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SYMPATRIC SEA SHELLS ALONG THE SEA'S SHORE: THE GEOGRAPHY OF SPECIATION IN THE MARINE GASTROPOD *TEGULA*

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Abstract.—Uncertainty and controversy surround the geographical and ecological circumstances that create genetic differences between populations that eventually lead to reproductive isolation. Two aspects of marine organisms further complicate this situation: (1) many species possess planktonic larvae capable of great dispersal; and (2) obvious barriers to movement between populations are rare. Past studies of speciation in the sea have focussed on identifying the effects of past land barriers and on biogeographical breakpoints. However, assessing the role such undeniable barriers actually play in the initial divergence leading to reproductive isolation requires phylogenetic studies of recent radiations living in varying degrees of sympatry and allopatry to see which barriers (if any) tend to separate sister species. Here I infer phylogenetic relationship between 23 species of the marine snail *Tegula* using DNA sequences from two regions of the mitochondrial genome: cytochrome *c* oxidase I (COI) and the small ribosomal subunit (12S). These snails possess planktonic larvae with moderate dispersal capabilities and have speciated rapidly, with over 40 extant species arising since the genus first appeared in the mid-Miocene (about 15 M.Y.B.P.). Trees constructed from the COI and 12S regions (which yielded 205 and 137 phylogenetically informative sites, respectively) were robust with respect to tree-building method, bootstrapping, and the relative weightings of transitions, transversions, and gaps. Within clades where all extant species have been sampled, five of six identified sister species pairs broadly coexist on the same side of biogeographical boundaries. These data suggest strong geographical barriers to gene flow may not always be required for speciation in the sea; transient allopatry or even ecological barriers may suffice. A survey of the geographic distributions of marine radiations suggests that coastal distributions may favor the sympatry of sister taxa more than island distributions do.

Key words.—Cytochrome oxidase I, marine biogeography, *Tegula*, 12S rRNA.

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The evolution of reproductive isolation requires the genetic differentiation of populations formerly capable of interbreeding. The geographical circumstances that permit this initial genetic divergence remain mysterious. At one extreme, populations may diverge initially in complete allopatry (Mayr 1970). Given sufficient time, drift may cause such isolated populations to diverge, but even limited gene flow can hinder such differentiation in the absence of disruptive selection. At the other end of this spectrum of geographic isolation, coexisting populations that exploit different resources can diverge sympatrically, but the resource or microhabitat fidelity and differential performance must be strong (Bush 1969; Rice 1987; Rice and Hostert 1993).

These geographic models of speciation stem from work on terrestrial organisms. The successful transfer of these terrestrial models to a benthic marine setting faces challenges from two fundamental differences between terrestrial and marine organisms. First, most free-living benthic marine organisms pass through a larval life-history stage. During this period, larvae develop in the plankton, where ocean currents may carry them far from their birthplace. Just how far larvae can go should depend on how long they can sustain themselves while floating. Larvae that rely on yolk reserves should have more limited dispersal potential than those capable of capturing food as larvae (Jablonski and Lutz 1983; Hellberg 1996). In the latter case, even though adults may possess limited dispersal abilities, larvae may be able to move such great distances that the globe itself seems insufficient to isolate populations (Palumbi 1994). As a corollary to this challenge of larval dispersal, even a short planktonic dispersal phase can break associations between the microhabitats of parents and their offspring, thereby disfavoring the strong host fidelity seen in many insects and favorable to sympatric speciation.

Second, the ocean habitat lacks geographical barriers as obvious as the mountain ranges and rivers that can separate terrestrial populations. Consequently, studies of speciation in the sea often have focused on identifying barriers that could hold populations in isolation for sufficient time for incipient species to form. Mayr (1954) inferred geographical patterns of speciation from the distributions of several echinoid (sea urchins and sand dollars) radiations and concluded they were in agreement with terrestrial patterns. He named two large barriers capable of stemming the movement of larvae and thereby producing allopatric speciation: vast expanses of ocean and the rise of land barriers (particularly the Isthmus of Panama). Subsequent work has continued to focus on the impact of such large barriers, including the Isthmus of Panama dividing tropical biotas in the Caribbean and Eastern Pacific (Bermingham and Lessios 1993; Knowlton et al. 1993), warm water dividing temperate species with antitropical distributions (Lindberg 1991; Stepien and Rosenblatt 1996), the Arctic Ocean dividing biotas in the North Pacific and North Atlantic (Vermeij 1991; Palumbi and Kessing 1991; Cunningham et al. 1992; Rawson and Hilbish 1995), and the North Pacific dividing temperate biotas in East Asia and North America (Majima 1989; Vermeij 1989; Collins et al. 1996).

More subtle barriers have received increased attention of late due to heightened interest in recent climatic changes and the realization that molecular markers (particularly mitochondrial DNA) may carry the mark of historical demogra-

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phy. Avise (1994) reviews numerous examples of phylogenetic breaks within species that occur at Cape Canaveral, coincident with the abutting endpoints of sister taxa separated by this biogeographic boundary. The clustering of so many sharp changes most probably stems from the past separation of populations living in the Gulf of Mexico and Atlantic Ocean. Likewise, haplotype distributions within the European anchovy reflect the isolation of the Black Sea and the Aegean (Magoulas et al. 1996). Both of these examples are essentially special cases of the strong geographical barriers mentioned above, "ghosts of geographical barriers past."

No doubt strong geographical barriers can divide marine populations and lead to the formation of reproductively isolated species. But are such barriers a required, or even common, first step toward speciation in the sea? Several observations suggest such drastic barriers may not be necessary. First, the fossil record shows that newly forming snail species tend to appear within the same geographical region as their progenitors. This holds true for species both with nonfeeding larvae and feeding larvae (Jablonski 1986). Although such overlapping distributions may result from postspeciation dispersal, they nonetheless suggest that strong geographical isolation is not required for speciation. Second, Pleistocene climatic changes have repeatedly altered the geographical distributions of marine taxa (Valentine and Jablonski 1983; Hellberg 1994; Roy et al. 1996) and shifted biogeographic endpoints along coastlines. Such climatic fluctuations may facilitate the formation of new species in transiently isolated populations (Valentine and Jablonski 1983). Third, although the biogeographical boundary at Cape Canaveral divides many sister taxa, marine biogeographic breakpoints are not always marked by exceptional intraspecific genetic divergence or peripatric sister species (Burton 1998). Finally, as more long-standing marine taxa are becoming recognized as complexes of sibling species, more examples of sister taxa not separated by any obvious geographical barrier have become evident (Knowlton 1993). Instead, sister taxa often occur sympatrically at different depths (Knowlton et al. 1992; Miya and Nishida 1997) or on different substrates (Duffy 1996).

The role played by geographical barriers in the initial differentiation leading to reproductive isolation should be reflected in the geographical distributions of closely related taxa. If large geographical barriers (either past or present) are critical to forming new species, sister taxa should be separated by such barriers. This appears to be the case for geminate species separated by the Isthmus of Panama and for Gulf of Mexico/Atlantic sister taxa currently separated by Cape Canaveral. However, if ecological barriers (e.g., microhabitat specialization) provide the impetus for initial divergence, or if species form during transient allopatry, then sister taxa should occur along the same coastlines and be undivided by an obvious geographical barrier. Such predictions assume that the relative geographical distributions of taxa have changed little since their formation, a more likely situation among relatively recent radiations than older ones.

Marine snails of the genus *Tegula* first appear in the fossil record in California in the mid-Miocene, about 15 M.Y.B.P. (Addicott 1970; Hickman and McLean 1990). Despite this recent origin, over 40 extant species of *Tegula* have been described. Consistent with their brief history and their nonfeeding larvae (Moran 1997), the genus *Tegula* is geographically restricted. About half of all *Tegula* species are small, cryptic forms (subgenus *Agathistoma*) found in the subtropical and tropical waters of the eastern Pacific and the Caribbean. The remaining species are prominent members of rocky intertidal and shallow subtidal habitats in temperate regions of East Asia and of the Pacific coasts of North and South America. These cool-water *Tegula* have featured prominently in discussions of large-scale geographic barriers presented by the North Pacific (Vermeij 1989) and warm equatorial waters (Lindberg 1991). In addition, ecological studies have detailed differences in microhabitat distribution (Reidman et al. 1981) and response to predators (Dayton et al. 1977; Schmitt 1981; Watanabe 1983) among coexisting *Tegula*. Thus, a phylogenetic hypothesis for relationships among *Tegula* would allow insights not only into the geographical circumstances under which species initially formed, but also into whether the ecological innovations promoting coexistence evolved sympatrically.

Here I report a gene phylogeny based on segments of two mitochondrial genes that I used to infer phylogenetic relationships among species of *Tegula*. Sister taxa coexisted in five of six cases where entire clades were sampled. In no case were sister taxa divided by a broad geographical barrier. These findings suggest that although broad geographical barriers (across oceans or thermal barriers) may have spurred new radiations within *Tegula*, most speciation in this genus occurred along single coastlines, perhaps facilitated at times by sympatric ecological differentiation. A brief survey of the distributions of other marine radiations suggests that coastal distributions may favor the sympatry of sister taxa more than island distributions do.

Materials and Methods

Table 1 lists the species analyzed, their approximate geographical ranges, and the localities from which they were collected (Fig. 1). The samples include every known species of temperate *Tegula* (with the exception of the Chilean species *T. luctuosa*) and about one-third of the tropical subgenus *Agathistoma*.

I prepared a DNA template for amplification by grinding a small sample of radular muscle in 200 μl of 20% Chelex suspension on ice, boiling for 8 min, and then centrifuging to pellet the Chelex beads. One to two microliters of the resulting supernatant was used as a template for a 50 μl polymerase chain reaction consisting of 5 μl 10TS *Taq*-Extender Buffer, 0.3 μl 10 mM dNTPs, 0.4 μl *Taq* polymerase, and 0.4 μl *Taq*Extender PCR Additive (Stratagene), along with 4.6 μl each of the appropriate primers (see below) at 5 mM concentrations. Amplification conditions for both mitochondrial regions consisted of 50 sec at 94°C, 90 sec at 50°C, and 90 secs at 72°C for 25 cycles, with a final cycle under identical conditions but with an 8 min extension time at 72°C.

To amplify and sequence a portion of cytochrome *c* oxidase (COI), I used the primers of Folmer et al. (1994): HCO2198 (5′-TAACTTCAGGGTGACCAAAAAATCA-3′) and LCO-1490 (5′-GGTCAACAAATCATAAGATATTGG-3′). I am-

TABLE 1. Collection information and geographic ranges for species examined in this study. Geographic ranges from McLean (1969, 1978), Keen (1971), Kuroda et al. (1971), Marincovich (1973), Abbott and Haderlie (1980), and the author's observations of collections at the Los Angeles County Museum of Natural History.

Species	Geographic range	Collection locality
1. *Norrisia norrisi*	Point Conception, CA, to Isla Asuncion, BCS	Punta Baja, BC
2. *Tegula aureotincta*	Ventura Co., CA, to Bahia Magdalena, BCS	Bahia Asuncion, BCS
3. *T. brunnea*	Cape Arago, OR, to Santa Barbara Is., CA	Pacific Grove, CA
4. *T. montereyi*	Sonoma Co., CA, to Santa Barbara Is., CA	Pacific Grove, CA
5. *T. regina*	Catalina Island, CA, to Isla Asuncion, BCS	San Diego, CA
6. *T. pfeifferi*	Hokkaido, Japan, to southern Japan	Izu Peninsula, Japan
7. *T. rusticus*	Siberia to central China	Uchiura, Japan
8. *T. nigerrima*	Southern Japan to southern China	Hong Kong
9. *T. argyrostoma*	Honshu Island, Japan, to southern China	Hong Kong
10. *T. xanthostigma*	Honshu Island, Japan, to southern China	Uchiura, Japan
11. *T. funebralis*	Vancouver Is., BC, to central Baja California	Punta Santo Tomas, BC
12. *T. gallina*	Point Conception, CA, to southern Baja California	Punta Santo Tomas, BC
13. *T. rugosa*	Northern Sea of Cortez endemic	Bahia de Los Angeles, BC
14. *T. atra*	Southern Peru to southern Chile	Algarrabo, Chile
15. *T. quadricostata*	Chile	Pichidangui Beach, Chile
16. *T. tridentata*	Southern Peru to central Chile	Algarrabo, Chile
17. *T. excavata*	Caribbean Sea	Tierre-de-Haut, Guadeloupe
18. *T. pellisserpentis*	El Salvador to Colombia	Panama City, Panama
19. *T. eiseni*	Los Angeles Co., CA, to Bahia Magdalena	East Anacapa Island, CA
20. *T. fasciata*	U.S. to Brazil	Isla Utila, Honduras
21. *T. verrucosa*	El Salvador to northern Peru	Panama City, Panama
22. *T. corteziana*	Northern Sea of Cortez to Guaymas, SN	Puertocitos, BC
23. *T. mariana*	Sea of Cortez to Panama	Puertocitos, BC
24. *T. felipensis*	Northern Sea of Cortez endemic	San Felipe, BC
25. *T. pulligo*	Sitka, AK, to northern Baja California	Pacific Grove, CA

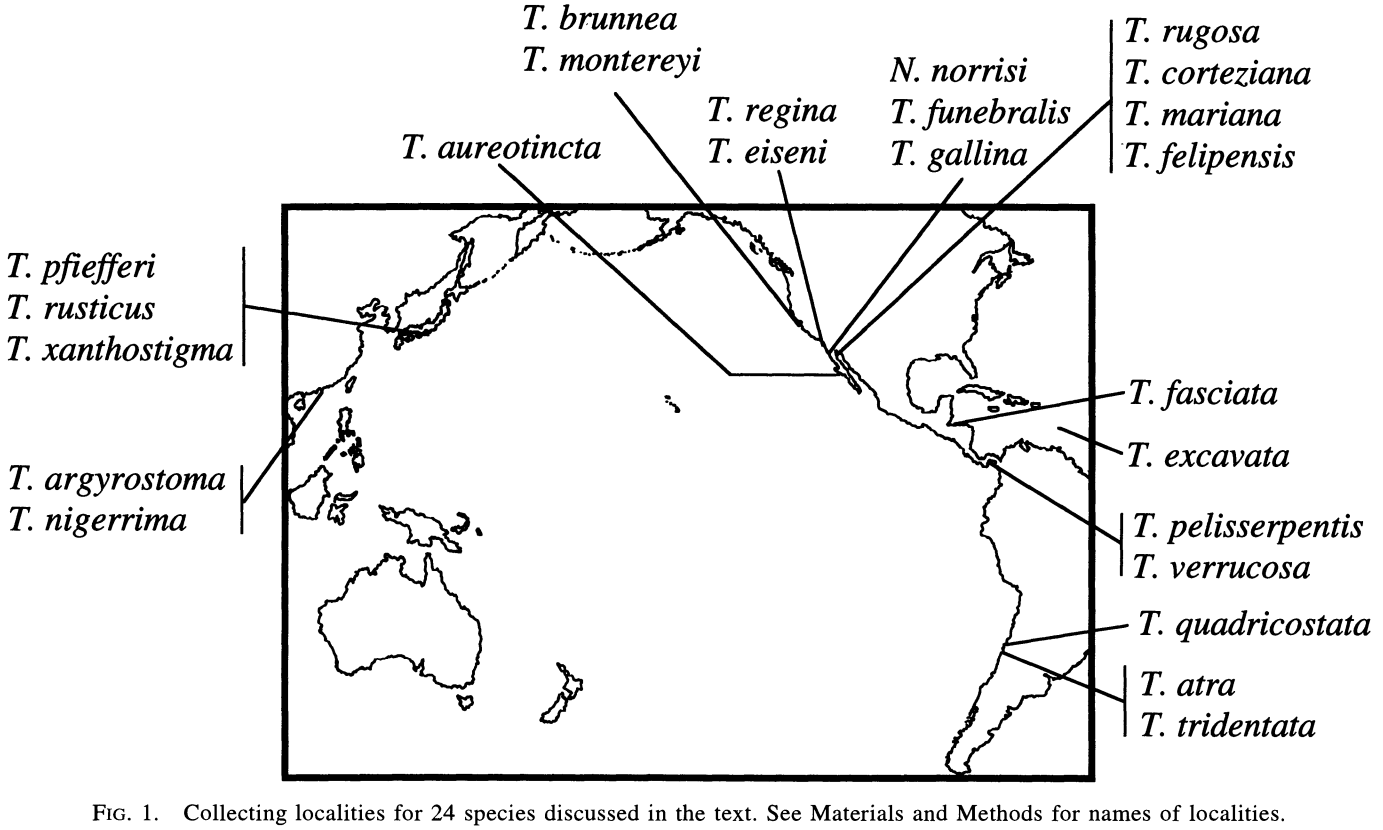

FIG. 1. Collecting localities for 24 species discussed in the text. See Materials and Methods for names of localities.

plified the small (12S) mitochondrial ribosomal subunit using 12SA of Kocher et al. (1989) (5′-AAACTGGGATTAGATACCCCACTAT-3′) and a second primer modified from 12SB in accordance with sequence from the chiton *Katharina tunicata* (Boore and Brown 1994) (12sBK: 5′-GAGAGTGACGGGCGATATGTA-3′). Preliminary screenings revealed that the 12SA/BK primer combination resulted in a product only when *Taq*Extender was used along with the polymerase. 12SA was useful for direct sequencing as well, but 12sBK was not. I designed two primers at the center of the amplified region, one forward (5′-GCTTGTATACCGTCGTCGTC-3′) and one reverse (5′-GGTAATCTGACGACGACGG-3′), which in combination with 12SA allowed me to sequence both strands.

Amplification products that produced a single band of the appropriate size were cleaned using Centricon-100 spin columns (Amicon). Purified products were cycle-sequenced using dye-labeled terminators and Ampli-*Taq*FS polymerase (Applied Biosystems). Dye-labeled products were purified using Centri-ceps columns and sequenced using an ABI 373 Automated DNA Sequencer (Perkin-Elmer Applied Biosystems, Foster City, CA).

Alignments of DNA sequences across species were made by eye. COI aligned unambiguously, as this region had no insertions or deletions (indels). To align the 12S sequences, which had indels, I first constructed models of secondary structure for each species using the folding model template of Hickson et al. (1996). These models assisted in aligning sites that, despite nucleotide differences between them, were probably homologous based on their position and base-pairing within the ribosomal molecule.

Phylogenetic trees based on the two gene regions separately and in combination were constructed using three different methods: maximum parsimony (MP), maximum likelihood (ML), and neighbor joining (NJ). MP analyses were performed using PAUP 3.1.1 (Swofford 1993). To evaluate the robustness of support for critical nodes with regard to the weighting of transversions (Tv) and transitions (Ts), I used Tv:Ts weighting ratios of 1:0 (transversion parsimony), 1:1, 4:1, 8:1, and 12:1 for both gene regions separately as well as for the combined dataset. I also either excluded gaps or set them equal in weight to transitions for the 12S and combined datasets. In all cases, I used heuristic searches with 20 random additions of taxa to find the MP tree or trees.

Pairwise counts of Ts and Tv between all taxa were computed using MEGA. The average Ts:Tv ratios between "close taxa" (arbitrarily defined as those with Kimura two-parameter distances for 12S/COI combined data < 0.05) were taken as the best estimates of the true Ts:Tv mutation bias (Hafner et al. 1994). I used the DNAML option in PHYLIP (Felsenstein 1993), with Tv:Ts weighting set to 12:1, to generate ML trees. I generated NJ trees (Saitou and Nei 1987) using MEGA (Kumar et al. 1991) using the two-parameter distance of Kimura (1980) because preliminary inspection of the data showed that Jukes-Cantor (1969) distances were moderate (> 0.05 for many pairwise comparisons, but always < 0.30) and the Ts:Tv ratio was greater than two.

To assess the statistical reliability of clades, I performed bootstrap analyses (Felsenstein 1985) on the data using MP (1000 replicates, five random additions, Tv:Ts = 12:1). I also determined interior branch confidence probabilities (P_C; Rzhetsky and Nei 1992) for the NJ tree. I used interior branch confidence probabilities because bootstrapping of NJ branches will tend to underestimate support when the number of taxa is large (Sitnikova et al. 1995).

Phylogenetically, the subfamily Tegulinae (which consists solely of the genera *Norrisia* and *Tegula*) has proven enigmatic, lying somewhere between the families Trochidae and Turbinidae (Hickman 1996). I used *N. norrisi*, the sole tegulinid outside of the genus *Tegula*, both as an outgroup for MP trees and for rooting NJ and ML trees. Rooting with *T. aureotincta*, the Recent species likely descended from the earliest *Tegula* reported in the fossil record (Addicott 1970), produced similar topologies.

Results

The combined dataset consisted of 1119 aligned nucleotide positions, 639 for COI and 480 for 12S. 12S sequences from individual species ranged from 445 (for *T. fasciata*) to 466 (for *T. eiseni*). COI varied at 239 sites, only two of which resulted in amino acid substitutions (both of these were in *T. excavata*). Of these sites, 205 were phylogenetically informative. Of the 195 variable sites in the 12S region, 137 were phylogenetically informative. GC content averaged 36.2% for all species and both gene regions combined and varied little across taxa (range = 34.7–38.2%). Pairwise Kimura two parameter distances between all species based on the two gene regions combined ranged from 0.6% (for *T. argyrostoma* and *T. xanthostigma*) to 19.3% (for *T. rugosa* and *T. excavata*; Table 2), except for *T. pulligo* (see below). The complete sequences for each gene region have been deposited in GenBank under accession numbers AF080625–AF080649 (12S) and AF080650–AF080674 (COI).

Inferred secondary structure of the 12S ribosomal RNA subunit (Appendix) fit well on to the template of Hickson et al. (1996). *Norrisia* and all *Tegula* (except *T. pulligo*, see below) possess a large insertion (approximately 50 nucleotides relative to the chiton *Katharina tunicata*) bounded by helix 38/38′. Hickson et al. (1996) noted a similarly large insertion in the bivalve *Mytilus edulis*. These insertions probably exhibit their own helical structure, as inferred pair bonds were easily recognized and created helices up to 14 pairs long (not shown). Inferred structures greatly facilitated alignment between species. Other trochids and turbinids sequenced showed extensive length variation in this region and could not be easily aligned with *Tegula* sequences (Hellberg, unpubl. data).

12S sequences for one of the species sampled, *T. pulligo*, were extremely different from those of other Tegulinae (26.6% to 32.5% nucleotide divergence using Kimura's two-parameter distance). The novel insertion present in all other Tegulinae could not be aligned with *T. pulligo*, which was six to 22 nucleotides shorter than all other *Tegula* in this region. Phylogenetic analyses consistently placed *T. pulligo* closer to either the turbinid *Astrea undosa* or to derived trochids (*Monodonta, Calliostoma* spp.) than to basal trochids including *Margarites* spp. or any other *Tegula* (Hellberg, unpubl. data). COI would not amplify for *T. pulligo* using the HCO2198 and LCO1490 primers. *Tegula pulligo* has previ-

TABLE 2. Kimura two-parameter distances (12S rRNA above diagonal, cytochrome *c* oxidase I below diagonal) for all pairwise comparisons of 23 *Tegula* species and *Norrisia norrisi*. Taxa numbered as in Table 1.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1		0.1086	0.1079	0.1211	0.1135	0.1078	0.1102	0.1049	0.1188	0.1107	0.1242	0.1683	0.1461	0.1261	0.1693	0.1862	0.1972	0.1650	0.1942	0.1635	0.1571	0.1631	0.1953	0.2048
2	0.1217		0.1204	0.1230	0.1097	0.1093	0.1090	0.0983	0.0957	0.0983	0.1217	0.1546	0.1163	0.1286	0.1686	0.1677	0.1892	0.1807	0.2016	0.1726	0.1814	0.1698	0.2019	0.2051
3	0.1121	0.1160		0.0292	0.0505	0.0725	0.0748	0.0799	0.0801	0.0725	0.1299	0.1458	0.1270	0.1360	0.1478	0.1759	0.1854	0.1623	0.1916	0.1659	0.1807	0.1633	0.1949	0.2044
4	0.1318	0.1258	0.0491		0.0480	0.0624	0.0697	0.0747	0.0825	0.0749	0.1381	0.1513	0.1352	0.1386	0.1424	0.1640	0.1736	0.1596	0.1913	0.1627	0.1865	0.1809	0.1983	0.2079
5	0.1238	0.1278	0.0594	0.0559		0.0651	0.0625	0.0700	0.0627	0.0602	0.1252	0.1353	0.1195	0.1230	0.1424	0.1702	0.1854	0.1564	0.1768	0.1631	0.1777	0.1663	0.1887	0.1980
6	0.1278	0.1452	0.1269	0.1174	0.1233		0.0201	0.0247	0.0583	0.0583	0.1142	0.1382	0.1114	0.1303	0.1559	0.1729	0.1738	0.1514	0.1793	0.1635	0.1663	0.1550	0.1793	0.1915
7	0.1163	0.1274	0.1174	0.1178	0.1100	0.0543		0.0133	0.0582	0.0607	0.1112	0.1379	0.1111	0.1273	0.1556	0.1814	0.1731	0.1625	0.1876	0.1629	0.1745	0.1631	0.1816	0.1938
8	0.1105	0.1254	0.1194	0.1197	0.1119	0.0491	0.0079		0.0533	0.0557	0.1032	0.1295	0.1084	0.1219	0.1527	0.1725	0.1672	0.1539	0.1876	0.1569	0.1774	0.1602	0.1816	0.1938
9	0.1262	0.1523	0.1316	0.1296	0.1300	0.1123	0.1087	0.1087		0.0111	0.1246	0.1347	0.1245	0.1411	0.1671	0.1784	0.1849	0.1742	0.1908	0.1601	0.1837	0.1782	0.1910	0.2035
10	0.1262	0.1481	0.1316	0.1296	0.1300	0.1123	0.1087	0.1087	0.0031		0.1274	0.1347	0.1300	0.1329	0.1642	0.1754	0.1849	0.1654	0.1848	0.1601	0.1717	0.1722	0.1910	0.2035
11	0.1391	0.1530	0.1289	0.1454	0.1429	0.1569	0.1389	0.1450	0.1389	0.1389		0.0896	0.0850	0.1469	0.1569	0.1651	0.1934	0.1625	0.1914	0.1715	0.1596	0.1542	0.1760	0.1851
12	0.1251	0.1464	0.1409	0.1413	0.1389	0.1227	0.1149	0.1092	0.1325	0.1365	0.1071		0.0954	0.1748	0.1764	0.1819	0.2301	0.1853	0.1908	0.1927	0.1975	0.1858	0.2186	0.2284
13	0.1593	0.1846	0.1574	0.1474	0.1532	0.1422	0.1385	0.1325	0.1369	0.1369	0.1328	0.1071		0.1382	0.1476	0.1644	0.2021	0.1707	0.1823	0.1687	0.1796	0.1624	0.1845	0.1936
14	0.1272	0.1325	0.1407	0.1310	0.1231	0.1350	0.1354	0.1374	0.1391	0.1391	0.1294	0.1271	0.1452		0.1366	0.1583	0.1867	0.1725	0.1880	0.1678	0.1948	0.1624	0.1957	0.2051
15	0.1643	0.1871	0.1522	0.1689	0.1522	0.1422	0.1462	0.1422	0.1603	0.1603	0.1686	0.1662	0.1686	0.1349		0.0937	0.1414	0.1229	0.1259	0.1356	0.1400	0.1320	0.1524	0.1611
16	0.1851	0.1977	0.1957	0.1875	0.1726	0.1875	0.1768	0.1747	0.1625	0.1625	0.1940	0.1957	0.1747	0.1774	0.1254		0.1484	0.1327	0.1640	0.1462	0.1786	0.1586	0.1914	0.2006
17	0.2187	0.2006	0.2070	0.2098	0.1964	0.2116	0.1986	0.1964	0.1942	0.1942	0.2053	0.1982	0.2031	0.1845	0.1682	0.1584		0.1336	0.1571	0.1513	0.1702	0.1702	0.1956	0.2052
18	0.1610	0.1740	0.1574	0.1661	0.1553	0.1708	0.1691	0.1629	0.1695	0.1653	0.1838	0.1794	0.1544	0.1477	0.1420	0.1708	0.1787		0.1455	0.1329	0.1314	0.1203	0.1605	0.1693
19	0.1670	0.1819	0.1648	0.1672	0.1552	0.1668	0.1714	0.1672	0.1689	0.1731	0.1589	0.1731	0.1668	0.1574	0.1544	0.1580	0.1772	0.1460		0.1377	0.1483	0.1599	0.1583	0.1700
20	0.1780	0.1475	0.1652	0.1896	0.1565	0.1640	0.1665	0.1622	0.1846	0.1800	0.1713	0.1709	0.1981	0.1652	0.1511	0.1947	0.1739	0.1771	0.1548		0.1157	0.1216	0.1471	0.1500
21	0.1678	0.1733	0.1860	0.1799	0.1613	0.1627	0.1672	0.1610	0.1634	0.1634	0.1799	0.1586	0.1586	0.1513	0.1412	0.1617	0.1746	0.1440	0.1574	0.1708		0.0815	0.1073	0.1100
22	0.1821	0.1930	0.1910	0.1802	0.1578	0.1819	0.1802	0.1716	0.1823	0.1866	0.1828	0.1845	0.1653	0.1685	0.1522	0.1640	0.1770	0.1561	0.1465	0.1578	0.1572		0.1155	0.1237
23	0.1853	0.1681	0.1791	0.1766	0.1728	0.1700	0.1682	0.1682	0.1620	0.1580	0.1668	0.1808	0.1808	0.1625	0.1492	0.1693	0.1862	0.1697	0.1516	0.1579	0.1475	0.1704		0.0154
24	0.1832	0.1660	0.1812	0.1787	0.1749	0.1721	0.1703	0.1703	0.1641	0.1600	0.1689	0.1787	0.1787	0.1605	0.1533	0.1735	0.1862	0.1697	0.1516	0.1579	0.1475	0.1747	0.0031	

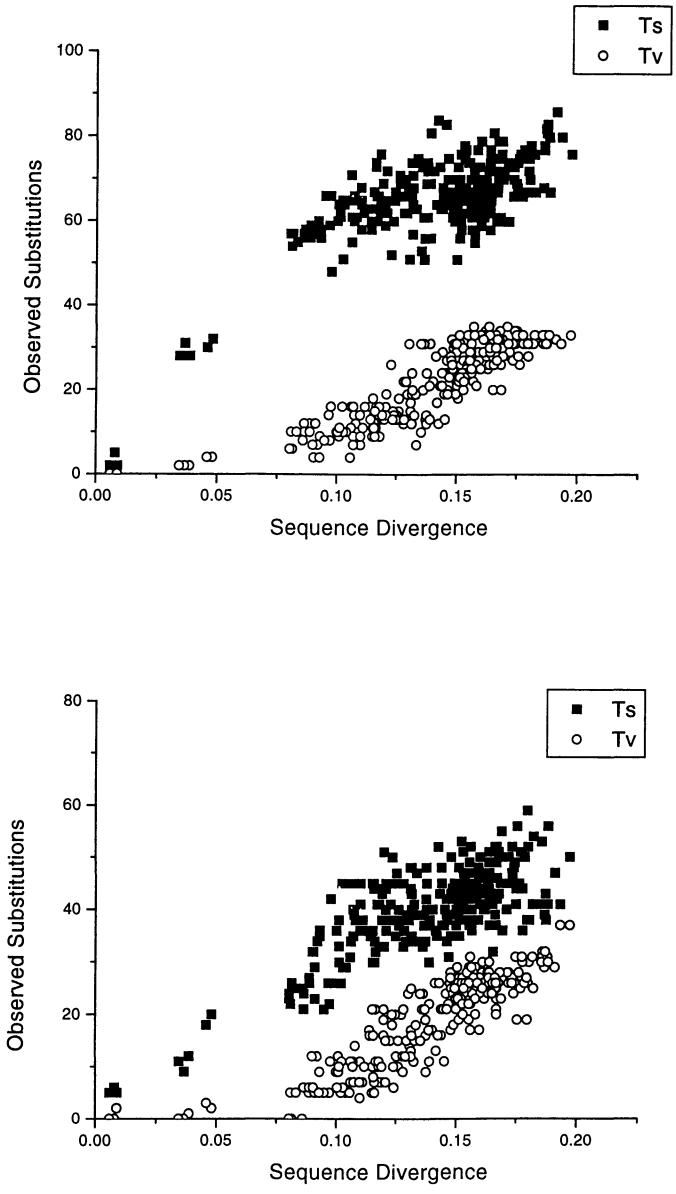

FIG. 2. Observed number of transitions (Ts) and transversions (Tv) within the COI segment (a) and the 12S segment (b) plotted against the percentage of nucleotide differences for combined COI + 12S datasets (corrected for multiple substitutions using Kimura's [1980] two-parameter method).

ously been placed in its own subgenus (*Promartynia*) within the genus *Tegula*, but these results suggest it is not closely related to the other Tegulinae and it was excluded from further analyses.

Observed ratios of Ts to Tv between all taxa ranged from a low of 1.73:1 (for *T. xanthostigma* vs. *T. mariana*) to a high of 5:0 (for *T. rusticus* vs. *T. nigerrima*) for the COI region and from a low of 1.11:1 (for *T. rugosa* vs. *T. excavata*) to a high of 25:0 (for *T. pfeifferi* vs. both *T. xanthostigma* and *T. argyrostoma*) for the 12S region. Plots of observed transversions against overall sequence divergence (Fig. 2) indicated a relatively linear relationship within both gene regions. The slope of observed COI transitions versus overall diver-

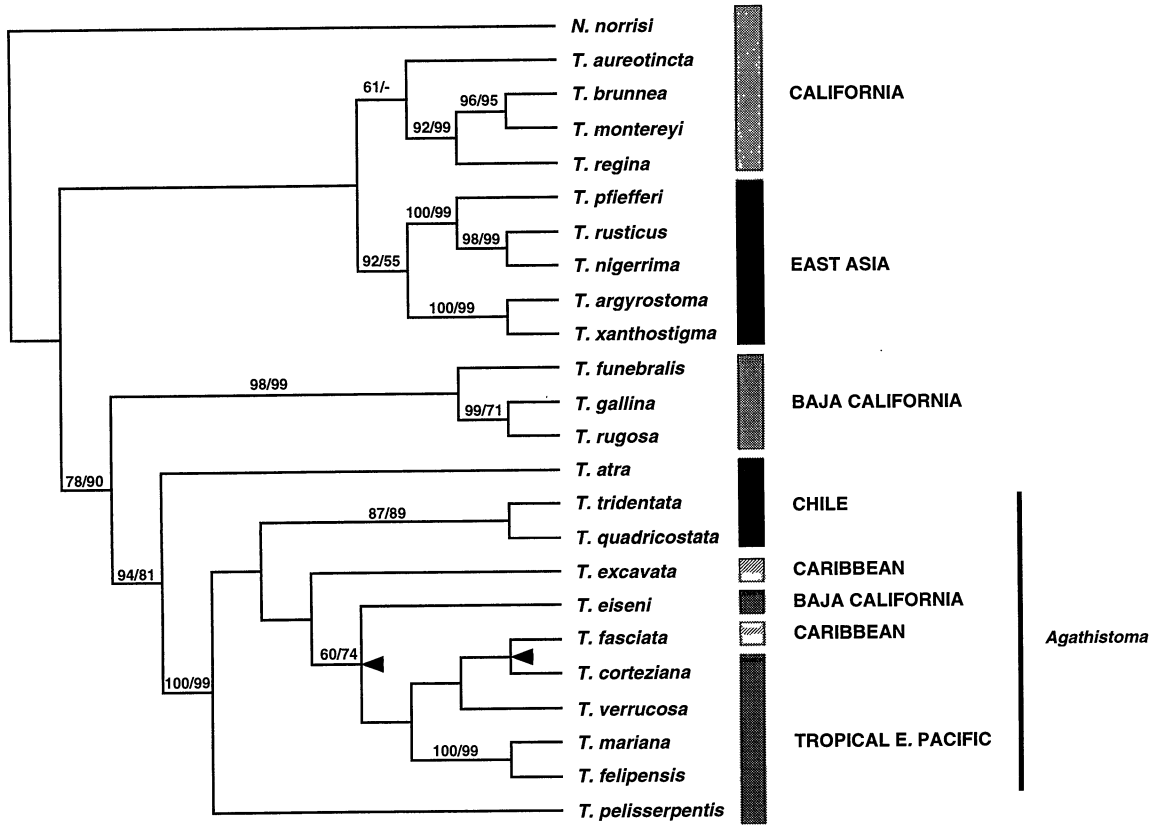

FIG. 3. The most-parsimonious (MP) phylogenetic tree for relationships of 23 species of *Tegula* based upon cytochrome *c* oxidase I (639 bp) and 12S (480 aligned positions) using a weighting scheme of 12:1 transversions to transitions. Two inferred exchanges between the eastern Pacific and Caribbean indicated with arrowheads. Bootstrap values for this MP tree are listed above the branches, followed by interior branch test values for a neighbor joining (NJ) tree using Kimura's two-parameter distances. In contrast to the tree shown above, the NJ tree supports *T. aureotincta* as the sister to all other *Tegula* shown (76%) and supports the monophyly of the California (less *T. aureotincta*) + East Asia clade (94%). All branches with MP bootstrap support greater than 50% are also supported by maximum-likelihood analysis ($P < 0.01$).

gence decreases markedly between 5% and 7.5% overall divergence, but still has not fully saturated even between the most distantly related taxa. The average Ts:Tv ratios between "close taxa" (Kimura two-parameter distances for combined COI/12S data < 0.05) were similar for the two gene regions (11.8:1 for COI, 7.6:1 for 12S), so the average Ts:Tv value for the combined datasets (0 = 11.97) was taken as the best estimate of the true Ts:Tv mutation bias. Two close species pairs (*T. rusticus* and *T. nigerrima, T. argyrostoma* and *T. xanthostigma*) were excluded because no transversions were observed between them for either gene region (observed Ts:Tv = 11:0 and 7:0, respectively).

Phylogenetic analysis of the combined COI and 12S data using a 12:1 Tv:Ts weighting scheme yields a single MP tree (Fig. 3). Bootstrap analysis of this tree shows strong support (\$ 93%) for the monophyly of three temperate three-species radiations: an East Asian radiation (*T. pfeifferi, T. rusticus*, and *T. nigerrima*), a subtidal Californian radiation (*T. regina, T. montereyi*, and *T. brunnea*) and a California/Baja California intertidal radiation (*T. funebralis, T. gallina*, and *T. rugosa*). Bootstrap values also indicate strong support (≥ 88%) for the sister group status of five coexisting species pairs: *T. brunnea* and *T. montereyi, T. rusticus*, and *T. nigerrima, T. argyrostoma*, and *T. xanthostigma, T. tridentata*, and *T. quadricostata*, and *T. mariana* and *T. felipensis*. The monophyly of all East Asian species receives strong support, as does the monophyly of a clade consisting of the Chilean species and the tropical *Tegula* (*Agathistoma* plus *T.* (*s. s.*) *pelisserpentis*). Relationships among four major lineages (*T. aureotincta*, the five East Asian species, the three subtidal Californian species, and the California/Baja + Chilean + tropical clade) are poorly resolved, as are relationships among the Neotropical *Tegula* (*Agathistoma*).

Trees based solely on the COI data or solely on the 12S data differ primarily in the relationships between major clades; both support the monophyly of crown groups. With transversions weighted 12 times more heavily than transitions (as in Fig. 3), two equally parsimonious COI trees (Fig. 4c) group *T. aureotincta* with the subtidal Californian radiation and indicate the California/Baja intertidal species are the sister to the Chilean/tropical clade. In contrast, the Tv:Ts 12:1 12S tree (Fig. 4b) places *T. aureotincta* as the sister to all other *Tegula* and the California/Baja intertidal clade as the sister to the five East Asian species. Both gene regions supported the monophyly of the East Asian, subtidal Californian, and intertidal California/Baja Californian radiations, as well as the sister species status of the four coexisting pairs mentioned above. The Tv:Ts 12:1 COI tree supports one more

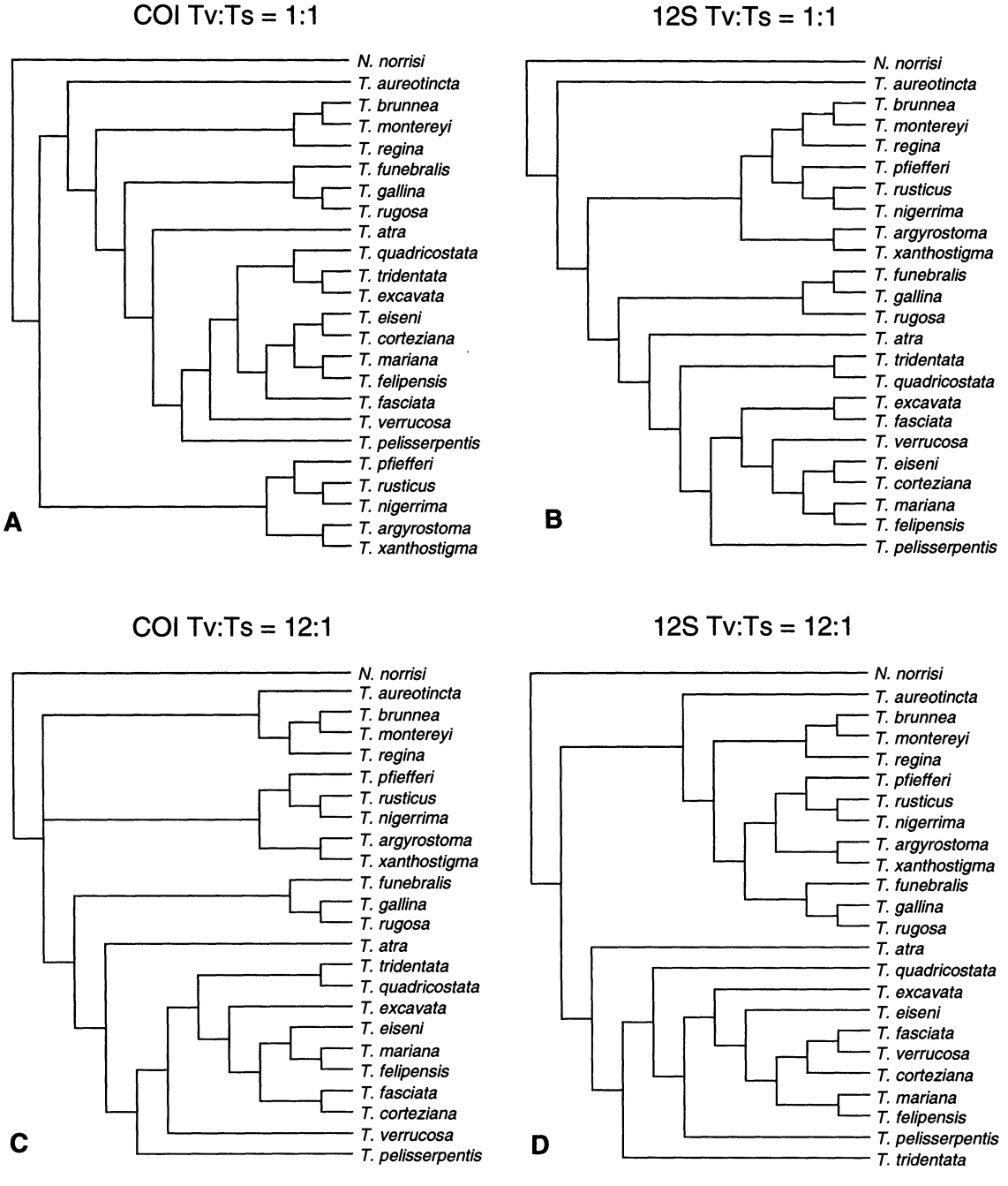

FIG. 4. Maximum-parsimony trees for 23 species of *Tegula* (plus the outgroup *N. norrisi*) based upon 639 bp of cytochrome *c* oxidase I subunit (left) and 480 aligned nucleotide positions of the 12S rRNA subunit (right). Transversions (Tv) were weighted either equally (above) or 12 times more heavily (below) than transitions (Ts). For the 12S data, gaps were weighted equal to transitions in the tree B, but given zero weight in tree D.

coexisting pair of sister species (the Chilean *T. tridentata* and *T. quadricostata*) that the similarly weighted 12S MP tree does not.

Support for the three temperate three-species radiations and four of the five coexisting sister taxa is robust with respect to the Tv:Ts:gap weighting scheme employed. MP analysis yielded a single tree (total length [TL] = 917, retention index [RI] = 0.478) when transitions and transversions were equally weighted for COI (Fig. 4a). Equally weighting Ts, Tv, and gaps for the 12S region also produced a single MP tree (Fig. 4b; TL = 672, RI = 0.650). Again, the trees differ primarily in the relationships of the major clades. Both equally weighted MP trees support the three temperate three-species radiations and four of the five coexisting sister taxa previously mentioned. In addition, the equally weighted 12S tree supports sister group status for two additional pairs of species (*T. funebralis* and *T. gallina*, and *T. tridentata* and *T. quadricostata*) not supported as sister taxa in the equally weighted

COI tree. The three temperate three-species radiations and the four of the five coexisting sister taxa are supported by all MP trees based on the COI region alone, the 12S region alone, and the COI and 12S regions combined for Tv:Ts weightings of 12:1. 8:1, and 4:1, with gaps treated as missing data. Most of these trees supported the sister species status of *T. tridentata* and *T. quadricostata* as well. When only transitions were used, the *T. rusticus/T. nigerrima* sister group was not resolved for either COI or the combined datasets (there are no transversions between them) and the 12S dataset supported only the sister group status of *T. brunnea* and *T. montereyi* and the three-species Californian/Baja intertidal clade.

MP trees based solely on 67 aligned positions in the insertion bounded by 12S helix 38 produced branching topologies that differed from trees based on the entire dataset primarily in relationships among, not within, the major clades. Excluding this region did not alter the results of the total dataset significantly; the single MP tree for the combined COI/12S dataset excluding the helix 38 insertion differs from the MP tree in Figure 3 only in relationships of the tropical *Agathistoma* (results not shown).

Discussion

The marine gastropod genus *Tegula* arose about 15 M.Y.B.P. and presently numbers over 40 species. Species of *Tegula* occur along the cool temperate shores of the northwestern Pacific (off of East Asia), northeastern Pacific (from British Columbia down to Baja California), and southeastern Pacific (off of Chile and southern Peru), as well as tropical waters of the Eastern Pacific and Caribbean. Strong geographical barriers separate all of these regions: vast expanses of uninhabitable Pacific Ocean and frigid Arctic shores isolate Asian and California species, warm tropical waters separate antitropical taxa in California and Chile, and the Isthmus of Panama has divided Eastern Pacific and Caribbean water since the late Pliocene. Further geographical barriers subdivide these five regions; for example, Point Conception marks a major biogeographical breakpoint in southern California. If the evolution of reproductive isolation in *Tegula* requires such major geographical barriers, then sister taxa should occur on different sides of barriers. Phylogenetic relationships between *Tegula* species based on mitochondrial DNA sequences presented here suggest otherwise. Five of six sister taxa supported by the MP tree overlap broadly in their geographical distributions (Fig. 3). Three well-supported, monophyletic clades in the northern Pacific are restricted to single coastlines. These results suggest that, although strong geographical barriers may play a vital role in initiating adaptive radiations, speciation in *Tegula* generally occurs along single coastlines.

Genetic Support for Sympatric Sister Taxa

Both the COI and 12S rRNA gene regions sequenced here supported the monophyly of the three coastal North Pacific radiations. Support for the three North Pacific clades was strong and consistent for both gene regions and for a variety of weighting schemes (Fig. 4), as well as for instances where a large, difficult-to-align insertion within the 12S region was deleted from the dataset. The monophyly of these North Pacific coastal clades was strongly supported both by bootstrap values and by interior branch confidence probabilities. Parsimony bootstrap values greater than 70% generally correspond to 95% confidence that clade is real (Hillis and Bull 1993), provided that internodal change is $< 20\%$ (as is true here) and that rates of nucleotide change do not vary greatly among clades. Here, bootstrap values for the three coastal North Pacific clades exceeded 92%, and the monophyly of all five East Asian species was equally well supported (92%). The interior branch-confidence probability for all three North Pacific clades was equal to 99% (Fig. 3).

Furthermore, support for coastal radiations was strongest where inference should be strongest, namely, in those parts of the tree where all species have been sampled (Lecointre et al. 1993). I was able to obtain samples of all temperate *Tegula* from the North Pacific, and support for coastal radiations was strongest among these taxa. Among tropical taxa, where I obtained samples from only about one-third of extant species, clear examples of coastal radiations were not evident.

Observed Ts:Tv ratios also supported the recent ancestry of coastal radiations in the North Pacific. Observed Ts:Tv should approach unity as the time since lineages split increases, due to the combined effects of transversion accumulations and back mutations of transitions. Among the North Pacific radiations, observed Ts:Tv for the combined COI and 12S data was high ($0 = 12.4$), especially for the East Asian radiation ($0 = 14.3$), which apparently arose quite recently. In contrast, the average observed Ts:Tv for pairwise comparisons of species found on different coasts was generally much lower ($0 = 2.8$).

The MP tree in Figure 3 included two instances of indicated sister taxa with allopatric geographic distributions. However, closer inspection of these two suggests neither lends strong support to speciation mediated by strong geographic barriers. *Tegula fasciata* and *T. corteziana* are unlikely to be true sister taxa because (1) support for the two as sisters is highly inconsistent (varies with weighting and gene region); (2) bootstrap support is weak ($< 50\%$); and (3) at least some of the many unsampled *Agathistoma* are probably closer to one species or the other than either is to the other. The sister pair of *T. gallina*, which inhabits the coast between southern California and the outer coast of southern Baja California, and *T. rugosa*, which is endemic to the Sea of Cortez, was well supported by bootstrap values in the combined 12:1 weighted dataset (Fig. 3), although not by the equally weighted 12S tree (Fig. 4b). These are the only sister species that do not presently share a region of geographical overlap, although they likely coexisted as recently as 1 M.Y.B.P., when a seaway divided the Baja Peninsula (Upton and Murphy 1997). Notably, they were also the most genetically distant of all the sister pairs in the tree (Table 2). This is precisely the opposite of the pattern expected if some strong geographical barriers (in this case, the warm waters around the tip of the Baja California peninsula) initiates speciation. Under the allopatric speciation scenario outlined by Mayr (1954), coexisting marine sister taxa should be *more* genetically distinct than allopatric ones. For *Tegula*, in the single instance allowing such a comparison, the observed pattern was exactly opposite that

expected for allopatric speciation mediated by strong geographic barriers.

By the same logic, the sister taxa that are genetically closest should offer the greatest insight into the geographical circumstances of speciation. *Tegula rusticus* and *T. nigerrima* presently share a region of sympatry that includes the opening to the Japan Sea in southwestern Japan. Past changes in sea level could have closed the Tsushima Strait, transiently isolating one of these two in the Sea of Japan. However, an isolated Sea of Japan would likely have been far colder and more anoxic than the open Sea of Japan of today, due to a cutoff of warm currents arriving from the south and the accumulation of freshwater from in-flowing rivers, respectively. Such conditions probably excluded many of the marine invertebrates that live there now (Oba 1991). Current patterns could provide an alternative means of isolating populations along a single coastline without imposing a land barrier. The divergent pathways of two northbound warm water currents that split off southern Kyusyu coincide with a major genetic division with *Turbo cornutus*, a marine snail with larval dispersal abilities similar to those of *Tegula* (Kojima et al. 1997).

Agreement with the Fossil Record

The known fossil record of *Tegula* generally agrees with the branching order of the mitochondrial phylogeny presented here. The earliest (mid-Miocene) recognized *Tegula* all appear most similar in shell morphology to the extant *T. aureotincta* (Upper Olcese Sands of Addicott 1970). Phylogenetic analyses presented here placed *T. aureotincta* as either the sister to all other *Tegula* or as sister to the subtidal Californian radiation, both consistent with its early appearance. *Tegula* appears in the fossil record of Japan soon after the first California examples (Hickman and McLean 1990), which is again consistent with the MP tree presented here. The early Pliocene was a time of rapid radiation within *Tegula*. Both *T. funebralis* and *T. gallina* of the Californian/Baja intertidal radiation first appear in the Pliocene. The Pliocene also marks the first appearance of *Tegula* in the Southern Hemisphere (Herm 1969), with larger species (*T. atra* and the as-yet-unsampled *T. luctuosa*) appearing first, followed shortly by smaller ones (such as *T. tridentata*). My phylogenetic analyses suggest these smaller, southeastern Pacific species may be the sister group to the tropical *Agathistoma*.

The oldest species clearly belonging to the tropical subgenus *Agathistoma* appear in the Pliocene Yorktown Formation of Virginia, and arose no more than 4 M.Y.B.P. (Campbell 1993). The absence of *Agathistoma* from earlier deposits that are rich with other trochid fossils (e.g., Brunet 1995) and the restricted geographical range of this subgenus attest to its relatively recent origin, a view consistent with its crown position within the mitochondrial phylogeny presented here. The early radiation of this subgenus was apparently quite explosive: No less than three species pairs of *Agathistoma* were split by the rise of the Isthmus of Panama (Vermeij 1978), which occurred about 3 M.Y.B.P., but began to divide some marine taxa well before that time (Knowlton et al. 1993). The MP tree in Figure 3 suggested at least two lineage splits between the Eastern Pacific and the Caribbean within *Agathistoma*, one of which likely predated the closing of the Isthmus. More species of *Agathistoma*, including geminate species pairs, will have to be sampled to ascertain the extent to which the more than 20 species of this subgenus have evolved along single coastlines.

Among the major radiations within *Tegula*, age rank (order of first appearance in the fossil record) correlates well with clade rank (branching order from the base of the tree; Norell and Novacek 1992). The notable exception to this pattern is the outgroup species, *Norrisia norrisi*. The earliest fossil *N. norrisi* date from the Pleistocene, however, plesiomorphic opercular features support this species' basal position within the Tegulinae (Hickman and McLean 1990).

The fossil record also provides information on how the geographical ranges of *Tegula* species have varied over time. Recurring climatic changes, which began in the Miocene, have shifted the ranges of temperate coastal species toward the equator during glacial episodes and of tropical coastal species poleward during interglacial periods (Rockwell et al. 1989; Ortleib and Diaz 1991; Roy et al. 1996). In *Tegula*, such latitudinal range shifts apparently do not disassociate sympatric sister taxa: *T. brunnea* and *T. montereyi* appear together in 80-K.Y.B.P. deposits in northern Baja California, a few hundred kilometers beyond their present southern endpoint (Rockwell et al. 1989).

Interglacial periods could conceivably result in longitudinal range shifts in *Tegula*, as distributions moved northward and Beringia provided stepping stones to trans-Pacific exchange. Subsequent cooling would push distributions southward, thereby sundering such amphi-Pacific species and creating disjunct sister species on Asian and American shores (Vermeij 1989). Although *Tegula* species have been held up as possible exemplars of such vicariance (Grant and Gale 1931; Vermeij 1989), the monophyly of the three North Pacific radiations supported by my phylogenetic analysis suggests all recent speciation within the genus has occurred along single coastlines. However, a trans-Pacific vicariant event near the Miocene/Pliocene boundary probably set the stage for the independent Asian and American radiations.

In general, present-day geographical distributions may not reveal whether species were sympatric or allopatric when reproductive isolation evolved, although they should be able to falsify sympatric speciation (Lynch 1989). The results of the phylogenetic analysis of *Tegula* presented here thus do not provide strong support for sympatric speciation, but they do strongly suggest that major geographical barriers (such as broad expanses of ocean or land bridges) are not required for speciation and that speciation often proceeds along single coastlines.

Sympatric Sister Taxa in Marine Radiations

Marine species with limited larval dispersal capabilities should exhibit patterns of speciation similar to those of terrestrial species: New species should arise in geographical proximity to their forebears (Palumbi 1992). Phylogenetic relationships among species with nonplanktonic larvae support this prediction (Kwast et al. 1990; Foltz et al. 1996; Marko 1998), although some sister species are divided by major barriers (Reid 1990; Collins et al. 1996). However, the planktonic larvae of many benthic marine animals provide

dispersal capabilities far beyond those available to most terrestrial animals. *Tegula funebralis* has nonfeeding larvae that disperse planktonically for five to 13 days (Moran 1997). Egg sizes in other *Tegula* species suggest such nonfeeding, planktonic larvae exist in other members of the genus as well. The three monophyletic North Pacific coastal radiations reported here suggest such moderate larval dispersal capability does not prevent speciation from existing along single coastlines.

Few published phylogenetic hypotheses bear on the geography of marine speciation; however, those available suggest that the pattern of sympatric sister species reported here for temperate *Tegula* may be common among coastal species distributed in one dimension, regardless of their mode of larval development. Coastal radiations in species with nonfeeding, planktonic larvae have arisen in horseshoe crabs (*Limulus*, Avise et al. 1994), in sponge-dwelling snapping shrimps (*Synalpheus*, Duffy 1996), and at least twice within abalone (*Haliotis*, Lee and Vacquier 1995), as well as in at least three radiations of *Tegula* reported here. More surprisingly, sympatric sister species also appear in the strongylocentrotid urchins (Kessing 1991), whose feeding larvae may spend over 10 weeks in the plankton (Strathmann 1987). Sister taxa from other coastal groups with feeding planktonic larvae (the urchin *Arbacia*, Metz et al. 1998; the sand crab *Emerita,* Tam et al. 1996) are not sympatric, but their geographic distributions generally abut, and are thus consistent with new species evolving along coastlines rather than across broad ocean or land barriers.

Certainly some marine sister species evolved while separated by strong geographical barriers. Such may be the case in the species-rich Indo-Pacific, where many marine species remain allopatric (e.g., butterflyfishes, McMillan and Palumbi 1995), while others presently coexist (*Echinometra* sea urchins, Palumbi et al. 1997) at some locations within their respective ranges. The Indo-West Pacific consists of many scattered islands, and coastlines. These Indo-Pacific examples suggest that the dispersion patterns (one-dimensional coastlines versus two-dimensional island arrays) of species may be as critical to whether sister taxa will coexist, as are the dispersal capabilities of larvae (Valentine and Jablonski 1983). If both insular and coastal species evolve while populations are transiently allopatric, we might expect to see more coastal sister species coexisting. Assuming that newly formed sister species have similar ecological requirements, that geographical ranges shift with climatic change, and that the life span of species is not short relative to the time scale of climatic change, coastal species will inevitably coexist due to postspeciational dispersal constrained by their one-dimensional distributions. In contrast, ecologically similar sister species formed on different islands in a broad, two-dimensional array of many islands could potentially colonize several new islands after speciation without necessarily ever coming into sympatry. The patchwork biogeographical pattern that would result has been observed in some Indo-Pacific radiations (e.g., Palumbi 1996). Strong support for a relationship between spatial distributions and sympatry would require comparisons that do not confound one- versus two-dimensional distributions with temperate versus tropical habitats (e.g., Northeastern Pacific vs. Indo-Pacific).

Alternatively, coexisting sister species may be the products of populations that have differentiated sympatrically through microhabitat specialization (Bush 1969; Rice 1987; Duffy 1996). Coexisting cryptic marine species often segregate by depth (e.g., the coral *Montastraea*, Knowlton et al. 1992; others in Knowlton 1993). Some of the closest coexisting sister species in *Tegula* also assort by depth: In central California, *T. montereyi* lives in slightly deeper water than *T. brunnea* (Reidman et al. 1981), and along the Penghu (Pescadores) Islands in the Strait of Formosa, *T. argyrostoma* ranges into the midintertidal, while *T. xanthostigma* tends to be lower (S.-M. Chao, pers. comm.). Such bathymetric segregation not only separates closely related species, but appears to have promoted sympatric morphological differentiation within some marine species (Johannesson et al. 1993). Such ecological speciation may be facilitated by geographical isolation: Host race speciation in snapping shrimp may be promoted by preferences for different sponge hosts in allopatry (Duffy 1996).

Ecological Differentiation: Sympatric Differentiation or Invasion?

Phylogenetic analysis may be used to infer whether differences between coexisting species evolved before or after first contact (Losos 1992; Richman 1996). Reexamination of two studies on the differential response of coexisting *Tegula* species to predation suggest instances of both sympatric differentiation and invasion. Watanabe (1983) found that three sympatric subtidal species of *Tegula* in central California used different behaviors to avoid predation by asteroids. Such different predator-evasion tactics may favor coexistence in the face of a common predator, which might otherwise lead to apparent competition between the prey species (Holt 1977). 12S sequences suggest one of these three species (*T. pulligo*) is only distantly related to all other *Tegula* (see Results). However, the sympatric sister taxa *T. brunnea* and *T. montereyi* differ markedly in evasive actions: The former flees asteroid predators, whereas the latter clamps down to the substrate and apparently relies on chemical defenses. My phylogenetic analysis suggests these differences may have evolved sympatrically. In the southern California intertidal, Schmitt (1981) found that *T. aureotincta* fled its predators, whereas *T.* (*Agathistoma*) *eiseni* did not, instead taking advantage of morphological defenses provided by its thicker shell. Such predator-resistant shell characteristics prevail among tropical molluscs (Vermeij 1978), and were probably present in the tropical ancestors of *T. eiseni* before it invaded the cooler waters of southern California. In this instance, interspecific differences in predator-evasion tactics most probably predate sympatry, because these two species are not closely related.

Conclusions

Although much work on speciation in the sea has focussed on the role of strong barriers, my results suggest that such barriers are not needed for new marine species to diverge. Species might form along single coastlines in a few different ways, but these alternatives do not seem equally likely. Parapatric speciation (Valentine and Jablonski 1983) seems un-

likely because climatic fluctuations (which have been rife since the Miocene) disrupt accumulation of genetic differences through isolation-by-distance (Hellberg 1994, 1995). Results presented here cannot exclude the possibility of sympatric speciation, and such in situ divergence may underlie the radiations of some marine groups with limited dispersal capabilities and strong habitat fidelity (Duffy 1996). However, such sympatric divergence in *Tegula* seems unlikely given their planktonic development period and generalist diet of microalgae.

Transient allopatry seems the most likely scenario for speciation in *Tegula*. The climatic shifts characteristic of the last several million years should repeatedly create temporally isolated populations in the wake of latitudinally shifting geographic distributions (Valentine and Jablonski 1983; Lindberg 1991). Shifting current patterns could also transiently isolate populations (Kojima et al. 1997). Such transiently isolated populations would have little time to accumulate the genetic differences that would confer reproductive isolation upon subsequent contact of incipient species. Strong diversifying selection on gamete recognition proteins may provide the necessary means of accelerating differentiation during brief periods of isolation (Palumbi 1992). Indeed, the sperm-borne protein lysin shows extensive divergence among *Tegula* species (Hellberg and Vacquier, unpub. data), as do sperm-borne proteins from other free-spawning marine invertebrates (Lee et al., 1995; Swanson and Vacquier 1995; Metz and Palumbi 1996).

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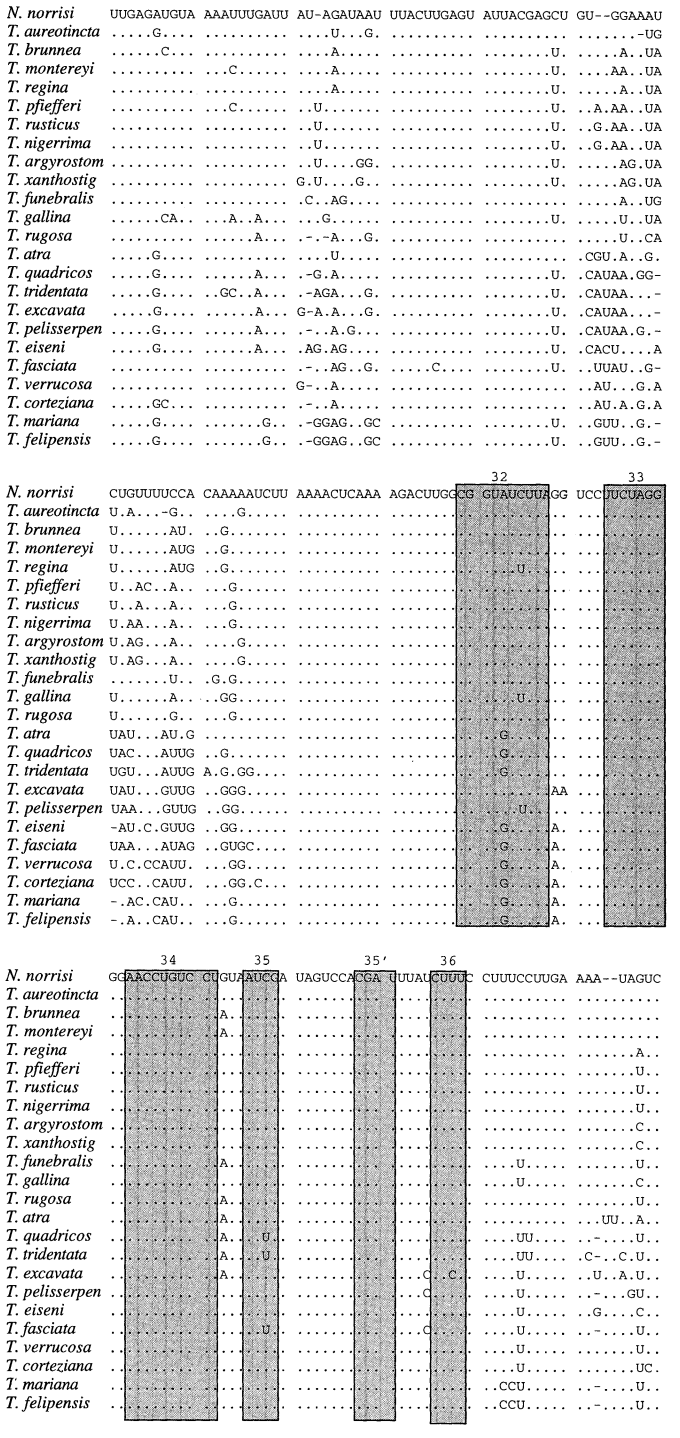
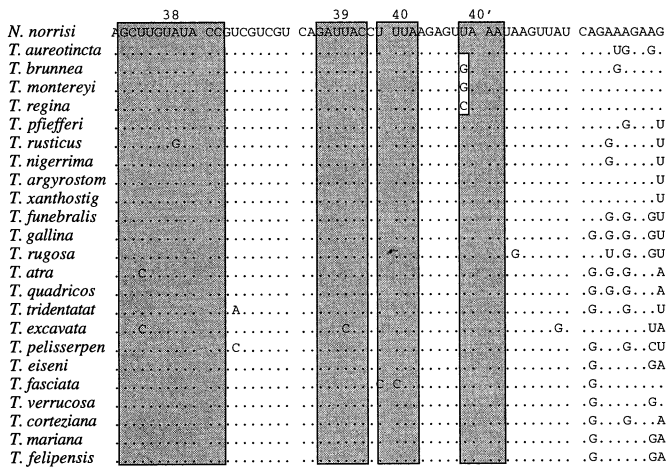
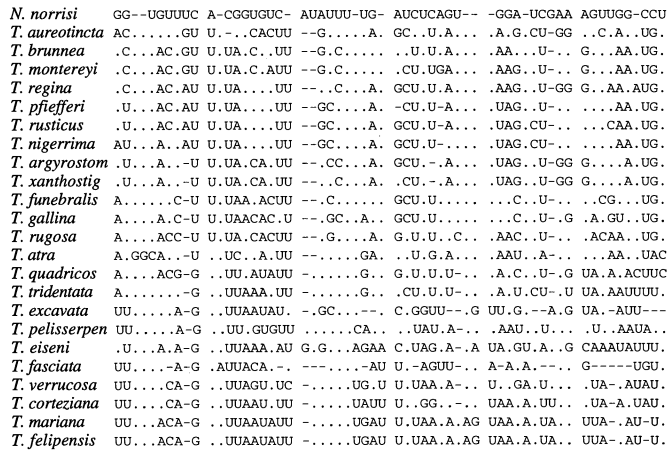
APPENDIX

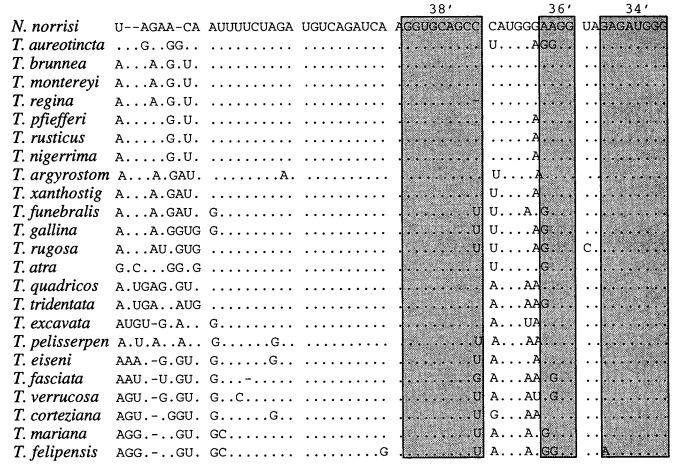
12S rRNA alignment and inferred secondary structure. Shaded regions indicate helices based on the folding template of Hickson et al. (1996).



APPENDIX. Continued.







APPENDIX. Continued.