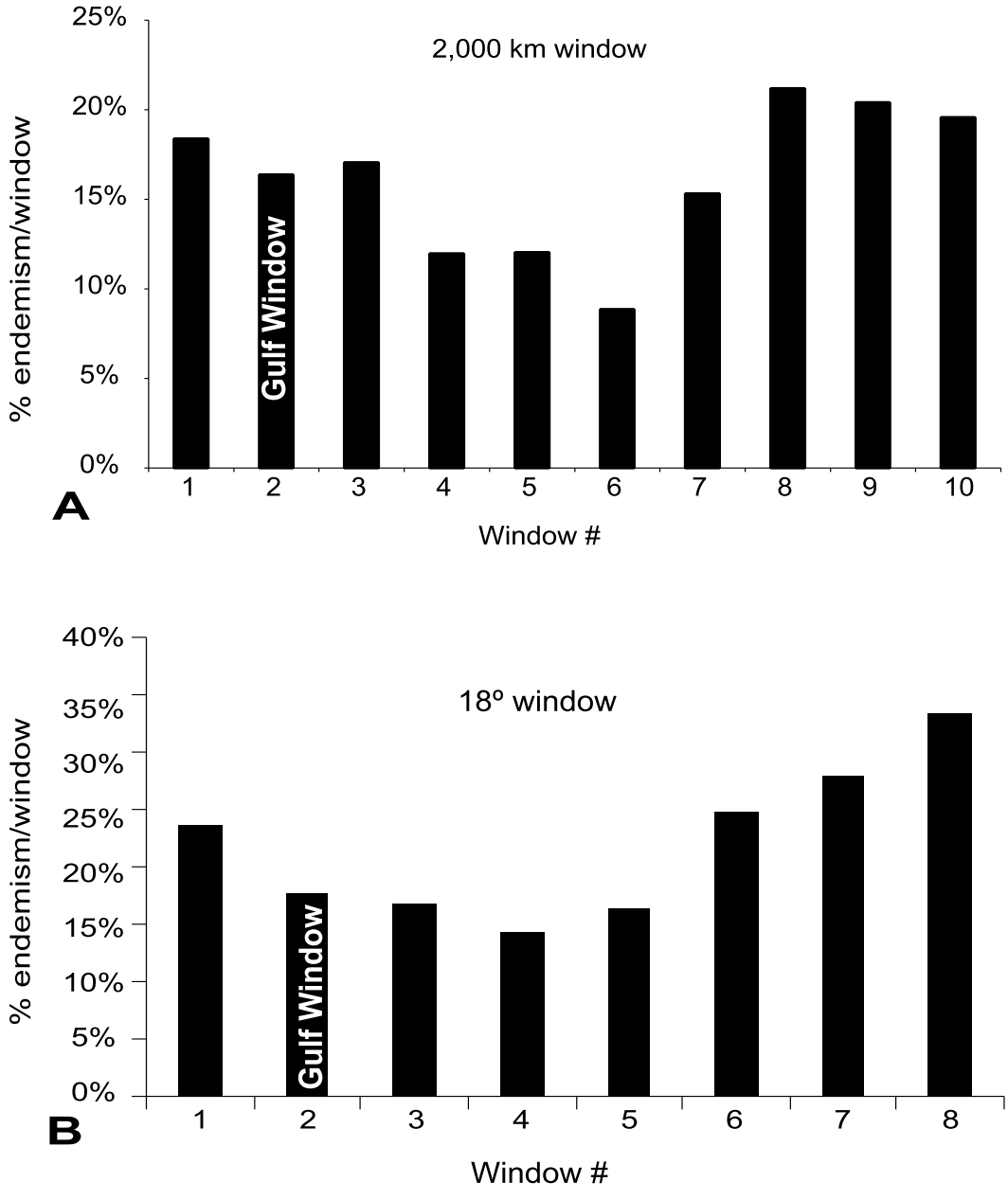

Figure 11. Plots from tables 4 & 5. This graph shows the comparison of total Gulf endemism to the rest of the TEP for both the 2,000 km analysis and the 18º analysis. The window sizes for each analysis approximate the total coastal length of the Gulf.

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Chapter Two

Data Compatibility and Evolutionary Relationships of New World Patellogastropds

Introduction

Gastropod taxonomy is as old as Linnean taxonomy itself; taxonomists have been studying snails for hundreds of years. Traditional malacologists have based taxonomic decisions on character systems that they thought best reflected the essentialistic “natural kinds“. For example, Cuvier (1817), Gray (1833), Edwards (1848) and Morch (1865) based their taxonomic decisions on the gastropod respiratory system; deBlainville (1824) used the reproductive system; von Ihering (1876) and Spengel (1881) used the nervous system; and Perrier (1889) used the renal system. Thiele (1903; 1929-31) was the first to synthesize the information from these different character systems to produce a single classification scheme. However, this was far from a total evidence approach, he used different character systems to classify different classification levels. Thiele’s classification still influences decisions about gastropod phylogeny (Bieler, 1992, 1992; Ponder & Lindberg, 1997).

The use of phylogenetic relationships and tree thinking in biological sciences continues to grow each year. This is particularly true in evolutionary biology. Few doubt the utility, or rather the necessity, of studying the patterns and processes of biology in light of evolutionary history. However, in the case of gastropod biology, most contemporary malacologists continue to study biology

through traditional methods, which have taught us most of what we know about gastropod biology. Their methods will continue to reveal important facts and make substantial contributions to malacology and biology in general. However, the current systematization of molluscan diversity utilizes the Linnean system, which is based on essentialistic principles and the immutability of species; the antithesis of what we know about evolution today. The most important requirement for malacology to join the Darwinian revolution is to understand the phylogenetic relationships of molluscs and use this information to advance our knowledge of one of the most diverse groups of organisms on earth. This study takes a small step towards this goal by using modern phylogenetic methods to reconstruct the phylogenetic history of New World limpets (Patellogastropoda).

Many new techniques like scanning electron microscopy, advances in histology, chemical and staining analyses, and developmental techniques have broadened our understanding of gastropod morphology, behavior, development, and evolution. While there will always be more to learn from further investigations of gastropod morphology, molecular data provide a new and independent source of information. Collins (1996) and Hellberg (1998) have recently shown that molecular data provide a new and robust source of phylogenetic information for gastropods. Because of the success of these studies and others, molecular data were employed here to study patellogastropod evolution.

Limpets serve as an ideal system for molecular studies because they are globally ubiquitous, easily collected, and morphologically simple. Their presence on every rocky shore of the world makes them an ideal model system for

phylogeographical studies. Limpets are primarily intertidal, diurnal, and they feed on algae of exposed rock surfaces making them very easy to collect. Morphologically, limpets are simple and difficult to differentiate. This has often led to artificial taxonomic groupings, which molecular studies may help to resolve.

A few morphological phylogenies have recently been published placing the Lottidae (Gray, 1840) genus Lottia (Sowerby, 1834) as sister to the Patelloida (Quoy and Gaimard, 1834) (Guralnick & Smith, 1999). The New World near shore limpets are almost exclusively Lottia, with the exception of the topical limpets *Patelloida pustulata, Patelloida semirubida,* and *Patella mexicana,* which are described as a Lottidae and Patellidae (Rafinesque, 1815) respectively. Patellidae are most common in the tropical south Pacific and throughout the east Atlantic. *P. mexicana*, the only New World Patellidae, may be extinct, there have been no published sightings since 1972 and in spite of 6 consecutive collecting seasons, I have never encountered a live specimen. *P*. *mexicana* is the largest of all limpets and has been reported as a common food source for humans along its tropical range (Keen, 1971) .

Based on published phylogenetic data, the New World lottiids are rooted with Patelloida exemplars from Australia and the Caribbean. The Ponder and Lindberg (1999) and the Guralnick and Smith (1999) morphological phylogenies of gastropods place the patellogastropod Cellanidae outside both Patelloida and Lottia, therefore, an Australian exemplar from the Cellanidae is included as an additional outgroup.

In choosing appropriate molecular markers for this project, many factors were considered. Cladistic methods of phylogenetic reconstruction require specific kinds of data and considerations. Markers must be homologous among all OTUs because of speciation events (orthologous) rather than gene duplication events (paralogous). It must also be ensured that the sequences have not been involved in the horizontal transfer of genetic material (xenologous). A paralogous history will result in a confusion of genealogy and phylogeny. Markers must be selected that are informative at the phylogenetic level of interest. In the case of eastern Pacific patellogastropods, two events bracket the time frame of interest. Fossil evidence indicates early Miocene origins (Lindberg and Marincovich, 1988) of the taxon, while the emergence of the Isthmus of Panama (3.5 Ma) played a more recent role in the biogeography of the eastern Pacific by dividing the formerly continuous New World Tropical Ocean and its fauna. Other considerations in choosing molecular markers are concerted evolution, gene conversion, lineage sorting, pseudogenes and ancestral polymorphism. Each of these phenomena can contribute to poor phylogenetic estimations.

Organellar and nuclear markers have different modes of inheritance and thus have different evolutionary characteristics. In metazoans, mitochondrial DNA (mtDNA) is maternally inherited, does not experience allelic segregation or crossover, generally evolves faster than nuclear DNA and is more useful for resolving relationships of closely related taxa.

Molecular systematists have already evaluated many genes in terms of their utility for particular phylogenetic questions and have published universal primer sequences. Primary literature searches of successful DNA extraction and amplification in gastropods and discussions with colleagues narrowed the search for useful genes to four prospects, the large (16S) and small (12S) subunit ribosomal RNA in mitochondria, cytochrome oxidase I, and cytochrome b. All of these are mitochondrial genes and have similar evolutionary rates. The decision to go with COI and 16S from the four prospective genes was based primarily on the greater amplification success of the universal primers for COI and 16S for patellogastropods.

There are still many debates about how different sources of phylogenetic data are to be treated in a phylogenetic analysis. There are two obvious approaches, use all the data in a single analysis (Kluge, 1989), or analyze the data sets separately (Miyamoto & Fitch, 1995). Often, phylogeneticists do both. However, the decision of what to do with the results of both approaches has only recently been dealt with. Bull et al. (1993), (de Queiroz, 1993), and (Rodrigo *et al*., 1993) have argued that the combinability of data from different sources should be evaluated prior to phylogenetic analyses. Here, COI and 16S are used to further investigate the utility of different combinability approaches with the added complication of how uneven sample sizes for the different data sets affect the final results.

Materials and methods

Collection and Preservation

Locality, habitat, date, preservation, and collector information were recorded and specimens were preserved in 500 ml Nalgene bottles or Ziploc bags half filled with 70% ETOH.

Sorting

To avoid introducing bias, a priori knowledge of limpet taxonomy was intentionally avoided during the collection phase. Limpets were collected based on the slightest differences in appearance and habitat and many specimens of each were collected. Specimens were sorted into morphological groups based on shared soft anatomy and shell features, which included radular differences, tissue features, shell texture, color, size, patterns, composition, and irregularities. Because of the possibility that any morphological grouping represents more than a single lineage, genomic DNA was extracted from at least three individuals from every morphological group. If there were three or fewer individuals per group, then genomic DNA was extracted from all available specimens. This sampling approach was used in the hope that any cryptic species, misnamed species or new species might be discovered. If specimens had been collected based on

published identification keys, a bias would have been introduced and there would thus have been little chance of discovering new taxonomic information.

DNA Extraction

Two equally successful DNA isolation protocols were used: Saturated salt/chloroform extraction, and CTAB/phenolchloroform extraction. For each extraction, pedal tissue was cut from the foot margin approximately 3-5 mm along the margin and 3-5 mm towards the center of the foot. The tissue was soaked in deionized water to remove any residual ethanol and finely diced using flame sterilized forceps and scalpel blades.

Saturated Salt/Chloroform Extraction

The diced tissue was digested in a 1.5 ml tube containing 250 µl isolation buffer (100 mM TRIS, 10m M EDTA and 400 mM NaCl), 60 µl 10% SDS, and 10 µl proteinase K. The mixture was then vortexed and stored in an incubated shaker at 37º C overnight. Following tissue digestion, 175 µl of saturated NaCl solution was added. The samples were inverted for 5 minutes and spun at 12g for 30 minutes. The supernatant was washed with chloroform using 2 times supernatant volume and mixed by inversion for 2 minutes. The supernatant DNA was precipitated using two volumes of ice cold 100% ethanol and spun at 12g for 15 minutes. The DNA pellet was washed twice with two volumes of 70% ethanol, and dried for five minutes in a speed vac. The DNA was eluted in 100 µl of double-distilled water and stored at -20ºC.

2xCTAB/Phenolchloroform Extraction

DNA extractions were performed using a CTAB/PHENOL extraction described by Palumbi (1996). The diced tissue was added to a 1.5 ml tube containing 600 µl 2xCTAB and 9 µl of proteinase K and then incubated at 37ºC for approximately 12 hours. 600 µl of phenol:chloroform:isoamyl alcohol (25:24:1) was then added to this solution and mixed via inversion for five minutes. The solution was then centrifuged at 12g for 15 minutes. The supernatant was added to 600 µl of chloroform:isoamyl alcohol (24:1), mixed for five minutes, and centrifuged at 12g for 15 minutes. DNA was precipitated using 600 µl isopropanol and stored at -20ºC for two hours. The precipitate was centrifuged at 12g for 30 minutes at 4ºC. The pellet was washed twice with 70% ethanol and centrifuged at 12g for 20 minutes. The DNA was dried using a speed vacuum for five minutes and eluted in 100 µl of de-ionized water. Three microliters of each sample was loaded on a 1% agarose electrophoreses gel for confirmation of extracted genomic DNA.

PCR

Amplification of portions of the mitochondrial genes 16S and cytochrome c oxidase Subunit I (COI) was achieved with the 16S-AR and 16S-BR primers (Palumbi, 1996), and (Kocher *et al*., 1989) and the HCO-2193 and LCO-1490

primers described by Folmer *et al*. (1994). In an attempt to amplify genomic samples that were not successfully amplified with the Folmer primers, the HCO and LCO primers were modified into degenerate primers, as follows:

Folmer et al. (1994) COI primers:

```
Codon position       231231231231231231231231
     LCO1490 5′-GGTCAACAAATCATAAAGATATTGG-3′
     HCO2198 5′-TAAACTTCAGGGTGACCAAAAATCA-3′
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Degenerated Folmer COI primers:

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Codon position         231231231231231231231231
     dgLCO1490 5′-GGTCAACAAATCATAAAGAYATYGG-3′
     dgHCO2198 5′-TAAACTTCAGGGTGACCAARAAYCA-3′
```

COI is a coding gene and the HCO and LCO primers lie within the coding region. A degenerative primer is essentially a mix of different oligonucleotides, each representing a different codon sequence for the amino acid selected to be degenerated. The extension phase of DNA duplication proceeds in the 5’ to 3’ direction. A mismatch of primer and template near the 3’ end of the primer is the most likely location for failed extension. If the 3’ end of the primer is weakly bound to the DNA template, the first nucleotide to be added will have difficulty binding to the primer and consequently result in the failure of the cyclic polymerase chain reaction. The HCO primer is the reverse compliment of the 3’ end of the HCO/LCO target region for COI, so the reverse compliment of the HCO must be evaluated in order to identify potential degenerate codons. For HCO, the extension end of the primer codes for tryptophan, which has a degeneracy level of two, TGA and TGG. The HCO primer uses the TGA codon.

A degenerative primer containing equal concentrations of oligonucleotides for both tryptophan codons was designed. Because it is impossible to know which primer is mismatched, a degenerative LCO primer was also degenerated.

Most PCR reactions followed the same reagent proportions. A series of solutions were added to an iced 0.5 ml tube: (1) 36.45 µl double-distilled water. (2) 5 µl 10x PCR buffer (Perkin Elmer). (3) 2.5 µl 10 µM dNTP's (Pharmacia). (4) 2.5 µl 25 µM $MgCl_2$ (Perkin Elmer). (5) 1 µl each of 10 µM 16S-AR and 16S-BR primers or 10 µM COI-HCO and COI-LCO primers. (6) 1 µl of template (usually undiluted, but occasionally at 1:100 dilutions). And (7) 0.25 µl of taq (Perkin Elmer). A negative control containing all of these reagents (excluding the template) was also prepared. All tubes were transferred to a Perkin Elmer 9600 geneamp thermal cycler.

The cycling parameters include an initial denaturation at 95ºC for two minutes followed by 36 cycles with three temperature plateaus of 95º C for 50 seconds, 39º-54º C for 50 seconds, and 72º C for 90 seconds. The cycling protocol ended with a seven minute extension at 72º C. Target amplification success was determined by running the reactions on a 1.5% agarose gel against a 100kb DNA ladder. If the reactions produced very weak or non-existent DNA bands, the annealing temperature was relaxed by several degrees depending on the initial annealing temperature and the strength of the weak bands. If the primers targeted more than one region, indicated by multiple bands in the electrophoresis gel, then the annealing temperatures were elevated to increase primer specificity. If neither of these strategies were successful, the primer or

template concentrations were varied. Occasionally the MgCl and dNTP concentrations were varied. If none of these adjustments were successful, new primers were designed.

This troubleshooting approach was very successful for the 16S primers, and moderately successful for the COI primers. Only about 64% of the genomic DNAs extracted for limpet specimens were successfully amplified for COI.

PCR products were purified using Wizard® PCR preps DNA Purification System.

The concentration of purified PCR products for accurate ABI® cycle sequencing was determined by running the purified DNA on a 1.5% agarose gel against a standardized DNA sizing ladder.

Cycle Sequencing

Direct double-stranded cycle sequencing of 20 to 30 ng of purified PCR product was performed in both directions using the ABI® BIG DYE cycle sequencing kit following a half reaction ABI® cycle sequencing protocol. The same primers were used for PCR and cycle sequencing. The cycle sequencing was performed using a Perkin Elmer 9600 geneamp thermal cycler. The cycling parameters included 25 cycles at 96ºC for ten seconds, 50ºC for five seconds, and 60ºC for four minutes. Cycle sequencing product was purified using a 75% isopropanol precipitation, then dried down in a speed vacuum. The dried, purified cycle sequencing product was re-suspended in 4.0 µl 5:1 loading solution of de-ionized Formamide and EDTA (25mM) /Blue Dextran(50mg/ml). 2.0 µl of sample

and loading solution were loaded in a 36 cm 4% acrylamide gel. The gel was run and analyzed on an ABI Prism® 377 DNA sequencer.

Alignment

The alignment of DNA sequences for phylogenetic analysis is essentially the establishment of putative homologies, in a Hennigian sense (i.e., shared, derived characters due to common descent), among the taxa for which sequences are being aligned.

As a starting point, all sequences were simultaneously aligned with the default gap penalty of 10 in the Clustal V alignment application embedded in ABI Sequence Navigator. The aligned sequences were copied into a NEXUS file. Gross alignment errors were adjusted by hand. The majority of the alignment for 16S was not problematic. However, there were five variable regions that needed further manual adjustments. Many systematists have chosen to exclude variable alignment regions, arguing that homology in these variable regions cannot be accurately assigned and therefore leads to misinformation and poor phylogenetic reconstruction. Others advocate the compartmentalized use of the variable regions (Mishler, 1994). That is to exclude the variable regions at a global level and include the variable regions at more exclusive levels. In the case of 16S for lottiids, the variable regions were very difficult to align as a whole; however, alignments in these regions among closely related groups were quite straightforward. Informative content, therefore, is clearly lost when the variable

regions are entirely excluded from the analysis, and can be gained when homology decisions can be made locally.

Another problem with the alignment process is the difficulty of independently reproducing the same alignment. In an attempt to produce a more reproducible alignment approach, a manual algorithm combining alignment programs and manual adjustments was designed. The procedure involves a 6-step process: 1) Initial alignment with an alignment program. 2) Manual correction of egregious alignment errors. 3) Simple parsimony analysis of alignment. 4) Randomization of taxon positions in alignment. 5) Manual adjustments to alignment. 6) Repeat steps 3, 4 and 5 until repeated cycles continually produce the same topologies. For step 1, I used the default settings of the Clustal alignment program bundled with ABI Sequence Navigator. The aligned sequences are individually copied and pasted into a PAUP NEXUS file, where obvious Clustal alignment errors are corrected. For step 3, a 10 replicate random addition parsimony analysis is performed. Because neighboring sequences heavily influence manual alignment manipulations, I felt it necessary to add step 4 and randomize the order of taxa in the alignment. The hope is that many alignment adjustments against varying neighbors will settle on a global best fit rather than localized best fits. As mentioned earlier, the alignment of variable regions for taxa within closely related subgroups is far easier than between more distantly related taxa. To monitor the integrity of these subgroup alignments, two to three exemplar sequences from each subgroup were duplicated and included in the alignment. These duplicates could then be

compared to the originals in terms of match, mismatch, and gap positions in order to evaluate the fidelity of the overall process. If the duplicate sequences remained relatively similar to the originals then I felt confident that the informative integrity of the subgroups remained intact. If, on the other hand, the duplicates differed from the originals, then I knew that the overall alignment process had failed.

Simple parsimony analyses followed each taxon randomization and alignment adjustment. This cycle was repeated until the parsimony trees continually settled on the same topology. The particular topologies were not considered, only whether continued alignment adjustments stopped producing different outcomes. To avoid any circularity due to subconscious preferences for a particular topology, random numbers replaced taxon names during the alignment process.

Conditional combinability analysis

Over 90% of the described species from the eastern Pacific and Caribbean were collected. In spite of several combinations of the Folmer COI primers, only 31 of 57 OTUs were successfully amplified for COI. This resulted in uneven sampling for COI and 16S; 31 of 57 OTUs are represented by COI and all 57 OTUs are represented by16S.

Bull et al. (1993), de Queiroz (1993), and Rodrigo et al. (1993), have argued that the combinability of data from different sources should be evaluated prior to phylogenetic analyses. Here 16S and COI are evaluated to determine

whether they have significantly different hierarchical signal and thus whether they should be combined in a single phylogenetic analysis. If the results of the combinability analysis demonstrate that these data sets are significantly different, it must then be determined, if possible, why the different data sets are conflicting and which source of data will recover the most likely hypothesis alone or whether they should be combined in a single phylogenetic analysis. To execute these objectives, a six-step strategy was employed:

1) Constructing Trees

There are four ways to use the 16S and COI matrix in a phylogenetic analysis (Fig. 1): 1) Include all of the data in the analysis (TE); 2) Include only the 16S partition (16S); 3) Include only those taxa for which both COI and 16S were successfully sequenced (16SCOI); 4) Include only the COI data (COI). Trees were generated from each of these partitions. Trees were also produced from a reduced 16S partition, which includes only those taxa represented by both COI and 16S (16S31). All of the trees were saved for the tree comparison steps of the following combinability analysis.

According to proponents of combinability tests, topologies should be compared. There has been considerable debate over the accuracy of the available methods of building phylogenetic trees (Miyamoto & Cracraft, 1991). Performing combinability analyses using trees from every available phylogenetic method would be laborious and redundant.

Among the most commonly used methods are the distance methods (distance and pair-group cluster analyses) and the character-based methods (Maximum Parsimony and Likelihood). The distance methods arrive at a single tree based on a derived distance matrix, whereas character-based methods construct many trees based on individual homologies and compare them based on criteria deemed appropriate in recovering evolutionary history. Several methods from both the distance and character-based methods were evaluated to find the most robust and efficient method for this analysis.

The distance methods (Neighbor Joining, UPGMA and the star decomposition method employed in PAUP* 4 (Swofford, 2000)) and the character-based methods (Maximum Parsimony (MP), Maximum Likelihood (ML) and Minimum Evolution (ME)) were used to generate trees for the combinability analysis.

2) Tree Evaluation

The robustness of the trees generated in step 1 must be evaluated. However, a contentious debate remains between bootstrap and decay analyses. By far the most common method has been nonparametric bootstrapping (Efron, 1979; Efron, 1982; Felsenstein, 1985a). However, the decay index (Bremer, 1994) has been gaining ground with increasing appearances in phylogenetic publications. Bootstrapping is a method of evaluating whether particular branches generated by a data set are well supported given different permutations

of the data. The method simply permutes the existing data set by resampling characters from the original matrix with replacement to generate new matrices the same size as the original. Trees from the replicate matrices are constructed and compared for support of common branches. If a particular branch is found in every replicate then that branch receives a bootstrap value of 100%, if it is found in half of the trees a value of 50% is assigned and so on. If there is little or no structure in the original data, then all branches collapse into a bush with no significant support for any branches.

The decay index is a measure of branch stability based on the difference between the original tree and the shortest parsimony tree that violates the monophyly of interest. The larger the decay values, the more robust the branch. However, like with the bootstrap, how large the value needs to be to be considered “good” support, is difficult to establish. Both of these methods were used to evaluate the quality of the trees generated in step 1.

3) Tree Comparison

Many tests have been devised to compare phylogenetic trees. I have selected five of the better-known tree comparison tests to compare COI and 16S trees.

I. Templeton’s (1993b) test compares the number of steps for each character for each partition as each partition is mapped onto each tree using a two-tailed Wilcoxon signed rank test.

II. The Winning sites test (Prager & Wilson, 1988) is a simplified version of Templeton's test, which assumes that the characters are weighted equally and do not change more than once for each tree. The test compares the total number of changes per tree against a binomial distribution.

III. The Kishino-Hasegawa test (1989) under MP calculates the difference between the two trees in terms of the number of nucleotide substitutions and treats it as a test statistic. This test statistic is compared to the expectation that there is no statistical difference between the two trees using a paired t-test. The K-H test may also be applied under likelihood criteria by using the differences in log likelihood scores as the test statistic.

IV. The Consensus Fork Index (CFI) measures tree similarity by calculating the number of shared clades between two trees divided by the total number of possible clades, which is the total number of taxa minus two (Colless, 1980, Swofford, 1991). Colless (1980) points out that CFI values can be equivalent for trees with very different levels of resolution. To correct for this, he normalizes the CFI calculation by weighting each fork by the number of OTU descendants branching from the fork. The Maximum score now becomes 1/2(n-1)(n+2), where n = the total number of OTUs.

V. The “compare two” T-PTP test implemented by PAUP* compares the difference in the number of steps between two trees to a null distribution of randomly generated differences.

Pairwise comparisons of many different trees were performed for all of the above methods (Table 1). While many of the individual comparisons are not informative by themselves, as a whole patterns may be searched for and consistency of methods can be evaluated.

Independent corroboration of one tree over another can contribute support for the preference of one topology over another. Distributional information mapped on competing trees may reveal biogeographical patterns, which can be compared statistically to determine the more parsimonious pattern. To examine the possibility that distributional data might favor one tree over another, the distributions of each OTU were mapped onto each of the trees from the five comparative partitions and the probabilities of the distributional patterns were calculated, herein referred to as the Mapped Biogeographic Distribution index (MBD). The probabilities were calculated using the binomial cofficient:

$$\binom{n}{r} = \frac{n!}{n!(n-r)!}$$

Where *n* is the total number of regional taxa and *r* is the subpopulation of *n* taxa, which belong to the same monophyletic biogeographical clade. For example, If there are 21 taxa found in the northeast Pacific (NEP) and 20 of them form a monophyletic clade, then *n*=21 and *r*=20. The probability that 20/21 NEP taxa form a monophyletic clade is determined by the binomial probability

$$P(X)=\binom{n}{r} p^{r} (1 - p)^{n-r}$$

Where $P(X)$ is the probability that r out of n regional taxa are monophyletic; p represents the fraction of all available regions (in this case there are four: northeast Pacific; Chile; tropics; and outgroups), so $p = 1/4$. The quantity $(1 - p)^{n-r}$ represents the probability that n-r taxa fell into one of the other regional clades. To calculate the distributional probabilities of the entire tree, a joint probability distribution was performed where the binomial probabilities of each regional clade were treated as independent events and multiplied together.

$$P(\text{NEP}) \times P(\text{Chile}) \times P(\text{tropics}) \times P(\text{outgroups}) = \text{MBD}$$

The MBD for each tree was compared. The lower the probability of a particular distribution, the greater the probability that the distribution is not random. Given the nature of evolution and lineage splitting, it should be expected that regional taxa be more closely related to one another than they are to taxa from distant regions (Hennig, 1966). This should be particularly true at ever-greater scales. Extinction and immigration can confound this pattern, but these should only be invoked as possible explanations when patterns of regional monophyly are not found. The expectation under evolutionary theory is regional monophyly. The more a tree resembles this expectation, the further it is from random, and therefore, the more likely it reflects phylogenetic structure. The MBD is a measure of how far the mapped distributions are from random, and therefore, a way to compare mapped distributional patterns among competing phylogenetic hypotheses.

4) Partition Comparison

In addition to comparing the trees supported by particular partitions, the partitions themselves can be compared to one another. Before the partitions were compared, they were tested for a lack of phylogenetic structure using a simple Permutation Tail Probability (PTP) test (Archie, 1989a; Archie, 1989b). To evaluate the congruence between 16S and COI, the partitions were subjected to the Partition Homogeneity (PH) test (Swofford, 1993) and the Incongruence Length differences (ILD) test (Farris *et al*., 1995).

The simple permutation tail probability (PTP) test incorporated in PAUP* was run to evaluate the existence of nonrandom structure in each partition within each character. This test performs repeated permutations of the original data matrix by randomizing the character states while holding the total number constant. This should destroy any correlation among states resulting from evolutionary processes. The null hypothesis of no phylogenetic structure can be tested by comparing the original tree length to the null distribution of tree lengths. If the tree length of the original data falls outside of the normal distribution of permuted tree lengths, then the null hypothesis that there is no structure in the data set is falsified. Of course, the structure does not necessarily have to be phylogenetic; it could be due to codon usage bias, convergence, nucleotide bias or other non-phylogenetic biological phenomenon.

The ILD test statistic, designed by Farris et al. (1995), separately computes the lengths of the most parsimonious trees for two partitions (Lx and

Ly) and the combined data set (Lo). The difference between the sum of the lengths of the x and y trees and the length of the combined tree is the test statistic ILD=|Lo-(Lx+Ly)|. Farris at al. reasoned that the difference between the sum of the lengths of the tree for x and y and the length of the trees generated by the combined analysis is a measure of the amount x and y differ. The rational being that if x and y produce very different trees, then the combined analysis will have much more homoplasy then either x or y and will have a much greater length than that of the summed length of x and y trees. Farris at al. came up with a sampling method, which produces a random distribution of ILD values. This is achieved by reproducing the x and y partitions by randomly drawing characters from the entire data set without replacement. This produces two new random partitions equal in size to the original x and y partitions. The Farris test statistic is calculated from the newly generated partition. This process is repeated to create a distribution of randomly generated ILD values. If the value of the original test statistic is greater than 95% of the values generated by the random sampling method, then there is more incongruence between x and y than would be expected from chance alone.

The PH test implemented in PAUP* is essentially the same as Farris' ILD test except that Swofford points out that the tree length of the combined analysis (Lo) will always be the same and thus can be ignored. Instead of evaluating the partitions of interest in terms of the difference between the sum of the tree length from the separate partitions and the length of the combined tree, PH compares the sums of the separate partition trees to the normal distribution of randomly

generated tree sums. One other difference between the ILD and PH is in the calculation of the P-value. Farris et al. calculate P as 1 - S/(W+1), where S is the number of random partition tree length sums that are smaller than that of the original partition, and W is the number of replicates. Swofford's P-value is the same except he does not incorporate the "+1" in the denominator. 1,000 replicates were performed so "+1" is of little significance.

Partitions have also been compared by what is called here the "combined consensus comparison" (CCC). This is a simple comparison of 16S and COI strict consensus trees from all phylogenetic methods (ME, MP, ML, NJ, UPGMA and Star Decomposition (SD)). While certain phenomenon, like convergence and long branch attraction may confound different phylogenetic methods and even bias them to a common incorrect topology, it is reasonable to assume that, if a particular partition results in the same topology over many relatively independent phylogenetic methods and another results in many very different topologies, the partition producing more congruent topologies may be considered more stable and therefore more reliable given the tested data.

By comparing consensus trees composed of trees generated by many phylogenetic methods, the consistency of 16S and COI structure can be compared. A partition composed of random noise would produce random trees, whereas a partition with less noise will produce fewer random trees. Six reconstruction methods (ME, MP, ML, NJ, UPGMA and SD) were applied to the COI and 16S31 partitions. All trees from each reconstruction method were combined as a strict consensus tree for each partition. The consensus trees were

evaluated for topological consistency using the consensus fork index and the Mickevich consensus information index.

5) Sources of Incongruence

If the above tests conclude that 16S and COI are significantly incongruent, it must then be determined why. There are two general sources of homoplasy, which can bias phylogenetic reconstruction, random and systematic. Random homoplasy is primarily due to sampling (eg. long branch attraction, high evolutionary rates). The range of potential bias introduced by random error tends to decrease with increased sampling. Systematic errors are violations of assumptions within a particular model (eg. reticulation, convergence). In the case of phylogenetics, systematic homoplasy occurs when assumptions of an evolutionary model behind the criteria for reconstructing phylogenies are violated by the actual evolutionary process.

Because this data set contains two partitions with different numbers of OTU's, taxon sampling must be considered as a possible source of incongruence. There are many possible systematic errors, which can account for differences. For example, one or both of the genes may have been involved in a paralogous or xenologous event; the tempo and mode of evolutionary processes may be different for each gene rendering them informative and potentially misinformative at different phylogenetic levels; convergence may be affecting

one or both genes; it may also be possible that some or all of the sequences are from sources other than limpets.

To examine whether the differences between 16S and COI are a sampling issue, the complete 16S MP tree was compared to the 16S31 MP tree and the 16S26 MP tree using the same battery of topology tests described in step 3 (Table 1, row x and row ag). In order to compare the larger 16S tree to the smaller 16S trees, the 16S tree was pruned to match the taxa in the 31-OTU tree (Pr16S) and the 26-OTU tree (Pr16S26) while maintaining its general topology (Fig. 2). If the phylogenetic signal is distorted by small sample size, then the tree comparisons should produce inconsistencies among the trees. If the smaller16S partitions are affected by sample size then it is possible that the COI partition is also affected by sample size.

Testing for a systematic source of incongruence is made simpler by the fact that both 16S and COI are mitochondrial. The mode of inheritance for nuclear and mitochondrial genomes are quite different and they do not experience all of the same evolutionary processes. For example, it would be very unlikely that hybridization could be detected by examining only mitochondrial data. While paternal leakage in metazoans has been suggested in mitochondrial inheritance, it is generally accepted that, for metazoans, inheritance is maternal. The introduction of extraspecific DNA is restricted to the nuclear genome and is unlikely to be affecting the uniparental line of mitochondrial inheritance. Because mtDNA does not undergo meiosis, it is not susceptible to cross-over events, gene conversion or concerted evolution, all of which can confound phylogenetic

signal. Substitutions rates of metazoan mtDNA have been shown to be, at times, ten times that found in the nuclear genome (Brown *et al*., 1979). Most believe this to be due to a lack of efficient repair mechanisms and exposure to elevated levels of mutagens in the mitochondria. The advantages of the mitochondria for phylogenetic analyses are that the high rates permit the investigation of recent evolutionary divergences and DNA repair does not obscure evolutionary events. The disadvantages are that rapid evolution can quickly saturate variable regions, thereby resulting in random homoplasy and eventually eliminating phylogenetic signal altogether.

To test for incompatibilities in evolutionary rate, 16S and COI were compared by their Ti/Tv substitution ratios, saturation curves, and bootstrap analyses.

The weighted Ti/Tv averages of absolute distances for all pairwise comparisons for both partitions were calculated. The weighted Ti/Tv values were used to compare the outgroup versus ingroup, and the northeast Pacific versus the Chilean clade for all partitions. This analysis can reveal that one partition or elements of a partition are closer to random Ti/Tv values than another partition, thus inferring a greater evolutionary rate.

Transition and Transversion saturation curves were plotted separately for 16S31 and COI against the16SCOI partition. Each codon position of COI was also plotted because the majority of synonymous substitutions is known to occur at the third position and may be a source of phylogenetic noise. Therefore, 16S31 was also compared to COI without the third position. Saturation curves

can provide information about the overall substitution levels, and where most changes are occurring. Relative rates of change can be inferred from the observed number of changes plotted against the estimated number of changes. If the observed numbers of changes plateau against the expected number of changes, it is assumed that many of the changes occurring are superimposed changes, thus preventing further observable substitutions. To achieve such a state requires a great deal of time, a greater evolutionary rate, or change restricted to certain sites.

Bootstrap 50% majority-rule consensus trees for each partition were compared using the Consensus Fork (CFI) and the Mickevich (1978) Consensus Information (MCI) Indicies. The Mickevich CI index is a measure of the difference between an Adam's (1972) consensus tree and an unresolved bush. If the substitution rates of a partition are too high for the divergences of the given OTU's, Ti/Tv and the bootstrap CFI and MCI values will be very low.

6) Analysis of Results

Once the source(s) of incongruence were identified, this information, along with the information from the tree, partition, and mapped distribution comparisons, was used to justify a preference for a combined or partial analysis of the 16S and COI data set.

16S phylogenetic analysis

Based on the results of the combinability analysis, COI has been excluded from the global phylogenetic analysis of New World patellogastropods. Here, 16S hypotheses are generated from six different phylogenetic methods and from five different MP weighting schemes. These hypotheses are then compared by examining mapped biogeographical distribution patterns and comparing ML and MP scores.

Six methods used to generate competing hypotheses

The six methods used to generate trees for comparison, were NJ, ME, UPGMA, star decomposition, MP, and ML. For NJ and ME, several substitution models are available, which use various formulas to correct differences in Ti/TV values and unobserved superimposed substitutions. The uncorrected P (uncP) and the Kimura 2-Parameter (K2P) substitution models were employed for these distance analyses; this produced two sets of trees for each method (Fig. 3).

Because of the computational time requirements of ML analyses, every effort was made to minimize the cost in processor time, yet maximize the fit of the ML model to the data. ML options permit likelihood estimations for Ti/Tv, the percentage of invariable sites, the nucleotide frequencies, and gamma shape parameters; however, this requires exceedingly long computation times even on fast G3 and Pentium III processors. One addition-sequence replicate under these conditions for the 16S partition did not finish after a month of uninterrupted processor time on a 350Mhz Macintosh G3 computer. In order to minimize the

computational requirements of the ML analysis, average values for Ti/Tv, the percentage of invariable sites, nucleotide frequencies, and gamma shape parameter were set based on estimations of these values from the other methods (Table 2).

To estimate Ti/Tv values, the percentage of invariable sites, the nucleotide frequencies, and gamma shape parameters, trees from the other methods were loaded into memory and the values were computed under the likelihood option for tree scores (Table 2). The likelihood settings were set to "estimate" for Ti/Tv, the percentage of invariable sites, the nucleotide frequencies, and gamma shape parameter.

Weighted MP hypotheses

The *a priori* weighting of partitions has been controversial, but the fact is, non-weighting is an *a priori* assumption that all substitutions across all partitions are equal, which is rarely the case. However, Albert et al. (1993) detailed a thorough approach to differential character-state weighting that employs estimations of Ti/Tv and λ (the expected number of changes per character per branch) in the assignment of differential weighting schemes. Their conclusions support similar finding of Hillis and Dixon (1991) and Albert et al (1992) that with large OTU sampling, weighting schemes provide negligible improvement in phylogenetic estimations over equal weighting MP schemes. While these studies demonstrate that weighting schemes may have little affect on phylogenetic estimations, they state that this is true for large sample sizes. This study has 57

OTUs and may or may not constitute a large sample size. To examine whether a simple Ti/Tv weighting scheme affects phylogenetic estimates with this data set, simple Ti/Tv matrices were differentially applied to the partitions. For MP analyses, PAUP* permits the differential assignment of character state changes by using step matrices that reflect differential costs of transitions and transversions. There are five variable regions in the 16S partition (loops 1-5) where homology is particularly equivocal. By accounting for potential Ti/Tv bias in these variable regions, potentially misleading transition signal may be dampened, thereby improving the robustness of the trees. On the other hand, muting the more rapidly evolving characters may compromise resolution at the tips of the trees. In an attempt to resolve this conundrum, five different Ti/Tv weighting schemes were applied to the 16S partition based on Ti/Tv values estimated from the other methods (table 2).

Each of these Ti/Tv weighting schemes provides a competing hypothesis, which are compared and used to evaluate the utility of correcting for rate heterogeneity. The five weighting schemes were as follows: 1) All characters equally weighted; 2) All loop regions with the same Ti/Tv matrix; 3) Stem and loop regions weighted differently; 4) Each loop region weighted differently; and 5) Each loop and stem region weighted differently. Loading trees generated by the other phylogenetic methods into PAUP's memory and including only those characters for which Ti/Tv values were desired estimated Ti/Tv weights. For example, to obtain Ti/Tv estimates for loop 1, a tree from one of the other methods, say ME, was loaded into PAUP's memory, and all characters except

those comprising loop 1 were excluded from the analysis. A Ti/Tv value was estimated from the Likelihood tree scores option. This was repeated for all loop and stem regions for all trees. The average Ti/Tv from all trees for each loop and stem was calculated (Table 2). The trees from each of the five weighting schemes were compared by mapping distributions and comparing MP and ML scores.

RESULTS

PCR and degenerate primers

The degenerate primers were successful for only a small number of taxa and only in the primer combination of HCO1490 + dgLCO2198. Specific internal COI primers from the sequences of the most closely related taxa based on a preliminary 16S phylogeny were also designed. For example, a 16S phylogeny places *L. turveri*, *L. acutapex*, *L. scabra*, and *L. conus* as a monophyletic group in the northeast Pacific clade. *L. turveri* and *L. acutapex* were successfully amplified using the Folmer COI primers and *L. scabra*, and *L. conus* were not. Conserved regions between the Folmer primer regions of *L. turveri* and *L. acutapex* were identified and used as potential primers to amplify a shorter segment of COI for *L. scabra*, and *L. conus.* These primers paired together and in combinations with the Folmer primers were never successful.

Phylogenetic Methods

The combinability analysis requires tree comparisons. In order to compare trees, some method of generating trees must be adopted. Distance methods have been shown to be poor models for recovering evolutionary relationships (Swofford, Olsen et al. 1996). The computational time requirements of large ML analyses on computers currently available obviate its use under most circumstances. For example, a ML analysis of all 16S and COI data with transition/transversion biases and rate heterogeneity estimated was halted after 900 hours during the first of ten replicates on a Macintosh G3 350Mhz processor. Another ML analysis for only 16S was halted after three replicates and 300 hours. For these reasons, I chose to use MP for the 16S and COI combinability analysis.

1) Tree construction

The COI-16S sequence matrix (Fig. 1) was divided into six comparative partitions; One for COI, one for 16S and COI (16SCOI), three for 16S (16S26, 16S31 and 16S) and one for the total matrix (TE). Parsimony analyses using random sequence addition and 1,000 replicates for all of the partitions except for 16S26 partition produced five sets of trees (Fig 4). In order to compare trees from analyses of different OTU sizes the larger trees (full 16S and the total evidence)

were pruned to match the number of OTU's in the smaller trees (Pr16S and PrTE) (Fig. 2).

2) Tree Evaluation

To evaluate the robustness of trees generated from each partition, Bootstrap and decay analyses were performed for each tree. A 1,000 replicate bootstrap analysis for 16S produced a 50% majority-rule consensus tree with 22 out of 29 possible nontrivial branches. The 16SCOI partition produced 21 out of 29 possible branches and the COI partition produced 22 out of 29 possible branches. Normalized CFI values for each partition are 0.786, 0.75, and 0.393 with MCI scores of 0.276, 0.305, and 0.081 respectively, (Fig. 5).

The decay analysis of the COI and 16S31 trees is difficult to interpret; they share very few clades, the number of informative characters are vastly different, the tree lengths are different, and the decay values are generally low for most branches on the trees (Fig. 6). However, when the COI and 16S31 partitions are combined, some of the decay values increase dramatically (Fig. 6, 16SCOI).

3) Tree Comparisons

Every combination of tree-to-tree comparison for all five partition combinations was performed for the five topology tests (Templeton, WS, KH-parsimony, KH-likelihood, "compare-2" T-PTP). However, the comparisons of the COI topology to the 16S31 topology provide the most telling comparisons (Table 1, rows e & o). All five tests produce P values of less than 0.05 when 16S and

COI topologies are directly compared. However, when the third position is excluded from the COI partition, the KH test produces significant differences in all of the comparisons except when the 16S31 trees and the 16SCOI-3 trees (the tree generated using 16SCOI partition excluding the third codon position) against the 16S31 partition and when the 16S tree is compared to the TE-3 trees (trees generated from all of the data excluding codon position three) against the 16S partition (Table 3).

The KH test reveals that the elimination of codon position three from the COI partition produces trees that are more similar than when position three is included in an analysis, but the results of this KH test are ambiguous and must be interpreted cautiously.

Independent corroboration

To examine the possibility that distributional data corroborates one tree over another, the biogeographical distributions of each OTU were mapped onto each of the four MP trees (Fig 7). For the complete 16S tree, clear biogeographical patterns emerged. All but one of the northeast Pacific taxa and all but one of the Chilean taxa form monophyletic clades, all of the tropical new world taxa and all of the outgroups form unique monophyletic clades. For all other partition combinations, the monophyly of at least one of the regional clades was lost.

The probability that the regional distributions would fit onto the observed monophyletic groupings due to chance alone is exponentially low. Compared to the total evidence tree (Fig. 7), the MBD for the16S tree indicates that the biogeographical distribution is $6.2x10^{5}$ times less probable ($5.14x10^{-26}$ vs. $8.19x10^{-32}$). Additionally, the NEP clade in the TE tree broke into two separate clades separated by the Chilean clade. The extreme probability against nearly all of the expected taxa from the northeast Pacific, Chile, and the New World tropics forming monophyletic clades corroborates the other tests, which favors the phylogenetic hypothesis inferred from the 16S data.

4) Partition Comparison

The simple PTP test implemented in PAUP* was performed on the COI and 16S31 partitions. Both partitions produced trees over 400 steps shorter than the shortest of 1,000 random trees, with P scores of 0.001 each (Fig. 8).

The Incongruence Length Difference (ILD) test and the Partition Homogeneity (PH) test produce equivalent results (Fig. 9). Both the ILD and PH tests were used to compare the COI partition with the equivalent number of OTUs for the 16S partition (16S31). A null distribution of test statistic values was generated from one thousand replicates of random trees. P values of 0.0001 were obtained for the test statistic of the original partitions against the null distribution of test statistics. This indicates that the two partitions differ far more than would be expected from chance alone.

The 16S31 CCC strict consensus tree scored a normalized CFI of 67.9% agreement of all possible branches compared to 39.3% for the COI consensus. The Mickevich information index favored the 16S31 consensus tree by a score of 0.305 to 0.081. Biogeographically, the phylogenetic relationships of the 16S31 CCC tree maintained the monophyly of the northeast Pacific, the Chilean, and the outgroup clades (Fig. 10). The tropical New World clade became paraphyletic but maintained its relative position between the temperate clades and the outgroups. The COI CCC tree collapsed to only about a third of all possible branches.

Strong phylogenetic signal should produce consistent topologies over different phylogenetic methods, while poor signal will produce results that are more random. This test demonstrates an inconsistency in the information content of the COI partition for this data set.

5) Sources of Incongruence

All of the above analyses provide evidence that the 16S and COI partitions are significantly different and produce incompatible trees. Why are they so different? To answer this, the possible sources of incongruence were identified and tested.

Sampling:

The COI partition includes 31 OTUs and the 16S partition includes 57 OTUs. If the differences between 16S and COI are due to sampling differences,

then the 16S31 partition, which includes the same 31 OTUs as COI, should be incongruent with the full16S partition as well. I used the same tree comparison tests in step 3 to compare the 16S31 tree to the full 57 OTU 16S tree. To compare the 31 OTU trees to the 57 OTU trees, I used the Pr16S tree. Every tree comparison test agreed that the differences between 16S31and Pr16S trees are within what can be expected from chance alone (Table 1, row d). Partition D (16S26) (Fig. 1) was also compared with the Pr16S26 tree with the same conclusion (Table 1, row ag). These tree comparisons suggest that the differences between COI and 16S are not likely due to sampling.

Rate Heterogeneity:

A pairwise comparison of all 31 16SCOI OTU's revealed that the COI partition has a weighted Ti/Tv average 16% less than the 16S partition (Table 2). And, when the Ti/Tv ratios are compared between the outgroup and ingroup, COI ranges from 41% to 19% less than 16S with a combined weighted average of 31.6% less than 16S. The expected ratio of transitions to transversions for random data is 1:2 (0.5), because there are twice as many substitution opportunities for transversions than there are for transitions. However, in mitochondrial DNA, transitions have been reported to occur as much as ten times more often than transversions (Futuyma, 1986, pp448-450). For COI, the two most distant outgroup members, *P. pustulata* and *C. turbata*, the Ti/Tv ratio against the entire ingroup is .67 each, very near the expectation of random data. Whereas, 16S has a Ti/Tv ratio for these comparisons of 1.14 and 1.06

respectively. A comparison of the northeast Pacific clade and the Chilean clade reveals an average Ti/Tv ratio of 1.06 for COI and 1.22 for 16S, a difference of 13% (Table 2). These results indicate that COI is evolving at a much higher rate than 16S and that many of the COI characters are changing very rapidly. The proximity of Ti/Tv values to the expectation of random for COI indicates that the resolution asked of COI to resolve is inappropriate.

By tallying transversions and transitions for all pairwise comparisons of 16SCOI taxa, the relative contribution of changes by 16S and COI can be compared. Transversions, transitions, and the total number of substitutions were plotted against a Kimura 2-Parameter correction of all pairwise comparisons (Fig. 11). The numbers of substitutions were converted to percents to correct for the difference in 16S and COI partition sizes. All three plots show that COI contributes a far greater percentage of substitutions than 16S. This demonstrates that COI has experienced far more evolution than 16S, which likely results in incompatible levels of phylogenetic resolution.

CFI and MCI indices were used to compare bootstrap 50% majority-rule consensus trees from the COI and 16S31 partitions. The bootstrap consensus tree from the COI partition produced a tree with 11 out of 29 possible bifurcating clades (normalized CFI=0.393), while the 16S tree has 22 out of 29 possible clades (normalized CFI=0.786)(Fig. 5). This test demonstrates, for this particular study, that COI has considerably less phylogenetic content than16S. Like the Ti/Tv analysis, the bootstrap analysis reveals considerable differences in evolutionary rates between COI and 16S and casts doubt on the appropriateness

of using COI to resolve global level phylogenetic relationships for these limpets. However, COI is less variable than 16S when the third codon position from the COI partition is excluded from the analysis (Fig. 12). Nevertheless, the slopes of the saturation curves for the first and second positions are flatter than those of 16S, particularly between ingroup and outgroup comparisons (Fig. 12). This implies that the number of observed substitutions are fewer than would be expected given the substitution rate estimated by the Kimura 2-parameter model.

16S phylogenetic analysis

Competing hypotheses

Figure 13 shows eight trees with mapped biogeographical distributions, ML scores and MP tree lengths. Four of these trees (MP, ME using the Kimura2-parameter substitution model (MEK2P), ME using an uncorrected P substitution model (MEuncP) and NJ using the uncorrected p model (NJuncP)) are monophyletic for each of the northeast Pacific, Chilean, and tropical New World faunas. The ML tree is nearly identical to the MP tree except for the placement of two tropical OTUs at the base of the northeast Pacific/Chilean clade and the placement of the unstable Chilean OTU *Scurria orbignyi.* The ML placement of the tropical OTUs, *K. mesoleuca* and *K. stipulata*, at the base of the northeast Pacific/Chile clade breaks up the otherwise monophyletic tropical clade. The

UPGMA tree is monophyletic for the northeast Pacific and Chilean faunas, but is paraphyletic for the tropical OTUs with *Scurria orbignyi* further breaking up the tropical OTUs. The tree generated by the Saitou and Nei (1987) star decomposition method has the worst correlation between monophyly and biogeography; none of the biogeographical regions are monophyletic.

Among the four trees with monophyletic biogeography, the MP tree has a clear north to south pattern that emerges in the northeast Pacific clade (Fig. 14). The most northerly OTUs are the most basal in the clade and the most southerly (Gulf of California endemics) are the most derived of the clade with a north to south gradient in between. None of the other trees has any discernable biogeographical pattern for this clade. The ML tree, which is not one of the four biogeographically monophyletic trees, does have the same north to south correlation found in the MP tree. The only difference between the northeast Pacific clades of ML and MP trees is the placement of *L. paradigitalis* from one middle clade to another. Its range and placement in either the MP or ML tree does not alter the north to south pattern found in the northeast Pacific clade.

Three trees (NJ-uncP, ME-uncP, and MP) all share the tropical clade that includes, *K. fascicularis, K. albicosta, K. biradiata, K. paleacea, K. mesoleuca,* and *K. stipulata*. All of these taxa are exclusively Pacific except *K. albicosta*, which I suspect is a trans-Panamic cognate to *K. fascicularis* (discussed in Ch. 4).

Four of the eight trees agree on the monophyly of the regional faunas, two trees agree on a north to south, and basal to derived correlation in the northeast

pacific clade, and three trees agree on the monophyly of the tropical Pacific clade. The only tree common to all three of these patterns is the MP tree. Additionally, the MP tree has the best ML score (except for the ML tree), while the ML tree has the shortest MP tree length (except for the MP tree) (Fig. 13). However, none of the trees can be said to have significantly better MP or ML scores. The scores are relatively similar and therefore cannot justify the preference of one tree over another based on these scores.

Parsimony weighting

Of the weighted MP trees, only the unweighted MP tree maintained biogeographical monophyly. However, all of the other trees are strict consensus trees composed of more than one most parsimonious tree, so if any of the equally most parsimonious trees are biogeographically monophyletic, monophyly was lost in the consensus. Tree 1 (Fig. 3) as a consensus tree is not monophyletic for biogeography, but four of the six most parsimonious trees are. Tree 1, which is a strict consensus of 6 most parsimonious trees, was generated with a Ti/Tv weighting scheme of 2.1 for all five loop regions and no Ti/Tv weight applied to the rest of the partition. Two of the six trees from this weighting scheme were only four steps longer than the unweighted MP tree. Four of the six trees maintained the monophyletic biogeography, and all of the trees preserved the north to south pattern in the northeast Pacific clade of the unweighted MP tree.

Tree 3 (Fig. 3) is a strict consensus of four most parsimonious trees with an average length under unweighted conditions of 3324. This tree was produced under MP with all loops weighted at 2.1 and all non-loop regions weighted 2.73 (Table 2). All four trees were monophyletic for both the northeast Pacific and Chilean clades, but none were monophyletic for the tropical fauna.

Most weighting schemes maintained the general north to south pattern seen in the MP and ML trees, but there is an inverse relationship between the level of weighting complexity and the fidelity of the north to south pattern in the northeast Pacific clade (Fig. 15). Trees 2 and 4, which were produced with unique weights applied to each loop region and the stem regions, recovered the weakest north to south pattern. The most consistent difference between the unweighted tree and the weighted trees is the placement of *S. orbignyi*. All trees from weighted MP analyses place *S. orbignyi* basal to the northeast Pacific/Chile clade, whereas, the unweighted MP tree places *S. orbignyi* basal to the entire ingroup (Fig. 3).

MP scores were calculated for each tree under unweighted conditions and not surprisingly, the trees with the least weighting had scores the closest to that of the unweighted MP tree. However, that was not true for ML scores. The best ML score of the six most parsimonious trees of analysis 1 was better than that of the unweighted MP tree (tree 5) (Fig. 3). That tree was nearly identical to the unweighted MP tree except for the placement of *S. orbignyi* as described previously.

Discussion

Based on the arguments of Bull et al. (1993), de Quieroz (1993), and Rodrigo et al. (1993), the combinability analysis performed herein suggests that the 16S and COI partitions are incongruent and should not be processed in a single phylogenetic analysis. Topological tests, partition comparisons, bootstrap, rate comparisons, and biogeography support the exclusion of COI as a globally informative data set for this phylogenetic study.

The strongest indication that COI and 16S should not be combined in a phylogenetic analysis of New World Patellogastropods is the differences in biogeographical distributions found on the individual and combined trees. The trees from the combined analysis of COI and 16S (TE) break up the monophyly of the NEP taxa. It is, of course, possible that this represents the true phylogeny, but it is far more parsimonious to accept the biogeographical monophyly of the 16S tree (Fig. 7). Consider the following thought experiment: Suppose there are four differently colored buckets (blue, red, yellow, and black), each representing a biogeographical region, and we have 57 balls representing OTUs, each colored to represent its historical affiliation with a correspondingly colored bucket (21 blue, 9 red, 20 yellow, and 7 black). If the balls were randomly distributed among the buckets, the expected distribution of balls would be a random assortment of 14.5 (57/4) colored balls in each bucket (5.25 blue balls/bucket, 2.25 red balls/bucket, 5 yellow balls/bucket, and 1.75 black balls/bucket). The combined probability that 20/21 blue balls would land in the blue bucket, 8/9 red balls would

land in the red bucket, 20/20 yellow balls would land in the yellow bucket, and 7/7 black balls would land in the black bucket is 8.19×10^{-32}.

Based on the substitution rates estimated for COI and 16S, the level of divergence for New World patellogastropods is more appropriately resolved by 16S. I have shown that COI has had so many substitutions between particular OTUs that substitutions are nearly random and therefore uninformative at a global level. It has been argued (Barrett *et al*., 1991) and demonstrated (Kluge, 1989; Mishler *et al*., 1994) that even if the trees from different sources are significantly different, the combination of sources will tend to cancel out misleading homoplasy by the additive accumulation of underlying information. However, there is no a priori reason to believe that this is true for all possible combinations of data. An extreme example, suppose we have two data sets, a perfect data set designed to produce a fully bifurcating tree with zero homoplasy and a random data set. Combining the two data sets can never result in a better topology because we already have the right topology. The addition of the random data can never improve the results; it can only lead to the wrong tree. While COI is not a collection of random data, it is nearly random between geographic regions and therefore cannot possibly contribute to the resolution of these regional clades. In fact, we see that the inclusion of COI in a total evidence analysis breaks up these regional clades. However, the support for excluding COI from the overall analysis does not preclude its utility at less inclusive levels. For example, a more complete sampling of COI could be used in a

compartmentalized approach and COI was used to resolve a patellogastropod cryptic species complex in the genus *Notoacmea* (Simison and Lindberg, 1999).

The independent corroboration of biogeography and monophyly for the MP tree and its superior ML scores relative to trees from all other methods provides the most support for confidence in the MP tree. However, all of the weighted MP trees and the ML tree agree on the placement of the otherwise unstable *S. orbignyi*. The placement of *S. orbignyi* on the unweighted MP tree is suspect, especially in light of the agreement of its placement by ML and other MP analyses. While the sampling for the northeast Pacific and tropical taxa is nearly complete, the Chilean fauna is only represented by 60% to 75% of all named species. Further sampling of the Chilean intertidal may stabilize the placement *S. orbignyi.* Until then, I will consider its position as equivocal.

The biogeographical patterns of the weighted parsimony comparison favors the unweighted MP tree over all other weighting schemes because it was the only tree that shared in every discernable pattern.

Given that no one tree can be statistically demonstrated as the one and only possible hypothesis for New World limpets, the unweighted MP phylogeny is nonetheless used throughout the rest of this dissertation to infer the phylogenetic relationships of New World limpets. This preference is based on the combined analyses, comparative phylogenetic analyses, and biogeographical support (Fig. 16).

Tables

Table 1 P scores for tree comparison tests.

Comparison[1]	Partition[2]	# TAXA[3]	best tree	Templeton	WS	KH Pars.	KH Likelihood	T-PTP
a-16S v TE	16S	57	16S	0.0001	0.0005	0.0001	<0.0007	0.001
b-16S v TE	TE	57	TE	<0.0004	<0.0009	0.0003	0.002	0.001
c-16S31 v PrTE	16S31	31	16S	0.0003	0.0008	0.0003	0.0003	0.001
d-16S31 v Pr16S	16S31	31	16S	0.0977	0.1138	0.0971	0.2533	0.022
e-16S31 v COI	16S31	31	16S	<0.0001	<0.0001	<0.0001	<0.0001	0.001
f-16S31 v 16SCOI	16S31	31	16S	0.0125	0.0192	0.0112	0.0019	
g-COI v PrTE	16S31	31	PrTE	<0.0001	<0.0001	<0.0001	<0.0001	
h-COI v Pr16S	16S31	31	Pr16S	<0.0001	<0.0001	<0.0001	<0.0001	
i-COI v 16SCOI	16S31	31	16S+COI	<0.0001	<0.0001	<0.0001	<0.0001	
j-Pr16 v PrTE	16S31	31	Pr16S	0.0075	0.0057	0.0068	0.0005	
k-16SCOI v PrTE	16S31	31	16S+COI	0.0381	0.0165	0.0359	0.146	
l-16SCOI v Pr16S	16S31	31	Pr16S	0.3275	0.4882	0.3331	0.043	
m-16S31 v PrTE	COI	31	PrTE	<0.0001	<0.0001	<0.0001	<0.0001	
n-16S31 v Pr16S	COI	31	16S	<0.1809	<0.1019	<0.1764	<0.0192	
o-16S31 v COI	COI	31	COI	<0.0001	<0.0001	<0.0001	<0.0001	0.001
p-16S31 v 16SCOI	COI	31	16S+COI	<0.0001	<0.0001	<0.0001	<0.0001	
q-COI v PrTE	COI	31	COI	0.0294	0.159	0.0358	0.3603	
r-COI v Pr16S	COI	31	COI	<0.0001	<0.0001	<0.0001	<0.0001	
s-COI v 16SCOI	COI	31	COI	0.0094	0.0132	0.0067	0.1513	
t-Pr16 v PrTE	COI	31	PrTE	<0.0001	<0.0001	<0.0001	<0.0001	
u-16SCOI v PrTE	COI	31	PrTE	0.4665	0.8623	0.4488	0.5997	
v-16SCOI v Pr16S	COI	31	16S+COI	<0.0001	<0.0001	<0.0001	<0.0001	
w-16S31 v PrTE	16SCOI	31	PrTE	<0.0001	<0.0001	<0.0001	<0.0012	
x-16S31 v Pr16S	16SCOI	31	16S	>0.0474	>0.063	>0.0474	0.5652	
y-16S31 v COI	16SCOI	31	COI	>0.5062	>0.3409	>0.517	0.3322	0.713
z-16S31 v 16SCOI	16SCOI	31	16S+COI	<0.0001	<0.0001	<0.0001	<0.0001	
aa-COI v PrTE	16SCOI	31	PrTE	0.0007	0.0002	0.0005	<0.0001	
ab-COI v Pr16S	16SCOI	31	COI	0.8044	0.1886	0.7669	0.484	
ac-COI v 16SCOI	16SCOI	31	16S+COI	<0.0001	<0.0001	<0.0001	<0.0001	
ad-Pr16 v PrTE	16SCOI	31	PrTE	<0.0001	<0.0001	<0.0001	<0.0001	
ae-16SCOI v PrTE	16SCOI	31	16S+COI	0.4663	0.1824	0.4766	0.7819	
af-16SCOI v Pr16S	16SCOI	31	16S+COI	<0.0001	<0.0001	<0.0001	<0.0001	
ag-16S26 V Pr16S	16S26	26	16S	0.1014	0.0452	0.0971	0.3914	
ah-16S26 v PrTE	16S26	26	16S	<0.001	<0.0078	<0.0009	<0.0041	
ai-Pr16 v PrTE	16S26	26	Pr16S	<0.0417	<0.0282	<0.0406	<0.0045	

[1]The two trees compared in each test (see Fig. 1). Trees preceeded by"Pr" are pruned trees (see Fig. 5).
[2]The partition against which the trees were compared (see Fig. 1).
[3]The number of taxa included in the trees and partition being compared.

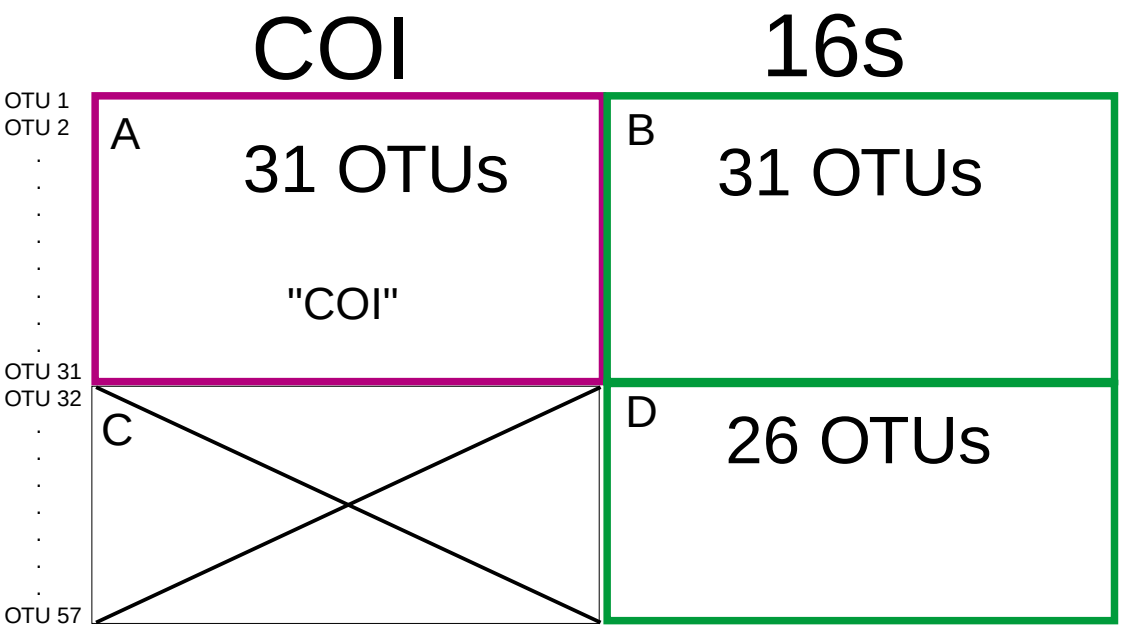
Table 2. ML Ti/Tv estimates for loop and stem partitions of 16S.

Partition	UPGMA	Star	NJ-uncP	NJ-K2P	ME-uncP	ME-K2P	MP	ML	AVE
16S	2.449	2.457	2.466	2.498	2.473	2.467	2.446	2.485	2.468
all loops	2.192	2.125	2.171	2.138	2.173	2.156	2.137	2.194	2.161
loop1	2.360	2.735	2.353	2.060	2.156	2.169	2.479	2.519	2.354
loop2	2.857	2.885	2.890	2.886	2.993	3.019	2.792	2.989	2.914
loop3	12.488	9.283	10.424	11.594	9.243	11.693	8.068	8.054	10.106
loop4	2.799	2.769	2.828	2.834	2.793	2.876	2.758	2.663	2.790
loop5	3.423	3.185	3.570	3.330	3.618	3.790	3.545	3.659	3.515
stems	2.709	2.728	2.741	2.770	2.751	2.735	2.709	2.722	2.733

Table 3. Kishino-Hasegawa test for trees generated with out codon position three

Comparison	Partition	# OTUs	P	
COI-3 v 16S31	COI-3	31	<0.0001	
COI-3 v TE31-3	COI-3	31	<0.0001	
16S31 v TE31-3	COI-3	31	<0.0015	
COI-3 v 16S31	16S31	31	<0.0001	
COI-3 v TE31-3	16S31	31	<0.0001	
16S31 v TE31-3	16S31	31	0.1405	*
16S v TE-3	COI-3	57	0.0001	
16S v TE-3	16S	57	0.484	*

* Values significantly different at P <0.05.
COI-3 = the COI partition excluding the third codon position.
TE31-3 = the 16SCOI partition with out the third codon position.
TE-3 = all of the data excluding the third codon position.



A = COI partition (COI)
B = 31 OTU 16s partition (16s31)
D = 26 OTU 16s partition (16s26)

A+B = 31 OTU 16s+COI partition (16sCOI)
B+D = Full 16s partition (16s)
A+B+C+D = Total Evidence (TE)

Figure 1. A partitioned representation of the complete COI and 16S aligned molecular data set for New World patellogastropods.

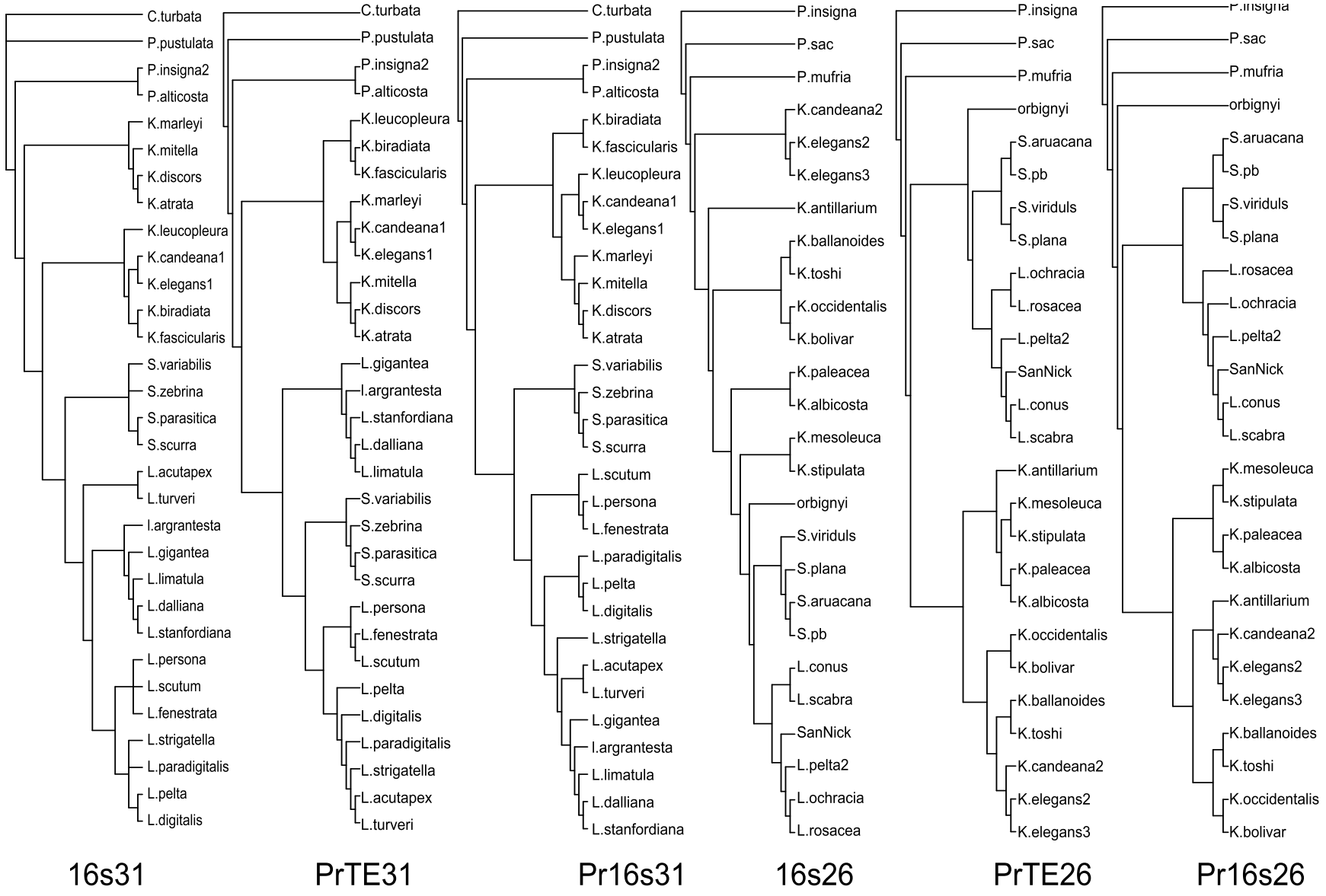
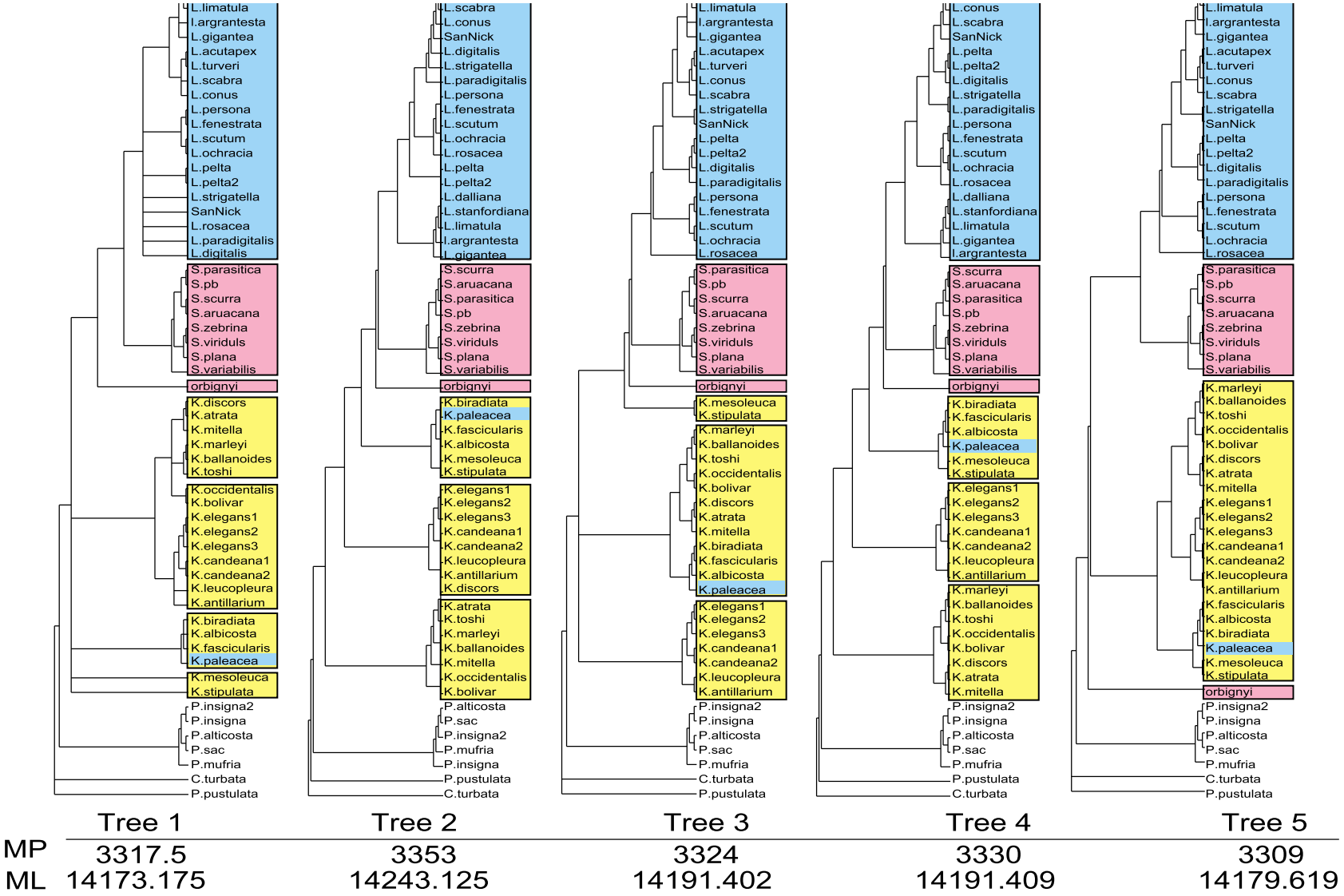


Figure 2. Total evidence and 16S trees pruned (Pr) to match the 31 OTU and 26 OTU partitions (Figure 1) for comparative analyses.



	Tree 1	Tree 2	Tree 3	Tree 4	Tree 5
MP	3317.5	3353	3324	3330	3309
ML	14173.175	14243.125	14191.402	14191.409	14179.619

Figure 3. Weighted strict consensus MP trees. Tree 1 is the result of all loop regions equally weighted. Tree 2 is a result of equal weights applied to stems and different equal weights applied to loops. Tree 3 is a result of each loop region weighted differently. Tree 4 is a result of unique weights applied to all stems and loops. Tree 5 is unweighted.

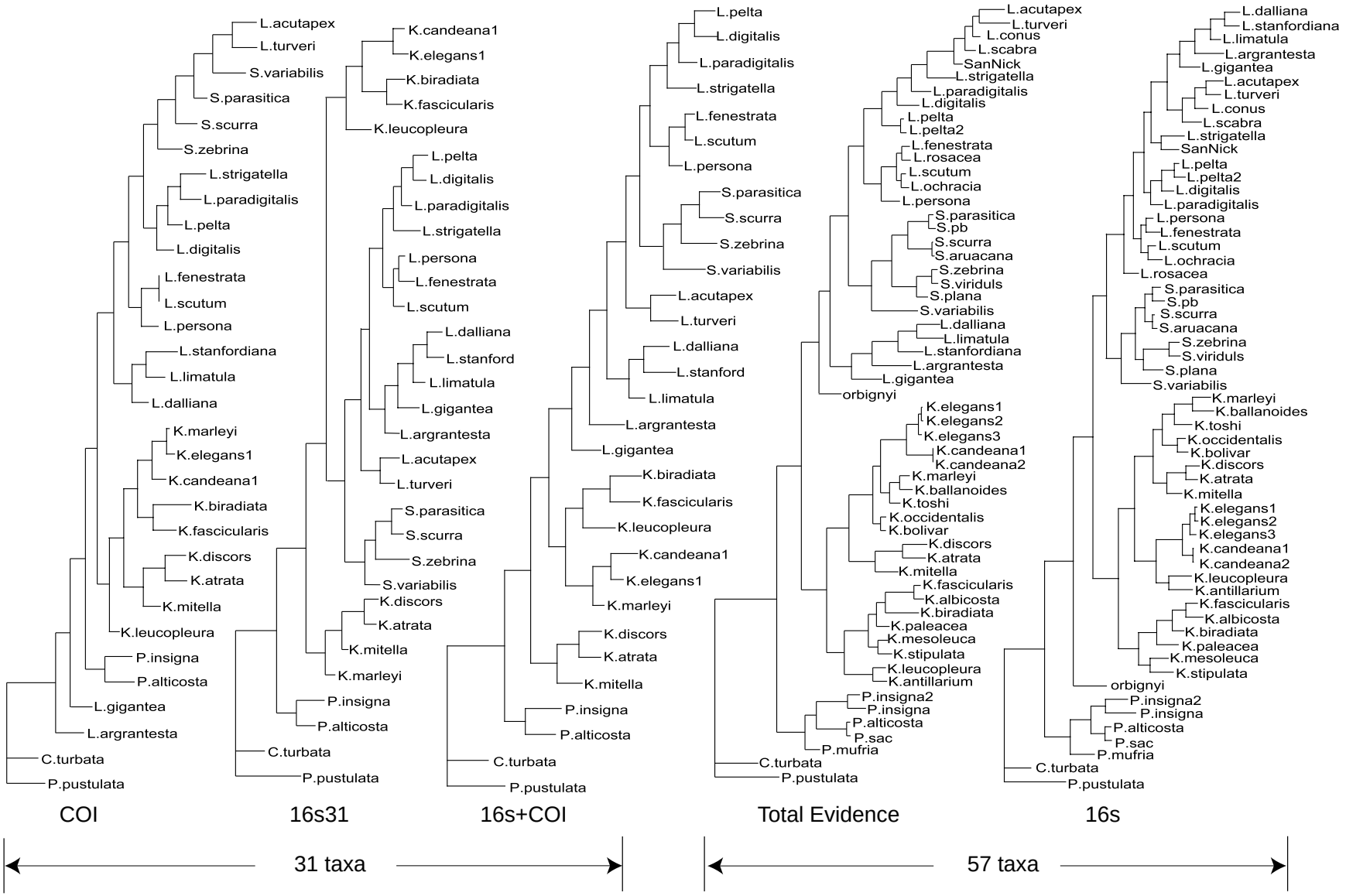
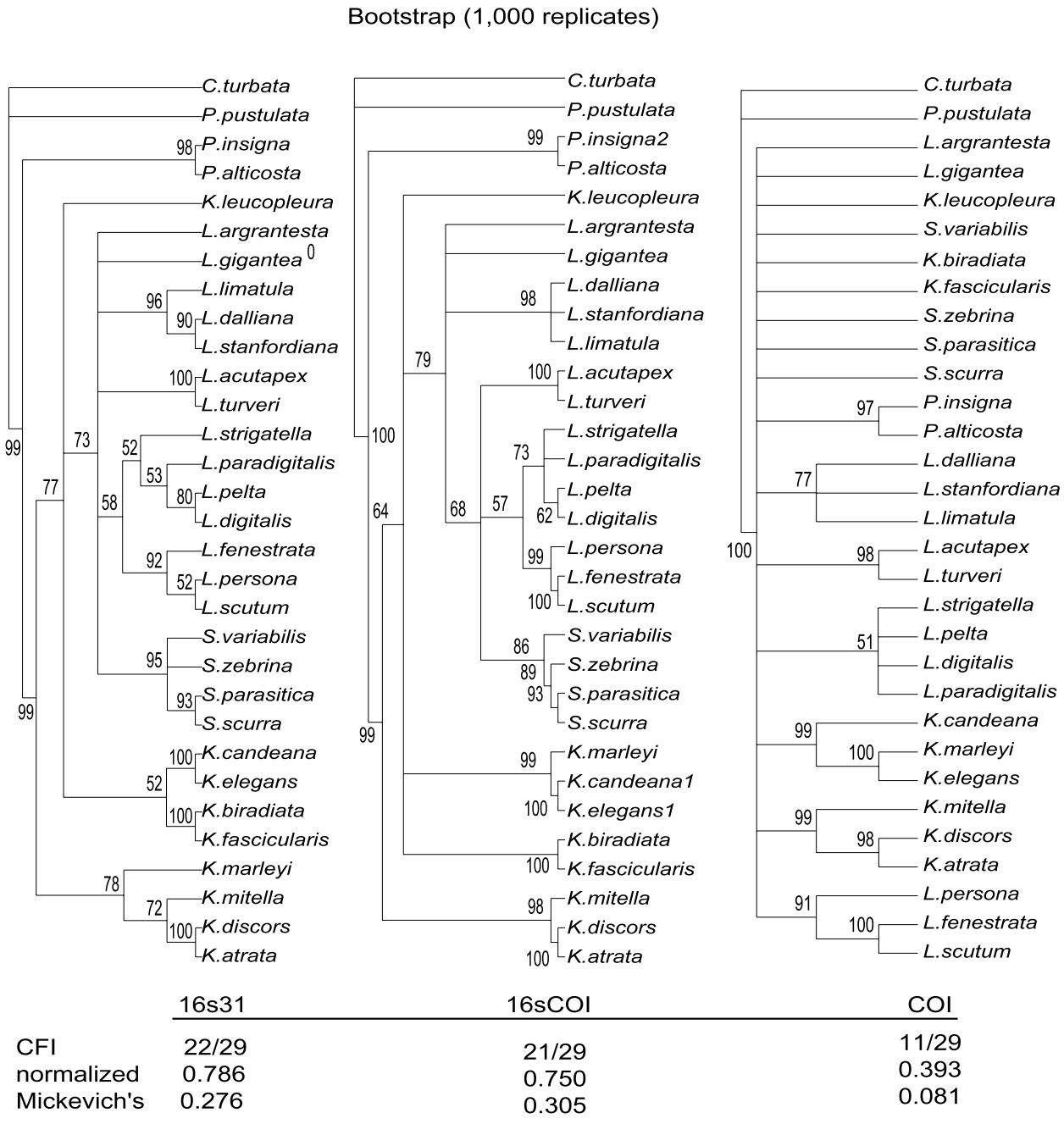


Figure 4. MP trees from the five partitions listed in figure 1.



	16s31	16sCOI	COI
CFI	22/29	21/29	11/29
normalized	0.786	0.750	0.393
Mickevich's	0.276	0.305	0.081

Figure 5. 50% majority consensus bootstrap trees from the 16S31, 16SCOI, and COI partitions with values for the Consensus Fork Index (CFI) and Mickevich's consensus index.

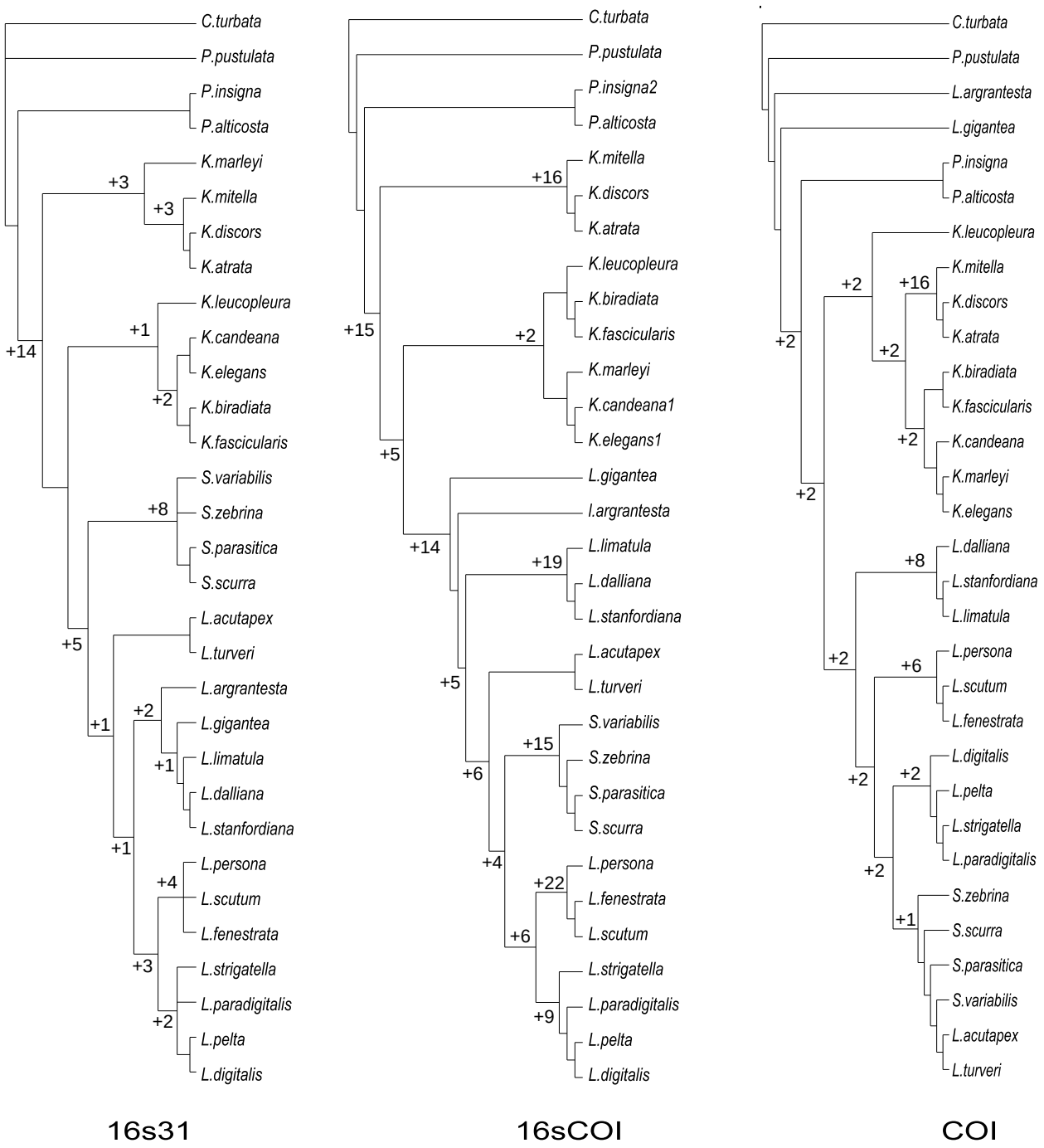


Figure 6. Decay values for MP trees from the 16S31, 16SCOI, and COI partitions.

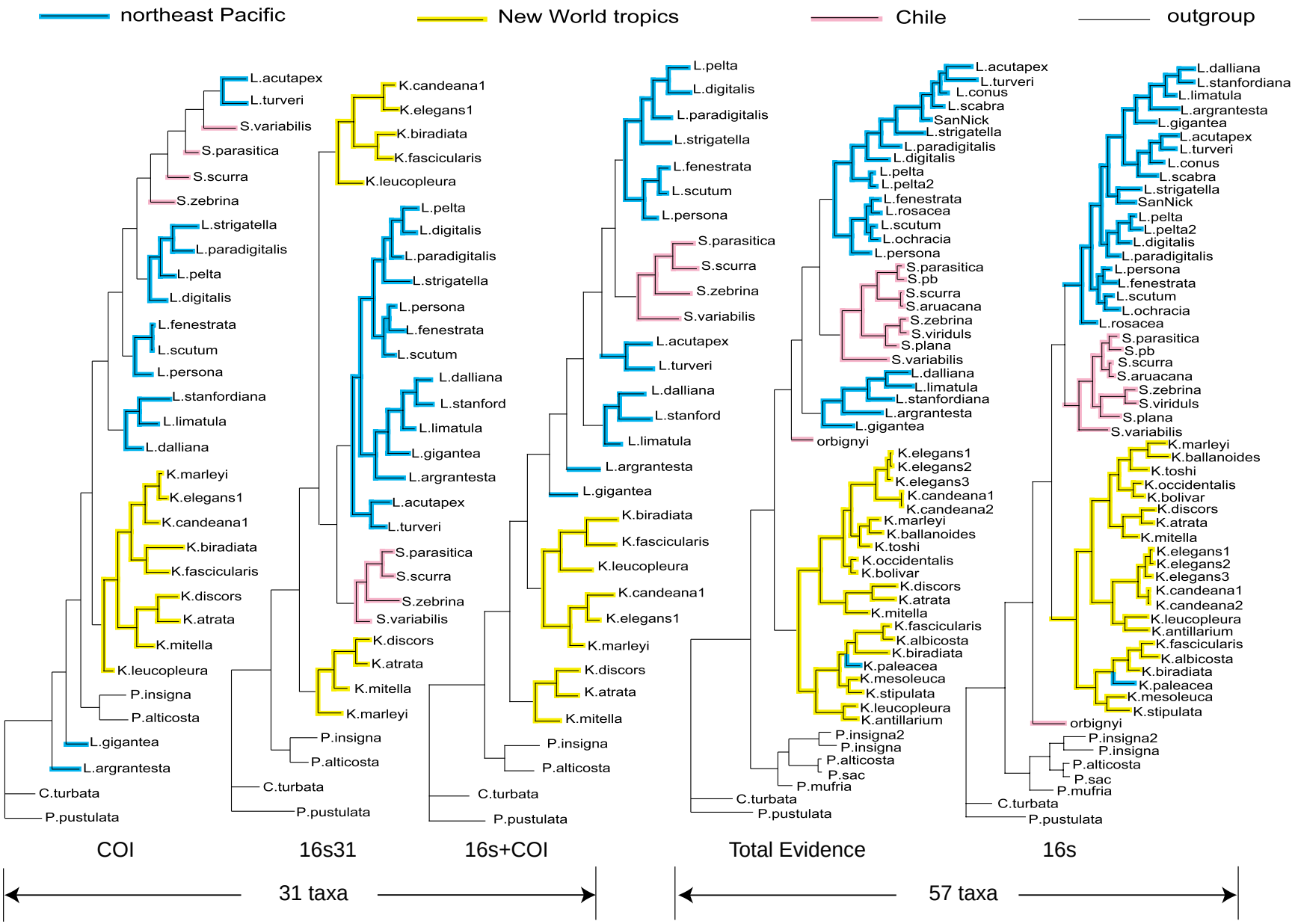


Figure 7. MP trees from the five partitions listed in figure 1 with mapped biogeographical distributions.

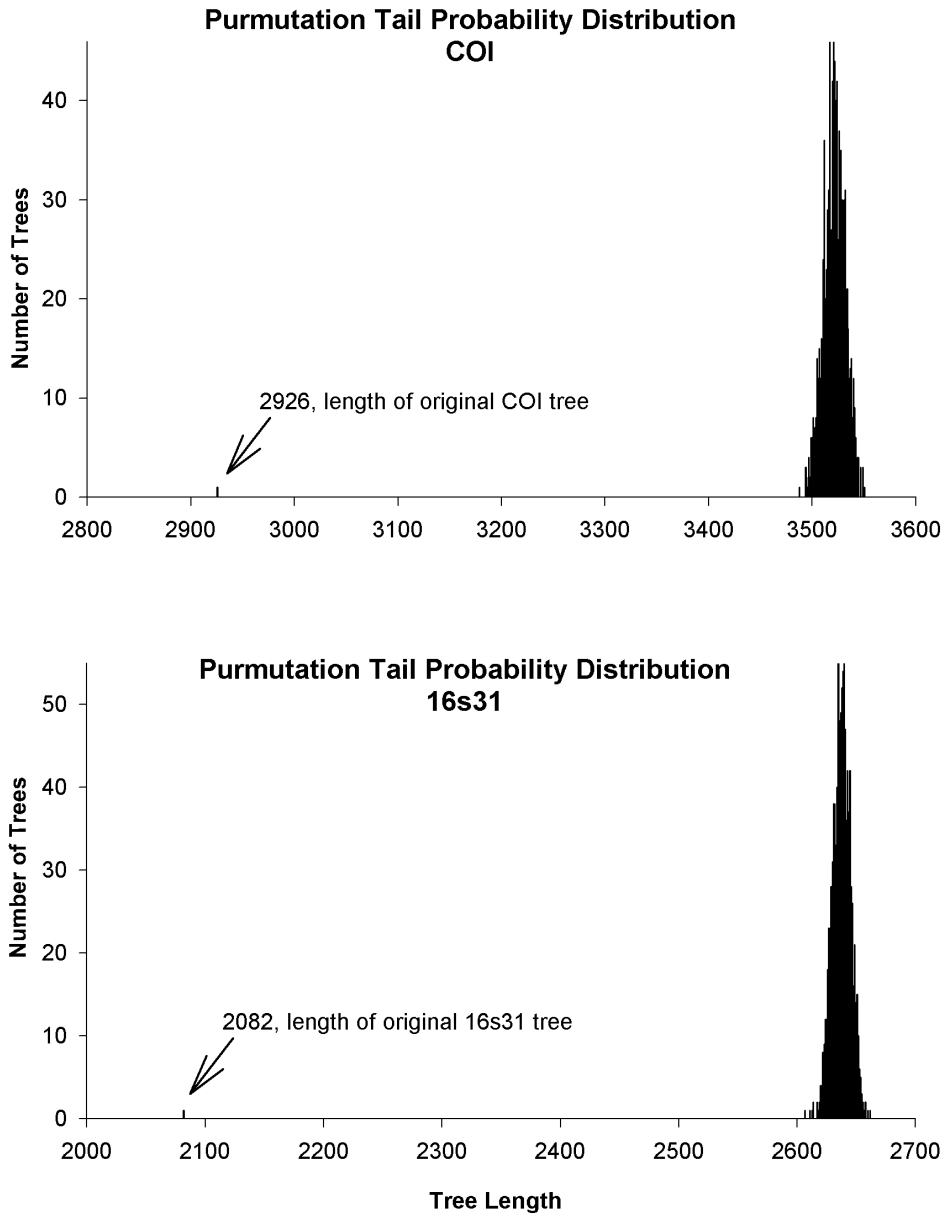


Figure 8 Plots of Purmutation Tail Probability tests performed on the 16S31 and COI partitions. This tests for the null hypothesis that the partitions contain no phylogenetic signal.

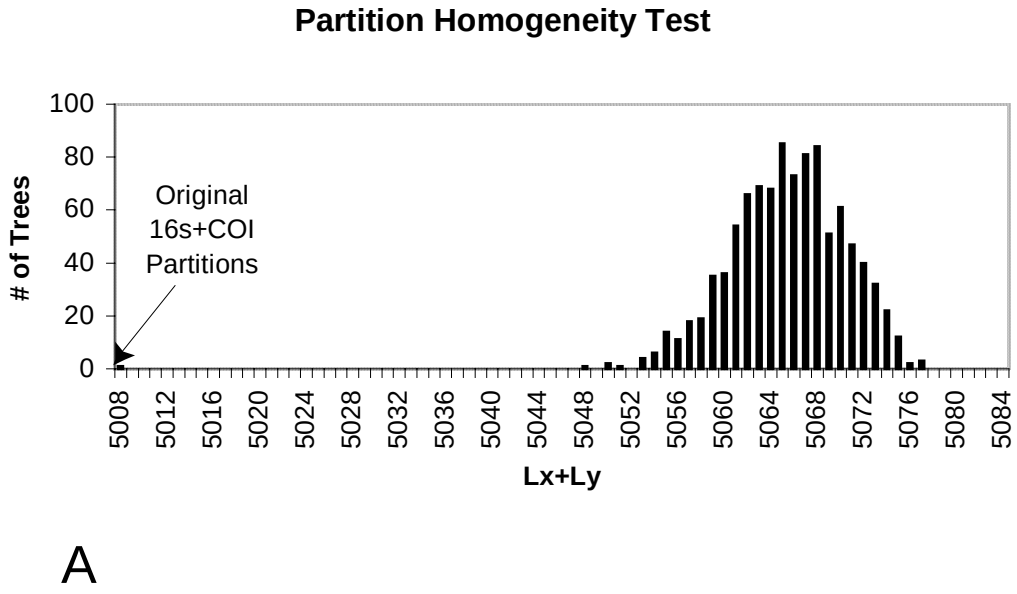


Figure 9. Plots of the Partition Homogeneity test (swofford, 1995) (A) and the Incongruence Length Difference test (ILD) (Ferris, 1995) (B). Lo = tree length of combined analysis of partitions x and y, Lx = tree length from partition x, Ly = tree length from partition y.

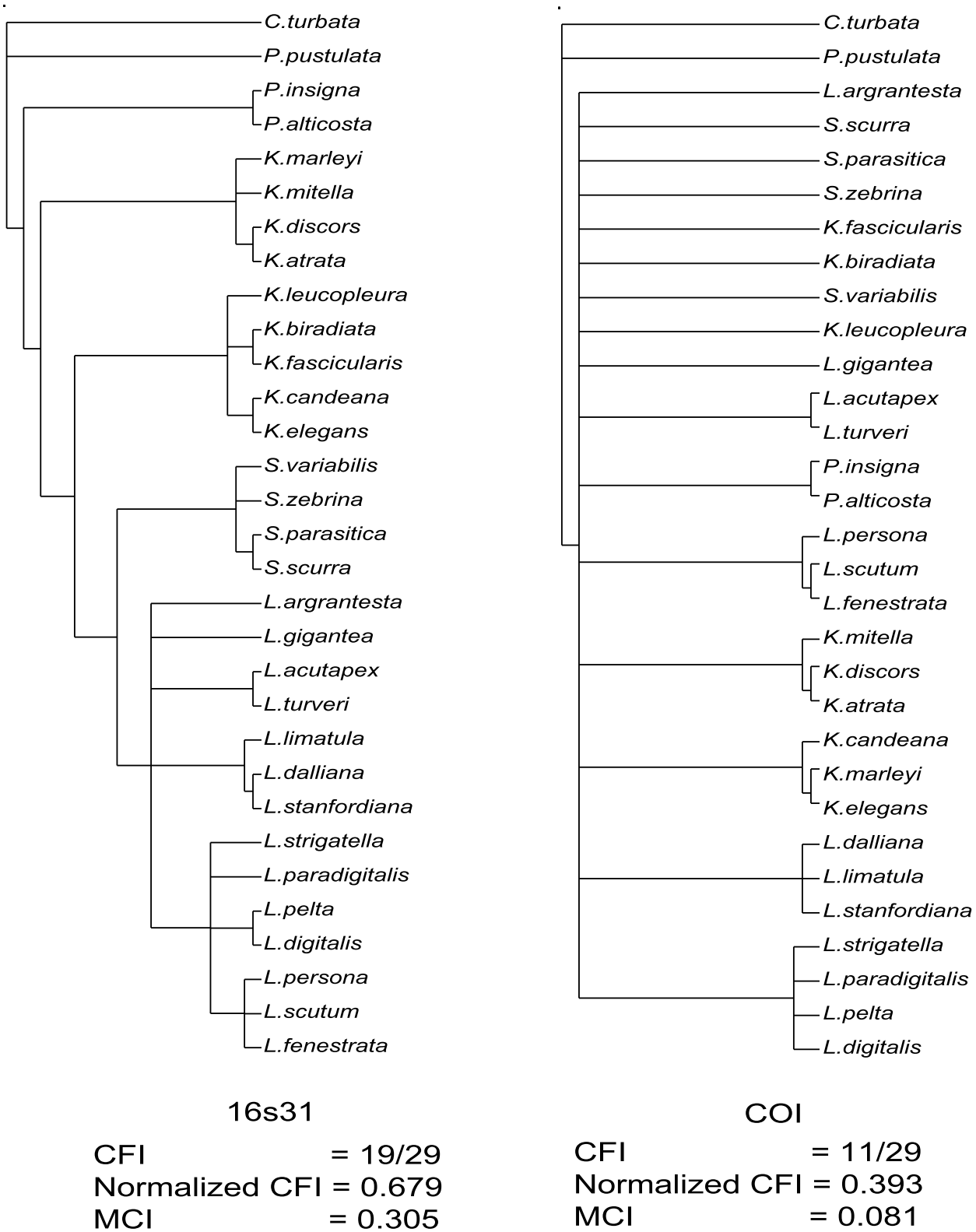


Figure 10. Strict consensus trees of trees generated from the COI and 16S31 partitions by MP, ML, NJ, UPGMA, and star decomposition methods.

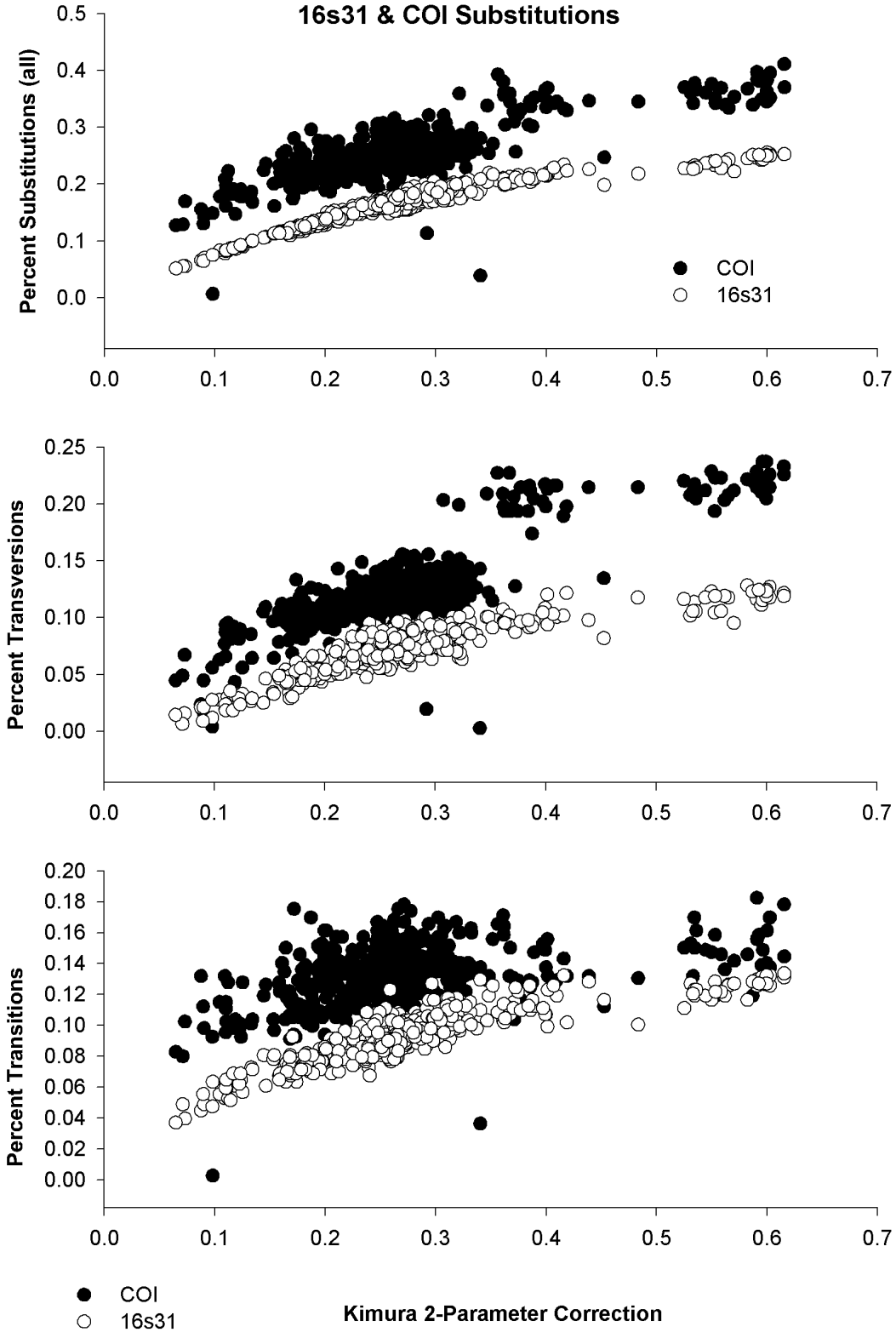


Figure 11. Substitution plots of observed all substitutions, transversions, and transitions against a Kimura 2-parameter substitution correction model.

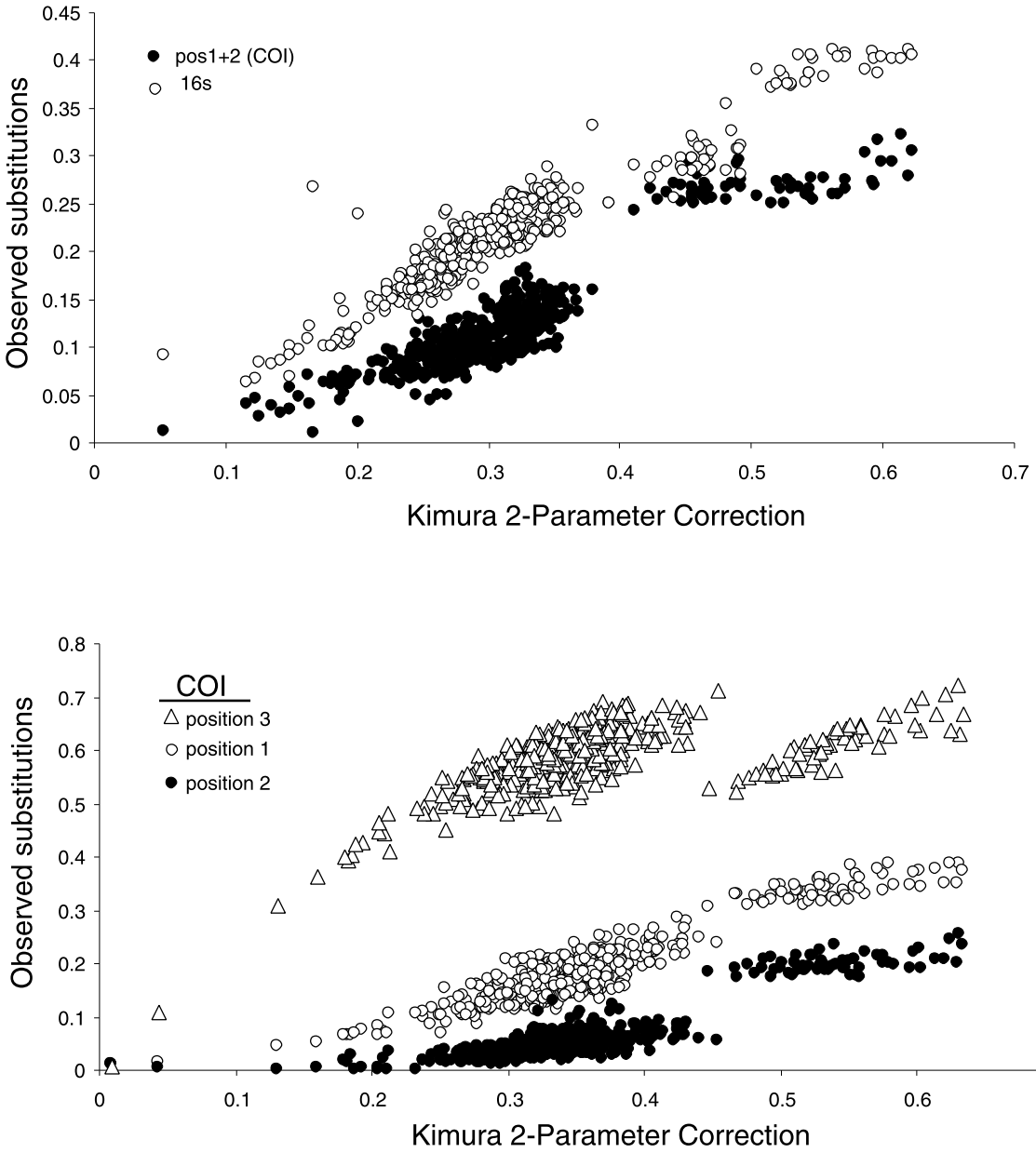
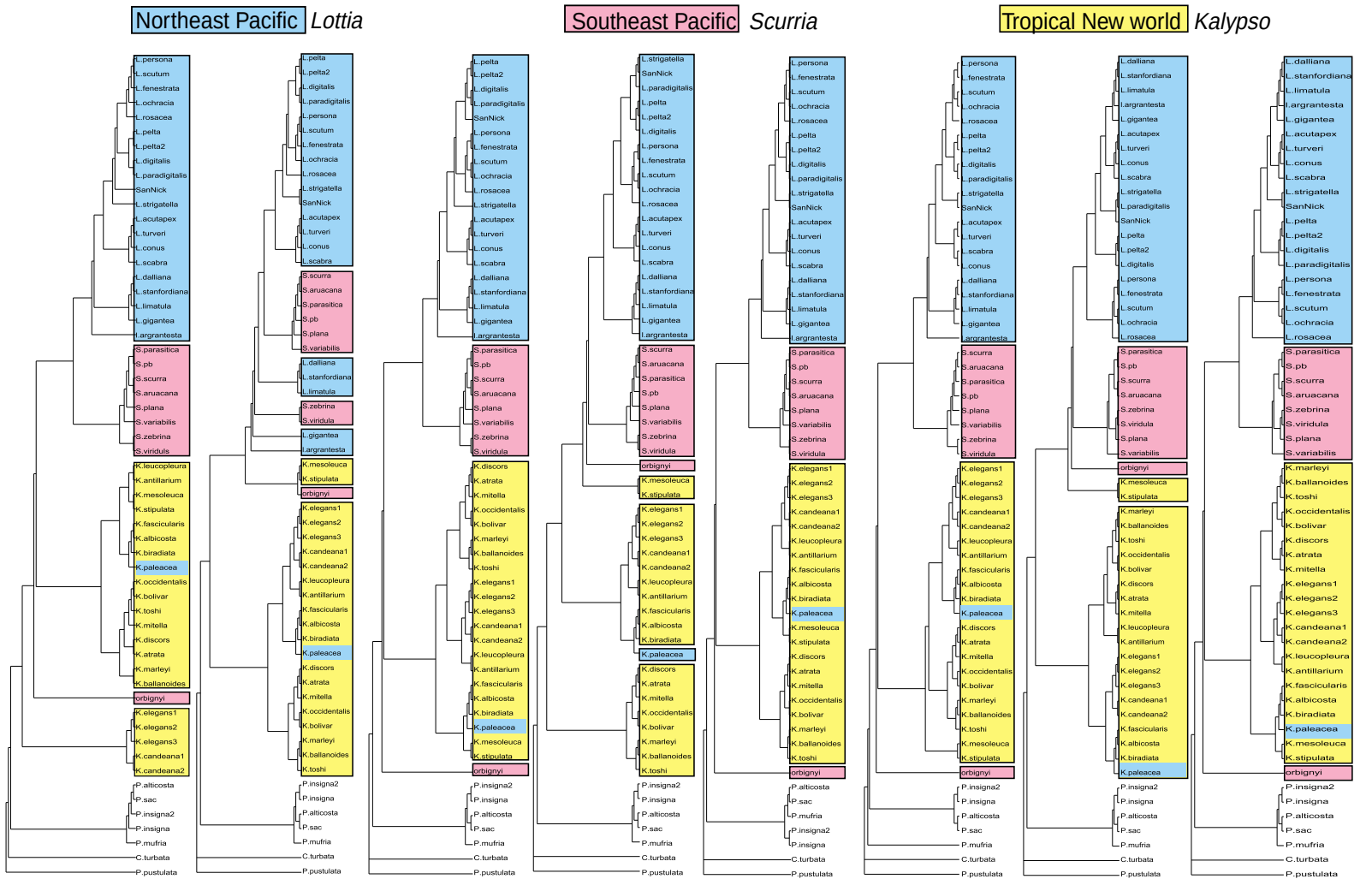


Figure 12. Substitution plots of observed substitutions against a Kimura 2-parameter substitution correction model. The top plot compares 16S and COI substitutions with the 3rd codon position eliminated for COI. The lower plot compares all three codon positions.



	UPGMA	Star	NJ-uncP	NJ-K2P	ME-uncP	ME-K2P	Likelihood	Parsimony
ML	14271.67324 (6)	14271.768 (7)	14209.47897 (3)	14188.0169 (2)	14214.6889 (4)	14224.71778 (5)	14164.593	14179.169 (1)
MP	3398 (7)	3388 (6)	3341 (2)	3342 (3)	3348 (4)	3352 (5)	3333 (1)	3309

Figure 13. Trees generated from UPGMA, Star decomposition, NJ, ME, ML, and MP with mapped biogeographical distributions and MP

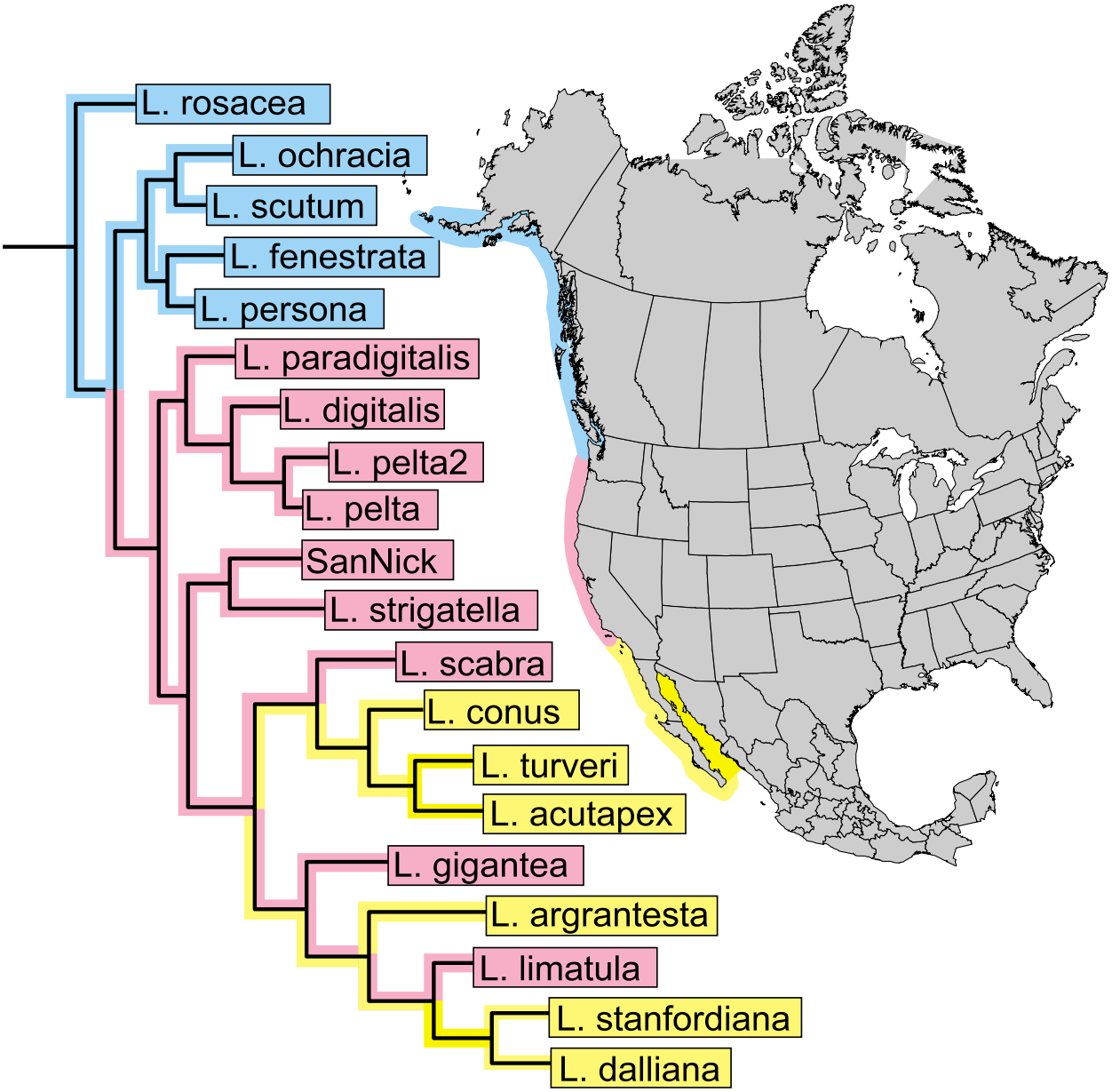


Figure 14. The northeast Pacific clade from the 16S MP tree with mapped biogeographical distributions. The mapped distributions reveal a north to south correlation with basal to derived OTUs. The colored OTUs are found along the correspondingly colored coastlines.

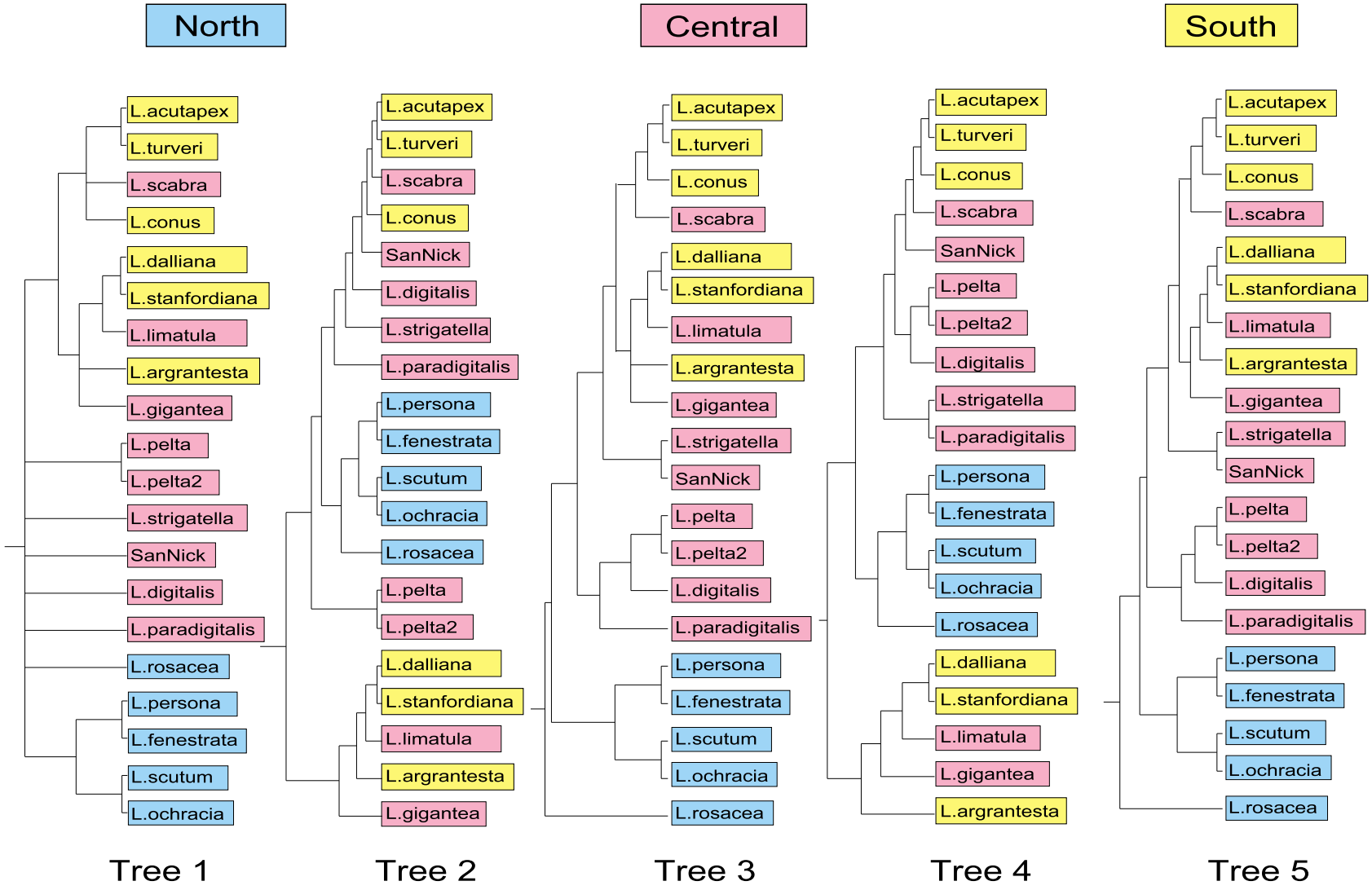


Figure 15. The northeast Pacific clade from the weighted MP trees in Figure 13. The northeast Pacifc is divided into three regions, north, central and South which correspond to the map in Figure 13. The northeast pacific regions are color coded and mapped onto each tree.

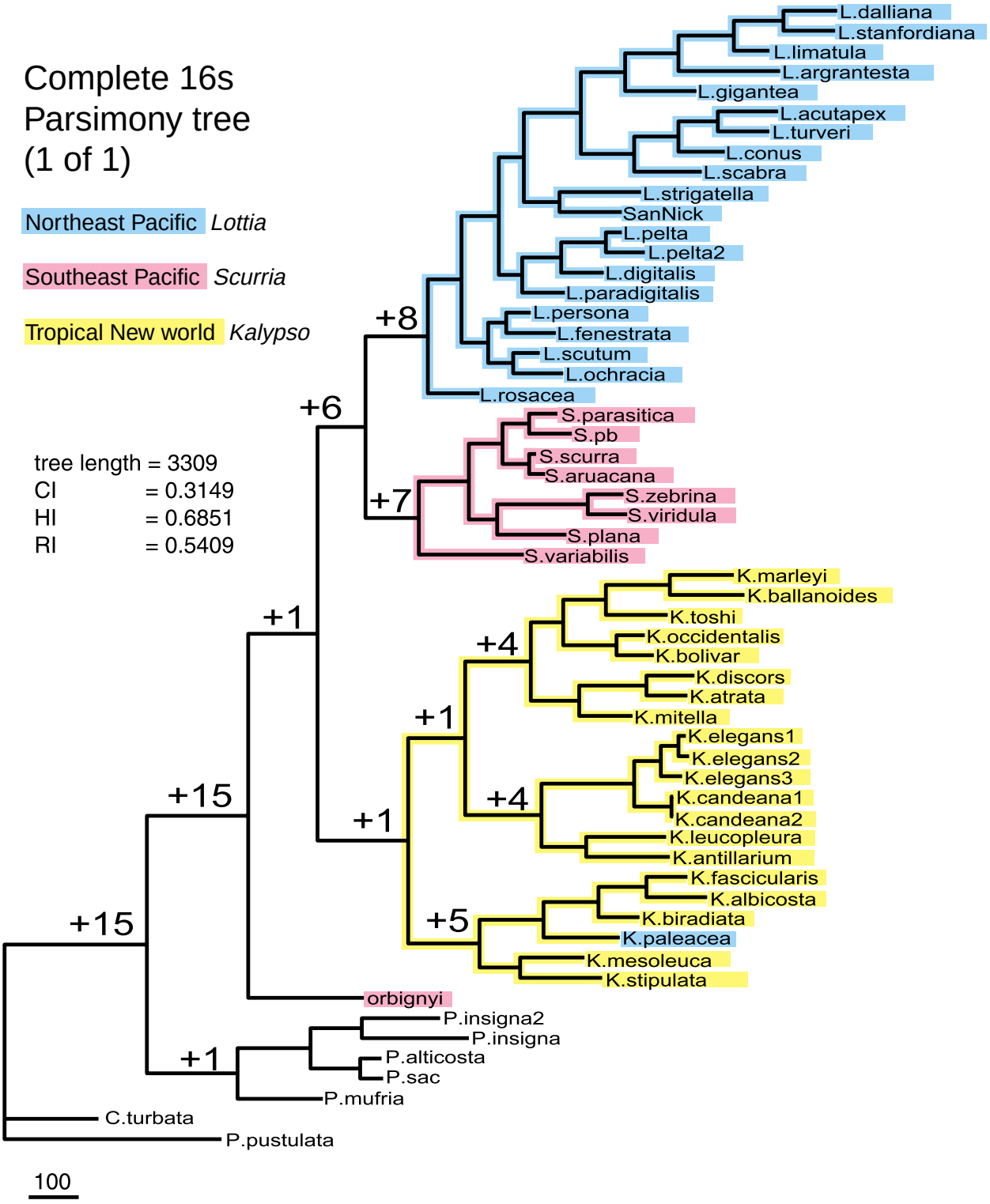


Figure 16. The 16S MP tree with Bremer support values and mapped biogeographical distributions indicated with color.

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Chapter Three

MORPHOLOGICAL AND MOLECULAR RESOLUTION OF A PUTATIVE CRYPTIC SPECIES COMPLEX: A CASE STUDY OF *NOTOACMEA FASCICULARIS* (GASTROPODA: PATELLOGASTROPODA)

Abstract

Evidence that *Notoacmea fascicularis* (Menke, 1851) is a complex of at least two distinct taxa of species rank is ambiguous. A discriminant function analysis of conchological data shows a weak geographic effect, while radular morphology clearly delineates two sympatric groups with rare intermediates. Lastly, molecular data (mt cytochrome c oxidase subunit I) suggests a single species and a geographic effect. We consider *N. fascicularis* to be a single taxon, variable for radular lateral tooth morphology. In the past these two different radular morphologies would be indicative of generic rank. Our knowledge of the intraspecific variability of most gastropod characters is poor, and this makes specific identifications or groupings based on single character systems such as the radula precarious. Adequate sampling and evaluation of population-level character states (conchological, anatomical and molecular) is needed to identify as well as falsify cryptic species complexes.

Introduction

Cryptic or sibling species are taxa that are difficult or impossible to distinguish on morphological characters alone (Mayr & Ashlock, 1993), and they have confused and troubled systematists since the late 1800's (Mayr, 1963). In the marine realm, they have been noted in almost every major phylum; Knowlton (1993) lists over 130 marine invertebrate examples of sibling species, including 21 molluscan cases. Since Knowlton's listing, numerous additional gastropod examples have been investigated, for example by Crossland et al. (1993), Rolan-Alvarez, et al. (1995), Kool (1995), Zaslavskaya (1995) and Gofas & Jabaud (1997). Within the Patellogastropoda, sibling species have been discussed in Patellidae (Acuna & Munoz, 1995) and Lottiidae (Test, 1946; Lindberg & McLean, 1981).

While Mayr (1948) used the existence of sibling species to argue against the morphological species concept, he also contended that additional data sets (morphometric, genetic, ecological) could successfully resolve sibling species complexes. Like Mayr, Knowlton (1993) emphasized that many of the taxonomic problems associated with sibling species resulted from a failure to use all available characters. However, she also pointed out that acquisition of comprehensive data sets is often difficult for many marine invertebrate groups. This is particularly true for molluscan studies where the bulk of specimens in research collections are shells (Brusca & Brusca, 1990).

The development of molecular techniques for sampling genomic characters has substantially increased the power of discovery and resolution of sibling

species complexes, and may ultimately increase the number of marine species by an order of magnitude (Knowlton, 1993). However, these same techniques also furnish data sets that can refute putative sibling species and instead reveal cases of polymorphism or plasticity within taxa.

Here we examine a putative example of cryptic species in the patellogastropod limpet *Notoacmea fascicularis* (Menke, 1851). Like most gastropods, this species was initially recognized and delineated on conchological characters. Although it was a variable species with respect to shell color and pattern (Carpenter, 1857), the range of variation within these characters did not substantially overlap with other tropical eastern Pacific taxa, and thus the identity of *N. fascicularis* was never viewed as problematic (Dall, 1871; Pilsbry, 1891; Keen, 1958). During the 1960's, McLean (pers. Comm.) examined radulae of more than 25 specimens of *N. fascicularis* from 11 localites between Cabo Pulmo, Baja California Sur, Mexico and Costa Rica (Table 1). Within these specimen lots, he discovered two distinct radular morphologies (Table 1, Fig 1). Based on this discovery, McLean (1971:327) concluded that "There is some indication that this is a complex involving more than one species; some populations have the three lateral teeth of nearly equal size, while in others the outer lateral is reduced. As yet no shell characters have been found that correlate with the radular differences."

McLean's suggestion that *N. fascicularis* was a cryptic species complex of at least two species demonstrates the taxonomic weight afforded the patellogastropod radula. Heavy reliance on radular characters in the diagnosis of

patellogastropod species has a long and noteworthy history in gastropod taxonomy (Thiele, 1866-1893; Dall, 1871; Oliver, 1926; Koch, 1949; McLean, 1966; Powell, 1973; Christiaens, 1973, 1975a,b; Lindberg & McLean, 1981; Lindberg, 1988). For over 25 years, McLean's suspicion of a cryptic species complex within the taxon *N. fascicularis* has remained untested. With the advent of molecular techniques, it is now possible to produce a third data set, which may resolve this question. We used partial sequences of cytochrome c oxidase subunit I (COI) to examine the correlations between phylogeny, conchology, and radular morphology in *N. fascicularis.*

We examined 59 specimens of *N. fascicularis*; 29 of these specimens were from collections available to McLean when he observed radular variation in this species; WBS collected the remaining 30 specimens between 1995-1997 for this study (Table 1). Institutional abbreviations used herein are as follows: LACM – Natural History Museum of Los Angeles County, Los Angeles, CA; UCMP – Museum of Paleontology, University of California, Berkeley, CA. Localities along the west coast of Mexico are abbreviated as: CSL - Cabo San Lucas, Baja California Sur; COL - Colima; MAN - Manzanillo; MAZ - Mazatlán; PVA - Puerto Vallarta.

Shell morphology

Digital images of the ventral, dorsal and profile of each shell were captured with a digital camera connected to a Scion LG-3 Scientific Frame Grabber system. Taking the maximum shell length obtained with dial calipers

and converting this measurement to pixels scaled a baseline for each image. Once the images were scaled for size, measurements of projected shell area, shell length, shell width (at widest point), apex position (from anterior and posterior ends), projected shell muscle scar area, and shell height was determined (Fig. 2).

Five ratios were also calculated: (1) distance from anterior edge to apex/distance from posterior edge to apex, (2) projected shell muscle scar area/projected shell area, (3) shell height/shell width, (4) shell height/shell length, and (5) shell width/shell length (Fig. 2).

Data for localities with ≥ 5 specimens were partitioned into three data treatments for discriminant function analysis (DFA). The first data treatment consisted of all linear measurements, area measurements, and ratios (DFA ALL). The second data treatment consisted just of ratios (DFA RATIOS), while the third data treatment contained only log linear and area measurements [DFA LOG (*x*)]. These treatments were individually submitted to DFA (Systat 7.0®) to identify morphological attributes that would distinguish specimens relative to an independent classification variable. Previously, DFA has been used to separate members of a sibling species complexes when morphological characters show considerable overlap (Knowlton, 1993). Two independent classification variables were used: (1) locality and (2) tooth morphology. Thus, there are six DFA results; two classifications by three data sets (see Appendix).

Radular morphology

An anterior portion of the radular ribbon from each specimen was dissected from the head region posterior to the odontophore and placed in a 10% sodium hypochlorite solution for ≤ 5 minutes and rinsed in distilled water. The radular ribbon was placed on an ElectroScan Model E3 Environmental Scanning Electron Microscope (ESEM) stub with cusps oriented upwards. The stubs were then placed in the ESEM vacuum chamber where 150x to 265x digital images of each radula were captured. Assignment to equal, unequal, or intermediate radular categories was based on the criteria illustrated in Fig. 1.

Molecular sequence data

To detect the presence of molecular divergence between individuals with equal and unequal radulae, cytochrome c oxidase subunit I (COI) was partially sequenced and compared among 30 individuals from three localities. COI was chosen for this study based on its interspecific and intraspecific levels of variation found among sequences of eastern Pacific patellogastropods (Wray; Simison; Clabaugh; Lindberg; all unpubl. data). All material was initially preserved in 70% ethanol.

Extraction

Two equally successful DNA isolation protocols were used: (1) saturated salt/chloroform extraction, and (2) CTAB/phenolchloroform extraction. For each extraction, pedal tissue was cut from the foot margin approximately 3-5 mm

along the margin and 3-5 mm towards the center of the foot. The tissue was soaked in deionized water to remove any residual ethanol and finely diced. For the saturated salt technique, the diced tissue was placed in a 1.5 ml tube containing 250 µl isolation buffer (100 mM TRIS, 10m M EDTA and 400 mM NaCl), 60 µl 10% SDS, and 10 µl proteinase K. The mixture was then vortexed and stored on a shaker at 37º C overnight. Following tissue digestion, 175 µl of saturated NaCl solution was added. The samples were inverted for 5 minutes and centrifuged at 13k rpm for 30 minutes. The supernatant was washed with chloroform using 2 times supernatant volume and mixed by inversion for 2 minutes. The supernatant DNA was precipitated using two volumes of ice cold 100% ethanol and spun at 13k rpm for 15 minutes. The DNA pellet was washed twice with two volumes of 70% ethanol, and dried for five minutes in a speed vac. The DNA was eluted in 100 µl of double-distilled water and stored at -20ºC.

For the CTAB technique, diced tissue was added to a 1.5 ml tube containing 600 µl 2XCTAB and 9 µl of proteinase K, and then incubated at 37ºC overnight. 600 µl of phenol:chloroform:isoamyl alcohol (25:24:1) was added to the tissue mixture and mixed via inversion for 5 minutes. The solution was then centrifuged at 13k rpm for 15 minutes. The supernatant was added to 600 µl of chloroform:isoamyl alcohol (24:1), mixed for 5 minutes and centrifuged at 13k rpm for 15 minutes. DNA was precipitated using 600 µl isopropanol and stored at -20ºC for 2 hours. The precipitate was centrifuged at 13k rpm for 30 minutes at 4ºC, and then the pellet washed twice with two volumes of 70% ethanol. It

was then centrifuged at 13k rpm for 20 minutes before being dried by speed vac for 5 minutes and eluted in 100 µl of deionized water.

Amplification

Amplification of a section of the coding region of COI was achieved with the HCO-2193 and LCO-1490 primers described by Folmer *et al*. (1994). In a 0.5 ml gene amp tube, on ice, 36.45 µl double-distilled water, 5 µl 10x PCR buffer (Perkin Elmer), 2.5 µl 10 µM dNTP's (Pharmacia), 2.5 µl 25 µM $MgCl_2$ (Perkin Elmer), 1 µl each of the 10 µM HCO and LCO primers, 1 µl of template, and 0.25 µl of taq (Perkin Elmer) were combined. A negative control containing all reagents except the template was run in parallel. The tube was then transferred to a Perkin Elmer 9600 geneamp. The cycling parameters began with an initial denaturation at 95ºC for 2 minutes followed by 36 cycles with three temperature plateaus of 95º C for 50 seconds, 45º C for 50 seconds, and 72º C for 90 seconds, ending with a 7 minute extension at 72º C. PCR products were purified using Wizard® PCR preps DNA Purification System.

Sequencing

Direct double-stranded cycle sequencing of 20 to 30 ng of COI PCR product was performed in both directions using the ABI® cycle sequencing kit following a half reaction ABI® cycle sequencing protocol. Cycle sequencing was performed using a Perkin Elmer 9600 geneamp. The cycling parameters were 25 cycles at 96ºC for 10 seconds, 50ºC for 5 seconds, and 60ºC for 4 minutes.

Cycle sequencing product was purified using Princeton Separations Centrisep spin columns, then dried in a speed vac. The dried, purified cycle sequencing product was resuspended in 2.5 µl loading solution of 5:1 deionized Formamide:25mM EDTA with 50mg/ml Blue Dextran. 1.5 µl of sample and loading solution was loaded on a 36 cm 4% acrylaminde gel. The gel was run and analyzed on an ABI Prism® 377 DNA sequencer.

Alignment and Anaylsis

All sequences were aligned by hand using ABI® Sequence Navigator. Because COI is a coding region, alignments were not problamatic; all of the intraspecific substitutions were synonomous, and very few gaps were necessary in the interspecific alignments. Intraspecific sequences were compared among 30 *N. fascicularis* individuals. Intraspecific distances were calculated for three species of Chilean *Scurria* limpets (*Scurria ceciliana*, *Scurria boehmita*, and *Lottia viridula*) and interspecific distances calculated for seven Chilean limpet species.

A pair-wise distance matrix and parsimony-based phylogeny of the *N. fascicularis* alignment data were produced using PAUP 3.1.1 (Swofford, 1993). The phylogeny was then used to map the distribution of radular types to detect possible correlations between equal and unequal radular groups or localities.

Shell morphology

The results of the DFA support McLean's (1971) assertion that shell characters are not correlated with the radular differences. Wilks' lambda for the

three data sets with tooth type as the classification variable range between p = 0.043 and 0.094 (Table 2) and only 61%-68% of the shells can be correctly assigned to their respective radular group based on shell parameters (Table 2). The results of the second series of DFA using locality as the classifying variable appear to provide better discrimination of the taxa (Fig. 3). Wilks' lambda for the three data sets was less than P = 0.0010 (Table 2).

The different treatments in the DFA analysis [DFA ALL, DFA RATIOS, and DFA LOG (x)] produced different patterns of discrimination. DFA ALL provided the greatest discrimination by locality, but also had the highest correlation between sample size and the percentage of specimens correctly classified (Table 2). The distribution of group centroids for DFA ALL and DFA LOG (x) were similar to one another, and these treatments had the greatest range of canonical functions, primarily in the size variables (see appendix). The range of canonical functions from the DFA ALL treatments were substantially smaller than those from the DFA ALL and DFA LOG (x) treatments. Furthermore, in the DFA ALL treatment, only the APEXPOS ratio was found to be significant in the discrimination of the groups, the remaining four ratio-based variables were not significant in the discrimination (Appendix). These results suggest that size is important in discriminating between groups in DFA ALL and DFA LOG (x) (see also Reist, 1985).

Radular morphology

Radular morphologies consisted of 10 equal and 18 unequal specimens with two intermediate forms (see Fig. 1). Based on examination of the collections and radular preparations in LACM, we estimate that McLean examined 26 specimens of *N. fascicularis* from 11 lots prior to suggesting the possibility of sibling species. Only one of the 11 lots apparently examined by Mclean contained specimens with both radular morphologies (Manzanillo in 1963), although at Zihuantenejo a single specimen with unequal morphology and one with the intermediate condition were present (Table 1). The remaining nine lots exhibited only a single radular morphology, either equal or unequal. Among the new collections, both extreme radular forms and intermediates were present at Cabo San Lucas and Mazatlán, while only equal morphologies were present at Puerto Vallarta (Table 1).

Molecular sequence data

Without suggesting that species be defined by some arbitrary molecular distance, we observe that distances found among *N. fascicularis* individuals fall well within the range of distances found among individuals of three Chilean *Scurria* species, and well outside the range of distances found between species of the Chilean clade. Differences range between 0-14 base pairs per 710 positions for *N. fascicularis* (0-2.0%) (Table 3) and between 5-9 base pairs per 639 positions within Chilean species (0.8-1.4%). In contrast, interspecific

distances calculated amongst the seven Chilean species ranged from 40-157 base pairs per 632 positions (6.3-24.8%).

The molecular phylogeny of *N. fascicularis* (Fig. 1) reveals clear geographic groupings with a random distribution of radular types across the groups. Although further populational level studies are needed, our analysis suggests that the radular differences found in *N. fascicularis* are intraspecific variation and are not sequestered in independent evolutionary lineages.

Discussion

Evidence that *N. fascicularis* is a complex involving two separate taxa of species rank is ambiguous. Conchological data subjected to a discriminant function analysis (DFA) show a weak geographic discrimination by shell characters. However, the discrimination appears to be primarily size-based and sample size further confounds this result. Radular morphology clearly delineates two distinct groups with rare intermediates, while molecular data suggests a single species and clear geographical associations. Thus, shell morphology and molecular data support the presence of a single species rank taxon, while radular data is consistent with the presence of a cryptic species complex (for a similar example in the hydrobiid *Tatea* see also Ponder *et al*., 1991). Based on these results we suggest that the taxon *N. fascicularis* is variable for radular lateral tooth morphology.

Historically, malacologists have placed substantial emphasis on radular characters in taxonomic studies, and McLean's suggestion that *N. fascicularis* was a cryptic species complex is consistent with this emphasis, as well as the then known range of variation in patellogastropod radular characters. While radular teratologies and variation in tooth cusp number have long been known (Jackson, 1907; Pelseneer, 1928; Peile, 1922; Eslick, 1940), the frequency and strongly bimodal distribution of radular morphologies seen in *N. fascicularis* were unprecedented.

Heavy reliance on radular characters in the diagnosis of patellogastropod can be traced back at least to the work of Dall (1871). With few exceptions (*e.g.*, Grant, 1937; Eslick, 1940; Sasaki & Okutani, 1993, 1994), most workers have viewed the patellogastropod radula as invariant at the species level and thus an important and conservative character in determining and grouping species-level taxa (Dall, 1871; Oliver, 1926; McLean, 1966; Lindberg, 1981; Ponder & Creese, 1982; Lindberg & McLean, 1981). Moreover, the differences in the size of the third lateral teeth, such as documented here in a single putative species, were often regarded as characters indicative of generic rank (McLean, 1966; Christiaens, 1985a, b; Lindberg, 1981).

Grant's (1937:38) aversion to the use of radular tooth morphology in patellogastropod systematics did not originate from insights into possible variation in tooth shape, but rather her perception that unequal wear, abrasion and injuries associated with feeding were likely to deform individual teeth. Instead of tooth morphology, she proposed that characters of the radular

membrane were diagnostic at specific and higher rankings. Sasaki & Okutani's (1993) example of variation in the patellogastropod radula is ontogenetic; uncini initially present at the edges of the radular membrane disappeared with growth in *Nipponacmea nigrans* (Kira, 1961) and *Nipponacmea teramachii* (Kira, 1961). Eslick (1940) also observed ontogenetic change in lateral tooth morphology of *Patella* species in England. However, none of these examples is of the magnitude of differences observed in the radula of *N. fascicularis*.

This, and a recent example in the littorinid *Lacuna* (Padilla, 1998), strongly suggests that our knowledge of the range of intraspecific variability of the gastropod radula is rudimentary at best, and that rigorous population level evaluation of radular morphology should be undertaken prior to its use in any phylogenetic analysis or species definition. It also shows the danger of basing identifications or groupings on single character systems. As Knowlton (1993) cautioned, reliance on a single character to distinguish sibling species is precarious, because single characters that delimited species in some taxa represented intraspecific variability in others. This insightful statement precisely describes the condition in *N. fascicularis.* Without the additional data sets and study, *N. fascicularis* might otherwise have been broken into separate species or subspecies based on radular morphology.

An additional caution is present in the results of our morphological study; population-level evaluation of character states must include sufficient samples to ensure that the results are unbiased. Figure 4 shows that the percentage of specimens correctly classified in the DFA is highly correlated with sample size.

As sample size increased the number of correctly classified specimens would stabilize, while with smaller sample sizes up to 100% of the specimens would be "correctly" classified.

The basis of this radular variability remains unexplored in these patellogastropods. However, there is a statistically significant size effect associated with the radular types at Mazatlan (Table 2), where individuals with equal teeth are significantly larger than individuals with unequal teeth (t = 4.235, p value = 0.001). Sample sizes were not adequate to explore this relationship at other localities and this association is not present in the pooled data. Its absence in the pooled data most likely results from the confounding of different habitat-specific growth rates, a common feature of patellogastropod taxa (Giesel, 1969; Branch, 1974; Lindberg & Wright, 1985; Brown & Quinn, 1988; Liu, 1994). Adequate sampling of additional populations is needed to test further this hypothesis.

The pooled data also show no clinal variation in the occurrence of radular morphologies. Both radular morphologies occur in male and female limpets and individuals with different radulae can occur on the same rock in juxtaposition (WBS, pers. obs.). Gut contents appear identical in specimens with both tooth types, suggesting that unlike the phenotypic plasticity in *Lacuna* (Padilla, 1998), radular morphologies in *N. fascicularis* are neither habitat nor food induced.

This study and its findings calls into question past uncritical use of radular characters and single character taxonomies in patellogastropod classifications, and is likely applicable to other gastropod groups as well. Prior systematic work

in the patellogastropods requires re-evaluation. For example, the sibling species pairs described from the Galapagos (Lindberg & McLean, 1981) are certainly suspect and, in the case of the patellogastropods, further detailed studies of putative species and complexes will undoubtedly reduce estimates of diversity in some faunas while increasing it in others.

Tables

Table 1. Specimen lots of *Notoacmea fascicularis.* 1-11 examined by McLean and 1-14 this study. Conchological and radular data sets were available for lots 1-14; molecular data sets for lots 12-14 only. Unless otherwise noted all localities are within Mexico.

	Locality Nos.	Locality & Date	Lateral Tooth Morphology
1	LACM 66-41	Acapulco, Guerrero. 1966.	3 unequal
2	LACM A.7079.70	Bahia Santiago, Colima. 1967	7 unequal
3	LACM 65-13	Cleotus, Tres Marias Ids.. 1965	1 unequal
4	LACM 63-10	Manzanillo, Colima. 1963	2 equal; 2 unequal
5	LACM 152423	Manzanillo, Colima. 1965	1 equal
6	LACM 63-11	Mazatlan, Sinaloa. 1963	2 unequal
7	LACM A.8981.68	Mazatlan, Sinaloa. 1966	2 equal
8	LACM 65-20	Zihauntenejo, Guerrero. 1965	1 unequal; 1 intermediate
9	LACM 70-65	Playas de Coco, Costa Rica. 1970	1 equal
10	LACM 66-7	Pulmo Reef, Baja CA Sur. 1966	1 unequal
11	LACM 67-97	Salina Cruz, Oaxaca. 1967	2 unequal
12	UCMP 15201	Cabo San Lucas, Baja CA Sur. 1995-97	2 equal; 1 intermediate; 7 unequal
13	UCMP 15202	Mazatlan, Sinaloa. 1995-97	5 equal; 1 intermediate; 11 unequal
14	UCMP 15203	Puerta Vallarta, Jalisco. 1995-97	3 equal

Table 2. Statistics for discriminant function analysis and classification based on shell and tooth morphology.

Shell morphology	*n*	DFA ALL Correctly Classified Wilks' lambda p = 0.000	DFA Ratios Correctly Classified Wilks' lambda p = 0.001	DFA Log(x) Correctly Classified Wilks' lambda p = 0.000
Cabo San Lucas	10	70%	50%	70%
Colima	7	86%	57%	86%
Manzanillo	5	100%	40%	60%
Mazatlan	18	44%	17%	44%
Tooth morphology		Wilks' lambda p = 0.094	Wilks' lambda p = 0.043	Wilks' lambda p = 0.053
Equal	18	67%	61%	67%
Unequal	31	68%	65%	68%

Table 3. Absolute pairwise distances between specimens of *N. fascicularis* from Mazatlan (MAZ), Cabo San Lucas (CSL) and Puerto Vallarta (PVA), Mexico; cytochrome c oxidase subunit I, 639 base pairs.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1 EC1	-																													
2 EC2	2	-																												
3 EC3	1	1	-																											
4 EC4	1	1	0	-																										
5 EC5	1	1	0	0	-																									
6 EC6	2	2	1	1	1	-																								
7 EC7	1	1	0	0	0	1	-																							
8 EC8	3	3	2	2	2	3	2	-																						
9 EC9	3	3	2	2	2	3	2	4	-																					
10 EC10	0	2	1	1	1	2	1	3	3	-																				
11 EC11	1	1	0	0	0	1	0	2	2	1	-																			
12 EC12	4	4	3	3	3	4	3	5	1	4	3	-																		
13 EC13	0	2	1	1	1	2	1	3	3	0	1	4	-																	
14 EC19	1	1	0	0	0	1	0	2	2	1	0	3	1	-																
15 EC20	1	1	0	0	0	1	0	2	2	1	0	3	1	0	-															
16 EC21	2	2	1	1	1	0	1	3	3	2	1	4	2	1	1	-														
17 EC22	1	1	0	0	0	1	0	2	2	1	0	3	1	0	0	1	-													
18 LJM001	7	9	8	8	8	9	8	10	10	7	8	9	7	8	8	9	8	-												
19 LJM004	7	9	8	8	8	9	8	10	10	7	8	9	7	8	8	9	8	0	-											
20 LJM005	8	10	9	9	9	10	9	11	11	8	9	10	8	9	9	10	9	1	1	-										
21 CSL001	9	11	10	10	10	11	10	12	12	9	10	11	9	10	10	11	10	2	2	3	-									
22 CSL002	8	10	9	9	9	10	9	11	10	8	9	9	8	9	9	10	9	3	3	4	1	-								
23 CSL003	8	10	9	9	9	10	9	11	10	8	9	9	8	9	9	10	9	3	3	4	1	0	-							
24 CSL008	8	10	9	9	9	10	9	11	11	8	9	10	8	9	9	10	9	3	3	4	1	0	0	-						
25 CH1	9	11	10	10	10	11	10	12	12	9	10	11	9	10	10	11	10	2	2	3	0	1	1	1	-					
26 CH2	10	12	11	11	11	12	11	13	13	10	11	12	10	11	11	12	11	3	3	4	1	2	2	2	1	-				
27 CH3	8	10	9	9	9	10	9	11	11	8	9	10	8	9	9	10	9	3	3	4	1	2	2	2	1	2	-			
28 SM1	10	12	11	11	11	12	11	13	13	10	11	12	10	11	11	12	11	3	3	4	1	1	1	2	1	2	2	-		
29 SM2	11	13	12	12	12	13	12	14	14	11	12	13	10	12	12	13	11	4	4	5	4	5	5	5	4	5	5	5	-	
30 SM3	9	11	10	10	10	11	10	12	12	9	10	11	8	10	10	11	9	2	2	3	2	3	3	3	2	3	3	3	2	-

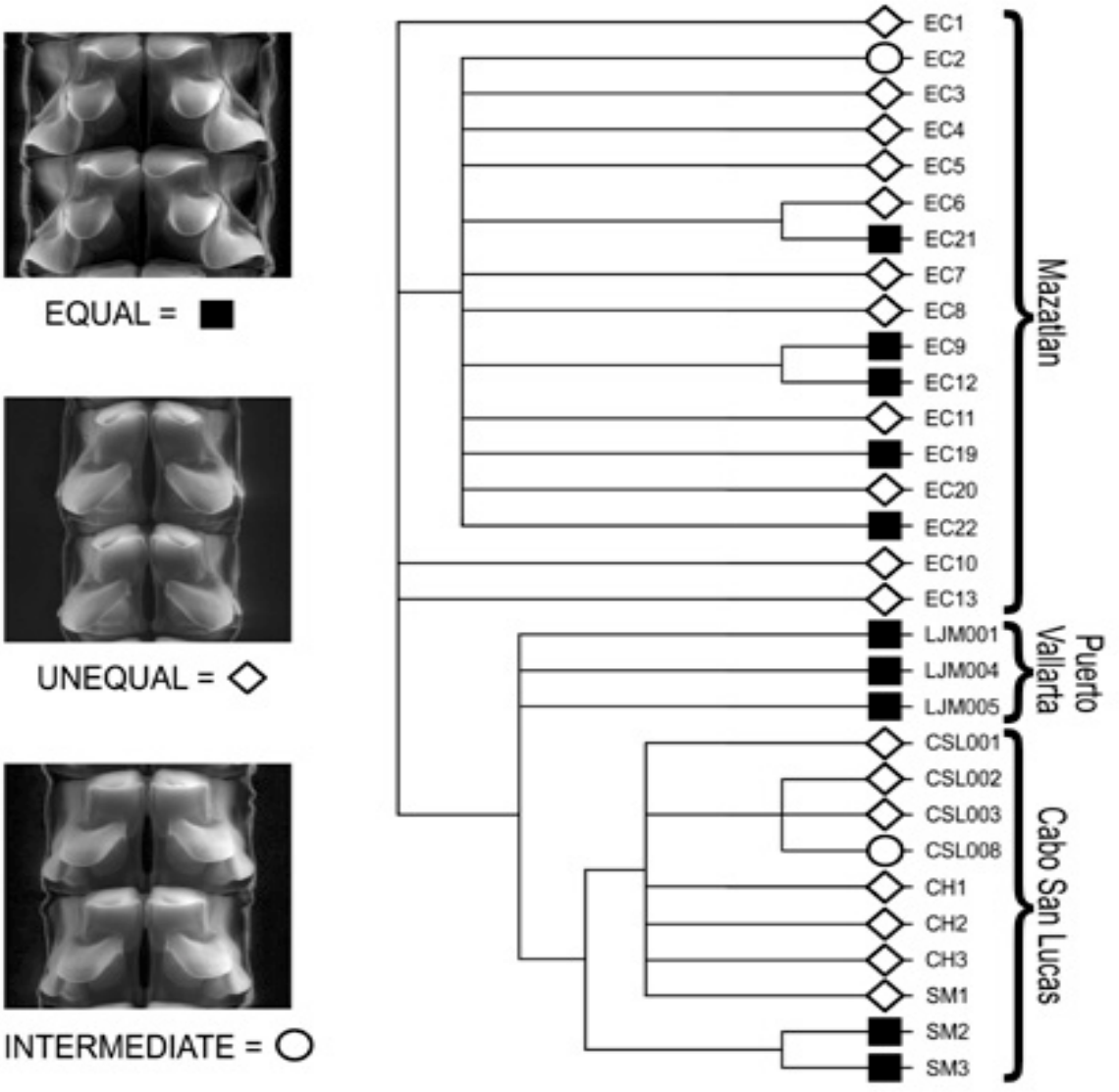

Figure 1. Cladogram of *Notoacmea fascicularis* (Menke, 1851) based on parsimony analysis of COI molecular data. Geographic distributions and radular variability are mapped on to tree.

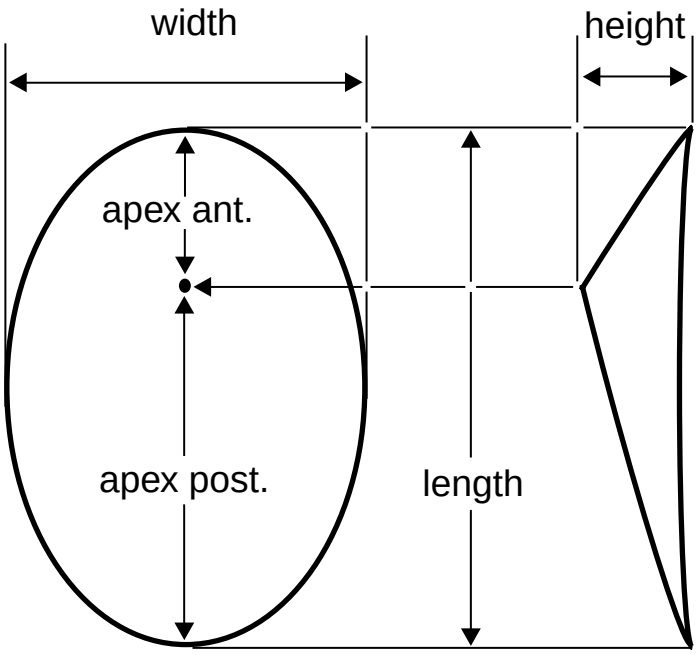

Figure 2. Shell measurements used in discriminant function analysis.

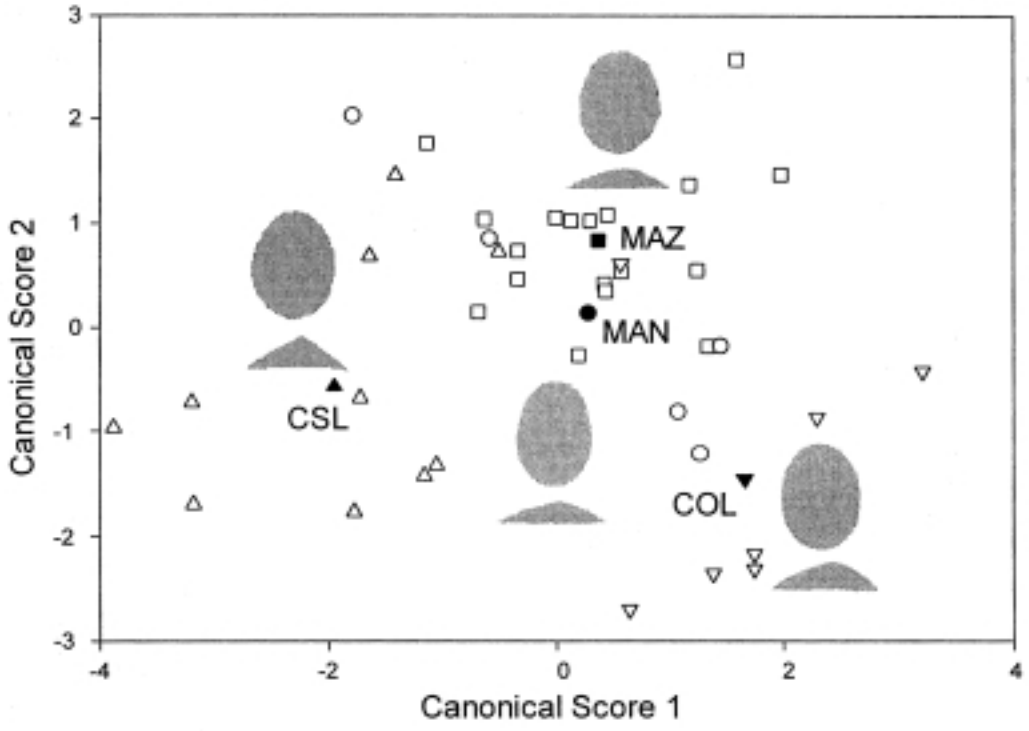

Figure 3. Scatterplot of first and second canonical scores from discriminant function analysis of *Notoacmea fascicularis* shell measurements. Open symbols are individual scores; solid symbols represent group means; shell silhouettes from arbitrarily chosen specimens from each locality. ▲ = Cabo San Lucas, ■ = Mazatlan, ● = Manzanillo, ▼ = Colima.

APPENDIX

Standardized canonical discriminant function (CDF) scores for locality and radular morphology classifications. DFA ALL = linear measurements, area measurements, and ratio data, DFA Ratios = ratio data alone, DFA Log (*x*) = log transformed linear and area measurements. ns = not significant in discrimination, N/A = not applicable.

LOCALITY	DFA ALL		DFA Ratios		DFA Log(x)	
	CDF 1	CDF 2	CDF 1	CDF 2	CDF 1	CDF 2
SHELLAREA	1.517	0.501	N/A	N/A	1.785	0.021
SHELLLENGTH	-3.498	-3.085	N/A	N/A	1.405	-6.452
SHELLWIDTH	ns	ns	N/A	N/A	ns	ns
APEXANT	5.862	-1.522	N/A	N/A	-1.334	2.559
APEXPOST	ns	ns	N/A	N/A	ns	ns
SCARAREA	-1.267	5.328	N/A	N/A	0.236	5.113
APEXHT	-1.019	-1.791	N/A	N/A	-1.538	-1.413
APEXPOS	-1.830	0.905	0.255	0.735	N/A	N/A
SCARSHELL	ns	ns	0.882	-0.515	N/A	N/A
HTWIDTH	ns	ns	-0.843	-1.571	N/A	N/A
HTLENGTH	ns	ns	0.060	0.770	N/A	N/A
WIDTHLENG	ns	ns	Ns	ns	N/A	N/A

Radular classification

VARIABLE	DFA ALL	DFA Ratios	DFA Log(x)
	CDF 1	CDF 1	CDF 1
SHELLAREA	0.318	N/A	0.670
SHELLLENGTH	1.109	N/A	2.204
SHELLWIDTH	ns	N/A	Ns
APEXANT	3.191	N/A	3.149
APEXPOST	ns	N/A	Ns
SCARAREA	-5.593	N/A	-5.572
APEXHT	1.219	N/A	-0.546
APEXPOS	ns	0.769	N/A
SCARSHELL	ns	-0.804	N/A
HTWIDTH	ns	0.599	N/A
HTLENGTH	-0.765	0.873	N/A
WIDTHLENG	ns	ns	N/A

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Chapter Four

ON THE IDENTITY OF

LOTTIA STRIGATELLA (CARPENTER, 1864)

(PATELLOGASTROPODA : LOTTIIDAE)

Abstract

Nomenclatural confusion has surrounded the northeastern Pacific lottiid currently referred to by the specific names strigatella or paradigitalis for 135 years. Much of this confusion has resulted because of the supposed range of this nominal taxon (Gulf of California to the Gulf of Alaska), its morphological variation within this range, and its overt similarity to several earlier named taxa. Here we examine the relatedness and distribution of these taxa from localities between Guaymas, Mexico and Bodega Bay, California. Relatedness is established by a maximum parsimony analysis of partial sequences of cytochrome c oxidase I and 16S mtRNA genes. The results of this analysis provides unequivocal evidence of the distinctness of Lottia strigatella, Lottia paradigitalis, and the presence of a third previously unrecognized taxon, Lottia argrantesta n. sp. Moreover, these taxa are not members of a species complex, but rather members of three distinct subclades within the northeastern Pacific Lottiidae. Lottia strigatella and Lottia paradigitalis show characteristic Californian distributions with apparent range end points in the vicinity of Point Conception, California. These data and the evolutionary history they reveal provide a compelling demonstration of the levels of morphological homoplasy present in the Patellogastropoda.

Introduction

Nomenclatural confusion has surrounded the northeastern Pacific lottiid currently referred to by the specific name strigatella or paradigitalis for 135 years. Much of this confusion has resulted because of the apparent extensive range of this nominal taxon (Gulf of California to the Gulf of Alaska), its morphological variation within this range, and its overt similarity to several other earlier known taxa. Understanding the extent of its distribution in the northern portion of this range is further complicated by the presence in Alaska of the morphologically similar Lottia borealis (Lindberg, 1982).

The tortured nomenclatural history began with the proposal of two similar specific names for a single nominal taxon - strigillata for the California population and strigatella for the Gulf of California population by P. P. Carpenter in the 1860's. Palmer (1958) and McLean (1966) give detailed discussions of the subsequent nomenclatural confusion.

In summary, Carpenter (1864a) proposed Acmaea strigatella for a limpet from Cabo San Lucas, Baja California Sur, Mexico. In a second paper (1864b) this specific name was erroneously spelled strigillata. Carpenter (1866:334) proposed Acmaea patina Var. b. strigillata for a second nominal taxon from the Vancouver-Californian provinces. He compared it to small specimens of Lottia pelta and remarked on the difficulty in distinguishing it from "the A. strigatella of Cape St. Lucas." Burch (1946) erroneously referred to the northern species as Acmaea persona strigillata, noting the similarity between it and small specimens of Lottia persona. Smith and Gordon (1948) and Abbott (1974) followed Burch.

Grant (1933) placed A. persona strigillata in synonymy with L. digitalis, but illustrated specimens of Burch's A. persona strigillata as "Acmaea persona." Four years later Grant (1937) illustrated the same shells as supposed hybrids between L. digitalis and L. pelta, but the name A. persona strigillata remained in synonymy with L. digitalis. It is interesting to note that Grant, who originally suggested that this taxon was a hybrid, never discussed this decision in any of her texts. The hybrid designation only appeared in figure captions without further comment (see also Light, 1941; Smith et al., 1954).

The name Acmaea paradigitalis was proposed by Fritchman (1960) after a study of the radular basal plate morphology of L. digitalis, L. pelta, and the supposed hybrid. McLean (1966) synonymized L. paradigitalis with the Panamic species L. strigatella based on the similar shell characters of the two taxa. The similarities had been noticed first by Carpenter (1866) but were subsequently ignored by most workers. McLean's treatment was followed by later workers including Seapy & Hoppe (1973), Carlton & Roth (1975), Christiaens (1975), and Morris et al. (1980). This nomenclature remained relatively stable until Lindberg (1981:75) revived the use of the specific name paradigitalis for northern California specimens of L. strigatella based on radular differences that distinguished the northern and southern California taxa from one another.

The advent of molecular techniques provides new data to examine levels of relatedness and to determine the distributions of populations and species-rank taxa. The strigatella/paradigitalis question is an ideal problem for such study. The debate has been ongoing for 135 years and character analysis of

morphological characters as well as ecological studies have provided conflicting answers to the distinctness and distributions of these nominal species. Obviously, a new data set is needed to address these questions.

Here we examine the relatedness and distribution of the lottiid taxa formerly known as strigatella and paradigitalis from localities between Guaymas, Mexico and Bodega Bay, California. Relatedness was established by maximum parsimony analysis of partial sequences of the mitochondrial genes cytochrome c oxidase I and16S mtRNA. After delimiting these taxa with molecular characters, shell and radular characters were examined to determine the range of morphological variation within each taxon. These morphological characters were then used to identify and delimit the regional distribution of the taxa and associate existing type specimens with specimens of known genotypes.

MATERIALS & METHODS

In the course of this study we examined over 1500 putative specimens of Lottia strigatella and Lottia paradigitalis from the Gulf of Alaska to the Gulf of California, Mexico. In addition, nearly 100 specimens from ten arbitrary localities between Guaymas in the Gulf of California, Mexico and Bodega Bay, California were collected for molecular sequencing (Fig. 1). Specimens collected for sequencing were biased to represent as much morphological variation as possible from each locality. All specimens were labeled with a locality-based code and preserved in 70% ethanol (ETOH).

In the laboratory the coded specimens were sorted into morphologically similar groupings irrespective of locality and several specimens were then randomly chosen from each group for sequencing. This approach increases the possibility that all phenotypes present in a taxon will be sampled as well as providing multiple sequences for similar individuals in each "lot". After reconstituting the groupings by locality it was discovered that 20 specimens from ten localities had been selected for sequencing (Table 1).

Institutional abbreviations used herein are as follows: LACM-- Malacology Section, Natural History Museum of Los Angeles County, Los Angeles, California; UCMP-- Museum of Paleontology, University of California, Berkeley, California; and USNM-- Division of Mollusks, U. S. National Museum of Natural History, Washington, D. C.

Molecular sequence data

Cytochrome c oxidase I (COI) and16S mtRNA genes were partially sequenced and compared among 14 and 20 individuals respectively from ten localities (Table 1). Sequences from 50 temperate and tropical eastern Pacific lottiids were also available for comparison to the L. strigatella and L. paradigitalis sequences (Simison, unpublished data). COI and 16S were used in this study based on their interspecific and intraspecific levels of variation found among sequences of other eastern Pacific patellogastropods (Simison, 2000). Both the 16S and COI sequences were combined in the same data set and hand aligned.

A default maximum parsimony analysis using 100 random stepwise-addition replicates was performed using PAUP* 4.0b3a (Swofford, 2000).

Extraction

Two equally successful DNA isolation protocols were used: (1) saturated salt/chloroform extraction, and (2) CTAB/phenolchloroform extraction. For each extraction, pedal tissue was cut from the foot margin approximately 3-5 mm along the margin and 3-5 mm towards the center of the foot. The tissue was soaked in deionized water to remove any residual ETOH and finely diced to bits. For the saturated salt technique, the diced tissue was placed in a 1.5 ml tube containing 250µl isolation buffer (100mM TRIS, 10mM EDTA and 400mM NACL), 60µl 10% SDS, and 10µl proteinase K. The mixture was then vortexed and stored on a shaker at 37º C overnight. Following tissue digestion, 175µl of saturated NaCl solution was added. The samples were inverted for 5 minutes and centrifuged at 13k rpm for 30 minutes. The supernatant was washed with chloroform using 2 times supernatant volume and mixed by inversion for 2 minutes. The supernatant DNA was precipitated using two volumes of ice cold 100% ETOH, centrifuged at 13k rpm for 15 minutes and discarded, the remaining pellet was washed twice with two volumes of 70% ETOH. The 70% ETOH wash was discarded and the pellet dried for five minutes in a speed vac. The DNA was eluted in 50µl of double-distilled water and stored at -20ºC.

For the CTAB technique, diced tissue was added to a 1.5 ml tube containing 600µl 2XCTAB and 9µl of proteinase k then incubated at 37ºC overnight. 600µl

of phenol:chloroform:isoamyl alcohol (25:24:1) was added to the tissue mixture and mixed via inversion for 5 minutes. The solution was then centrifuged at 13k rpm for 15 minutes. The supernatant was added to 600µl of chloroform:isoamyl alcohol (24:1), mixed for 5 minutes and centrifuged at 13k rpm for 15 minutes. DNA was precipitated using 600µl isopropanol and stored at -20ºC for 2 hours. The precipitate was centrifuged at 13k rpm for 30 minutes at 4ºC. The supernatant was discarded and the pellet washed twice with two volumes of 70% ETOH and centrifuged at 13k rpm for 20 minutes. The ETOH was discarded and the pellet dried by speed vac for 5 minutes and eluted in 100µl of deionized water.

Amplification

Amplification of a section of the coding region of mt cytochrome c oxidase subunit I (COI) was achieved with the HCO-2193 and LCO-1490 primers described by Folmer et al. (1994). For the 16S mtRNA region, amplification was targeted using the 16Sar and 16Sbr primers described by (Palumbi, 1996; Kocher et al., 1989). In a 0.5 ml gene amp tube, on ice, 36.45µl double-distilled water, 5µl 10x PCR buffer (Perkin Elmer), 2.5µl 10µM dNTP's (Pharmacia), 2.5µl 25µM MgCl2 (Perkin Elmer), 1µl each of the 10 µM primers, 1µl of template, and 0.25µl of taq (Perkin Elmer) were combined. A negative control containing all reagents except the template was run in parallel. The tube is then transferred to a Perkin Elmer 9600 geneamp. The cycling parameters began with an initial denaturation at 95ºC for 2 minutes followed by 36 cycles with three temperature

plateaus of 95ºC for 50 seconds, 45ºC for 50 seconds, and 72ºC for 90 seconds, ending with a 7 minute extension at 72ºC. PCR products were purified using Wizard® PCR preps DNA Purification System.

Cycle Sequencing

Direct double-stranded cycle sequencing of 20 to 30 ng of PCR product was performed in both directions using the aforementioned primers and the ABI® cycle sequencing kit following a half reaction ABI® cycle sequencing protocol. Cycle sequencing was performed using a Perkin Elmer 9600 geneamp. The cycling parameters were 25 cycles at 96ºC for 10 seconds, 50ºC for 5 seconds, and 60ºC for 4 minutes. Cycle sequencing product was purified using Princeton Separations Centrisep spin columns, then dried in a speed vac. The dried, purified cycle sequencing product was resuspended in 2.5µl loading solution of 5:1 deionized formamide: 25mm MEDTA with 50mg/ml Blue Dextran. 1.5 µl of sample and loading solution was loaded on a 36 cm 4% acrylaminde gel. The gel was run and analyzed on an ABI Prism® 377 DNA sequencer.

Alignment & Anaylsis. - All sequences were aligned by hand using the ABI® Sequence Navigator.

Morphology

Digital images of the ventral, dorsal, and profile views of the shells of the eighteen molecular specimens were captured with a digital camera connected to a Scion LG-3 Scientific Frame Grabber system.

An anterior portion of the radular ribbon from nine specimens (Table 1) was dissected from the head region posterior to the odontophore and placed in a 0.5% sodium hypochlorite solution for 5 minutes or less to dissolve associated organic material and rinsed in distilled water. The radular ribbon was examined using a ElectroScan Model E3 Environmental Scanning Electron Microscope (ESEM).

Results

Molecular sequence data

Three discreet COI and 16S genotypes were identified in the specimens currently referred to as L. strigatella and L. paradigitalis. These three genotype groups are not allopatric. Based on genotype and their associated morphologies, the San Francisco Bay group (L. paradigitalis) appears to overlap with the Baja California group (L. strigatella) in southern California, while the Baja group co-occurs with the Gulf group (L. argrantesta n. sp.) in the southern Gulf of California (Fig. 1).

Morphology

Examination of radular and shell morphologies of taxa sorted by genotype revealed previously unsuspected morphological differences, especially between Gulf specimens of L. strigatella and L. argrantesta. Although both taxa have a wide range of shell pattern variation, specimens of L. argrantesta (Figs. 16-19) tend to be lower in profile than specimens of L. strigatella (Figs. 9-15). Lottia strigatella specimens also tend to have more convexed posterior shell profiles. Both taxa have variegated forms that are similar in shell color and pattern (cf. Figs 9 & 16) as well as dark tessellate forms with random white markings (cf. Figs. 12 & 19). In many cases L. argrantesta can be distinguished from L. strigatella by the presence of low coarse ribs on its shell, but relatively smooth specimens also occur (Fig. 17). L. argrantesta appears to lack strongly demarcated shell patterns such as found in L. strigatella (e.g., Figs. 11, 13, & 12).

Lottia paradigitalis and L. strigatella are substantially more similar to one another than either is to L. argrantesta. Both taxa have a wide range of overlapping shell pattern variation (cf. Figs. 7, 8 with 9 13), strongly demarcated shell patterns (cf. Figs. 3, 5 with 11, 15), and dark tessellate forms [cf. Fig. 5 (central area) and 12]. A solid, yellow-tan form has been found only in L. strigatella (Fig. 14). Both taxa lack ribbing and primarily concentric growth lines texture the exterior shell surface although microscopic radial treads are sometimes present; shell profiles are virtually identical in both taxa. One discernible difference between L. paradigitalis and L. strigatella shell color

patterns are the stronger bifurcating patterns of the white markings present in L. paradigitalis (cf. Figs. 3, 5, 7 with 9, 11, 13).

The radula of L. argrantesta is readily distinguisable from both L. paradigitalis and L. strigatella. In L. paradigitalis (Figs. 26-28) and L. strigatella (Figs. 20-22) the inner margins of the second lateral teeth appear concave, while in L. argrantesta the edges appear concave (Figs. 23-25). This places the cusps of the second lateral teeth of L. argrantesta more distant of the cusps of the first lateral teeth than in either L. paradigitalis or L. strigatella. Lottia paradigitalis and L. strigatella radulae are very similar in overall morphology. One possible difference we noted was that radular segments in L. paradigitalis appear slightly shorter than in L. strigatella. There is minor radular polymorphism in L. paradigitalis (cf. Figs. 27 & 28), but it is not as marked as that reported in the Panamic taxon Lottia fascilularis (Simison & Lindberg, 1998).

DISCUSSION

Despite 135 years of conjecture in previous literature, the results of this study provide unequivocal evidence of the distinctness of Lottia strigatella, Lottia paradigitalis, and a third previously unrecognized taxon, Lottia argrantesta n. sp. Moreover, these taxa are not members of a 'species complex' or even sister taxa, but rather members of three distinct subclades within the northeastern Pacific Lottiidae (see below). These data and the evolutionary history they reveal

provide a compelling demonstration of the levels of morphological convergence present in the Patellogastropoda.

Without the molecular data Lottia argrantesta would likely have gone unrecognized. And while Lindberg (1981:75) revived the use of the specific name paradigitalis for northern California specimens of L. strigatella based on radular differences, it was thought at that time that Lottia strigatella and Lottia paradigitalis likely represented a species pair which transitioned at Point Conception, California. This scenario was consistent with the range of morphological shell and radular variation shared by these two taxa, their similar habitats, and their contiguous ranges. Moreover allopatric divergence during a glacial or interglacial period provided a plausible mechanism.

However, this scenario is falsified by the phylogeny derived from the molecular data. Instead, the shared morphology and habitats of these taxa appear to result from convergence not common ancestry, and the range division is characteristic of the larger, more inclusive clades to which each taxon belongs and not the outcome of a recent divergence from a common ancestor. While disconcerting relative to the more familiar scenario, this result suggests that deeper divergences are also affected by Pt. Conception. This barrier is possibly thermal in nature and acts to limit the distributions of either larvae or adults. For members of the Collisella and “A” subclades (Fig. 2) southern limiting temperatures occur near the southern California Bight; northern limiting temperatures do not appear to be reached until the northern Gulf of Alaska or Aleutian Islands. For members of the sister clade that contains L. strigatella,

Macclintockia, and Nomeopelta (Fig. 2), northern limiting temperature are seldom found north of central California, and the majority are south of the Bight. Most southern limiting temperatures in the L. strigatella + Macclintockia + Nomeopelta clade occur at the mouth of the Gulf of California. Thus ranges in Collisella + subclade A average about 7900 km, while ranges in the L. strigatella + Macclintockia + Nomeopelta clade average only about 1600 km. Moreover, these different thermal tolerances (and their potential relationship to range size) appear to be clade-level traits that first appeared in their respective common ancestors in the Pliocene; long before glacial and interglacial sequences provided a plausible mechanism for divergence. Subsequent divergences in both clades produced taxa with similar ranges suggesting that thermal tolerance was heritable in these clades and this trait constrained descendents to similar range sizes. This finding offers a deeper historical view of the potential makeup of latitudinal barriers and range size than is attainable through classical systematic studies. Moreover, the pattern has implications for clade selection (Lloyd & Gould, 1993; Vermeij, 1996).

SYSTEMATICS

Patellogastropoda Lindberg, 1986a

Lottiidae Gray, 1840

Although this taxon is the most diverse and abundant of all patellogastropod clades in the world, it is diagnosed by few characters, and most notably by an absence of calcitic foliated shell microstructures and the presence of fibrillar ones. Foliated shell structures are present in the Patelloidea, Nacelloidea and many Acmaeoidea, but are absent in the Lottiidae. The remaining anatomical and shell characters of the Lottiidae are all found in different combinations in one or more of the outgroups.

Two major subclades, Lottiinae and Patelloidinae, have been previously recognized on radular and shell microstructure characters; they are also delimited by molecular characters (Simison, 2000). Both groups contain numerous subclades that have been named, as well as previously unrecognized ones. In North America, Australia, Japan, and South America, members of the Lottiidae compose the vast majority of the species in the nearshore patellogastropod guilds. Unlike the Acmaeoidea, members of the Lottiidae are not found in the deep sea. Instead, they are primarily intertidal in habitat and rarely occur deeper than 30 meters. They occupy a wide range of intertidal heights and habitat types. Some species are tolerant of brackish water and can be found in estuarine habitats. Several species are associated with algae and marine angiosperms while others are found only on carbonate substrates.

The Lottiidae are distributed world wide with the exception of Antarctica. There are no strong biogeographical trends within the global distribution of Lottiidae, and different taxa in a single clade may range from cool temperate to subtropical environs. Members of the Lottiidae are identifiable in the Cretaceous based on shell microstructure and radular characters (Akpan, et al. 1982; Lindberg 1988). And by the Eocene, circulatory characters that diagnose living taxa are visible as impressions preserved on the interior of fossil shells (Lindberg and Squires 1990).

Lottia Gray, 1833

(Figure 2)

Lottia Gray, 1833: 800. Type species, by subsequent designation of Dall, 1871:

Lottia gigantea Sowerby, 1834. Northeastern Pacific.

Tecturella Carpenter, 1860: 3. Type species, by monotypy: "Gray" (= Sowerby, 1834) (not Stimpson, 1853: 36).

Tecturina Carpenter, 1861: 219. Type species by original designation: Tecturina grandis "Gray" (= Lottia gigantea Sowerby, 1834) (nomen nudum).

Lecania Carpenter, 1866: 343. Type species by original designation: Lottia gigantea Sowerby, 1834 (nomen nudum, published in synonymy with Lottia Sowerby, 1834).

Shell profile varies from high to low with the apex positioned anterior of center of shell.. Shell sculpture consists of combinations of ribs, riblets, and concentric growth lines. Radular configuration consists of three pairs of lateral teeth. If present, one pair of marginal teeth (or uncini) are located on the radular

membrane at the posterior edge of the ventral plates; they are substantially smaller than the third lateral teeth and non-mineralized. The first and second inner pair of radular teeth are approximately equal in height, but the second pair are usually wider than the first. The outermost third lateral teeth are typically reduced in size relative to the inner teeth. In coralline feeding species all three lateral teeth are approximately equal to one another in size and shape. The ventral plates underlying the lateral teeth are complex with distinct plates for each tooth; however the tooth plates for the second and third lateral teeth may be partially fused anteriorly. A complete or partial secondary gill may be present in the mantle groove. In most taxa, the fibrillar layer dominates the shell microstructure.

This temperate taxon reaches its zenith in the North Pacific especially in the northeastern Pacific. It moved to the North Atlantic by way of the Arctic Ocean in the Late Negoene. Some Australasian taxa have been assigned to the taxon Collisella (e.g., Ponder & Creese, 1980) – a subclade within Lottia. However, the presence of Collisella (or Lottia) taxa in Australia is problematic. The Australian taxa are clearly outliers and whether they share common ancestry with the Lottia of the North Pacific has not been convincingly demonstrated. Alternatively, they could represent an independent derivation from a distantly related Australasian lottiid ancestor.

A complete nomenclatural revision of the taxon Lottia is beyond the scope of this paper. However, there is sufficient data and sampling to present an overview of our current working classification. This classification provides a

framework upon which to place the taxa discovered, described, and discussed herein. It also resolves several long standing nomenclatural issues surrounding 'generic' assignments with the northeastern Pacific Patellogastropoda. A more detailed nomenclatural treatment will be published elsewhere.

In the northeastern Pacific we recognize five subclades within Lottia based on molecular and morphological characters (Figure 2). An unnamed taxon (Fig. 2, subclade A) is composed primarily of taxa previously assigned to the Notoacmea by McLean (1966) and Tectura by Lindberg (1986b). The taxon Collisella is restricted from its previous usage by McLean (1966) and others to correspond to those taxa that share a more recent common ancestor with Lottia paradigitalis than with Lottia strigatella or members of subclade A (e.g., Lottia persona). Another subclade currently consists of an undescribed species from the southern California Islands and Lottia strigatella. The crown group consists of two taxa – Macclintockia (Lindberg MS in Kozloff, 1987) and a clade comprised primarily of Californian taxa and the Nomaeopelta (Berry, 1958) of the Gulf of California, Mexico. The taxa formerly known collectively as Lottia strigatella and Lottia paradgitalis actually reside in three of these five clades. Based on examination of their shell and radular morphology it is surprising that they do not share a most recent common ancestor.

Equivalent, nested phylogenetic nomenclature for these taxa is as follows:

Linnean		**Phylogenetic**
Lottia strigatella	=	Lottia strigatella
Lottia paradigitalis	=	Lottia Collisella paradigitalis[1]
Lottia argrantesta	=	Lottia Nomaeopelta argrantesta

Lottia paradigitalis (Fritchman, 1960)

Figures 3-8, 26-28

The shell is moderately thin with the apex positioned approximately 1/3 of the way from the anterior end. The apex is often eroded and rounded, but on less eroded specimens the apex comes to a strong point and slightly protrudes towards the anterior. Both the anterior and posterior slopes from the apex to the margin are slightly convex. Shell height is medium in profile and the shell typically lacks radial ribbing. Fine and regular concentric growth lines are the predominate form of shell sculpture. The shell apex is typically erodes to white with either brown radial markings at the margins or a dark band at the apex margin (e.g., Figs. 7, 8). Less eroded specimens show a range of radial patterns that include tessellate green-brown apical areas with white radial lines leading to

[1] The trinomials used here should not be confused with the subgeneric rank of the Linnean classification scheme. Here they are clade names that provide additional hieratical information regarding relationships (e.g., see Fig. 2).

the shell margin (Figs. 3, 5). Specimens likely change substrates during their ontogeny and this is reflected in changes in the color and pattern of the shell (Fig. 5). White radial markings often bifurcate at the shell margin creating numerous short radial parallel lines along the apertural margin. This pattern is often mirrored on the interior of the shell as well.

The interior surface of the shell typically has very little dark staining. Usually there is a translucent white coating over the entire inner surface except at the very margins. The exterior color patterns clearly show through to the interior surfaces, particularly at the shell margins where the white layer is lacking. Occasional specimens have darkly stained interiors overlaying the translucent white layers.

Radula (Figs. 26-28): The first lateral teeth are have pointed cusps, and the anteromedial edges of the ventral attachment plates are roughly parallel. Then second lateral teeth are also pointed and the inner and outer tooth margins are convexed. The cusps lie lateral of the outer edges of the first lateral teeth. The third lateral teeth are reduced and pointed. They lie lateral and almost perpendicular to the bases of the second lateral teeth. The third lateral teeth are distinct from the second lateral teeth except at their bases. The third lateral cusps extend posterior to a position similar to that of the second lateral cusps. The uncini on the radular membrane are prominent and appear rounded.

Holotype dimensions: Length 16 mm, width 15mm, height 5.5 mm.

Type locality: (Fig. 1). UNITED STATES: California; Alameda County, Berkeley Marina (37° 52’ N, 122° 18’ W)

Type material: Holotype (USNM 611301), 5 paratypes (USNM 1611302). Although Fritchman (1960) extensively studied the radula of Acmaea paradigitalis, the type material consists entirely of shells; not a single radula associated with a type specimen was found.

Distribution: Neither the southern or northern range limits of this taxon have been established based on molecular data, only its identity at the type locality. Morphological comparisons places the northern most distribution along the Kenai Peninsula (59° 14'N, 151° 52'W) in the Gulf of Alaska (LACM No. 83-103), and the southern limit near Pt. Conception, California (34° 27'N, 120° 28'W), with a small scattering of individuals occurring at mainland and island localities within the southern California bight. Such a range is comparable and even slightly more limited than other members of the Collisella clade such as Lottia Collisella pelta (Rathke, 1833) and Lottia Collisella digitialis (Rathke, 1833).

Discussion

Lindberg (1981) unexpectedly noticed radular differences in Lottia paradigitalis that distinguished it from L. strigatella. These differences included the shorter and more compact ventral plate length and the shorter and blunter second lateral teeth. However, it is doubtful that these characters would have held up in a larger and statistically valid study. Fritchman's (1960) original study radular of "Acmaea paradigitalis" included both specimens of L. paradigitalis as well as L. strigatella. For example, Fritchman's figured specimens 8 and 9 (and

possibly the top specimen in Fig. 7) appear to be L. strigatella not L. paradigitalis. It is highly probable that his quantitative analysis of radular morphology confounds both L. strigatella and L. paradigitalis, especially in his “S of 34°” category.

Lottia strigatella (Carpenter, 1864)

Figures 9-15, 20-22

Acmaea strigatella Carpenter 1864: 474; Acmaea patina var. strigillata Carpenter, 1866: 334.

The shell is moderately thin with the apex positioned in the anterior third of the shell. The apex is often eroded and rounded, but on less eroded specimens the apex is anteriorly directed. Both the anterior and posterior slopes from the apex to the margin are slightly convex; the anterior slope may be straight in some specimens. Shell height is medium in profile. The shell exterior surface of the shell lacks prominent radial ribbing although evenly spaced, microscopic radial treads are often present. These threads are substantially weaker than the concentric growth lines that sculpted the exterior shell surface. Specimens of L. strigatella likely change substrates during their ontogeny and this is reflected in changes in the color and pattern of the shell (Fig. 11). Initially the protoconch is brown in color, but it is often eroded and the apex is white; sometimes with a small, darker spot at its center. In the northern part of its range (southern California and Baja California Norte) most specimens are olivaceous green with grayish white markings (Fig. 12). The markings surrounding the apex may

radiate outward as evenly spaced stripes, but they soon deteriorate into offset blotches of lighter shell material that maintain the radial pattern. This pattern maybe maintained to the shell margin or the blotches may elongate into stripes that then continue to the shell edge. It is not unusual for specimens to exhibit all three-color patterns, however the regular, radial white markings surrounding the aperture are the most distinctive. While the markings nearer the apex are more gray- or blue-white, the markings closer to the margin are whiter. In the southern part of its range and into the Gulf variegated patterns are more common (Figs. 9-13). In central Baja California a solid yellow-tan forma has also been found (Fig. 14), and juveniles may be dark with two lateral white flashes (Fig. 15).

The central area of the shell inside of the muscle scar is typically marked with a brown stain. In some specimens the coloration does not extend into the actual apical area which remains white. The intermediate area between the muscle scar and the shell margin ranges from blue to white. In darker specimens this may be suffused with brown. The interior margin is narrow and dark and reflects the exterior shell markings.

Radula (Figs. 20-22): The first lateral teeth are have sharply pointed cusps that flare out laterally. Then second lateral teeth are also pointed and the inner and outer margins convexed. The cusps lie lateral of the cusps of the first lateral teeth in the adjacent row. The third lateral teeth are reduced and also sharply pointed. They lie lateral and almost perpendicular to the bases of the second lateral teeth. The third lateral teeth are distinct from the second lateral teeth except at their bases. The third lateral cusps extend posterior to a position

slightly behind that of the second lateral cusps. The uncini on the radular membrane are prominent and appear rounded.

Type locality: (Fig. 1). MEXICO: Sonora; Guaymas (28° N, 111° W).

Type material: Six syntypes (USNM 12594).

Distribution: MEXICO: Sonora; Guaymas (28° N, 111° W) to UNITED STATES: California; southern California bight region (Fig. 1).

Discussion: Phenotypic variation present in Lottia strigatella has previously led to its being confused with other taxa most notably L. paradigitalis, L. persona and L. fenestrata – both relatively smoothed shell members of the Testudinalia clade. It is possible that over 140 years ago P. P. Carpenter saw through this variation and distinguished both L. strigatella and L. paradigitalis only to have 'modern' systematists confound his distinction because of the overall similarity shared by these taxa. However, Carpenter did not localize his nominal taxon Acmaea patina var. strigillata, but only stated that it was found in the Vancouver-Californian provinces. Jay (1852) indicated the locality as "Upper California", but this does not distinguish between the L. strigatella and L. paradigitalis in modern day central and southern California. Burch's Solomon-like division of L. strigatella for the southern taxon and L. strigillata for the northern one may have been correct. However, the fact that he thought both of these only to be forms of Lottia Testudinalia persona suggests even further nomenclatural confusion. Because of the lack of a locality or type specimens associated with the name strigitilla, we chose to use the name paradigitalis for this taxon. This nominal

taxon was well described, localized, and can be unequivocally associated with a genotype.

Test (1946:11) suggested that "Acmaea fenestrasta" represented one of "two polytypic species of the genus Acmaea known at the present time in North American waters…" While the northern form had a subcircular aperture with the interior of the shell suffused with brown, the southern form had an oviform aperture with a blue interior, and little if any brown coloring.

McLean (1966:105) also recognized this distinction between northern and southern specimens of Lottia fenestrata, but considered the differences to result from their occurrence in different habitats rather than geographical variation. McLean noted that both northern and southern forms were present in at some localities albeit in different habitats (i.e., sandstone reefs near sand vs. rubble-reefs, respectively). McLean (1966:81) also noted, "Color patterns of the rubble-reef living form of C. strigatella are closely approximated by those of C. fenestrata (with which it is always in association), but the interior lacks the brown suffusion of C. fenestrata."

The presence of brown interiors in specimens from Bahía Tortugas, Baja California Norte, Mexico that are molecularly identical to specimens of Lottia strigatella from the type locality of Guymas, suggests to us that the specimens of southern California rubble-reefs represent ecophenotypes of L. strigatella rather than L. fenestrata. As pointed out be McLean rubble-reefs are rare north of Point Conception, California as are specimens of L. strigatella. In contrast, Lottia

fenestrata is a northern taxon that is rare south of Point Conception and differs little throughout its northern range.

Lottia argrantesta Simison & Lindberg n. sp.

Figures 16-19, 23-25

Shell height ranges from relatively low to medium profiles. Shell ribbing typically consists of irregular ribs, and shells less than 10 mm in length tend to be smoother, but still have a knobby texture. The aperture and growth lines are irregular. The apical area erodes to white and the initial shell is dark with approximately 6 – 8 white rays radiating from the apex. Subsequent shell color varies from predominately black with radially drawn out white markings (Fig. 17) or predominately white with black radial markings corresponding to coarse irregular ribs (Fig. 14). In most cases the markings on both the white and black ground colors do not extend from the apex to the margin, but rather stop and restart in different positions. In the lighter shells the white areas are marked with brown markings; in darker specimens the brown markings are more sparse, but are often visible at the margins associated with the white markings. Occasional small specimens (less than 10 mm in length) are found that are completely brown in color (Fig. 15). The ribs are not regular but instead often form knuckles or knobs at irregular intervals from the apex to the margin, and do not protrude to form a crenulated margin; in smaller specimens that shells are typically smoother.

The inners surface of the shell is typically marked with a brown or yellow brown apical stain that clearly delineates the interior boundary of the shell

attachment muscle scar. Sporadic darker markings may also be present in the central area. The intermediate area ranges from blue to white and is often overlain by a yellow brown stain as well. The interior margin is broad and dark reflecting the outer white markings. In the small brown shells the entire interior surface is brown with the central area being slightly darker than the intermediate area and margin. The edge of the aperture is slightly reflected back.

Radula (Figs. 23-25): The first lateral teeth are have sharply pointed cusps that flare out laterally. Then second lateral teeth are also pointed and the inner tooth margins are concaved and the outer margins slightly convexed. The cusps lie close to the edge of the radular ribbon. The third lateral teeth are reduced and also sharply pointed. They lie lateral and almost perpendicular to the bases of the second lateral teeth. The third lateral teeth are distinct from the second lateral teeth except at their bases. The third lateral cusps extend posterior to a position similar to that of the second lateral cusps. The uncini on the radular membrane are prominent and appear rounded.

Holotype dimensions: Length 20 mm, width 16.5 mm, height 4.2 mm.

Type locality: (Fig. 1). MEXICO: Baja California Sur; Bahía de San Francisquito.

Type material: Holotype UCMP No. 157007, Paratypes UCMP Nos. 157003, 157006, 157007. Paratypes have also been deposited in LACM and USNM.

Distribution: MEXICO: Baja California Sur; Bahía de San Francisquito (28° 30'N, 112° 40'W) to La Paz (24° 10', 110° 21') and MEXICO: Sonora; Guymas (27° 56', 110° 54').

Material examined: 9 specimen lots, 33 specimens, 3 radula preparations.

Etymology: It is an honor for us to name this species for the first limpet systematist of the University of California at Berkeley, after Dr. Avery Ransome Grant Test, in recognition of her contributions to our knowledge of the Lottiidae.

Tables

Table. 1. Specimens and localities examined in the course of this study. Symbols and numbers refer to type and additional sampling localities, respectively. Shell and radula numbers refer to illustrated specimens and checkmarks to recovered molecular sequences.

Specimen No	Taxon	Locality	Fig. 1	Shell	Radula	COI	16S
UCMP No. 157001	*Lottia strigatella*	Guaymas, Sonora, Mexico	○	12		✓	✓
UCMP No. 157002	*Lottia strigatella*	Guaymas, Sonora, Mexico	○	15		✓	✓
UCMP No. 157003	*Lottia argrantesta*	Calfin, La Paz, BCS, Mexico	1	17	23	✓	✓
UCMP No. 157004	*Lottia strigatella*	Calfin, La Paz, BCS, Mexico	1			✓	✓
UCMP No. 157005	*Lottia argrantesta*	Tecolate, La Paz, BCS, Mexico	1			✓	✓
UCMP No. 157006	*Lottia argrantesta*	Tecolate, La Paz, BCS, Mexico	1	19		✓	✓
UCMP No. 157007	*Lottia argrantesta*	Bahía de San Francisquito, BCS, Mexico	△	16	25	✓	✓
UCMP No. 157008	*Lottia argrantesta*	Bahía de San Francisquito, BCS, Mexico	△	18	24		✓
UCMP No. 157009	*Lottia strigatella*	Santa Maria, Cabo San Lucas, BCS, Mexico	2		22		✓
UCMP No. 157010	*Lottia strigatella*	Cabo San Lucas, BCS, Mexico	2		21		✓
UCMP No. 157011	*Lottia strigatella*	Cabo San Lucas, BCS, Mexico	2	9			
UCMP No. 157012	*Lottia strigatella*	Cabo San Lucas, BCS, Mexico	2	11			
UCMP No. 157013	*Lottia strigatella*	Cabo San Lucas, BCS, Mexico	2	13			
UCMP No. 157014	*Lottia strigatella*	Chileno, Cabo San Lucas, BCS, Mexico	2		20	✓	✓
UCMP No. 157015	*Lottia strigatella*	Bahía Tortugas, BCN, Mexico	3	14		✓	✓
UCMP No. 157016	*Lottia strigatella*	Bahía Tortugas, BCN, Mexico	3			✓	✓
UCMP No. 157017	*Lottia strigatella*	Bahía Tortugas, BCN, Mexico	3			✓	✓
UCMP No. 157018	*Lottia paradigitalis*	San Francisco Bay, CA, USA	□	7	27	✓	✓
UCMP No. 157019	*Lottia paradigitalis*	San Francisco Bay, CA, USA	□	8	28	✓	✓
UCMP No. 157020	*Lottia paradigitalis*	San Francisco Bay, CA, USA	□	6	26	✓	✓
UCMP No. 157021	*Lottia paradigitalis*	Bodega Bay, CA, USA	4	5			✓
UCMP No. 157022	*Lottia paradigitalis*	Bodega Bay, CA, USA	4	3			✓
UCMP No. 157023	*Lottia paradigitalis*	Bodega Bay, CA, USA	4				✓

Figures

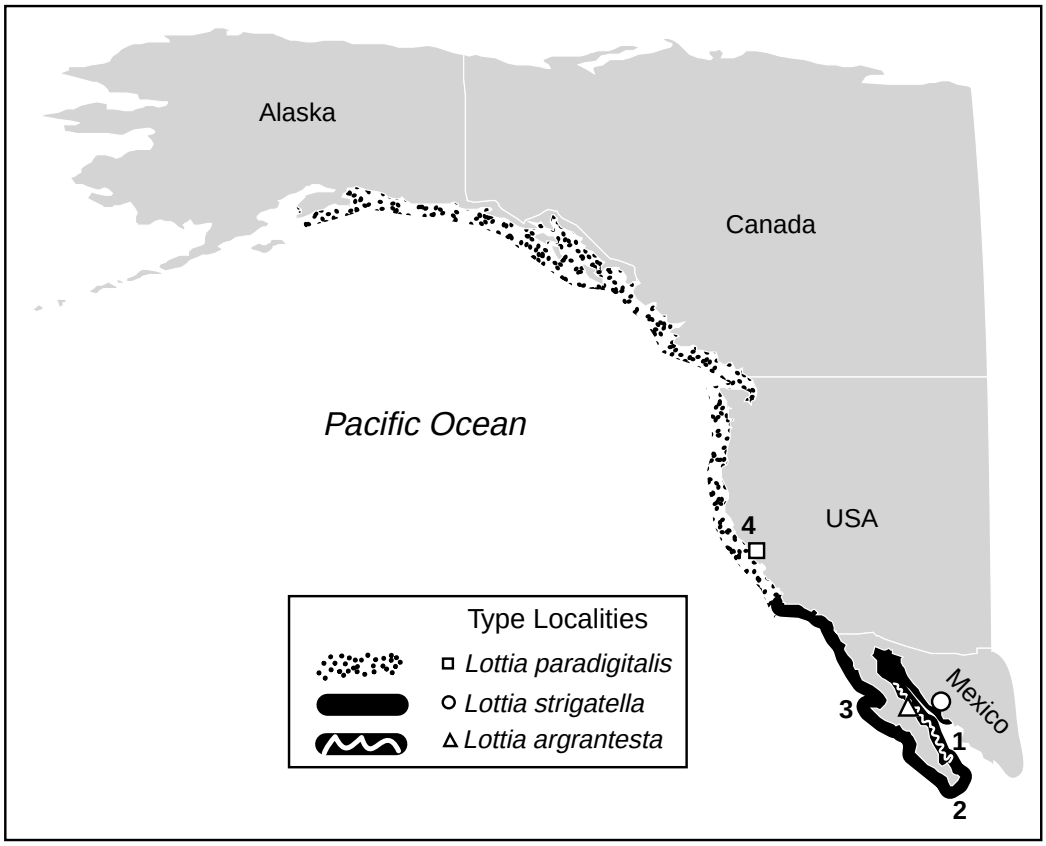


Figure 1. Sketch map of a section of temperate North America showing literature distribution of "Lottia strigatella", localities of molecular samples and expected distributions of genotype groups based on associated shell morphologies.

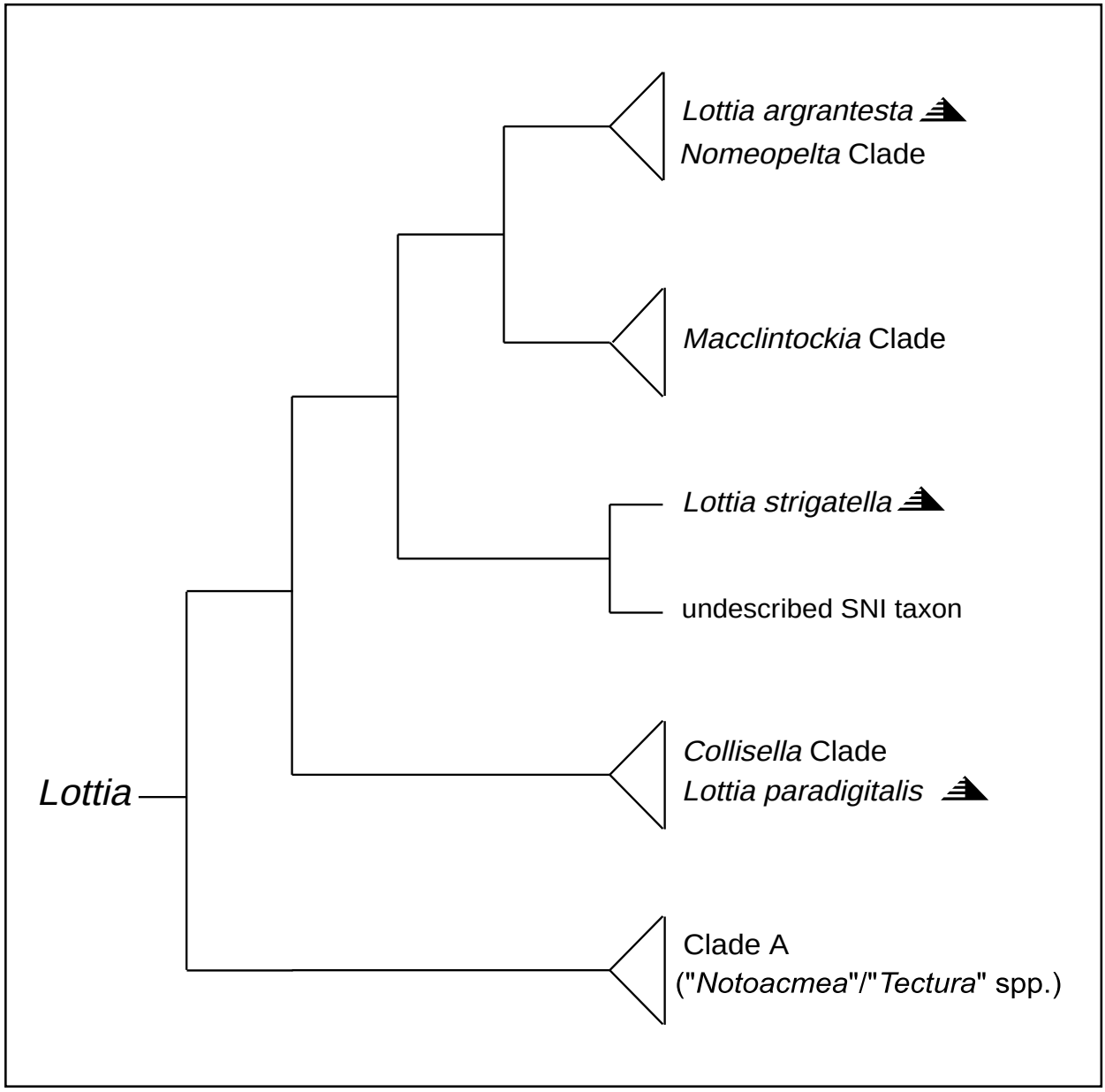
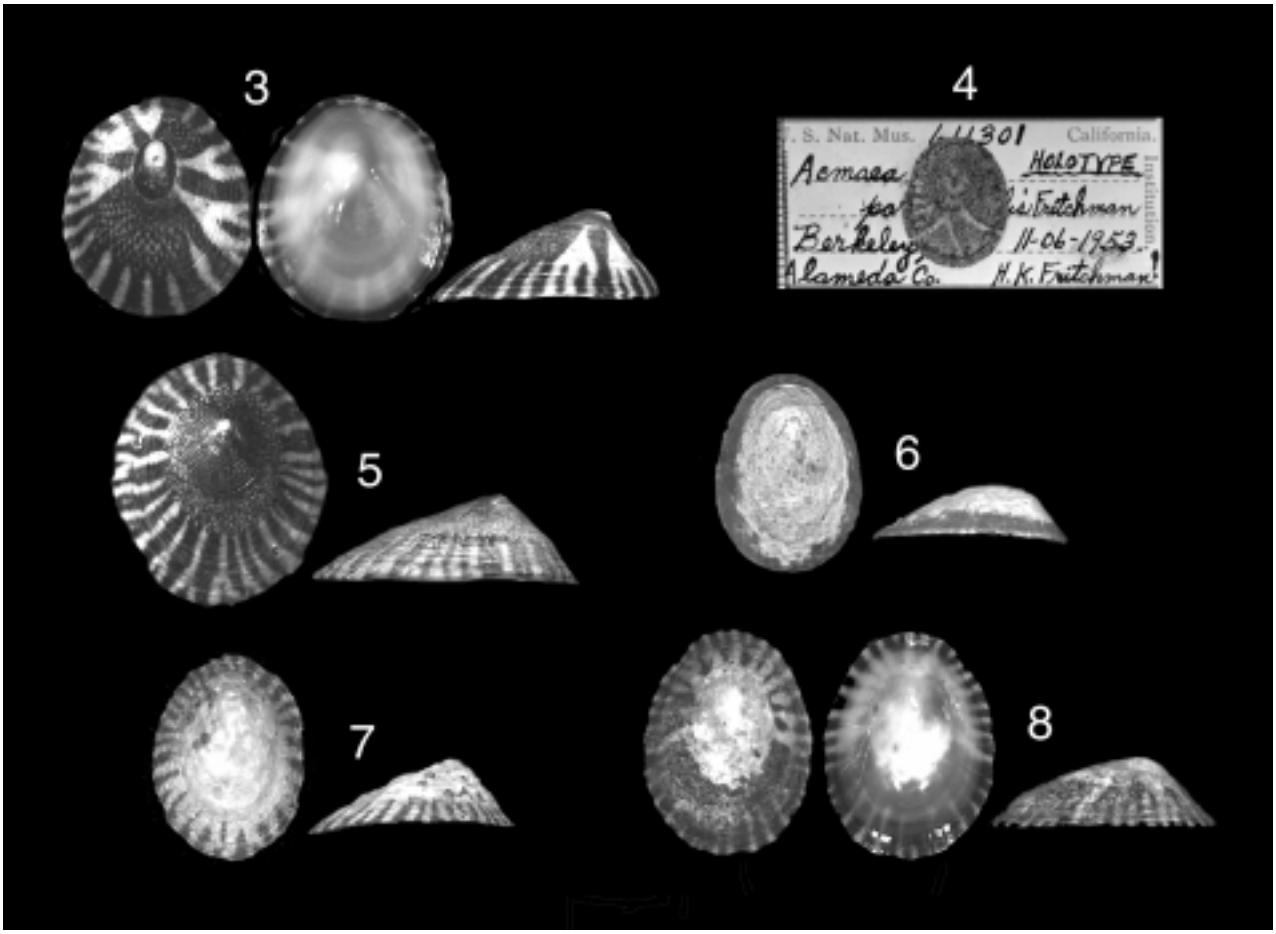
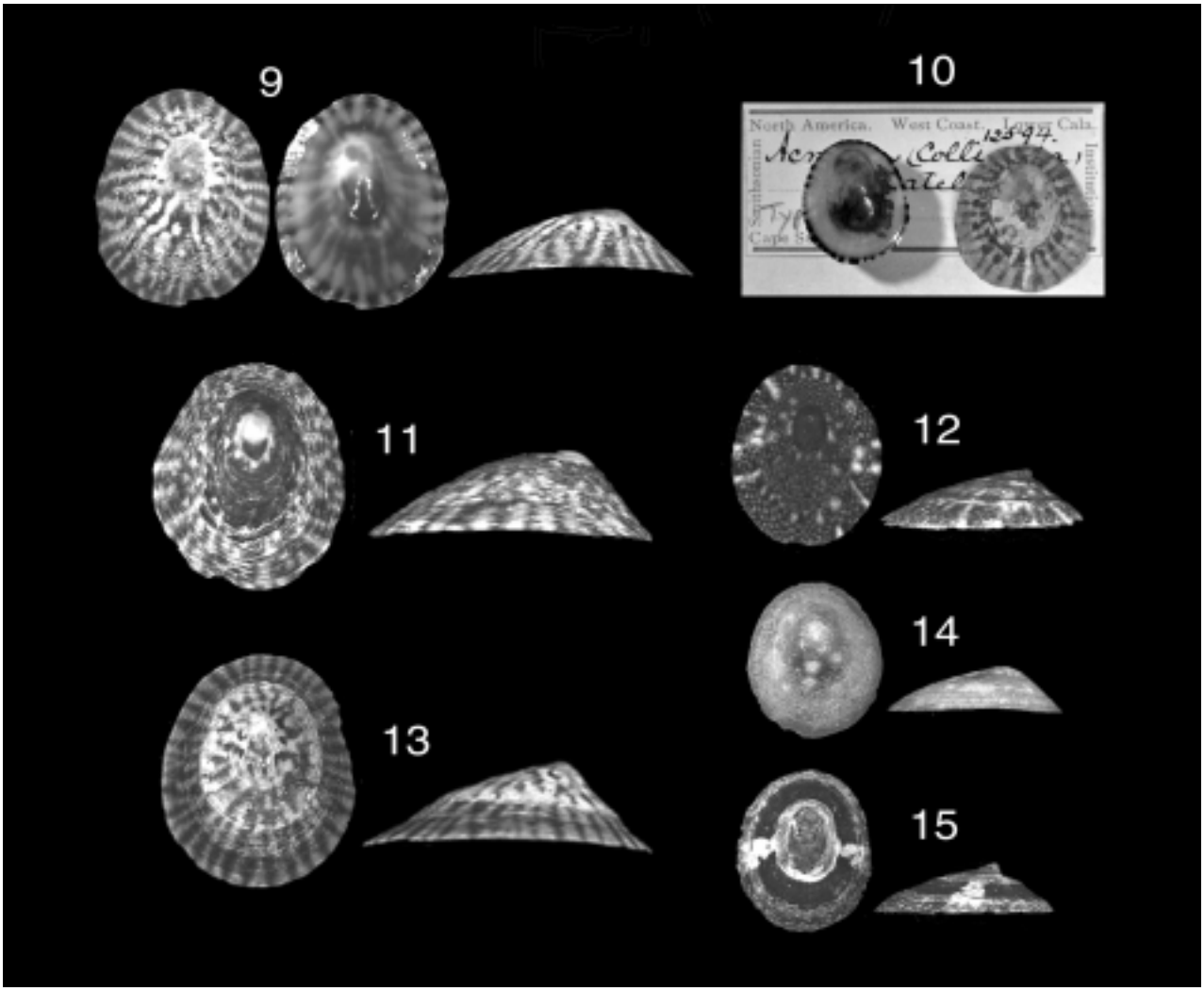


Figure 2. Phylogenetic hypothesis of the relationships among the major clades of temperate northeastern Pacific patellogastropod limpets based fig. 16, chapter 2. Data from Simison (2000) and Simison, Begovic & Lindberg (unpubl.).

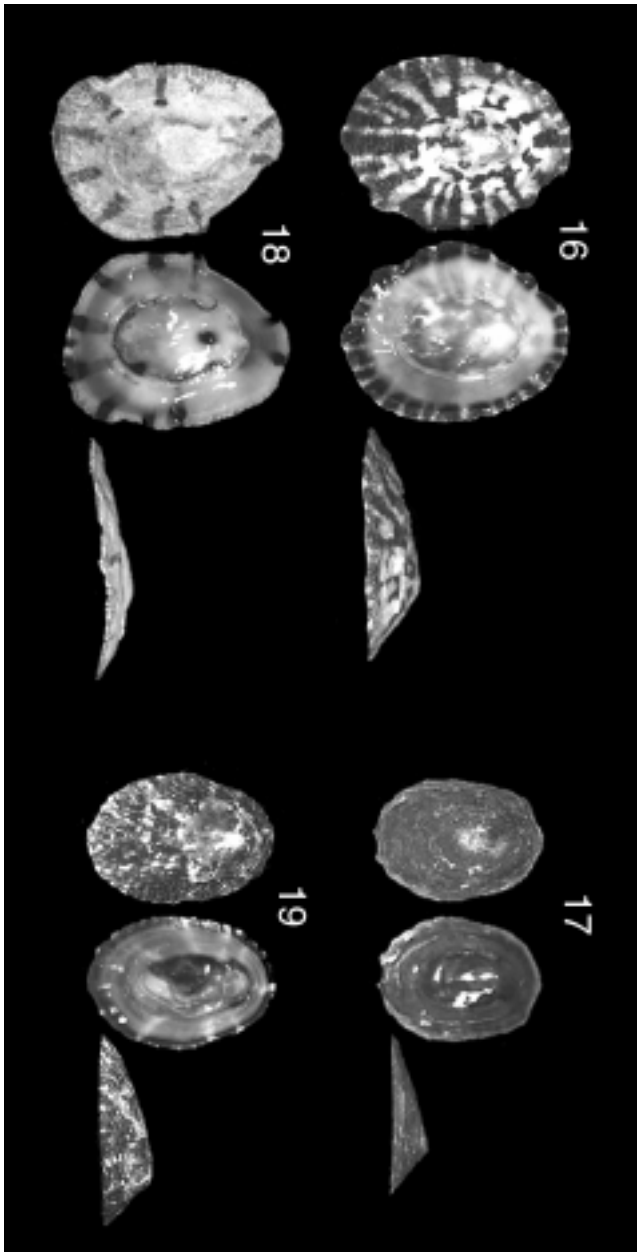


Figures 3-8. Shell morphology of Lottia paraditigalis (Fritchman, 1960) (Figs. 3-8) and Lottia strigatella (Carpenter, 1846) (Figs. 9-15). 3. UCMP XXXXX: Bodega Bay, Sonoma Co., California. 4. USNM 611301 [Holotype]: Berkeley Marina, Alameda Co., California. 5. Transitional shell morphology. UCMP XXXXX: Bodega Bay, Sonoma Co., California. 6. UCMP XXXXX: San Francisco, San Francisco Co., California. 7. UCMP XXXXX: San Francisco, San Francisco Co., California. 8. UCMP XXXXX: San Francisco, San Francisco Co., California.

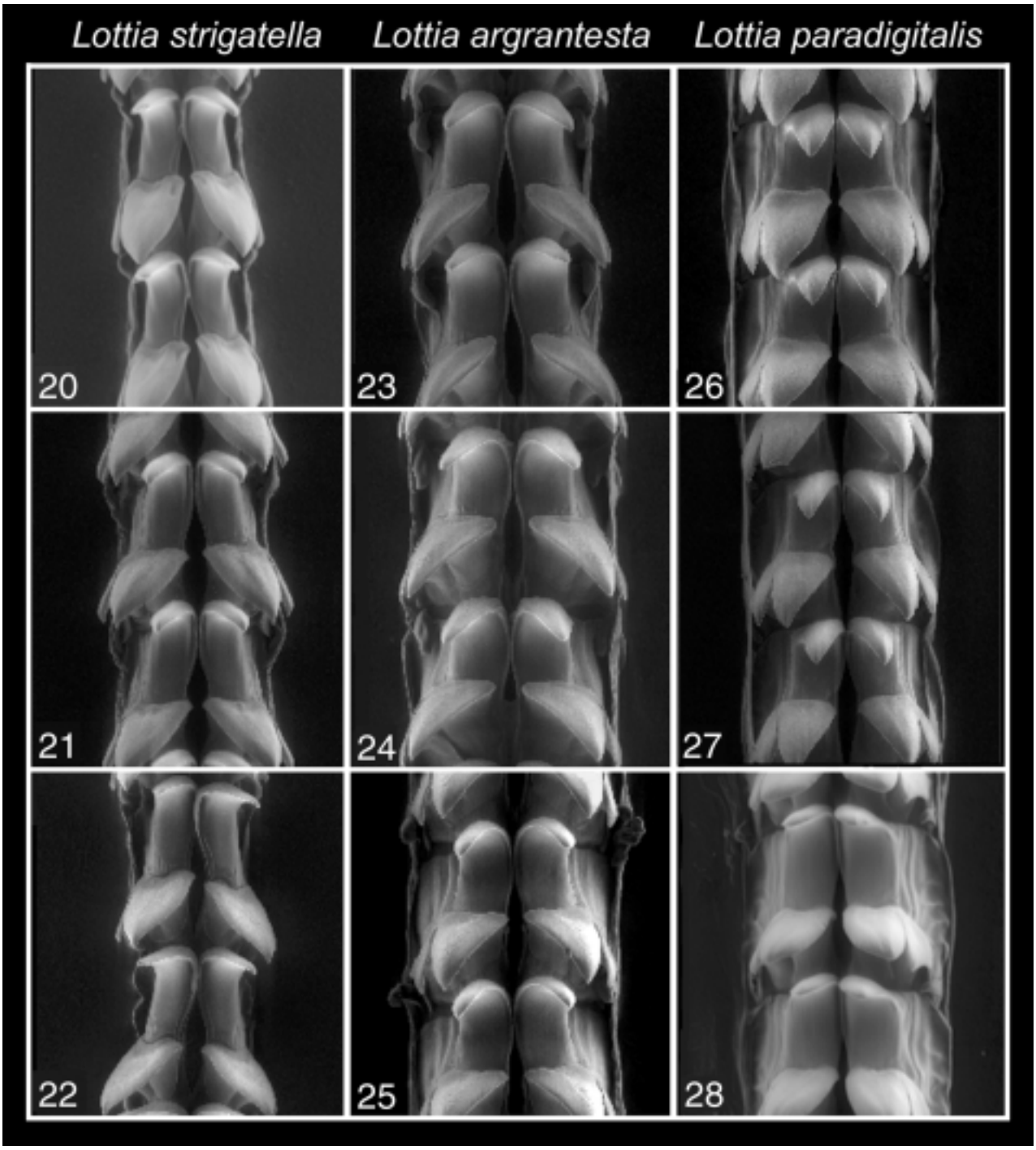


Figures 9-15. 9. UCMP XXXXX: Cabo San Lucas, Baja California Sur, Mexico. 10. USNM 12584 [Lecotype on right]: Cabo San Lucas, Baja California Sur, Mexico. 11. Transitional shell morphology. UCMP XXXXX: Cabo San Lucas, Baja California Sur, Mexico. 12. UCMP XXXXX: Guaymas, Sonora, Mexico. 13. UCMP XXXXX: Cabo

San Lucas, Baja California Sur, Mexico. 14. UCMP XXXXX: Bahía Tortugas, Baja California Norte, Mexico. 15. UCMP XXXXX: Guaymas, Sonora, Mexico.



Figures 16-19. Shell morphology of Lottia argrantesta Simison & Lindberg n. sp. 16. UCMP XXXXX [Holotype]: Bahía de San Francisquito, Baja California Sur, Mexico. 17. UCMP XXXXX [Paratype]: Califin, La Paz, Baja California Sur, Mexico. 18. UCMP XXXXX [Paratype]: Bahía de San Francisquito, Baja California Sur, Mexico. 19. UCMP XXXXX [Paratype]: Tecolate, Baja California Sur, Mexico



Figures 20-28. Radular morphology. Figs. 20-22. Lottia strigatella (Carpenter, 1846). 20. Chileno, Cabo San Lucas, Baja California Sur, Mexico. 21. Cabo San Lucas, Baja California Sur, Mexico. 22. Guaymas, Sonora, Mexico. Figs. 23-25. Lottia argrantesta

Simison & Lindberg, n. sp. 23. Califin, La Paz, Baja California Sur, Mexico. 24 -25. Bahía de San Francisquito, Baja California Sur, Mexico. Figs. 26-28. Lottia paradigitalis (Fritchman, 1960). San Francisco Bay, San Francisco Co., California.

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Chapter 5

Phylogeographical patterns of New World Patellogastropoda

Introduction

Charles Darwin realized the importance of geographic distributions only after he returned to England from his famous journey to the Galapagos Islands. He had collected many organisms from the many islands of the Galapagos, including the now famous finches. Unfortunately, he did not specify in his field notes exactly which island each specimen came from. Rather, he simply noted “Galapagos”. He soon realized after examining his finch specimens that there were significant morphological differences among the lot of “Galapagos” finches and that the difference may stem from their occupation of different islands. Fortunately for Darwin, he was not the only member of the H.M.S. Beagle crew who collected from the various Galapagos Islands. Others had collected finches and recorded which islands were associated with which specimens. He compared his specimens to those with more accurate locality information and the rest is history. Alfred Russel Wallace, the co-discoverer of natural selection, was the first true biogeographer. His extensive exploration of the world and his keen eye for morphological variation led him to quickly realize that geography plays a key role in the biological diversity we see on earth. The two men responsible for how we currently view biology intimately linked together geography, diversity, and evolution. Yet, not until Willi Hennig (1966) did evolutionary biologists consider examining biological distributions in an evolutionary (phylogenetic) context. This approach is referred herein as

“phylogeography”. The theory of island biogeography developed by MacArthur and Wilson (MacArthur & Wilson, 1967) opened new ways of examining biological distributions but it was devoid of phylogenetic history. This lack severely limits the explanatory power of island biogeography, because distributions are directly associated with phylogenetic history. For example, if closely related fish from five adjacent lakes have all diverged from one another it is impossible to determine a plausible distribution history based on the size, position, or level of isolation of the lakes. However, if the phylogeny of the fish were known it could be determined which lineages occupied which lakes and in what order.

The tectonic history of the New World is well known. Several significant biogeographical events have been documented, including the great biotic interchange between North and South America, which was facilitated by the connection of these continents by the emergence of the Isthmus of Panama (3.5 mya). The emergence of Panama also created a vicariant barrier between marine faunas of the Caribbean and tropical east Pacific. There have been numerous periods of extensive glaciation, which forced the wholesale realignment of ranges (Lindberg & Lipps, 1993). Nearshore patellogastropods have a paleontological record in the New World dating back to at least the Cretaceous and have occupied the continental margins throughout New World history. This makes patellogastropods ideal for studying New World historical biogeography.

There are six distinct patterns found in the phylogeographic distributions of New World patellogastropods (Fig. 1): (1) The phylogenetic relationships of the northeast Pacific (NEP) and Gulf of California limpets reveal a north to south correlation with the

most basal to the most derived OTUs (Fig. 2). (2) There are at least five limpets endemic to the Gulf of California. (3) The southern temperate Chilean clade is sister to the northeast Pacific temperate clade, thereby establishing an antitropical distribution (Fig. 3). (4) The tropical taxa are the basal clade in the New World phylogeny. (5) Within the tropical New World clade, there are three independent trans-Panamic relationships (Fig. 4 and 4b). (6) The tropical clade includes two unique NEP taxa.

In this study, each observed pattern is discussed at a regional level followed by a global synthesis of all regional patterns into a single unifying hypothesis. At the regional level, details of each pattern are described, hypotheses are made to explain each pattern, and tests are proposed for each hypothesis. For the global approach, a large-scale spatiotemporal hypothesis is presented, which accounts for the origins of each regional pattern, phylogenetic predictions for unstudied limpets are proposed, and a discussion of possible tests of the universal hypothesis is given

Regional patterns

North East Pacific Gradient

The most basal taxa of the northeast Pacific (NEP) clade have the most northerly distributions while the more derived taxa have the more southerly distributions (Fig. 2). This gradient pattern may be a result of random species migrations, however, the fossil evidence suggests that the ranges of the extant taxa have remained constant since the E. Pleistocene (Linberg & Lipps, 1993) (Table 1). The more parsimonious explanation is a northern ancestor, which may have migrated along the Aleutian Islands

from the northwest Pacific. The prediction from this hypothesis is that the northwest Pacific OTUs will be basal to all or most of the NEP OTUs. To test this, specimens from Kamchatka and Japan need to be collected and sequenced. Three Japanese specimens have been sequenced, which appear to have phylogenetic connections with the NEP and Chilean clades. However, it is clear that taxon sampling strongly influences phylogenetic position. For example, a phylogenetic analysis of a sub-sample of NEP and Chilean taxa produces a mix of NEP and Chilean taxa with no clear biogeographical associations (Fig. 5). Whereas, a more complete sampling of NEP and Chilean taxa produce a sister relationship between the Chilean and NEP clade. The same can be expected from a sub-sampling of Japanese taxa. If the Japanese taxa form a clade separate from the Chilean and NEP clades, then a sub-sample from the Japanese clade would necessarily have longer branches than those found among the Chilean and Californian clades. This would tend to artificially attract the Japanese taxa into either the California or Chilean clades (Felsenstein, 1978a). Clearly, a much larger sampling of Western Pacific limpets is required before any phylogenetic hypothesis about the western and eastern Pacific can be tested.

Gulf Endemics

There are four described species of limpets found exclusively in the Gulf of California, and, another (*L. argrantesta*) described in Chapter 4. The range of this potential endemic needs to be verified considering its proximity to the mouth of the gulf and thus will be treated tentatively as an endemic to the gulf. The phylogenetic positions of all five endemics are relatively terminal in the NEP clade (Fig. 2). Two events may

explain this pattern, a possible mid-peninsular seaway across Baja and the glacial compression of northeastern Pacific provinces.

A mid-peninsular seaway across the Viscaino desert of Baja, California has been suggested by Upton and Murphy (1997) based on mitochondrial differences found between *Uta* lizards found north and south of the desert. Based on mitochondrial evolutionary rates, they have estimated that the seaway existed 1 million years ago.

A mid-peninsular seaway connecting the Gulf of California to the Pacific Ocean may have provided an opportunity for taxa to invade the gulf from the temperate north. The Pacific opening of the projected seaway lies at the provincial boundary of temperate and neo-tropical waters. Recent periods of glaciation may have driven more southerly temperate species through this seaway and into the gulf. Because of the proximity of the provincial boundary to the proposed seaway, migration through the seaway may have also been possible without a glacial push.

While there is some Pleistocene evidence of marine flooding near Santa Rosalia, it lies on the western shores of the gulf at the base of the north-south Sierra Coyote mountain range. Marine deposits found near the coast and at the base of a long mountain range do not provide evidence that the mountain range was once under the sea. What is needed is stratigraphic evidence of shorelines and seafloors at higher elevations along the Viscaino range. Peter Lonsdale (Scripps Institute of Oceanography) does not know of any tectonic, geological, or paleontological evidence supporting a mid-peninsular seaway (pers. comm. 1998).

Phylogenetic and biogeographical studies of other temperate marine taxa may provide corroborative evidence of a seaway if disjunct distributions of temperate taxa

are currently found near the proposed gulf opening of the seaway. There are several described disjunct distributions between California and the Gulf of California (Present, 1987 and references therein), but none of these studies are phylogenetic and none describe ranges associated with the proposed location of the seaway. There are explanations that are more parsimonious. For example, birds have been known to carry organisms great distances, ships and boats transport many marine adults and larvae, organisms could enter the gulf from the north around the tip of the Baja peninsula, migrating whales and drifting flotsam are known to transport life, these and other explanations compete with the seaway hypothesis.

The glacial compression of NEP provinces may have provided temperate taxa the opportunity to enter the gulf around the cooled tip of the Baja peninsula. The subsequent warming of the mouth of the gulf after the glacial retreat could have isolated taxa resulting in disjunct populations and eventual speciation. There have been many "ice ages" but only a few large enough to cool the neo-tropical waters of the mouth of the gulf. (Lindberg & Lipps, fig. 7.2, 1996,). Testing this hypothesis, like the mid-peninsular seaway hypothesis, is difficult, but changes in provincial boundaries during glacial periods have been demonstrated (Valentine, 1966; Stanley, 1986) and would certainly permit the movement of taxa into the Gulf of California.

Antitropical Pattern

The New World phylogeny of patellogastropods reveals a sister relationship between limpets of the temperate waters of Chile in the Southern Hemisphere and the NEP in the Northern Hemisphere (Fig 3). This "antitropical", "trans-equatorial" or

“bipolar” distribution is not unique to limpets. Representatives from nearly every Class of terrestrial and marine organisms have been described as having antitropical distributions (Hubbs, 1952; Ekman, 1953; Nelson, 1985; White, 1986; Lindberg, 1991). Several dispersal hypotheses have been presented explaining how organisms might have crossed tropical equatorial waters to form antitropical distributions. Lindberg (1991) proposed a Pleistocene island-hoping model, based on migrating cells of cool marine upwelling, which appeared at varying latitudes depending on changing sea levels. He cited evidence (Emerson, 1952 and Emerson, 1956; Valentine, 1955) that thermally anomalous faunas were associated with regions of upwelling in warmer waters and that these dynamic regions of upwelling could, over time, provide refugial stepping stones for temperate taxa across the equatorial warm water barrier. Smith (1970) suggested that dispersal across the tropics was overcome by a combination of currents and submergence below the warm surface of the tropics. While both hypotheses are reasonable, Smith’s can only account for subtidal organisms and the expected pattern from either scenario would be a phylogenetic mosaic of Chilean and NEP taxa in temperate habitats.

Not until now has there been a phylogenetic approach to this question. The phylogenetic relationship between the northern and southern temperate limpets clearly falsifies either exchange model, for all of the NEP limpets share a common ancestor, as do all of the limpets from the temperate south. White (1986) and Briggs (1987) have proposed different relictual models. Each has suggested that the antitropical distributions are relicts of once continuous clades. White presented evidence that tropical extinction occurred due to Miocene warming of the tropics. This model predicts

a recent recolonization of the tropics and therefore shallower and more recent divergences in the tropical clades than in the temperate clades. This model would also predict an ancestral-descendant gradient from lower to higher latitudes, or, given enough time, no latitudinal association with phylogeny. The New World phylogeny and paleontological record are inconsistent with this model. The paleontological record reveals pre Miocene evidence of extant tropical molluscs (Jackson et al., 1993) and the phylogeny reveals that the tropical taxa are basal and have deep trans-Panamic clades. These suggest that the tropical limpets have resided in the tropics well before the Miocene and certainly as long, if not longer, than the temperate clades. The north to south ancestral-descendant pattern found in the NEP limpet clade is also inconsistent with White's relictual model.

Briggs' model includes the displacement of tropical eastern Pacific faunas by the rich tropical Indo-West Pacific faunas. Briggs' model is inconsistent with evidence supporting the strong east to west barrier of the deep Pacific (Ekman, 1953; Grigg & Hey, 1992). Invasion from the Indo-West Pacific may have occurred, however, the wholesale displacement of most tropical taxa would require a continuous flow of many different taxa across the east Pacific Barrier and the successful occupation of many different habitats. Ekman (1953), who identified and named the east Pacific Barrier, examined 240 species of Indo-West Pacific echinoderms and found that only two percent were represented on the East Pacific shelf. Cox and Moore (Cox, 1993) showed that Hawaii and the tropical east Pacific only share 6% of their shore fishes. Given the current paleontological evidence, and the known affinities with the Atlantic (Grigg and Hey, 1992), it seems highly improbable that a regiment of Indo-West Pacific faunas

displaced most tropical east Pacific faunas. It is unlikely that New World tropical ancestors would leave no tropical descendants and that all tropical descendants would be replaced by other Indo-West Pacific lottiid limpets, which are rare in the tropical Indo-West Pacific. None of the published models explaining antitropicality are consistent with the New World limpet fossil record or phylogeny.

I propose that the antitropical distribution of lottiids is the result of a common ancestral clade from the Western Pacific with independent migrations from the north and south. This scenario avoids the crossing of the East Pacific Barrier and is consistent with the north-south ancestral-descendant pattern found in the NEP clade.

If the NEP and Chilean clade had their origins in the Western Pacific and have invaded from the north and south, then it should be expected that the temperate taxa occupying the lowest latitudes along the eastern Pacific should also be the most derived taxa in the their respective clades. This is certainly the case for the NEP clade as discussed above. In fact, there is a discernable north to south association with basal and terminal taxa in the NEP clade (Fig. 2). Without a phylogenetic perspective for the western Pacific limpets, it is impossible to determine whether they too display an antitropical distribution. However, there is no reason to assume that they do. The geological history of the west Pacific does not include a comparable vicariant event such as the closure of the Panamanian Seaway with the associated disruption of oceanic currents and weather. It is quite possible that the west Pacific limpets have a continuous phylogenetic relationship along the entire western margin. Three 16S sequences from Japanese limpets show an affinity for the California and Chilean clades. These preliminary affinities are viewed cautiously here because, as described

earlier, sampling bias and Long Branch attraction are documented problems in phylogenetic analyses (Felsenstein, 1987a). A more complete sampling of Japanese and other west Pacific localities is required before any hypothesis can be fully examined. I predict that the western Pacific limpet phylogeny will show a single clade with separate branches leading to the NEP clade from the north and to the Chilean clade from the south.

Basal tropics

Trans-Panamic

The New World tropical clade is the most basal of the New World patellogastropod phylogeny (Fig 1) and includes OTUs restricted to either side of the Isthmus of Panama. Three separate trans-Panamanian relationships exist (Fig 4), indicating that the Caribbean and tropical eastern Pacific limpet clades predate the emergence of the Isthmus of Panama. The Isthmus of Panama provides an ideal setting for studying vicariance and allopatric speciation. The isthmus emerged from the Panamanian Seaway approximately 3.5 million years ago, connected North and South America, and physically divided the New World tropical seas into the Caribbean and Pacific (Vermeij, 1993). This surely isolated once continuous populations of many different taxa into separate habitats.

The three distinct trans-Panamic relationships in the tropical New World clade are described as follows (Fig. 4): 1) The most basal trans-Panamic relationship is between the Pacific *K. stipulata* clade and the rest of the tropical clade. 2) The second

trans-Panamic relationship is between the Pacific *K. mitella* clade and the Caribbean *K. occidentalis* clade. 3) The third and probably the most recent tran-Panamic split is between *K. fascicularis* and *K. albicosta*.

Stipulata **clade**

This T-P relationship is the most complex of the three, for it is the deepest phylogenetically and it contains the more recent T-P relationship existing between *K. fascicularis* and *K. albicosta*. The deep T-P divergence and the inclusion of the *K. fascicularis* and *K. albicosta* relationship suggests that this primarily pacific clade has been isolated from the Caribbean well before the emergence of Panama. All of the Caribbean taxa but *K. albicosta* are sister to this clade and the sister Caribbean clade also contains a nested T-P relationship. The presence of T-P relationships in the Caribbean and Pacific clades of this most basal T-P association further suggests that the isolation of these two clades predates the emergence of Panama. There may have been a pre-Panamanian barrier between the Caribbean and Pacifc.

Mitella **clade**

This T-P clade is phylogenetically more recent than the *stipulata* clade and involves eight taxa, three of which form a Pacific clade.

***fascicularis-albicosta* clade**

The *K. fascicularis-K. albicosta* T-P relationship is the closest phylogenetically and presents the best opportunity to study the effects of a recent vicariance. *K. fascicularis* is restricted to the Pacific shores and *K. albicosta* is restricted to the Caribbean. *K. fascicularis and K. albicosta* are not only phylogenetically similar but are also very similar morphologically. Shell characteristics and coloration patterns are similar and they both share the very unusual presence of two distinct radular morphologies. This radular characteristic has been fully described by Simison and Lindberg (1999) for *fascicularis* and by Padilla for *albicosta* (unpublished data). The ranges of both limpets include Panamanian shores, and almost certainly are an example of a once continuous population that was split by the emergence of Panama. The two radular types may be an example of the maintenance of a polymorphism of at least 3.5 million years. More needs to be done to establish that the two radular morphologies represent a true polymorphism, but weather it turns out to be a polymorphism or not, this T-P relationship serves as an ideal subject for future studies of vicariance and other associated evolutionary processes.

***Paleacea-depicta* clade**

Of all of the NEP OTUs only one, *Tectura paleacea* (Gould, 1853), was not included in the exclusively monophyletic NEP clade (Fig. 1). *T. paleacea* nests with the tropical Pacific clade that includes *K. stipulata* and *K. fascicularis*, and is the sole NEP representative in the tropical New World clade. *T. paleace* and *Notoacmea depicta*

(Hinds, 1842), are the only NEP or tropical New World OTUs that feed exclusively on angiosperm marine grasses. *N. depicta* has only recently been sequenced for 16S (Begovic, unpublished data). A preliminary MP analysis using the 16S sequence for *N. depicta* places it sister to *T. paleacea* in the tropical New World clade. The curious phylogenetic position of these two temperate NEP taxa in an exclusively tropical clade presents a biogeographical puzzle, for there is only a single described tropical species that feeds on sea grasses and that is believed to be a morph of *Patelloida pustulata*, which is a clear outgroup to the entire New World patellogastropod clade. A clue to this puzzle may come from the extinct temperate Atlantic limpet *L. alveus*, which was shown to die out during a "wasting disease" of its host plant *Zostera marina* (Carlton *et al*., 1991). A detailed paleontological survey of tropical and temperate sea grasses and limpets might reveal a historical scenario where sea grass limpets once ranged throughout the New World and eventually experienced extinctions throughout the tropics. Until then, *T. paleacea* and *N. depicta* will be considered temperate descendants of the New World tropics.

Global hypothesis

The basal position of the tropical clade, the antitropical association of the NEP and Chilean clades, and the latitudinal gradient in the NEP clade all provide clues to the origins of the New World patellogastropods. As previously mentioned, it has been hypothesized here that the antitropical pattern of the temperate clades is the result of a common ancestral clade from the west Pacific, with independent migrations from the

north and south. The global distribution of known lottiids is temperate and tropical in the New World and almost exclusively temperate everywhere else. The distribution of the phylogenetic sister group to the lottiids, the patelloidids, is primarily tropical, with only two representatives in the New World; one in the Atlantic and one in the Pacific (Keen, 1971). The origins of the tropical New World clade is hypothesized here as Tethyan, with subsequent dispersal to the east and west facilitated by the break up and tectonic movement of Gondwana (Fig 6). The taxa that migrated west eventually established a tropical foothold in the New World tropics while the eastern taxa established temperate habitats in the western Pacific. Eventually the temperate northwest Pacific taxa migrated across the Aleutians into the NEP and the temperate southwest Pacific limpets migrated across the south via New Zealand and Antarctica and settled in South America. This scenario results in the temperate New World limpets reuniting with and flanking their Tethyan ancestors, which currently occupy the New World tropics.

To test this hypothesis, lottiids limpets as well as their reputed sister group, the patelloidids, from the western Pacific need to be collected and sequenced. It is predicted that the patelloidids and lottiids are indeed sister taxa, and that they have partitioned different habitats throughout the world. I also predict that the western Pacific lottiids will form either a single monophyletic group with the NEP and Chilean clades evolving from separate subclades, or they will be two distinct clades coming out of the tropical New World clade, each leading to the temperate NEP and Chilean clades.

Table 1. The paleontological record of NEP lottiid patellogastropods (Lindberg, unpublished data)

taxon	northern range	southern range	Occurance
testudinalis	Arctic circle	Pacific	L. Pleistocene
fenestrata	Alaska	Punta Pequeña, Baja	Recent
persona	Alaska	Morrow Bay, CA	L. Pleistocene
scutum	Aleutians	Japan	E. Pleistocene
ochracia	Aleutians	Cedros Is, Baja	L. Pleistocene
s	Alaska	Point Concepcion, CA	L. Pleistocene
digitalis	Kiska, Alaska	Southern Baja	Pliocene
pelta2	San Francisco		
pelta	Aleutians	Hokkaido, Japan and Northern Baja	E. Pleistocene
macleanii	Channel is.		
strigatella	point concepcion	Gulf of California	L. Pleistocene
scabra	Cape Arago, Oregon	Cabo San Lucas, Baja	
conus	point concepcion	Southern Baja	L. Pleistocene
turveri	Gulf of California		
acutapex	Gulf of California		
gigantea	Neah Bay, Washington	Tortuga, Baja	L. Pleistocene
argrantesta	?	Gulf of California	
limatula	Newport, Oregon	Southern Baja and Revillagigedos	E. Pleistocene
a	Gulf of California		
dalliana	Gulf of California		
rosacea	Ketchikan, Alaska	Northern Baja	Recent

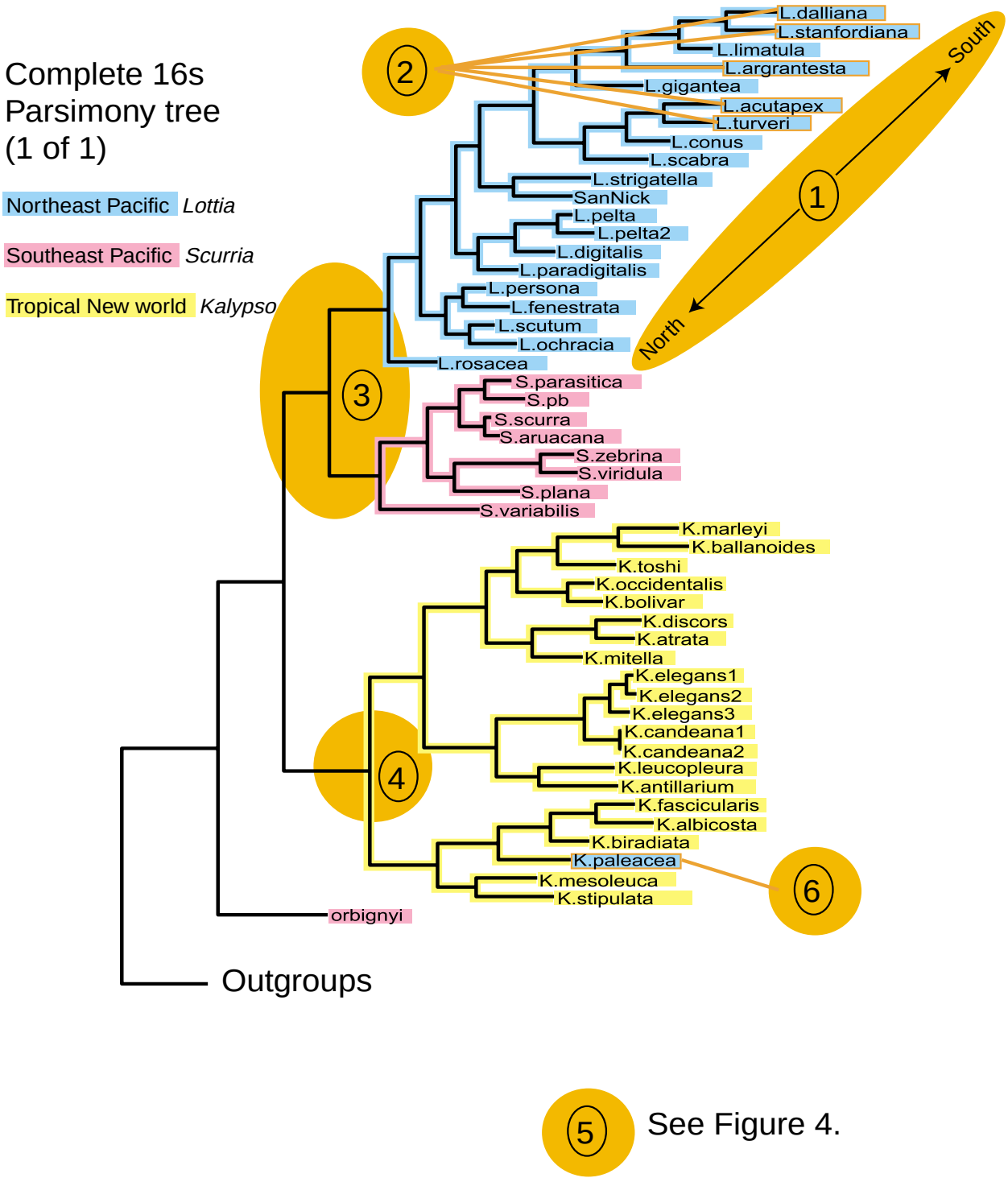

Figure 1. A 16S MP phylogeny with 6 biogeographical patterns identified. 1)A north to south gradient in the NEP clade. 2) Gulf of California endemics are most terminal in the NEP clade. 3) An antitropical sister relationship between the temperate north and south. 4) Tropical New World clade is the basal clade of New World patellogastropods. 5) Fig. 4. 6) A single NEP representative in the tropics.

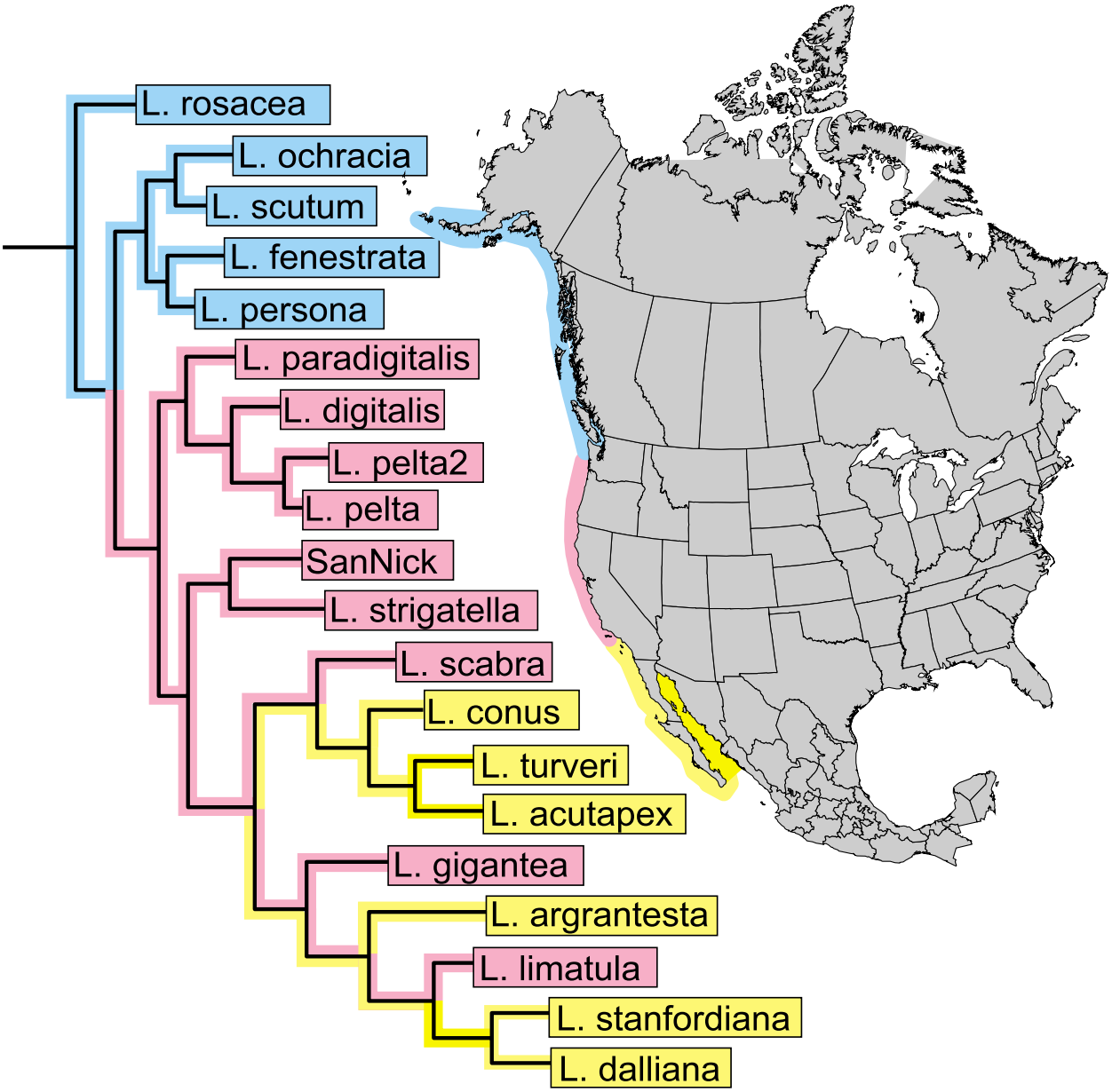

Figure 2. The NEP clade from the 16S MP phylogeny reveals a north to south progression. The most ancestral OTUs are found in the north while the more derived are found in the south.

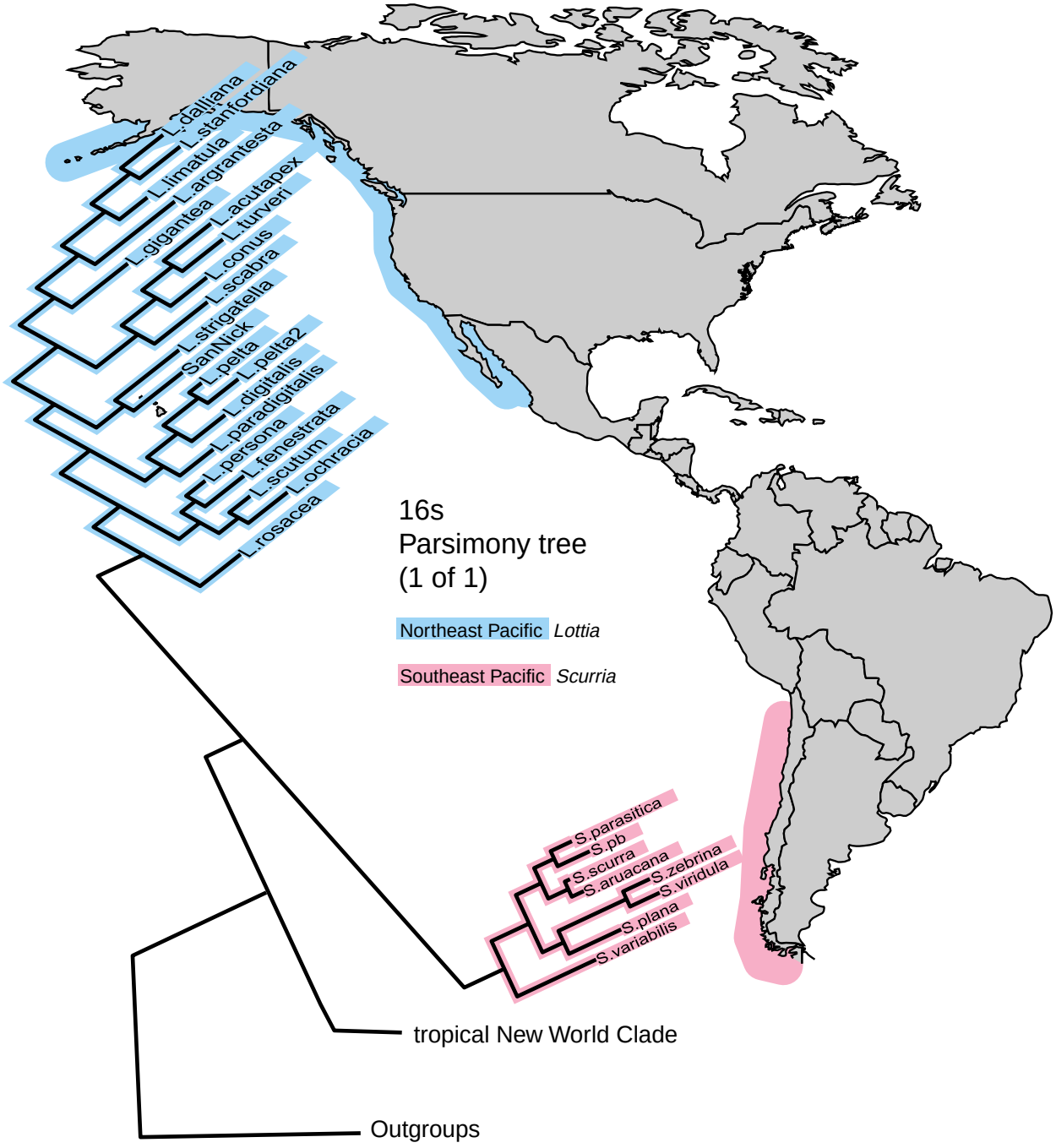

Figure 3. The temperate NEP clade form a sister relationship with the temperate southeast Pacific clade and are separated by the warm tropics of the equatorial Pacific.

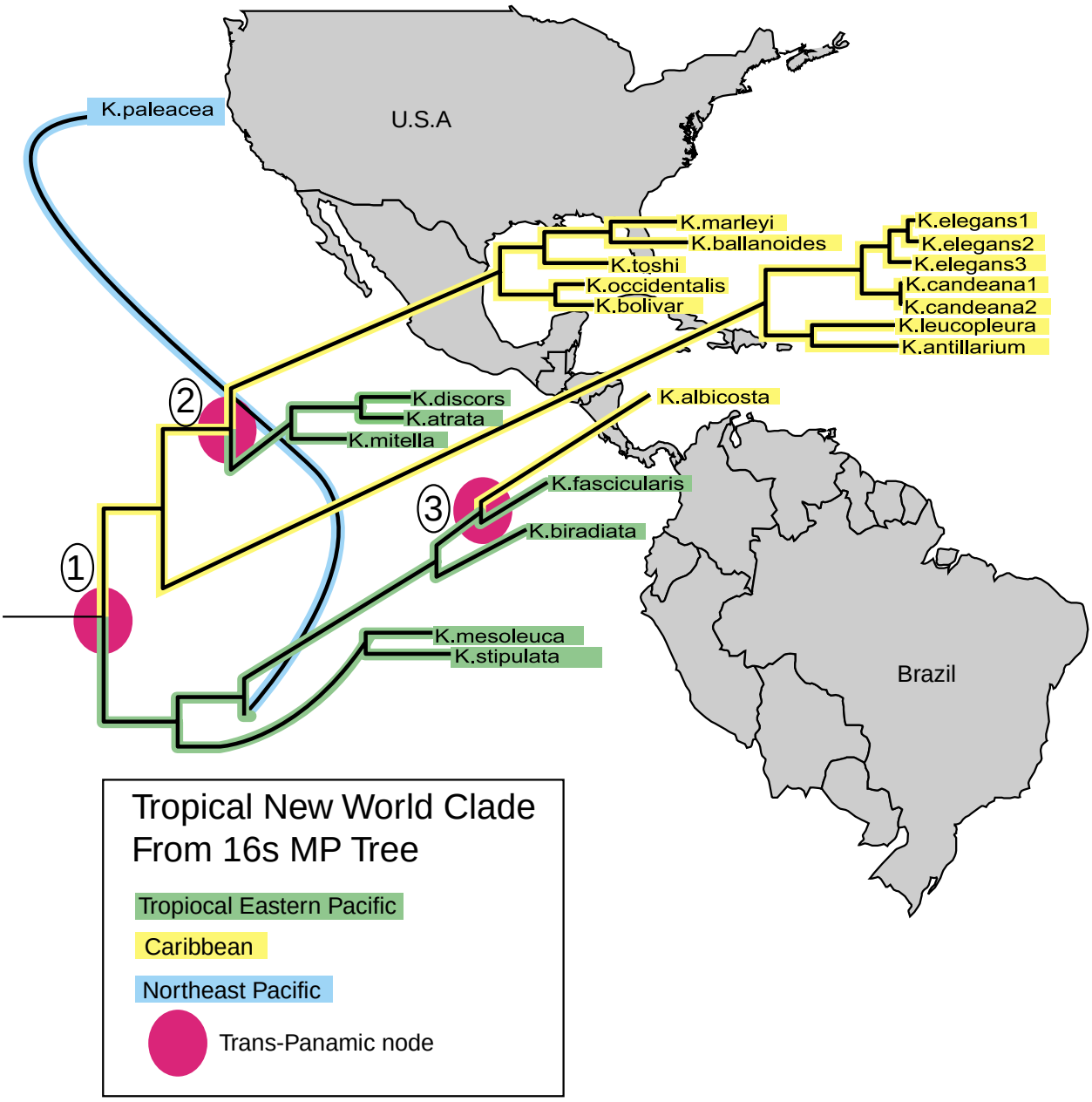

Figure 4. A graphical representation of the tropical New World clade, which contains three nodes spanning the Isthmus of Panama. Some OTUs are exclusively found on the Pacific side while others are only found in the Caribbean.

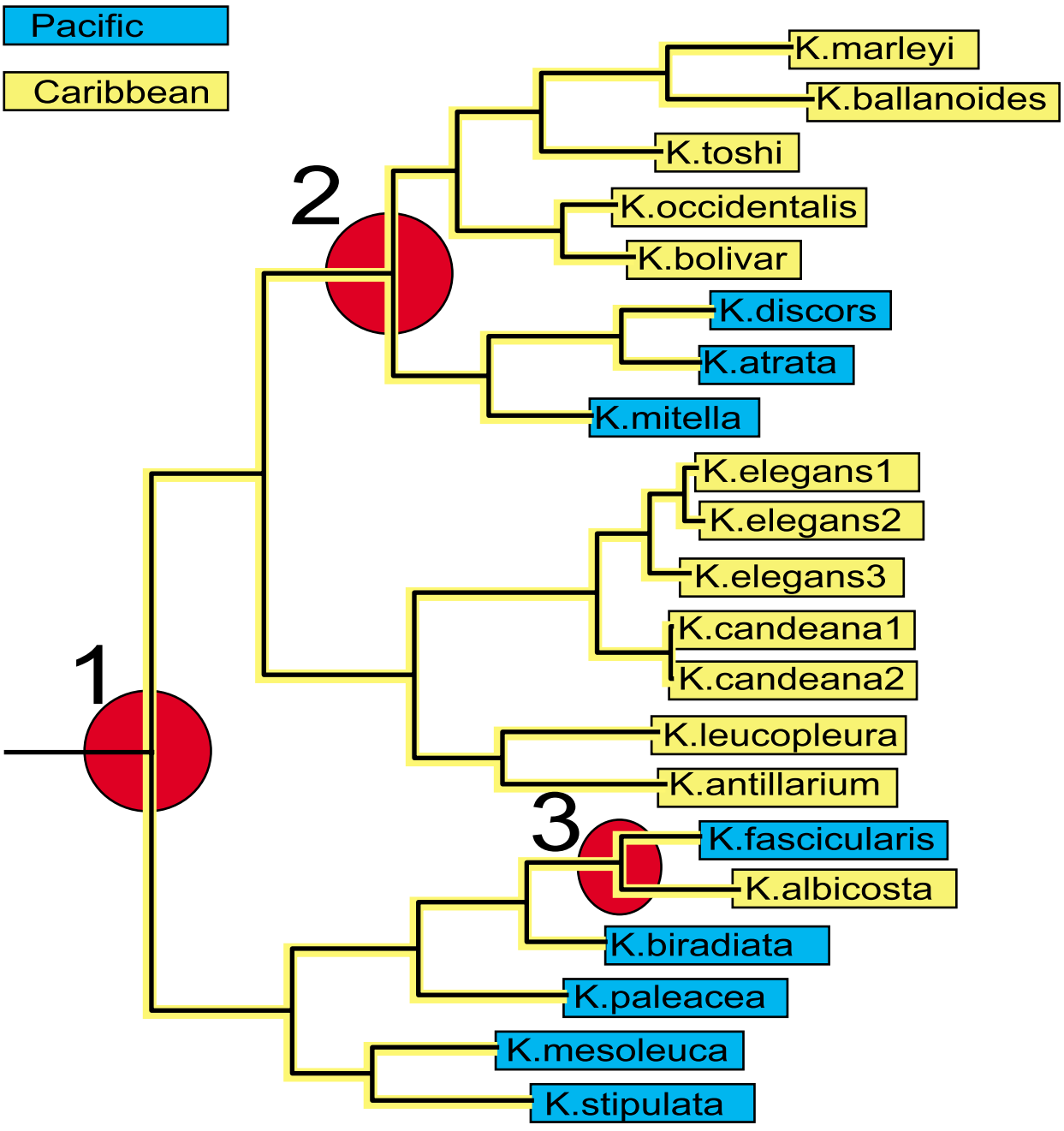

Figure 4b. A tree view of the tropical New World clade and its three trans-Panamanian nodes.

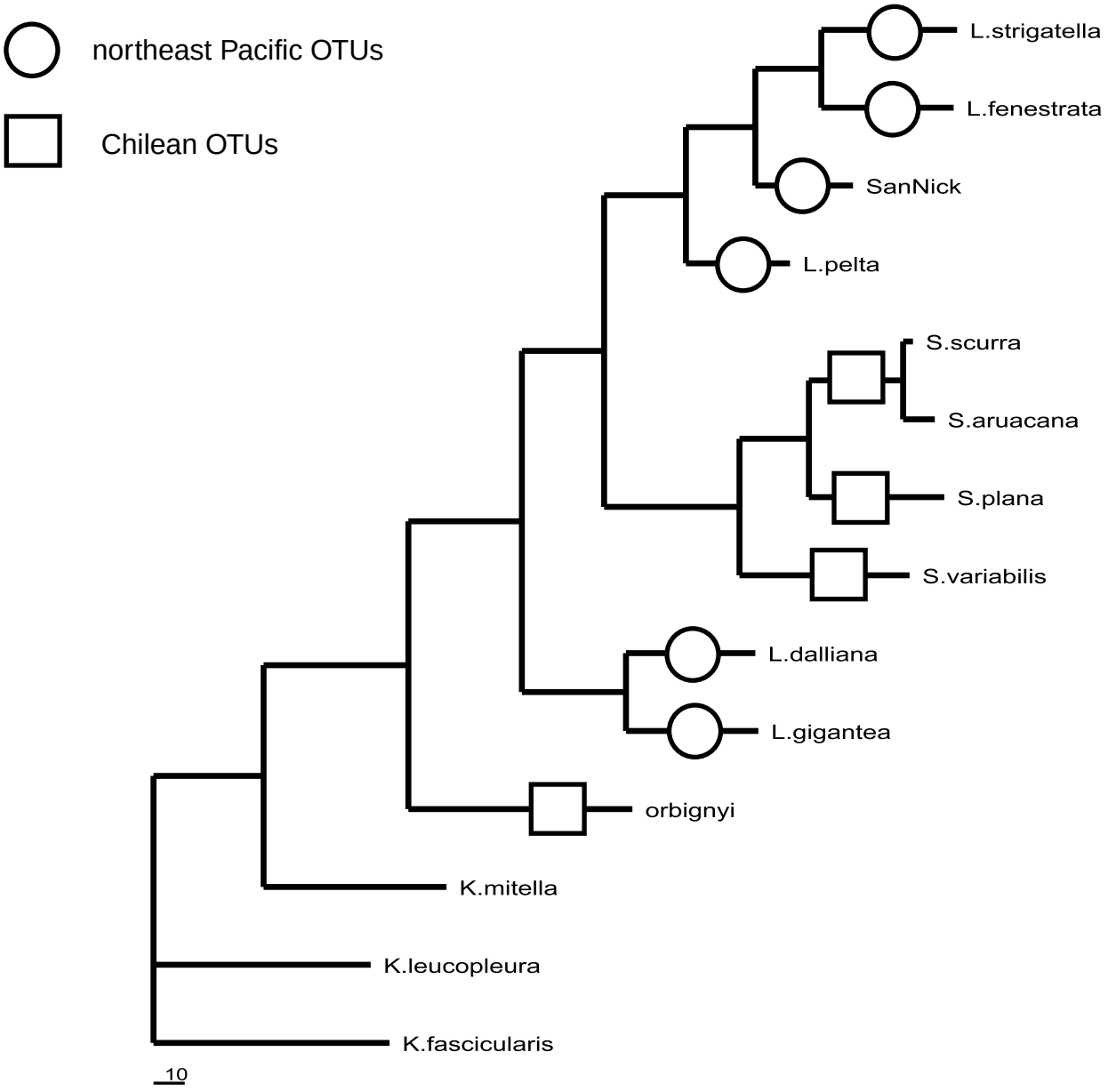

Figure 5. A simple MP tree generated from a subsample of NEP and Chilean OTUs demonstrating the misleading effect of using exemplars. Here the Chilean clade jumps into the NEP clade and breaks up the monophyly of the exclusive NEP clade.

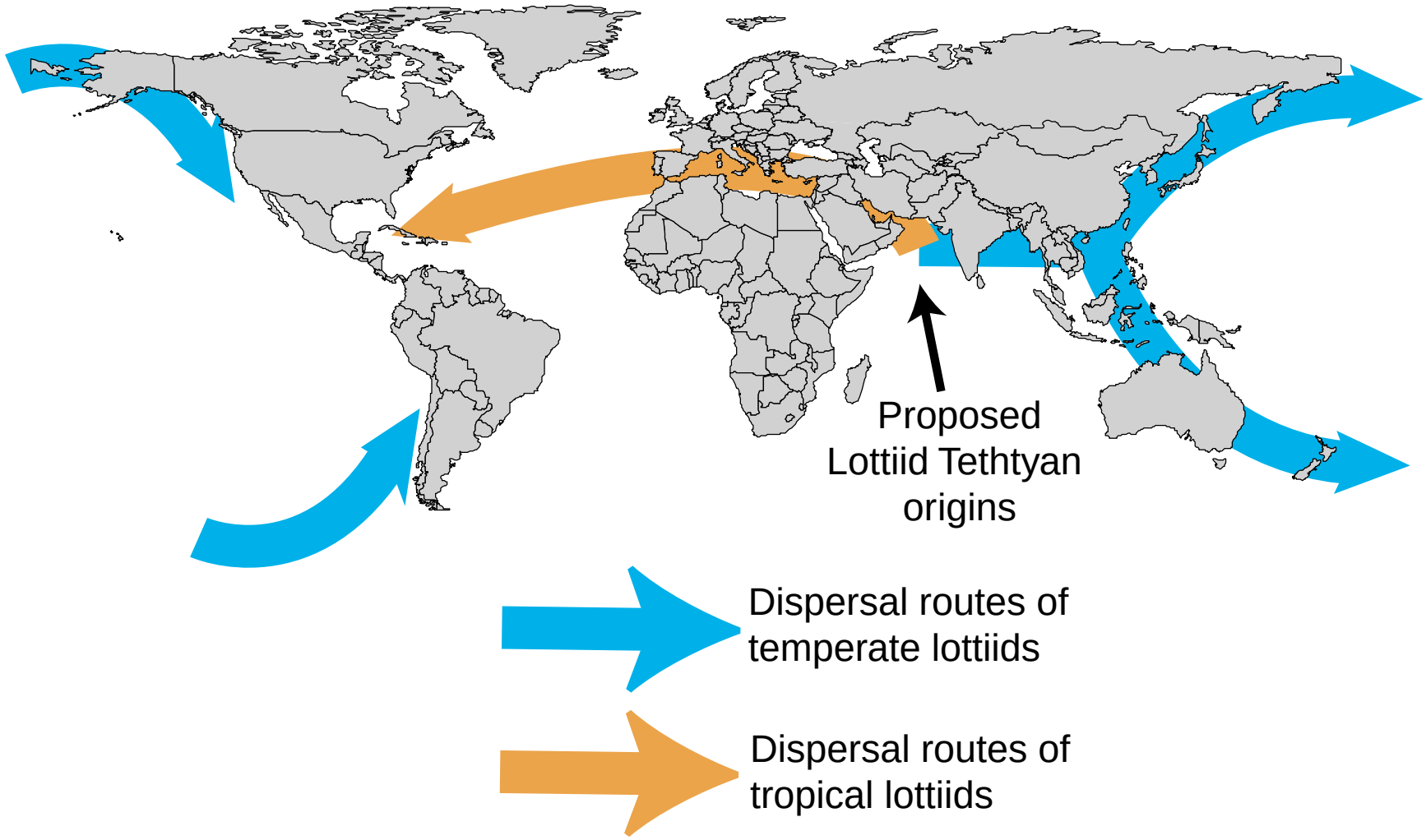

Figure 6. A graphical representation of the hypothesized Tethyan origins and subsequent dispersal routes of the temperate and
tropical patellogastropods.

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