



Species-level phylogeography and evolutionary history of the hyperdiverse marine gastropod genus *Conus*

Thomas F. Duda Jr.^{a,b,*}, Alan J. Kohn^b

^a *Naos Marine Laboratory, Smithsonian Tropical Research Institute, Apartado 2072, Balboa, Ancon, Panama*

^b *Department of Biology, University of Washington, Seattle, WA 98195, USA*

Received 21 April 2004; revised 27 September 2004

Available online 19 November 2004

Abstract

Phylogenetic and paleontological analyses are combined to reveal patterns of species origination and divergence and to define the significance of potential and actual barriers to dispersal in *Conus*, a species-rich genus of predatory gastropods distributed throughout the world's tropical oceans. Species-level phylogenetic hypotheses are based on nucleotide sequences from the nuclear calmodulin and mitochondrial 16S rRNA genes of 138 *Conus* species from the Indo-Pacific, eastern Pacific, and Atlantic Ocean regions. Results indicate that extant species descend from two major lineages that diverged at least 33 mya. Their geographic distributions suggest that one clade originated in the Indo-Pacific and the other in the eastern Pacific + western Atlantic. Impediments to dispersal between the western Atlantic and Indian Oceans and the central and eastern Pacific Ocean may have promoted this early separation of Indo-Pacific and eastern Pacific + western Atlantic lineages of *Conus*. However, because both clades contain both Indo-Pacific and eastern Pacific + western Atlantic species, migrations must have occurred between these regions; at least four migration events took place between regions at different times. In at least three cases, incursions between regions appear to have crossed the East Pacific Barrier. The paleontological record illustrates that distinct sets of *Conus* species inhabited the Indo-Pacific, eastern Pacific + western Atlantic, and eastern Atlantic + former Tethys Realm in the Tertiary, as is the case today. The ranges of <1% of fossil species ($N = 841$) spanned more than one of these regions throughout the evolutionary history of this group.

© 2004 Elsevier Inc. All rights reserved.

Keywords: Biogeography; Molecular phylogeny; Fossil record; Evolutionary history; *Conus*

And just as the final aim of taxonomic research is not the graduated scale per se but the unraveling of the historical (phylogenetic) relationships between the taxonomic categories and thus the history of the animal kingdom, in the same way the final aim of zoogeography is not the graduated regional system in itself but the history which this system reflects, that is the history of the faunas.

Sven Ekman (1953, p. 372)

1. Introduction

Concordant distributions of large groups of species within the major tropical oceanic regions (Briggs, 1974, 1995; Ekman, 1953) suggest that similar forces influenced the speciation and divergence of tropical marine organisms and thus their high modern biodiversity. Barriers to dispersal such as the final closure of the Tethys Sea, emergence of the Isthmus of Panama, East Pacific and Mid-Atlantic Barriers, and presence of cold waters around polar or temperate margins of continents must have played important vicariant roles in the evolutionary history of tropical marine taxa. Phylogenetic analyses are essential to determine patterns of species

* Corresponding author. Present address: Department of Ecology and Evolutionary Biology, University of Michigan, 1109 Geddes Avenue, Ann Arbor, MI 48103, USA.

E-mail address: tfduda@umich.edu (T.F. Duda).

origination and divergence and the significance of potential and actual barriers to dispersal. Phylogenies of large monophyletic assemblages of taxa that occur throughout all of the world's tropical oceans can most clearly elucidate these patterns.

The gastropod genus *Conus* (Family Conidae) contains over 500 species and is widely distributed throughout all tropical oceans. Roughly 60% of these (Röckel et al., 1995) occur in the Indo-Pacific region (IP), comprising the tropical and subtropical Indian Ocean and the tropical western and central Pacific Ocean (Ekman, 1953). This is by far the largest marine biogeographic province, extending longitudinally halfway around the world and through about 60° of latitude (Briggs, 1974) and comprising one-fourth of the world's ocean area. More than 30 *Conus* species are reported from each of three other main marine provinces: the eastern Pacific (EP), extending from Baja California to Ecuador, the western Atlantic (WA), from Bermuda, southern Florida and the western Gulf of Mexico to near Rio de Janeiro, and the eastern Atlantic (EA), from Cape Verde and the Cape Verde Islands to Angola (Abbott, 1974; Briggs, 1974; Keen, 1971; Pin and Leung Tack, 1995; Röckel et al., 1980; Walls, 1979). About 10 species occur in the South African region, from western Transkei to the western Cape (Kilburn and Rippey, 1982).

Several large-scale historical-geological events are critical to the biogeography and paleobiogeography of tropical marine organisms. A broad, deep, island-free expanse of ocean, the East Pacific Barrier has formidably inhibited dispersal between the islands of the central Pacific and the offshore islands and coast of the Americas since the Mesozoic (Grigg and Hey, 1992; Scotese, 1999). It thus long antedates the earliest known fossil *Conus*, from the Lower Eocene about 55 million years ago (mya) (Kohn, 1990). Very few modern shallow water marine taxa have distributions that span the Indo-Pacific and eastern Pacific (see Lessios et al., 1996), including only three of the more than 300 IP *Conus* species (Keen, 1971). Similarly, the Mid-Atlantic Barrier separates the Atlantic Ocean into western and eastern regions and grew in strength as the Atlantic widened during the Cretaceous and Cenozoic. The tropical Atlantic Ocean is narrower than the East Pacific Barrier, so trans-Atlantic migration is probably more common than across the East Pacific Barrier (Briggs, 1995). Nonetheless, the Atlantic seems to have been as effective a barrier to *Conus* as the East Pacific, as only one of probably >100 Atlantic *Conus* species occur on both its west and east sides.

The Middle Miocene closure of the Tethys seaway between about 17 mya (Harzhauser et al., 2002) and 11 mya (Vrielynck et al., 1997), and the Upper Pliocene emergence of the Isthmus of Panama about 3 mya (Coates and Obando, 1996) imposed more recent obstacles to dispersal. The times of appearance of these

obstructions provide a chronological framework for using phylogenetic analyses and calibration of rates of divergence to examine how they affected spatial and temporal patterns of diversification in *Conus*.

Duda and Palumbi (1999), Duda et al. (2001), and Espiritu et al. (2001) reconstructed phylogenies from mitochondrial and nuclear gene sequences of 75 largely IP species of *Conus*. They tested hypotheses about the evolution of life histories, feeding modes, and radulas, and roughly examined the evolutionary history of *Conus*. However the limited geographic focus of these studies precluded investigating biogeographic processes affecting diversification of the entire genus. Here we present new phylogenetic analyses with sequence data from nearly twice as many taxa, including species from all major provinces (see Appendix), that permit a more thorough, though still preliminary, assessment. To gauge the influence of migration between biogeographic regions and vicariant events or barriers to dispersal on modern distributions of *Conus*, we examined patterns and dates of divergence of lineages determined from molecular clock examination of phylogenetic reconstructions and the fossil record. Phylogenetic and paleontological analyses of this hyperdiverse genus allow insights into the processes that have shaped its evolutionary history and perhaps also those of other marine taxa.

2. Materials and methods

2.1. Specimens

We collected *Conus* specimens in the field with SCUBA, snorkeling or a dredge and preserved tissue in 70–95% alcohol. Some specimens were obtained from colleagues or museums as listed in the acknowledgments. We identified species using Röckel et al. (1995) for Indo-Pacific, Keen (1971) and Walls (1979) for eastern Pacific, Walls (1979) and Abbott (1974) for western Atlantic, and Walls (1979) and the assistance of Emilio Rolán for eastern Atlantic. Identification of most specimens collected or received was assigned with high confidence. However, one specimen collected from the western Atlantic could not be placed with certainty into any of the previously described species and is referred to as CspWA-1. Voucher specimens are currently held at the Smithsonian Tropical Research Institute, but will be permanently deposited at the Museum of Zoology, University of Michigan.

2.2. DNA extraction and sequence determination

We extracted DNA and amplified mitochondrial 16S fragments and a calmodulin locus that contains an intron with methods of Duda and Palumbi (1999) and

Duda et al. (2001). We sequenced mitochondrial fragments directly with an ABI 377 automated sequencer. We cloned nuclear fragments with a t-tailed cloning kit (Invitrogen) and sequenced inserts from two positive colonies from each specimen.

We obtained approximately 450 basepairs (bp) of a fragment of the mitochondrial 16S gene from 40 species and we combined these with published sequences from 98 *Conus* (data from Duda et al., 2001 and Espiritu et al., 2001) and two outgroup species (Appendix). We also obtained a fragment of a nuclear calmodulin locus that contains 52 bp of exon sequence and approximately 230 bp of intron sequence from 30 species and we combined these with published sequences from 70 other *Conus* species (data from Duda and Palumbi, 1999 and Duda et al., 2001) (Appendix). The mitochondrial and nuclear datasets overlap for 97 species for the mitochondrial 16S and calmodulin data that were sequenced. Calmodulin sequences were not obtained for 38 of the *Conus* species and the two outgroups that have described 16S sequences and 16S sequences were not obtained for three of the *Conus* species that have described calmodulin sequences.

2.3. Phylogenetic reconstruction

We aligned nucleotide sequences by eye. Models of nucleotide substitution for the datasets were chosen with Modeltest 3.06 (Posada and Crandall, 1998) and these models were used to construct phylogenies with neighbor-joining and maximum parsimony methods with PAUP* 4.0 (Swofford, 2002). Levels of support for branches were determined by resampling the data (1000 times) using bootstrap methods with PAUP* (Swofford, 2002); branches with bootstrap support less than 50% were collapsed. *Strombus luhuanus* and *Terebra subulata* were used as outgroups in phylogenetic reconstruction with the 16S data. To determine whether trees derived from the 16S and calmodulin datasets are congruent, we used the Kishino and Hasegawa (1989) and Shimodaira and Hasegawa (1999) tests as implemented in PAUP*.

2.4. Molecular clock hypotheses testing and clock calibration

To determine whether a molecular clock could be applied to the phylogenies, we compared log likelihood scores of trees constructed with and without enforced molecular clocks for each dataset and for subsets of these datasets; likelihood scores were calculated with PAUP*. Because analyses of complete datasets gave *P* values less than 0.05, based on the derived phylogenies we divided the datasets into subsets of species. Because sequences of particular species may show different rates of evolution that could result in the significant difference of likelihood scores of clock and non-clock trees, in cases

where *P* values were between 0.001 and 0.05, we excluded single species from these subsets to determine whether exclusion of these species give *P* values greater than 0.05.

Rates of sequence divergence in the trees constructed with enforced molecular clocks were calibrated using the time of separation of species that are closely related and have the oldest fossil records. Because these lineages may have diverged earlier than their times of appearance, the calibration overestimates divergence rates. Rates of sequence divergence were also calibrated with divergence of the geminate pairs separated by the Isthmus of Panama taken as 3 mya. We considered geminate pairs to be those that contain species from the eastern Pacific and western Atlantic that are each other's closest relative according to DNA sequence analyses.

2.5. Paleontological analyses

A database of known paleontological records of *Conus* was examined to determine first appearance dates of modern taxa used for molecular clock calibration and to identify species' distributions that occur in single or span more than one biogeographic region. The database (available from the authors on request) contains over 2700 entries from more than 450 reports. However, the data have not been completely critically evaluated and species identifications and stratigraphy were generally accepted as reported. The numbers of taxa per region per epoch were also determined from this database. We combined records from eastern Africa, Asia, Australia, Indian Ocean, and Oceania to represent the Indo-Pacific region (*IP*), records from the Caribbean and North and South America to represent the eastern Pacific plus western Atlantic region (*EA + WA*), and records from areas in the eastern Atlantic and former Tethys Realm (sensu Harzhauser et al., 2002) to represent the former Tethys Realm plus eastern Atlantic region (*TR + EA*). The designators of these Cenozoic regions are italicized to distinguish them from present-day regions, indicated in Roman type. Kohn (1985) previously reported numbers of species per region per epoch, but the database has been updated considerably since then.

3. Results

3.1. Phylogenetic reconstructions

The phylogenies of *Conus* derived from mitochondrial 16S and nuclear calmodulin intron sequences show three main features: (1) a deep split that groups species into two clades, the larger of which contains about 90% of the species analyzed, (2) many well-resolved subclades within the larger of the two main clades, and (3) strikingly different geographic distributions of their species (Figs. 1 and 2). The calmodulin tree was rooted to the

clade comprising *C. arcuatus*, *C. californicus*, *C. jaspideus*, *C. mahogani*, *C. mindanus*, *C. perplexus*, *C. puncticulatus*, *C. tornatus*, and *C. ximenes*. A calmodulin tree rooted at the midpoint is identical to that presented in Fig. 2. Despite the size disparity between the two main clades, the smaller comprises mainly (16S; Fig. 1) or entirely (calmodulin; Fig. 2) EP and WA species, while 78% of the species in the large clade (both trees) are from the IP. All of these differences are highly significant ($P < 0.001$; G tests for independence).

How do the topologies constructed from each dataset differ? First, branching patterns within the subclades that are shared among phylogenies are not identical; for example *C. chaldaeus* and *C. ebraeus* are sister species in the calmodulin phylogeny (Fig. 2) but are simply members of the same subclade in the 16S phylogeny (Fig. 1). The 16S and calmodulin tree topologies also show different positions for *C. frigidus* (Figs. 1 and 2). Moreover, six subclades in the calmodulin tree are not supported in the 16S tree: (1) *C. araneosus* through *C. pennaceus* (bootstrap support = 68%); (2) *C. barthelemyi* through *C. striolatus* (98%); (3) *C. biliosus* and *C. boeticus* (77%); (4) *C. coffeae*, *C. glans*, and *C. tenuistriatus* (95%); (5) *C. geographus*, *C. obscurus*, and *C. tulipa* (74%); and (6) *C. jaspideus* through *C. ximenes* (55%) (Figs. 1 and 2). Only the subclade that contains *C. emaciatius*, *C. flavidus*, *C. moreleti*, *C. terebra*, and *C. virgo* (92%) is supported in the 16S tree but not in the calmodulin tree (Figs. 1 and 2). Levels of divergence of 16S sequences are on average 1.7 times greater than those of calmodulin intron sequences. These observations suggest that calmodulin sequences better resolve phylogenetic relationships of *Comus* than do 16S data, possibly due to slower rates of evolution of calmodulin sequences in comparison to those of 16S sequences.

Kishino and Hasegawa (1989) and Shimodaira and Hasegawa (1999) tests reveal that the topologies that include species with both 16S and calmodulin sequences ($n = 97$) differ significantly ($P < 0.01$), so the datasets are not congruent. In general, the tree topologies for these 97 species are similar to those presented in Figs. 1 and 2, and the main differences among them are as reported above for the larger datasets.

3.2. Molecular clock application and calibration of rates of evolution

The trees reconstructed with and without enforced molecular clock show significant differences in log likeli-

hood scores except for those built with calmodulin data while excluding *C. varius* and members of the small clade (see Fig. 2: *C. arcuatus* through *C. ximenes* and *C. californicus*) ($P = 0.08$) (Figs. 2 and 3) and while excluding members of the large clade (see Fig. 2) and *C. californicus* ($P = 0.17$) (Fig. 4). Thus a molecular clock was only applied to the trees constructed with calmodulin data for the large clade excluding *C. varius* (Fig. 3) and the small clade excluding *C. californicus* (Fig. 4).

Dates of divergence of lineages in the first set were estimated from the calmodulin data based on the divergence of *C. lividus* and *C. quercinus* at 11 mya (Fig. 3), the only pair of extant species that are close relatives having Miocene fossil records. The oldest known specimens of *C. quercinus* are from the Tjilang Bed of Java (Van der Vlerk, 1931), assigned to foraminiferal zone N15 at the juncture of the Middle and Upper Miocene (Shuto, 1975), about 11 mya (Odin et al., 1997). The oldest record of *C. lividus* is from the Vigo Shale, Philippines (Dickerson, 1921), also probably Middle or Upper Miocene (Shuto, 1975), and aged 5.2–12 mya (Odin et al., 1997). Thus the lineages that gave rise to *C. lividus* and *C. quercinus* separated at least 11 mya. The *lividus quercinus* rate calibration gives times of divergence for the lineages of the large clade from 0.0 to 33.0 mya (Fig. 3). If this calibration is correct, most speciation events occurred during the Miocene, but overall speciation rates peaked during the Upper Miocene and Lower Pliocene (Fig. 3).

The fossil calibration gives times of divergence of the transisthmian species *C. bartschii*/*C. brunneus* and *C. regius*, *C. gladiator* and *C. mus*, and *C. ermineus* and *C. purpurascens* at 14.3, 6.9, and 7.3 mya, respectively (Fig. 3). If the molecular clock tree is instead calibrated with the divergence of the geminate pair that shows the least sequence divergence (*C. gladiator* and *C. mus*) at 3 mya, this gives a maximum divergence of *C. lividus* and *C. quercinus* at 4.8 mya, a date that implies a much more recent splitting of these species than the fossil record suggests. Several transisthmian pairs of marine crustaceans, bivalves, and other gastropods show levels of divergence that reflect separation much earlier than 3 mya (Collins et al., 1996; Knowlton et al., 1993; Knowlton and Weigt, 1998; Marko, 2002). As Knowlton and Weigt (1998) and Marko (2002) caution, the molecular data indicate that divergence in several marine taxa preceded the final closure of barriers like the Isthmus of Panama, so the use of vicariant events in calibrating

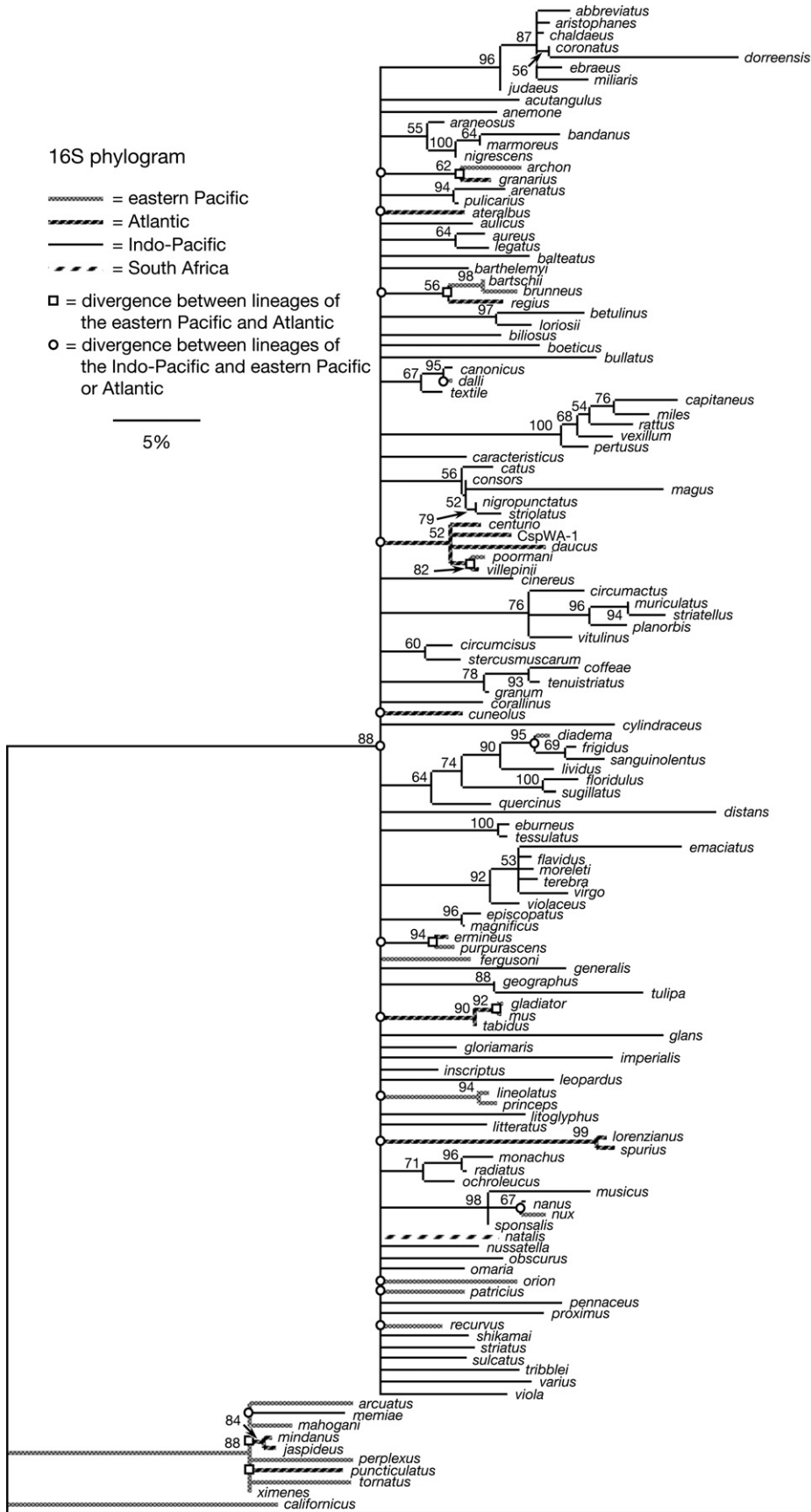
Fig. 1. Phylogeny of 135 *Comus* species reconstructed with neighbor-joining of maximum likelihood distances of a fragment of the mitochondrial 16S gene. Maximum likelihood settings correspond to the general time-reversible model (Lanave et al., 1984 and Rodriguez et al., 1990) (base frequencies: A = 0.393, C = 0.113, G = 0.155, and T = 0.340; substitution rate matrix: A–C = 1.067, A–G = 9.371, A–T = 1.477, C–G = 1.471, C–T = 9.371, and G–T = 1.000) with rates assumed to follow a gamma distribution with shape parameter α of 0.534 and proportion of invariable sites of 0.370. Bootstrap values are indicated on nodes when values are greater than 50%. The tree was rooted to the outgroups *Strombus luhuanus* and *Terebra subulata*. Geographic ranges of lineages (see Appendix) are indicated in the figure. Divergence of lineages from the Indo-Pacific and eastern Pacific + western Atlantic and eastern Pacific and western Atlantic are indicated on branches as described in figure.

16S phylogram

- = eastern Pacific
- = Atlantic
- = Indo-Pacific
- = South Africa

- = divergence between lineages of the eastern Pacific and Atlantic
- = divergence between lineages of the Indo-Pacific and eastern Pacific or Atlantic

5%



Terebra subulata

Strombus luhuanus

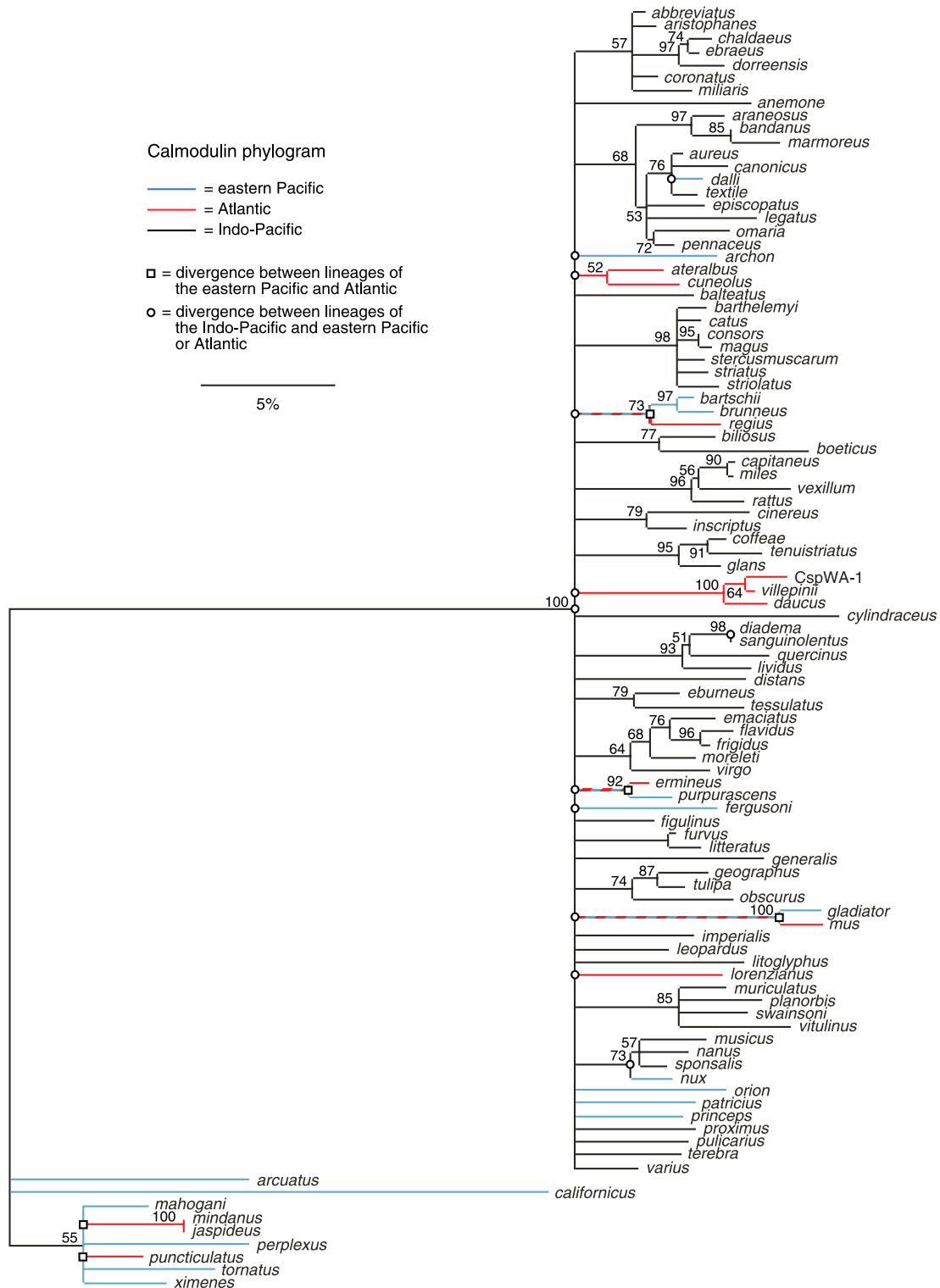


Fig. 2. Phylogeny of 100 *Conus* species reconstructed with neighbor-joining of maximum likelihood distances of exon and intron regions of a nuclear calmodulin locus. Maximum likelihood settings correspond to the Hasegawa et al. (1985) model (base frequencies: A = 0.254, C = 0.202, G = 0.221, and T = 0.323; transition to transversion ratio = 1.446) with shape parameter alpha of 2.942 and proportion of invariable sites of 0.106. Bootstrap values greater than 50% are indicated on nodes when values are greater than 50%. The tree was rooted to the small clade. Geographic ranges of lineages (see Appendix) are indicated in the figure; although unlabeled due to its short branch length, *C. diadema* occurs in the eastern Pacific (see Appendix). Divergence of lineages from the Indo-Pacific and eastern Pacific + western Atlantic and eastern Pacific and western Atlantic are indicated on branches as described in figure.

rates of evolution can overestimate these rates, and taxa probably diverged earlier than estimated. Thus we feel more confident estimating the calibration from the fossil data than from transisthmian divergence.

Only eastern Pacific species in the small clade have records into the Pliocene (Durham, 1950; Powell, 1989). The eastern Pacific *C. perplexus* and western Atlantic *C. puncticulatus* are predicted transisthmian sister species (Vermeij, 1978) that presumably diverged at least 3 mya based on the final closure of the Isthmus of Panama (Coates and Obando, 1996). Application of the transisthmian calibration does not contradict the fossil appearance date of *C. arcuatus* and *C. puncticulatus* in the small clade and gives times of divergence of lineages between 0.0 and 4.5 mya (Fig. 4). Although the Indo-Pacific *C. memiae* was excluded from the clocked tree due to the lack of calmodulin data for this species, it diverged from the other members about 4.5 mya based on its relationship to other members of this clade (Fig. 1) and the transisthmian rate calibration.

3.3. Paleontological analyses

Partitioning of taxa by major biogeographic region shows that *Conus* occurred throughout all of the world's tropical oceans during the Eocene, with greatest number of species reported from former Tethys Realm and eastern Atlantic region (*TR+EA*) (Table 1). During the Oligocene, numbers of *TR+EA* and *IP* *Conus* dropped by about 50%, but *EP+WA* numbers changed little. *Conus* radiated within all major parts of its distribution during the Miocene; numbers of species increased more than threefold in all regions during this epoch. Although fewer species occurred in all regions in the Pliocene and Pleistocene compared to the Miocene, the *TR+EA* decline was most severe (Table 1).

Only 7 of the 841 fossil *Conus* species are reported to occur in more than one major biogeographic region. Four of these, *C. brevis*, *C. ineditus*, *C. lotoisii*, and *C. subbrevis*, range across the *TR+EA* and *IP*. While three occur in deposits as far east as Burma, *C. lotoisii* is reported from Miocene deposits as far east as the Philippines; today *C. lotoisii* occurs in the eastern Indian Ocean and western Pacific (Röckel et al., 1995). Two species, *C. marginatus* and *C. orbigny*, are reported, respectively, from Miocene and Pleistocene deposits from both the *EP* and *IP*. Only one species, *C. catenatus*, is reported from both *WA* and *TR+EA* Miocene deposits, of the 276 Miocene species identified from these two regions. Although our database only includes seven records of *Conus* from western Africa that are likely to represent eastern Atlantic species, five of these that occur in Miocene deposits from Angola and Principe Island, *C. grateloupi*, *C. intermedius*, *C. puschi*, *C. sharpeanus* and *C. tarbellianus*, also occur in Miocene *TR* deposits.

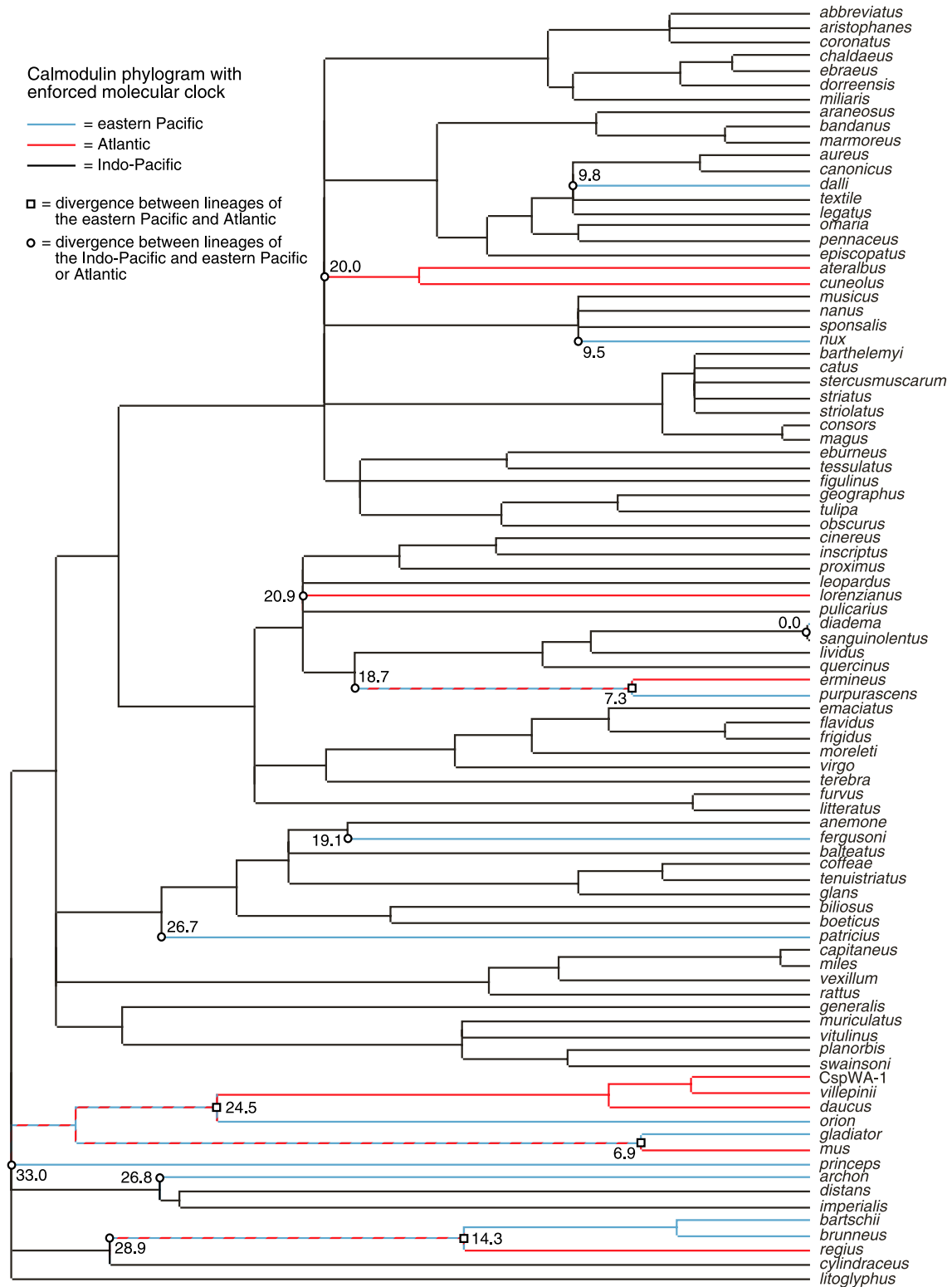
4. Discussion

4.1. Early divergence of ancestral lineages of *Conus*

From its earliest documented occurrences about 55 mya in the Lower Eocene of present-day Europe, *Conus* has evolved into the most species-rich marine animal genus, with well over 500 extant species throughout the world's tropical oceans. Phylogenetic reconstructions of 138 *Conus* species based on nuclear and mitochondrial sequence data reveal two patterns related to this unusually rapid diversification (Kohn, 1990; Stanley, 1979). First, between its origin and at most 33 my later, lineages of *Conus* separated into two divergent clades. Second, these major clades differ markedly in size and in their proportions of taxa from different geographic regions (Figs. 1 and 2). Although the data reported here are limited to about 25% of extant species and these patterns are thus subjected to change with future increases in the database, we predict that they are likely to persist. The smaller major clade contains mostly eastern Pacific (*EP*: $n=6$) and western Atlantic (*WA*: $n=3$) species, but also one species from the Indo-Pacific (*IP*). The larger contains species from all geographic regions surveyed. As outlined in the following section, these patterns suggest that migration of *Conus* between these regions has been severely impeded since at least the Lower Oligocene and possibly as early as the Eocene. Limited dispersal between the western Atlantic and Indian Oceans likely drove the early divergence of lineages that gave rise to the two modern *Conus* clades, but it is unclear whether obstruction of the Tethys seaway or between western and eastern Atlantic regions caused the divergence. To explain the mixed geographic assemblages of species in the two main clades (Figs. 1 and 2), members of several ancestral lineages must have migrated between the *IP* and *EP* or Atlantic.

The composition of the two major clades may suggest the cause of their separation. While most of the smaller clade's species are *EP* and *WA*, most of the larger's are *IP*. Hence, the clades likely derived from lineages in the *EP+WA* and *IP*, respectively, and geographic separation of lineages between these regions promoted divergence. Vermeij (2001) and Vermeij and Rosenberg (1993) suggest that the modern Indo-Pacific shallow water marine biota may have originated from lineages that migrated eastward from the Americas during the early Cenozoic. Our results suggest that *Conus* diversity in the *IP* and *EP+WA* arose in situ with some instances of migration between regions (see below), but we cannot reject the hypothesis that the *IP* clade arose from a lineage that invaded this region from the *EP+WA*.

Geographic separation of *IP* and *EP+WA* lineages requires two barriers to dispersal in the Eocene or Lower Oligocene, one between the central and eastern Pacific and the other between the western Atlantic and Indian

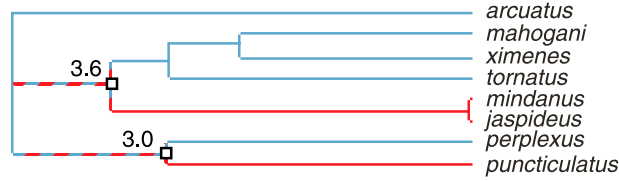


Oligocene		Miocene			Pliocene		Plei	Epoch
Lower	Upper	Lower	Middle	Upper	L.	U.		sub-epoch
9	8	20	15	24	9	1	3	number of speciation events
1.5	1.3	2.9	2.5	4.0	5.0	0.6	1.8	speciation rate
								time (mya)

Calmodulin phylogram with enforced molecular clock

- = eastern Pacific
- = Atlantic
- = Indo-Pacific

□ = divergence between lineages of the eastern Pacific and Atlantic



Pliocene		Pleistocene	Epoch
Lower	Upper		sub-epoch
3	3	1	number of speciation events
1.7	1.7	0.6	speciation rate
4	3	2	time (mya)

Fig. 4. Phylogeny of eight *Conus* species reconstructed with neighbor-joining of maximum likelihood distances of a fragment of a nuclear calmodulin locus that includes both exon and intron sequence. Maximum likelihood settings correspond to the Hasegawa et al. (1985) model (base frequencies: A = 0.296, C = 0.167, G = 0.219, and T = 0.318; transition to transversion ratio = 2.237). The tree was midpoint rooted. Geographic ranges of lineages are indicated as described in the figure (see Appendix). Time scale was calibrated by the divergence of the eastern Pacific *C. perplexus* and western Atlantic *C. puncticulatus* at 3 mya. The numbers of speciation events were determined as the number of nodes within particular time periods; polytomies were considered to have number of nodes equal to the number of branches emanating from it less one. Divergence of lineages from the eastern Pacific and western Atlantic are indicated on branches as described.

Table 1

Number of described species occurring in fossil deposits from the eastern Pacific and western Atlantic (*EP + WA*), the former Tethys Realm and eastern Atlantic (*TR + EA*), and Indo-Pacific (*IP*) during the evolutionary history of *Conus* as determined from an uncritical analysis of the paleontological literature

Epoch	<i>EP + WA</i>	<i>TR + EA</i>	<i>IP</i>
Eocene	28	65	29
Oligocene	30	31	14
Miocene	100	171	131
Pliocene	73	44	90
Pleistocene	57	11	111

Oceans. While the East Pacific Barrier has likely thwarted dispersal between the central and eastern Pacific since the early Cenozoic (Grigg and Hey, 1992), the broad Tethyan connection between the Atlantic and Indian Oceans persisted until the Middle Miocene (Harzhauser et al., 2002; Vrielynck et al., 1997). Moreover, the Benguela cold current and upwelling system around the southwestern tip of Africa, that currently limits migration of tropical marine organisms between the eastern Atlantic and Indian Oceans, did not originate until the Miocene (Diester-Haass and Schrader, 1979; Meyers et al., 1983; Siesser, 1980) and did not intensify until the Upper Pliocene (Meyers et al., 1983; Shannon, 1985). Also, because of the continued widening of the Atlantic during the Cenozoic (Adams, 1981), the Mid-Atlantic Barrier was presumably somewhat weaker in the Eocene and Oligocene than today. Hence, although the East Pacific Barrier likely explains the break between the cen-

tral and eastern Pacific, the location or means of the Atlantic-Indian Ocean barrier is ambiguous.

Analyses of Caribbean fossil reef corals and squids suggest that the Mid-Atlantic Barrier did cause western and eastern Atlantic taxa to diverge. Origination rates of coral taxa in the western Atlantic decreased during the Cenozoic due to declining migration of corals from the Mediterranean, and the western Atlantic coral fauna differentiated from the eastern Atlantic fauna by the Lower Miocene (Budd, 2000). Anderson (2000) attributes the separation of ancestral lineages of *Loligo* and *Sepioteuthis* squids between the EP+WA and the EA+IP to the widening of the Atlantic during the Cretaceous.

On the contrary, histories of Oligocene divergence of other marine taxa across the Tethys Realm suggest that the geographic break that delimited ancestral *Conus* lineages may have occurred between the eastern Atlantic

Fig. 3. Phylogeny of 90 *Conus* species reconstructed with neighbor-joining of maximum likelihood distances of exon and intron regions of a nuclear calmodulin locus. Maximum likelihood settings correspond to the Hasegawa et al. (1985) model (base frequencies: A = 0.246, C = 0.223, G = 0.211, and T = 0.321; transition to transversion ratio = 1.413) with shape parameter α of 1.561. The tree was midpoint rooted. Geographic ranges of lineages are indicated as described in the figure (see Appendix). Time scale was calibrated by the divergence of *C. lividus* and *C. quercinus* at 11 mya. The numbers of speciation events were determined as the number of nodes within particular time periods; polytomies were considered to have number of nodes equal to the number of branches emanating from them less one. Divergence of lineages from the Indo-Pacific and eastern Pacific + western Atlantic and eastern Pacific and western Atlantic are coded on branches as indicated.

and western Indian Oceans and not in the Mid-Atlantic. Eels of the genus *Anguilla* and killifish of the genus *Aphanius* separated into western and eastern Tethyan counterparts between 30 and 45 mya (Hrbek and Meyer, 2003; Tsukamoto and Aoyama, 1998); these dates well precede the final closure of the Tethyan seaway and are strikingly similar to the estimated date of divergence for the two main *Conus* clades between 33 and 55 mya. Furthermore, disparity between the gastropod faunas of the western and eastern reaches of the Tethys Realm increased dramatically between Oligocene and Lower Miocene (Harzhauser et al., 2002). This was sufficient for these authors to propose the origin of two biogeographic provinces within this region during the Oligocene: the Mediterranean-Iranian Province in the western Tethys Realm and the western Indian–eastern African Province in the eastern Tethys Realm. Thus, concordance in dates of divergence across the Tethys Realm among several marine taxa suggests a geographic break situated between the eastern Atlantic and western Indian Oceans rather than at the Mid-Atlantic.

Phylogenetic results and analyses of the *Conus* fossil record suggest that extant EA species are more closely allied to IP than to WA species. First, all three EA species examined belong to the IP not the EP+WA clade (Figs. 1 and 2). However, absence of EA species from the EP+WA clade could be a sampling artifact due to the small number examined from this region, and the presence of EA species in the IP clade may reflect migrations of lineages from the IP to the EA (see below). Second, although potential biases have not been considered, more fossil *Conus* show ranges that span the former Tethys Realm and Indo-Pacific ($n=4$) and Tethys Realm and eastern Atlantic ($n=5$) than have or have had ampho-Atlantic distributions ($n=2$: *C. catenatus* and *C. ermineus*). Thus based on available data, the Mid-Atlantic Barrier appears to have played a role in the separation of IP and EP+WA *Conus* species.

4.2. Migrations between the Indo-Pacific and eastern Pacific or Atlantic

Of the 34 EP and Atlantic species examined, 28 occur in the large, predominantly IP clade (Figs. 1 and 2). Only one IP species, *C. memiae*, clusters with the smaller EP+WA clade (Fig. 1). Vicariance of IP and EP+WA lineages likely caused the two main clades to diverge, and the geographic distributions of modern species reflect the outcome of migrations among regions.

Within the EP+WA and IP clades, 17 nodes in the 16S and 12 in the calmodulin trees join lineages from the IP and either the EP or WA (Figs. 1 and 2). If polytomies are conservatively assumed to represent single divergence events, then the 16S and calmodulin trees show only five and four such nodes, respectively. Molecular clock-based dates of divergence of IP and EP or WA lin-

eages estimate the times of migration between these regions from 0.0 (*C. diadema* and *C. sanguinolentus*), possibly 4.5 (*C. memiae* and the other members of EP+WA clade; estimated from the maximum divergence of members of this clade), 9.5 (*C. nux* and other members of its subclade) and 9.8 mya (*C. dalli* and other members of its subclade) (Figs. 3 and 4). These times are all well after final closure of the Tethys seaway 17–11 mya, so we attribute these divergences to migrations and not vicariant events. Divergence events that are part of polytomies in the 16S and calmodulin phylogenies (Figs. 1 and 2) show separation of lineages between about 19 and 33 mya (Fig. 3) and so may represent either migrations or divergence due to vicariance.

Grigg and Hey (1992) found that corals invaded the EP from the IP more frequently than the opposite way. The occurrence of just one IP *Conus* species in the EP+WA clade and many EP, WA, and EA species in the IP clade suggests that migration between the IP and EP has been more common from west to east than the reverse. However, the proportion of EP and Atlantic species in the IP clade relative to the total number of species in this clade (28/125) is statistically identical to that of Indo-Pacific species in the EP+WA clade (1/10) (χ^2 test; $P>0.5$). Thus, although more *Conus* species migrated across the East Pacific Barrier from the west, this pattern may be a sampling artifact or biased due to the larger number of potential candidates for migration across this barrier in the IP than in the EP+WA clade.

Modern distributions of the three *Conus* species common to both the Indo-Pacific and eastern Pacific, *C. chaldaeus*, *C. ebraeus*, and *C. tessulatus*, and biogeographic patterns of other marine taxa, e.g., corals (Grigg and Hey, 1992), cowries (Meyer, 2003), and the sea urchin genera *Diadema*, *Echinothrix*, and *Tripneustes* (Lessios et al., 1996, 1998, 2003) demonstrate that the East Pacific Barrier is penetrable. Three other *Conus* species probably crossed the East Pacific Barrier to invade the EP. First, the EP *C. diadema* and the IP *C. sanguinolentus* (Figs. 1 and 2) share identical calmodulin intron sequences but are more than 2.5% divergent at mitochondrial 16S. The former result suggests that these species diverged in the Pleistocene (Fig. 3). *C. diadema* lacks a fossil record, so its EP appearance cannot be dated paleontologically. The alternative explanation, that the ancestor of *C. diadema* migrated around the southern tip of Africa and South America and either went extinct in the Atlantic or did not occur or was never detected there, seems highly unlikely.

Two other EP species, *Conus nux* and *C. dalli*, also have closest affinities to IP species, no likely WA sister species (Figs. 1 and 2), and no fossil record. This strongly suggests that the ancestors of these species also traversed the East Pacific Barrier, probably about 9–10 mya (Fig. 3). This is long before final closure of the connection between the EP and WA. The absence of these lineages

in the WA thus implies that either the dates of divergence are overestimated, these species went extinct in the WA, or an oceanic circulation barrier (Duque-Caro, 1990) or emerging Isthmus of Panama was able to thwart migration of *Conus* from the EP to WA in some cases as early as 10 mya. The latter explanation is consistent with the pre-isthmus divergence times of EP and WA species reported in *Conus* here and in other taxa by several workers (Collins et al., 1996; Knowlton and Weigt, 1998; Knowlton et al., 1993; Marko, 2002).

How the remaining EP and WA species with IP affinities spread is unclear, due to the incompleteness of the phylogenetic reconstructions or because they belong to subclades with both EP and WA representatives. *Conus* occurs around southern South Africa, and other tropical marine taxa have migrated via this route, e.g., the sea urchin genus *Diadema* (Lessios et al., 2001). Alternatively, IP-Atlantic migration prior to the Mid-Miocene Tethys closure may have split species with ranges that spanned the eastern Atlantic, Tethys Realm, and Indian Ocean. These scenarios also require east-to-west breaching of the Mid-Atlantic Barrier into the EP + WA.

Several subclades of the predominantly larger IP clade contain EP and WA representatives, at least some of which are probably sibling species pairs: *C. bartschii*, *C. brunneus*, and *C. regius*; *C. gladiator* and *C. mus*; *C. ermineus* and *C. purpurascens*; and *C. centurio*, *C. daucus*, *C. poormani*, *C. villepini*, and CspWA-1 (Figs. 1 and 2). This also indicates migration between the IP and EP or WA prior to emergence of the Isthmus of Panama. Phylogenetic reconstruction of calmodulin data with an enforced molecular clock indicates that these groups diversified much earlier than 3 mya. Furthermore, the oldest record of an extant EP or WA member of the Indo-Pacific clade is of the WA *C. spurius* in the Gatun Formation of Costa Rica (Olsson, 1922) and Panama (Woodring, 1970) that antedates the closure of the isthmus (Duque-Caro, 1990). Thus, incursions of IP *Conus* lineages into the EP + WA have occurred at different times throughout the evolutionary history of this group and could span the Lower Oligocene to present (Fig. 3).

The composition of the smaller main clade in the 16S phylogram suggests that the ancestor of *C. memiae*, a species restricted to the western Pacific, migrated into the IP from the EP or WA. To date we lack gene sequences of any of its several morphologically most similar congeners (Röckel et al., 1995), so we cannot propose a migration route. All three of the EA species examined, and the sole modern amphi-Atlantic *Conus*, *C. ermineus*, belong to the larger, mainly IP clade (Figs. 1 and 2). The EA *C. ateralbus* and *C. cuneolus* appear to have diverged from IP lineages about 20 mya (Fig. 3). Their distribution patterns represent at least one migration and subsequent isolation between the IP and Atlantic or vicariant splitting of lineages by the final closure of the Tethys seaway or onset of the Benguela cold water

system in the Miocene. On the other hand, the EA *C. tabidus* is a member of the subclade containing EP and WA species (Fig. 1) that may have diverged from IP ancestors 33 mya (Fig. 3). Its phylogenetic position and geographic distribution suggest that either the Mid-Atlantic Barrier severed the range of a former amphi-Atlantic species, or that its ancestor migrated between the WA and EA. Because calmodulin sequences were not available from *C. tabidus*, we cannot estimate its divergence date. Its relationship to *C. gladiator* and *C. mus* (Fig. 1) suggests that *C. tabidus* diverged earlier than these species diverged from each other (Fig. 3).

Conus ermineus diverged from its EP sister *C. purpurascens* approximately 7 mya, and the lineage that gave rise to these species diverged from IP lineages about 19 mya (Fig. 3). The presence of *C. ermineus* in the EA may reflect a more recent breaching of the Mid-Atlantic Barrier. This barrier may have been permeable to dispersal of *C. ermineus* after it diverged from *C. purpurascens*. Although this seems unlikely given its apparent impermeability to other *Conus* species, *C. ermineus* is known to have a planktonic veliger larva (Bandel, 1976). In contrast, most (about 80%; Kohn, unpublished data) WA *Conus* whose developmental mode in known lack a planktonic stage. Unfortunately the fossil record of *C. ermineus* is limited to Upper Pleistocene records from the Mediterranean region (Glibert, 1960 [as *C. testudinarius*]) and does not clarify its history.

EA and Indian Ocean *Conus* may have maintained genetic exchange or migrated between regions until the Tethys closure or initiation of the Benguela upwelling system. However, because no fossil *Conus* occur in both eastern Atlantic and Indo-Pacific deposits, the Tethys may have served a more important role in migration of *Conus* between the EA and Indian Ocean than did a possible connection around South Africa. Vermeij and Rosenberg (1993) cite a number of IP taxa that invaded the EA around the southern tip of Africa during the Pleistocene. Although we have not sampled very many EA species, our results suggest that any invasions from the IP would have occurred earlier than the Pliocene (Fig. 3) and could have been either via the former Tethys Realm or around South Africa.

4.3. Diversification patterns of *Conus*

Application of a molecular clock to phylogenies of *Conus* suggests that most extant species analyzed originated during the Miocene (Fig. 3). The fossil record indicates that more *Conus* species originated then than in any other epoch (Table 1; Kohn, 1985, 1990), but most of these are now extinct. When and where the many speciation events leading to the present hyperdiversity of *Conus* occurred remain to be determined, but only a small proportion likely resulted from major vicariant occurrences such as emergence of the Isthmus of Panama.

Phylogenies based on 16S sequences confirm five transisthmian sister species relationships that Vermeij (1978) predicted on the basis of morphology: EP *C. bartschii* and *C. brunneus* and WA *C. regius*, EP *C. gladiator* and WA *C. mus*, EP *C. poormani* and WA *C. villepini*, EP *C. perplexus* and WA *C. puncticulatus*, and EP *C. purpurascens* and Atlantic *C. ermineus*; the calmodulin trees confirm four of these (Figs. 1–4). In addition, the calmodulin tree constructed with an enforced molecular clock contains a subclade comprising the EP *C. orion* and three WA species (Fig. 3), but its bootstrap support is less than 50%. The molecular clock tree gives divergences ages of these transisthmian species of between 6.9 and 14.3 mya and possibly as early as 24.5 mya (Fig. 3), several to many millions of years prior to separation by the Isthmus of Panama. The divergence of *C. poormani* and *C. villepini* could not be dated due to the lack of calmodulin sequence data from *C. poormani*. However, because *C. villepini* and CspWA-1 apparently diverged 4.8 mya (Fig. 3) and *C. poormani* and *C. villepini* are more closely related than *C. villepini* and CspWA-1, based on 16S data (Fig. 1) divergence of *C. poormani* and *C. villepini* likely occurred more recently than 4.8 mya and as such would show the most recently diverged transisthmian *Conus*. The EP *C. archon* clusters with the WA *C. granarius* in the 16S phylogeny (Fig. 1; calmodulin sequences were not available from *C. granarius*). The degree of 16S divergence among these species (5.2%) falls within the range of the transisthmian species pairs mentioned above (0.7–6.1%) so this pair may also have diverged with the emergence of the isthmus.

One 16S subclade within the large major clade contains the EP *C. poormani* and the WA *C. centurio*, *C. daucus*, *C. villepini*, and CspWA-1 (Fig. 1). The available calmodulin sequences for three of these are generally congruent (Fig. 2). Although not supported by high bootstrap support, this group shows affinity to three EP and WA species, *C. gladiator*, *C. mus*, and *C. orion*, in the calmodulin tree constructed with an enforced molecular clock (Fig. 3). Hence, the ancestors of these species likely migrated into the EP or WA from the IP prior to the emergence of the Isthmus of Panama, perhaps in the early Oligocene, and then subsequently diversified.

4.4. Conclusions—evolutionary history of *Conus* as inferred from fossils and phylogenies

The earliest bona fide *Conus* fossils occur in Lower Eocene deposits of England and France (Kohn, 1990). The genus radiated rapidly during the Eocene and became widely distributed throughout the world (Table 1) (see also Kohn, 1985, 1990). The former Tethys Realm apparently was the center of Eocene *Conus* diversity but members of the genus also occurred in the IP and seas surrounding the Americas (Table 1). During the Oligo-

cene *Conus* experienced, as did other marine invertebrates (Raup, 1976a), a decline in diversity throughout much of its range (Table 1). Phylogenetic analyses and application of a rate calibration show that the splitting of *Conus* into EP + WA and IP counterparts commenced during the Eocene or Oligocene (Figs. 3 and 4). Very few *Conus* are known to have occurred in more than one major biogeographic region during any epoch, so interchange of lineages among regions has presumably been limited by barriers to dispersal since the origin of the genus. Molecular phylogenetic results also confirm that barriers to dispersal must have been strong to segregate lineages in the IP and EP + WA, but that they must have been breached at different times during the evolutionary history of the genus (Figs. 1–4).

The fossil record also demonstrates that *Conus* radiated rapidly throughout its distribution during the Miocene (Table 1; Kohn, 1990). Numbers of described *Conus* species more than doubled in the former TR + EA, the IP, and the EP + WA. During this epoch TR + EA *Conus* diversity was highest, and it was lowest in the Americas (Table 1). Phylogenetic reconstructions also suggest that origination events increased during the Miocene (Figs. 3 and 4). During the Pliocene, species richness of *Conus* decreased in all regions occupied by the genus (Table 1), but the brevity of the Pliocene compared to the Miocene and the limited area of Pliocene fossil deposits (cf., Raup, 1976b) undoubtedly influence these values. Species diversity declined sharply in the TR + EA during the Upper Pliocene and Pleistocene. Diversity decreased less severely in the Americas, and increased slightly in the IP, but these data are subject to the same paleontological biases.

In summary, molecular phylogenetic analyses and the fossil record show that assemblages of *Conus* species have been geographically segregated in the Indo-Pacific, eastern Pacific/western Atlantic, and possibly eastern Atlantic/former Tethys Realm throughout the evolutionary history of the genus. The East Pacific and Mid-Atlantic Barriers appear to be the main causes establishing unique *Conus* taxocenes in these regions. Nonetheless, the presence of modern and fossil taxa in more than one major biogeographic region and the phylogenetic results implying that ancestors of modern *Conus* species migrated between regions indicate that these barriers have occasionally been breached.

Acknowledgments

We thank H.A. Lessios, G.J. Vermeij, and L. Cortés Ortiz for comments on earlier drafts of the manuscript and discussions. We also thank those that have contributed specimens for this work including E. Rolán, D. Touitou, G.C. Fielder, G. Rosenberg, and the Academy of Natural Sciences, H. Chaney and the Santa Barbara

Museum of Natural History, P. Quiquadon, F. Rodríguez, A. Calderón, C.P. Meyer, and H.A. Lessios. We wish also to thank the crew of the *R/V Uracaá* as well as H.A. Lessios, N. Knowlton, H. Fortunato, and D.R.

Robertson for allowing TFD to participate on their research cruises. Supported by NSF Grant 0316338 and by a Tupper Fellowship from the Smithsonian Tropical Research Institute to T.F.D.

Appendix

Conus species used in phylogenetic reconstructions

Species	Author, date	Source	Province	16S-GAN	Cal-GAN
<i>Conus abbreviatus</i>	Reeve, 1883	Midway	IP	AF174140	AF113252
<i>Conus acutangulus</i>	Lamarck, 1810	Philippines	IP	AF160718	—
<i>Conus anemone</i>	Lamarck, 1810	Australia	IP	AF174141	AF113253
<i>Conus araneosus</i>	[Lightfoot], 1786		IP	AF174142	AF113254
<i>Conus archon</i>	Broderip, 1833	Panama	EP	AY381995	AY382035
<i>Conus arcuatus</i>	Broderip & Sowerby I, 1829	Panama	EP	AY381996	AY382036
<i>Conus arenatus</i>	Hwass, 1792	Papua New Guinea	IP	AF174143	—
<i>Conus aristophanes</i>	Sowerby II, 1858	American Samoa	IP	AY381997	AY382037
<i>Conus ateralbus</i>	Kiener, 1849	Cape Verde	EA	AY381998	AY382038
<i>Conus aulicus</i>	Linné, 1758	Papua New Guinea	IP	AF174144	—
<i>Conus aureus</i>	Hwass, 1792	Guam	IP	AF174145	AF113255
<i>Conus balteatus</i>	Sowerby I, 1833	Papua New Guinea	IP	AF174146	AF113256
<i>Conus bandanus</i>	Hwass, 1792	Oahu, USA	IP	AY381999	AF113257
<i>Conus barthelemyi</i>	Bernardi, 1861	India	IP	AY382000	AY382039
<i>Conus bartschi</i>	Hanna & Strong, 1949	Panama	EP	AY382001	AY458018
<i>Conus betulinus</i>	Linné, 1758	Papua New Guinea	IP	AF174147	—
<i>Conus biliosus</i>	(Röding, 1798)	Papua New Guinea	IP	AF17418	AF11329
<i>Conus boeticus</i>	Reeve, 1843	Guam	IP	AF174148	AF113258
<i>Conus brunneus</i>	Wood, 1828	Mexico	EP	AF174149	AF113259
<i>Conus bullatus</i>	Linné, 1758	Guam	IP	AF174150	—
<i>Conus californicus</i>	Reeve, 1844	California	EP	AF174151	AY382040
<i>Conus canonicus</i>	Hwass, 1792	Papua New Guinea	IP	AF174152	AF113261
<i>Conus capitaneus</i>	Linné, 1758	Papua New Guinea	IP	AF17415	AF11329
<i>Conus characteristicus</i>	Fischer, 1807		IP	AF126017	—
<i>Conus catus</i>	Hwass, 1792		IP	AF174154	AF113260
<i>Conus centurio</i>	Born, 1780	Panama	WA	AY382002	—
<i>Conus chaldaeus</i>	(Röding, 1798)	Oahu	IP, EP	AF174155	AF113262
<i>Conus cinereus</i>	Hwass, 1792	Papua New Guinea	IP	AF174156	AF113263
<i>Conus circumactus</i>	Iredale, 1929		IP	AF144001	—
<i>Conus circumcissus</i>	Born, 1778		IP	AF144002	—
<i>Conus coffeae</i>	Gmelin, 1791	Papua New Guinea	IP	AF174158	AF113265
<i>Conus consors</i>	Sowerby I, 1833	Papua New Guinea	IP	AF174159	AF113267
<i>Conus corallinus</i>	Kiener, 1845		IP	AF143995	—
<i>Conus coronatus</i>	Broderip & Sowerby, 1833	Papua New Guinea	IP	AF174160	AF113268
<i>Conus cuneolus</i>	Reeve, 1843	Cape Verde	EA	AY382003	AY382041
<i>Conus cylindraceus</i>	Broderip & Sowerby, 1833	Guam	IP	AF174161	AF113269
<i>Conus dalli</i>	Stearns, 1873	Panama	EP	AY382004	AY382042
<i>Conus daucus</i>	Hwass, 1792	Panama	WA	AY382005	AY382043
<i>Conus diadema</i>	Sowerby I, 1834	Panama	EP	AY382006	AY382044
<i>Conus distans</i>	Hwass, 1792	Papua New Guinea	IP	AF174162	AF113270
<i>Conus dorreensis</i>	Péron, 1807	Australia	IP	AF174163	AF113271
<i>Conus ebraeus</i>	Linné, 1758	Oahu, USA	IP, EP	AF17416	AF113272
<i>Conus eburneus</i>	Hwass, 1792	Papua New Guinea	IP	AF174165	AF113273
<i>Conus emaciatus</i>	Reeve, 1849	Papua New Guinea	IP	AF174166	AF113274
<i>Conus episcopatus</i>	Hwass, 1792	Papua New Guinea	IP	AF174167	AF113275
<i>Conus ermineus</i>	Born, 1778	Cape Verde	WA, EA	AY236860	AY236861
<i>Conus fergusonii</i>	Sowerby III, 1873	Panama	EP	AY382007	AY382045
<i>Conus figulinus</i>	Linné, 1758	Philippines	IP	—	AF113276
<i>Conus flavidus</i>	Lamarck, 1810	Oahu, USA	IP	AF174168	AF113277
<i>Conus floridulus</i>	Adams & Reeve, 1849		IP	AF160705	—
<i>Conus frigidus</i>	Reeve, 1848	Papua New Guinea	IP	AF174169	AY382046
<i>Conus furvus</i>	Reeve, 1843	Philippines	IP	—	AF113278
<i>Conus generalis</i>	Linné, 1767	Guam	IP	AF174170	AF113279

(continued on next page)

Appendix (continued)

Species	Author, date	Source	Province	16S-GAN	Cal-GAN
<i>Conus geographus</i>	Linné, 1758	Papua New Guinea	IP	AF174171	AF113280
<i>Conus gladiator</i>	Broderip, 1833	Panama	EP	AY382008	AY382047
<i>Conus glans</i>	Hwass, 1792	Guam	IP	AF174172	AF113281
<i>Conus gloriamaris</i>	Chemnitz, 1777		IP	AF126168	—
<i>Conus granarius</i>	Kiener, 1845	Panama	WA	AY382009	—
<i>Conus granum</i>	Röckel & Fischöder, 1985		IP	AF126169	—
<i>Conus imperialis</i>	Linné, 1758	Papua New Guinea	IP	AF174173	AF113282
<i>Conus inscriptus</i>	Reeve, 1843	India	IP	AY382010	AY382048
<i>Conus jaspideus</i>	Gmelin, 1791	Panama	WA	AY382030	AY382059
<i>Conus judaeus</i>	Bergh, 1895	Okinawa	IP	AY382011	—
<i>Conus legatus</i>	Lamarck, 1810	Guam	IP	AF174174	AF113283
<i>Conus leopardus</i>	(Röding, 1798)	Oahu, USA	IP	AF174175	AF113284
<i>Conus lineolatus</i>	Valenciennes, 1832	Panama	EP	AF480307	—
<i>Conus litoglyphus</i>	Hwass, 1792	Papua New Guinea	IP	AF174176	AF113285
<i>Conus litteratus</i>	Linné, 1758	Papua New Guinea	IP	AF174177	AF113286
<i>Conus lividus</i>	Hwass, 1792	Papua New Guinea	IP	AF174178	AF113287
<i>Conus lorenzianus</i>	Dillwyn, 1817	Panama	WA	AY382012	AY382049
<i>Conus loriosii</i>	Kiener, 1845		IP	AF126171	—
<i>Conus magnificus</i>	Reeve, 1843	Fiji	IP	AY382013	—
<i>Conus magus</i>	Linné, 1758	Indonesia	IP	AF174179	AF113288
<i>Conus mahogani</i>	Reeve, 1843	Panama	EP	AY382014	AY382050
<i>Conus marmoreus</i>	Linné, 1758	Palau	IP	AF174180	AF113289
<i>Conus memiae</i>	(Habe & Kosuge, 1970)	Philippines	IP	AF160723	—
<i>Conus miles</i>	Linné, 1758	Papua New Guinea	IP	AF174182	AF113291
<i>Conus miliaris</i>	Hwass, 1792	Papua New Guinea	IP	AF174181	AF113290
<i>Conus mindanus</i>	Hwass, 1792	Panama	WA	AY382015	AY382051
<i>Conus monachus</i>	Linné, 1758		IP	AF126172	—
<i>Conus moreleti</i>	Crosse, 1858	Papua New Guinea	IP	AF174183	AF113292
<i>Conus muriculatus</i>	Sowerby I, 1833	Papua New Guinea	IP	AF174184	AF113293
<i>Conus mus</i>	Hwass, 1792	Panama	WA	AY382016	AY382052
<i>Conus musicus</i>	Hwass, 1792	Papua New Guinea	IP	AF174185	AF113294
<i>Conus nanus</i>	Sowerby I, 1833	Oahu, USA	IP	AF174199	AF113309
<i>Conus natalis</i>	Sowerby II, 1858	South Africa	SA	AY382017	—
<i>Conus nigrescens</i>	Sowerby II, 1859	Samoa	IP	AY382018	—
<i>Conus nigropunctatus</i>	Sowerby II, 1858		IP	AF086614	—
<i>Conus nussatella</i>	Linné, 1758	Seychelles	IP	AY382019	—
<i>Conus nux</i>	Broderip, 1833	Mexico	EP	AF174186	AF113296
<i>Conus obscurus</i>	Sowerby I, 1833	Oahu, USA	IP	AF174187	AF113297
<i>Conus ochroleucus</i>	Gmelin, 1791		IP	AF036528	—
<i>Conus omaria</i>	Hwass, 1792	Guam	IP	AF174188	AF113298
<i>Conus orion</i>	Broderip, 1833	Panama	EP	AY382020	AY382053
<i>Conus patricius</i>	Hinds, 1843	Panama	EP	AY382021	AY382054
<i>Conus pennaceus</i>	Born, 1778	Oahu, USA	IP	AF174190	AF113300
<i>Conus perplexus</i>	Sowerby II, 1858	El Salvador	EP	AY382022	AY382055
<i>Conus pertusus</i>	Hwass, 1792		IP	AF108827	—
<i>Conus planorbis</i>	Born, 1778	Papua New Guinea	IP	AF174191	AF113301
<i>Conus poormani</i>	Berry, 1968	Panama	EP	AY382023	—
<i>Conus princeps</i>	Linné, 1758	Mexico	EP	AF174192	AF113302
<i>Conus proximus</i>	Sowerby II, 1859	Guam	IP	AF174193	AF113303
<i>Conus pulicarius</i>	Hwass, 1792	Papua New Guinea	IP	AF174194	AF113304
<i>Conus punctulatus</i>	Hwass, 1792	Panama	WA	AY382024	AY382056
<i>Conus purpurascens</i>	Sowerby I, 1833	Panama	EP	AF480308	AF480311
<i>Conus quercinus</i>	[Lightfoot], 1786	Papua New Guinea	IP	AF174195	AF113305
<i>Conus radiatus</i>	Gmelin, 1791	Philippines	IP	AF160724	—
<i>Conus rattus</i>	Hwass, 1792	Papua New Guinea	IP	AF174196	AF113306
<i>Conus recurvus</i>	Broderip, 1833	Panama	EP	AY382025	—
<i>Conus regius</i>	Gmelin, 1791	Florida, USA	WA	AF174197	AF113307
<i>Conus sanguinolentus</i>	Quoy & Gairnard, 1834	Papua New Guinea	IP	AF174198	AF113308
<i>Conus shikamai</i>	Coomans, Moolenbeek & Wils, 1985	Philippines	IP	AF160720	—
<i>Conus sponsalis</i>	Hwass, 1792	Oahu, USA	IP	AY382026	AY382057
<i>Conus spurius</i>	Gmelin, 1791	Virgin Islands	WA	AY382027	—
<i>Conus stercusmuscarum</i>	Linné, 1758	Papua New Guinea	IP	AF174200	AF113310
<i>Conus striatellus</i>	Link, 1807	Philippines	IP	AF143994	—
<i>Conus striatus</i>	Linné, 1758	Papua New Guinea	IP	AF174202	AF113311

Appendix (continued)

Species	Author, date	Source	Province	16S-GAN	Cal-GAN
<i>Conus striolatus</i>	Kiener, 1845	Papua New Guinea	IP	AF174201	AF113312
<i>Conus sugillatus</i>	Reeve, 1844		IP	AF143990	—
<i>Conus sulcatus</i>	Hwass, 1792	Philippines	IP	AF160714	—
<i>Conus swainsoni</i>	Estival & von Cosel, 1986	Papua New Guinea	IP	—	AF113266
<i>Conus tabidus</i>	Reeve, 1844	Cape Verde	EA	AY382028	—
<i>Conus tenuistriatus</i>	Sowerby II, 1858	Papua New Guinea	IP	AF174203	AF113313
<i>Conus terebra</i>	Born, 1778	Papua New Guinea	IP	AF174204	AF113314
<i>Conus tessulatus</i>	Born, 1778	Palau	IP, EP	AF174205	AF113315
<i>Conus textile</i>	Linné, 1758	Papua New Guinea	IP	AF174206	AF113316
<i>Conus tornatus</i>	Sowerby I, 1833	Panama	EP	AY382029	AY382058
<i>Conus tribblei</i>	Walls, 1977	Philippines	IP	AF160716	—
<i>Conus tulipa</i>	Linné, 1758	Okinawa	IP	AF174207	AF113317
<i>Conus varius</i>	Linné, 1758	Papua New Guinea	IP	AF174208	AF113318
<i>Conus vexillum</i>	Gmelin, 1791	Oahu	IP	AF174209	AF113320
<i>Conus villepini</i>	Fischer & Bernardi, 1857	Panama	WA	AY382031	AY382060
<i>Conus violaceus</i>	Gmelin, 1791	Seychelles	IP	AY382032	—
<i>Conus viola</i>	Cernohorsky, 1977	Philippines	IP	AF160719	—
<i>Conus virgo</i>	Linné, 1758	Papua New Guinea	IP	AF174211	AF113319
<i>Conus vitulinus</i>	Hwass, 1792	Oahu, USA	IP	AF174210	AF113321
<i>Conus ximenes</i>	Gray, 1839	Panama	EP	AY382033	AY382061
CspWA-1		Panama	WA	AY382034	AY382062
<i>Strombus luhuanus</i>	Linné, 1758	Papua New Guinea	IP	AF174212	—
<i>Terebra subulata</i>	Linné, 1767	Papua New Guinea	IP	AF174213	—

Sources of specimens analyzed and the biogeographic province(s) in which the species occurs are listed (EA, eastern Atlantic; EP, eastern Pacific; IP, Indo-Pacific; SA, South Africa; and WA, western Atlantic). Genbank accession numbers (GAN) are listed for mitochondrial 16S (16S) and calmodulin intron sequences (Cal) for each species ('—' no sequence).

References

- Abbott, R.T., 1974. *American Seashells: The Marine Mollusca of the Atlantic and Pacific Coasts of North America*, second ed. Van Nostrand Reinhold, New York.
- Adams, C.G., 1981. An outline of Tertiary palaeogeography. In: Cocks, L.R.M. (Ed.), *The Evolving Earth*. British Museum of Natural History, London, pp. 221–235.
- Anderson, F.E., 2000. Phylogeny and historical biogeography of the Loliginid squids (Mollusca: Cephalopoda) based on mitochondrial DNA sequence data. *Mol. Phylogenet. Evol.* 15, 191–214.
- Bandel, K., 1976. Spawning, development and ecology of some higher Neogastropoda from the Caribbean Sea of Colombia (South America). *Veliger* 19, 176–193.
- Briggs, J.C., 1974. *Marine Zoogeography*. McGraw-Hill, New York.
- Briggs, J.C., 1995. *Global Biogeography*. Elsevier, Amsterdam.
- Budd, A.F., 2000. Diversity and extinction in the Cenozoic history of Caribbean reefs (Invited review). *Coral Reefs* 19, 25–35.
- Coates, A.G., Obando, J.A., 1996. The geologic evolution of the Central American Isthmus. In: Jackson, J.B.C., Budd, A., Coates, A.G. (Eds.), *Evolution and Environment in Tropical America*. University of Chicago Press, Chicago, Illinois, pp. 21–56.
- Collins, T.M., Frazer, K., Palmer, A.R., Vermeij, G.J., Brown, W.M., 1996. Evolutionary history of northern hemisphere *Nucella* (Gastropoda, Muricidae): molecular, morphological, ecological, and paleontological evidence. *Evolution* 50, 2287–2304.
- Dickerson, R.E., 1921. Notes on a fauna of the Vigo Group and its bearing on the evolution of marine molluscan faunas. *Proc. Calif. Acad. Sci.* 11, 1–26.
- Diester-Haass, L., Schrader, H.J., 1979. Neogene coastal up-welling history off northwest and southwest Africa. *Mar. Geol.* 29, 39–53.
- Duda Jr., T.F., Kohn, A.J., Palumbi, S.R., 2001. Origins of diverse feeding ecologies within *Conus*, a genus of venomous marine gastropods. *Biol. J. Linn. Soc.* 73, 391–409.
- Duda Jr., T.F., Palumbi, S.R., 1999. Developmental shifts and species selection in gastropods. *Proc. Natl. Acad. Sci. USA* 96, 10272–10277.
- Duque-Caro, H., 1990. Neogene stratigraphy, paleoceanography and paleobiogeography in northwest South America and the evolution of the Panama seaway. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 77, 203–234.
- Durham, J.W., 1950. 1940 E.W. Scripps Cruise to the Gulf of California. Part II. Megascopic paleontology and marine stratigraphy. *Geol. Soc. Am. Mem.* 43, 177.
- Ekman, S., 1953. *Zoogeography of the Sea*. Sidgwick and Jackson, London.
- Espiritu, D.J.D., Watkins, M., Monje, V.D., Cartier, G.E., Cruz, L.J., Olivera, B.M., 2001. Venomous cone snails: molecular phylogeny and the generation of toxin diversity. *Toxicon* 39, 1899–1916.
- Glibert, M., 1960. Les Conacea fossiles du Cénozoïque étranger des collections de l'Institut Royal des Sciences Naturelles de Belgique. *Mém. Inst. R. Sci. Nat. Belg.* 2nd ser., 64, 1–132.
- Grigg, R.W., Hey, R., 1992. Paleoceanography of the tropical eastern Pacific Ocean. *Science* 255, 172–178.
- Harzhauser, M., Piller, W.E., Steininger, F.F., 2002. Circum-Mediterranean Oligo-Miocene biogeographic evolution—the gastropods' point of view. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 183, 103–133.
- Hasegawa, M., Kishino, H., Yano, T., 1985. Dating the human-ape split by a molecular clock of mitochondrial DNA. *J. Mol. Evol.* 22, 160–174.
- Hrbek, T., Meyer, A., 2003. Closing of the Tethys Sea and the phylogeny of Eurasian killifishes (Cyprinodontiformes: Cyprinodontidae). *J. Evol. Biol.* 16, 17–36.
- Keen, A.M., 1971. *Sea Shells of Tropical West America; Marine Mollusks from Baja California to Peru*. Stanford University Press, Stanford.
- Kilburn, R., Rippey, E., 1982. *Sea Shells of Southern Africa*. Macmillan, Johannesburg.
- Kishino, H., Hasegawa, M., 1989. Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA

- sequence data, and the branching order in Hominoidea. *J. Mol. Evol.* 29, 170–179.
- Knowlton, N., Weigt, L.A., 1998. New dates and new rates for divergence across the Isthmus of Panama. *Proc. R. Soc. Lond. B* 265, 2257–2263.
- Knowlton, N., Weigt, L.A., Solorzano, L.A., Mills, D.K., Bermingham, E., 1993. Divergence in proteins, mitochondrial DNA, and reproductive compatibility across the Isthmus of Panama. *Science* 260, 1629–1632.
- A.J. Kohn, 1985. Evolutionary ecology of *Conus* on Indo-Pacific coral reefs. In: *Proc. Fifth Inter. Coral Reef Congr.*, vol. 4, pp. 139–144.
- Kohn, A.J., 1990. Tempo and mode of evolution in Conidae. *Malacologia* 32, 55–67.
- Lanave, C., Preparata, G., Saccone, C., Serio, G., 1984. A new method for calculating evolutionary substitution rates. *J. Mol. Evol.* 20, 86–93.
- Lessios, H.A., Kane, J., Robertson, D.R., 2003. Phylogeography of the pantropical sea urchin *Tripneustes*: contrasting patterns of population structure between oceans. *Evolution* 57, 2026–2036.
- Lessios, H.A., Kessing, B.D., Wellington, G.M., Graybeal, A., 1996. Indo-Pacific echinoids in the tropical eastern Pacific. *Coral Reefs* 15, 133–142.
- Lessios, H.A., Kessing, B.D., Robertson, D.R., 1998. Massive gene flow across the world's most potent marine biogeographic barrier. *Proc. R. Soc. Lond. Ser. B* 265, 583–588.
- Lessios, H.A., Kessing, B.D., Pearse, J.S., 2001. Population structure and speciation in tropical seas: global phylogeography of the sea urchin *Diadema*. *Evolution* 55, 955–975.
- Marko, P.B., 2002. Fossil calibration of molecular clocks and the divergence times of geminate species pairs separated by the Isthmus of Panama. *Mol. Biol. Evol.* 19, 2005–2021.
- Meyer, C.P., 2003. Molecular systematics of cowries (Gastropoda: Cypraeidae) and diversification patterns in the tropics. *Biol. J. Linn. Soc.* 79, 401–459.
- Meyers, P.A., Brassell, S.C., Huc, A.Y., Barron, E.J., Boyece, R.E., Dean, W.E., Hay, W.W., Keating, B.H., McNulty, C.L., Nohara, M., Schallreuter, R.E., Sibuet, J.-C., Steinmetz, J.C., Stow, D., Stadner, H., 1983. Organic geochemistry of sediments recovered by DSDP/IPOD leg 75 from under the Benguela Current. In: Thiede, J., Suess, E. (Eds.), *Coastal Up-Welling: its Sedimentary Record. Part B. Sedimentary Records of Ancient Coastal Upwelling*. Plenum Press, New York, pp. 453–466.
- Odin, G.S., Montanari, A., Coccioni, R., 1997. Chronostratigraphy of Miocene stages: a proposal for the definition of precise boundaries. In: Montanari, A., Odin, G.S., Coccioni, R. (Eds.), *Miocene Stratigraphy: An Integrated Approach*. Elsevier, Amsterdam, pp. 597–629.
- Olsson, A.A., 1922. The Miocene of Northern Costa Rica. *Bull. Am. Paleontol.* 9, 1–168.
- Pin, M., Leung Tack, K.D., 1995. The Cones of Senegal. *La Conchiglia* 277, 1–55.
- Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14, 817–818.
- Powell, C.J., 1989. The Miocene and Pliocene Imperial Formation of Southern California and its molluscan fauna: an overview. *West. Soc. Malac. Annu. Rep.* 20, 11–18.
- Raup, D.M., 1976a. Species diversity in the Phanerozoic: a tabulation. *Paleobiology* 2, 279–288.
- Raup, D.M., 1976b. Species diversity in the Phanerozoic: an interpretation. *Paleobiology* 2, 289–297.
- Röckel, D., Korn, W., Kohn, A.J., 1995. *Manual of the Living Conidae. Vol. I, Indo-Pacific*. Christa Hemmen Verlag, Wiesbaden.
- Röckel, D., Rolán, E., Monteiro, A., 1980. *Cone Shells from Cape Verde Islands*. Feito, Vigo.
- Rodriguez, R., Oliver, J.L., Marin, A., Medina, J.R., 1990. The general stochastic model of nucleotide substitution. *J. Theor. Biol.* 142, 485–501.
- Scotese, C.R., 1999. Paleomap project. Available from: <<http://www.scotese.com>>.
- Shannon, L.V., 1985. The Benguela ecosystem. Part I. Evolution of the Benguela physical features and processes. *Oceanogr. Mar. Bio. Annu. Rev.* 23, 105–182.
- Shimodaira, H., Hasegawa, M., 1999. Multiple comparisons of log likelihoods with applications to phylogenetic inference. *Mol. Biol. Evol.* 16, 1114–1116.
- Shuto T, T., 1975. Preliminary correlation of the Neogene molluscan faunas in Southeast Asia. *Geol. Paleont. Southeast Asia* 15, 289–301.
- Siesser, W.G., 1980. Late Miocene origin of the Benguela upwelling system off northern Namibia. *Science* 208, 283–285.
- Stanley, S.M., 1979. *Macroevolution: Pattern and Process*. Freeman, San Francisco.
- Swofford, D.L., 2002. Paup*. *Phylogenetic Analysis Using Parsimony (* and other methods)*. Version 4. Sinauer, Sunderland.
- Tsukamoto, K., Aoyama, J., 1998. Evolution of freshwater eels of the genus *Anguilla*: a probable scenario. *Environ. Biol. Fishes* 52, 139–148.
- Van der Vlerk, I.M., 1931. *Caenozoic Amphineura, Gastropoda, Lamellibranchiata, Scaphopoda*. Leidsche Geologische Mededeeling 5, 206–296.
- Vermeij, G.J., 1978. *Biogeography and Adaptation. Patterns of Marine Life*. Harvard University Press, Cambridge.
- Vermeij, G.J., 2001. Community assembly in the sea Geologic history of the living shore biota. In: Bertness, M.D., Gaines, S.D., Hay, M.E. (Eds.), *Marine Community Ecology*. Sinauer, Sunderland, pp. 39–60.
- Vermeij, G.J., Rosenberg, G., 1993. Giving and receiving: the tropical Atlantic as donor and recipient region for invading species. *Am. er. Malac. Bull.* 10, 181–194.
- Vrielynck, B., Odin, G.S., Dercourt, J., 1997. Miocene palaeogeography of the Tethys Ocean: potential global correlations in the Mediterranean. In: Montanari, A., Odin, G.S., Coccioni, R. (Eds.), *Miocene Stratigraphy: An Integrated Approach*. Elsevier, Amsterdam, pp. 157–165.
- Walls, J.G., 1979. *Cone Shells: A Synopsis of the Living Conidae*. TFH Publications, Neptune City.
- Woodring, W.P., 1970. *Geology and paleontology of Canal Zone and adjoining parts of Panama: Description of Tertiary mollusks (Gastropods: Eulimidae, Marginellidae to Helminthoglyptidae)*. US Geological Survey Professional Paper 306-D 299–452, pls. 48–66.