



Origins of diverse feeding ecologies within *Conus*, a genus of venomous marine gastropods

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Received 10 October 2000; accepted for publication 15 March 2001

Specialized predators on polychaetes, fishes, hemichordates or other molluscs, members of the predominantly tropical gastropod genus *Conus* diversified rapidly during the Miocene to constitute the most species-rich modern marine genus. We used DNA sequence data from mitochondrial and nuclear loci of 76 *Conus* species to generate species-level phylogenetic hypotheses for this genus and then mapped known diets onto the phylogenies to elucidate the origins and evolutionary histories of different feeding specializations. The results indicate that dramatically new feeding modes arose only a few times, that the most derived feeding modes likely arose in the Miocene, and that much of the known diversity of *Conus* that was generated during Miocene radiations has survived to the present.

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ADDITIONAL KEY WORDS: Gastropoda – *Conus* – molecular phylogeny – feeding ecology – ecological diversification.

INTRODUCTION

Conus is an unusually species-rich genus of predatory, tropical marine gastropods with more than 500 extant and several hundred extinct species (Röckel, Korn & Kohn, 1995). Although rather invariant in shell morphology, species of *Conus* vary widely in life history and ecological attributes, especially feeding ecology and microhabitat use (Kohn & Perron, 1994; Kohn, 1998). Almost all species in the genus whose diets are known prey on members of only one of three general prey types: fishes, other gastropod molluscs, and worms, especially in the Indo-West Pacific region where the genus has been best studied (see Appendix). By far the largest number of *Conus* species eat polychaete annelids, but within this taxon their diets are diverse, with specializations on errant (Eunicidae, Nereidae, Amphinomididae and Glyceridae) or sedentary (Terebellidae, Capitellidae, Maldanidae, Cirratulidae, Chaetopteridae) families. A few are predators on hemichordates, and one is also known to prey on echinurans.

Because the first hypothesis of phylogenetic relationships among species in this genus has been proposed only recently (Duda & Palumbi, 1999a), very little is understood about the origins or evolutionary history of *Conus* species with different feeding ecologies. Have these diet specializations evolved many times or is diet evolution conservative with one or few origins of each diet type?

DIETS OF *CONUS*

The diets of many *Conus* species are known from gut content or faecal analyses by Kohn and others (Kohn, 1959, 1960, 1966, 1968, 1978a,b, 1981, 1987, 1997; Marsh, 1971; Kohn & Nybakken, 1975; Nybakken, 1970, 1978, 1979; Leviten, 1978; Reichelt & Kohn, 1985; Kohn & Almasi, 1993). Although diet diversity is great at the genus level, individual species tend to be specialized, particularly where large numbers of congeners co-occur (e.g. Kohn, 1959, 1968; Kohn & Nybakken, 1975), and most *Conus* species hunt prey of only one of the three types listed above.

However, the diets of a few species span more than one prey category. *Conus californicus* is the most notable exception with the broadest diet known of all *Conus* species; its diet includes fishes, molluscs (including bivalves as well as gastropods), polychaetes

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and crustaceans (Kohn, 1966). *C. bullatus* has been observed to feed on both fish and molluscs (McDowall, 1974 as cited by Röckel *et al.*, 1995). Also, several vermivorous species, including *C. arenatus*, *C. eburneus*, *C. miliaris*, *C. lividus*, and *C. sponsalis*, have been reported to prey on sedentary polychaetes, errant polychaetes and hemichordates in different geographic locations (see Appendix), and *C. eburneus* and *C. tessulatus* occasionally prey on fishes as well (Kohn & Nybakken, 1975; Reichelt & Kohn, 1985).

Species of the family Turridae, the group that presumably gave rise to *Conus* (Kohn, 1990), are largely polychaete-eaters (Bouchet & Warén, 1980; Taylor, 1985; Miller, 1989). Therefore it is likely that polychaetes are the ancestral prey of *Conus* and lineages with other diets arose from polychaete-eating *Conus* species. However, because the evolutionary relationships of *Conus* species are so unclear, we do not know when or how many times the unique feeding modes arose, or whether diets among vermivores are conservative traits. Two opposing hypotheses are: (1) Feeding mode is an evolutionarily labile trait and changes in diet have occurred many times among lineages during the evolutionary history of *Conus*. Similar feeding ecologies and the traits correlated with them (e.g. radular type, foraging behaviour, venom composition) could have evolved in parallel in many lineages. (2) Feeding mode is an evolutionarily conservative trait such that clades comprise species with similar diets, and molluscivorous and piscivorous diets each only evolved once and perhaps only during the early radiation of the group.

RADULAR CHARACTERS: HOMOLOGY OR HOMOPLASY?

Conus subdues prey by injecting a paralytic, neurotoxic venom through a single, detachable, tubular, barbed, chitinous radular tooth. These teeth are quite variable among species and appear to be related to feeding mode among species of *Conus* (Kohn, 1998; Kohn, Nishi & Pernet, 1999).

Molluscivorous *Conus* radular teeth are distinctive and consist of a long, narrow, and minutely serrated shaft, a slightly enlarged base, and a tip armed with two small barbs or a barb and a blade (Kohn *et al.*, 1999: fig. 7; Nishi & Kohn, 1999). All investigated molluscivores possess this type of tooth.

At least two types of radular teeth are common among piscivores: (1) teeth with a long, anteriorly serrated shaft and a slightly enlarged base, and a tip with a small blade on one side and a barb on the other; and (2) teeth with shaft a bit shorter than the first type and lacking serrations, a slightly enlarged base, and a tip with two opposing barbs and a third very large and outwardly protruding. The first type is characteristic of *Conus geographus* and *C. tulipa*, while

the second is characteristic of most other piscivorous species, including *C. achatinus*, *C. catus*, *C. cinereus*, *C. magus*, *C. stercusmuscarum*, and *C. striatus* (Endean & Rudkin, 1965; Nybakken, 1990; Rolán & Raybaudi Massilia, 1994; Kohn *et al.*, 1999: fig. 8).

The radular teeth of vermivores are variable among species and have been categorized into several distinct types by several authors beginning with Troschel (1866; Kohn *et al.*, 1999). In general, these teeth have much larger bases than those of piscivores and molluscivores (Endean & Rudkin, 1965; Kohn *et al.*, 1999). Five of these types are presently considered unique to single species: *Conus californicus*, *C. diadema*, *C. ebraeus*, *C. lividus*, and *C. tornatus*; the remainder are found in a variety of *Conus* species (Nybakken, 1990). For the most part, possession of a particular radular tooth type does not appear to be directly related to the types of worms that are consumed by a species. Indeed, some species with the same tooth type have different prey specializations (Nybakken, 1990). However, an exception is that the four species known to prey predominantly or exclusively on amphinomid polychaetes ('fire worms'), *C. brunneus*, *C. imperialis*, *C. regius*, and *C. zonatus*, all possess the same distinctive tooth type (Nybakken, 1970; Kohn & Hunter, 2001). Three other species whose diets have not been described, *C. archon*, *C. bartschi*, and *C. genuanus*, also possess this tooth type (Nybakken, 1990).

Overall radular tooth type appears to be correlated with diet among molluscivores, piscivores, and possibly vermivores that specialize on amphinomids. Do these relationships result from convergence of teeth appropriate to particular prey types, or do the shared traits demonstrate common ancestry among molluscivores, some piscivores, and amphinomid-eating species? Moreover, if tooth type is highly correlated with phylogeny, does the lack of correlation with diet among most vermivores suggest that vermivorous diets are labile? These types of questions can only be addressed in a phylogenetic framework.

EVOLUTIONARY AND GEOLOGICAL HISTORY

Based on interpretations of the fossil record (Kohn, 1990), *Conus* originated from a presently unknown lineage of turrids during the early Eocene, roughly 55 million years ago (Mya), with subsequent diversification through the end of the Eocene (35.4 Mya; about 40 fossil species are known). Further radiation occurred from the Upper Oligocene (29.3–35.4 Mya) until the Upper Miocene (5.2–10.4 Mya; about 150 species known) with species origination rates in excess of 0.2 Myr^{-1} (Kohn, 1990), three times the average origination rate of other gastropod groups ($= 0.067 \text{ Myr}^{-1}$; Stanley, 1979). The Lower Pliocene (3.4–5.2 Mya) was marked by apparent excessive extinction, with reductions in species numbers by 73%. Because of this

extinction only 11% of species present in the Miocene are estimated to be extant (Kohn, 1990). Following the Pliocene extinction, a second major radiation occurred with the rapid origination (rate $>0.3 \text{ Myr}^{-1}$) of several hundred species for which no fossil record prior to the Pleistocene is known (Kohn, 1990).

The deepest paleontological records of extant fish-eating *Conus* species are the piscivorous *Conus achatinus* and *C. magus* from the Middle Miocene of Indonesia (Beets, 1941). These fossils are now considered to be from the Serravalian Stage, about 11–12 Mya (Shuto, 1975; Odin, Montanari & Coccioni, 1997). The molluscivorous species *C. canonicus* and *C. textile* are also reported from the Upper Miocene (Martin, 1879–80; Van der Vlerk, 1931, *C. verriculum* is listed in Van der Vlerk (1931), but this name is a junior synonym of *C. textile*). The fossil record of *Conus* thus shows that fish and mollusc-eating lineages had appeared as early as the Miocene. Do estimates from other sources (e.g. divergence estimates from molecular sequence data) corroborate these dates or suggest an earlier origin? Approximately 25 molluscivorous and 20 piscivorous species likely exist today, although diets have been documented only for about half of these (Kohn, unpublished). If survival from the Miocene was so limited, are these species descendants of the Miocene lineages with diet specializations or are they more recently derived from worm-eating lineages?

PHYLOGENETIC SYSTEMATICS

Evolutionary relationships of *Conus* species have been difficult to estimate based on morphology largely due to the absence of resolution and possible convergence of shell and radula characters (Röckel *et al.*, 1995). Several non-phylogenetic infrageneric classification schemes, beginning with Linnaeus (1758), have been proposed for the genus. However, these schemes, based mainly on shell shape and sculpture, shell colour pattern, and radular morphology, cut across each other, none has received widespread acceptance, and recent works on *Conus* continue to refer to this group as a single genus (e.g. Walls, 1979; Kohn, 1990; Nybakken, 1990; Röckel *et al.*, 1995).

In order to estimate the evolutionary relationships among a selection of the 500 species *Conus*, we obtained molecular sequence data for 76 species from a region of the mitochondrial 16S rRNA gene and an intron located within a nuclear calmodulin locus. We then used these data to reconstruct the phylogeny of these taxa. The data presented here augment those of Duda & Palumbi (1999a). The species analysed included 13 piscivores, 11 molluscivores, 44 vermivorous species with described diets (of errant or sedentary polychaetes or hemichordates), 5 vermivorous species with undescribed diets, 2 with unknown diets and radular

teeth, and *C. californicus*, which has the most catholic diet of any known species (see Appendix).

OBJECTIVES

The objectives of this study were to provide species-level phylogenetic hypotheses for *Conus*, to investigate the origins of piscivory and molluscivory, and to analyse species-level relationships among the vermivorous species. We specifically address the following questions. (1) Are piscivores and molluscivores independent monophyletic groups, suggesting that these feeding modes each arose only once in the genus? (2) What were the feeding modes of the ancestral lineages that gave rise to fish- and mollusc-eating species? (3) Are vermivorous diets strictly static traits or is the exact nature of a vermivorous diet ever-changing? (4) When did molluscivory and piscivory evolve in *Conus* and what do molecular data tell us about the times of the origins of these traits?

MATERIAL AND METHODS

SPECIMENS

We obtained specimens from throughout the Indian and Pacific Oceans and a specimen of *Conus regius* from the western Atlantic. For Indo-West Pacific species, identifications are as in Röckel *et al.* (1995). Diet data for the species used in our analyses are summarized in the Appendix. *Strombus luhuanus* (Linné, 1758) and *Terebra subulata* (Linné, 1758) were used as outgroups. The former is presumably distantly related to *Conus*, while the latter is a member of the Superfamily Conoidea (Taylor, Kantor & Sysoev, 1993).

DNA MANIPULATION

We isolated DNA with a modified CTAB extraction protocol (Winnepenninckx, Backeljau & de Wachter, 1993). Tissue clips (~5 mg) from the foot were placed in 250 μl 2X CTAB buffer (100 mM Tris-HCl, pH 8.0; 1.4 M NaCl; 20 mM EDTA; 2% CTAB) and 0.5 mg proteinase K and incubated at 60°C for between 2 and 24 h. Samples were then centrifuged briefly to remove remaining tissue. Subsequent phenol/chloroform extractions and alcohol precipitations followed methods of Palumbi (1996a). DNA was resuspended in 50 μl water and 2 μl of the resuspension was electrophoresed and visualized on a 2% agarose gel.

We diluted the DNA 1:10 to 1:100 depending on the estimated quantity and quality of the extracted DNA. PCR conditions were 30–40 cycles at 94°C for 30 s, 52–55°C for 30 s, 72°C for 30 s for both 16S and calmodulin amplifications. General 16S primers (16sar = CGCCTGTTTATCAAAAAACAT and 16Sbr = ACGT-GATCTGAGTTCAGACCGG, Palumbi, 1996a) were

used to amplify a 540 bp region of the mitochondrial 16S gene. The 16Sar primer was biotinylated by the manufacturer (Operon, Inc.) for use in solid phase sequencing. Calmodulin primers (cal1=GCCAGCTGCARGAYATGATCAA, cal2=GTGTCCTTCATTTTNCKTGCCATCAT) were designed from exon sequences flanking a conserved intron position in *Aplysia californica* (Genbank accession number X64653 and X64654), *Drosophila melanogaster* (X05949 and X05950), and *Homo sapiens* (X52608). These primers span a conserved intron position flanked by 52 bp of exon sequence. Reactions were carried out in 50 and 25 μ l volumes for 16S and calmodulin amplifications respectively following methods of Palumbi (1996a).

We sequenced the 16S amplifications directly with solid phase sequencing (Palumbi, 1996b). Calmodulin amplifications yielded two loci, one containing a 300 bp intron and a second containing a 700 bp intron. Because it could be consistently amplified in most species, we targeted the smaller of the two loci. We ligated the amplification products from the calmodulin primers into T-tailed pBluescript II KS- (Marchuk *et al.*, 1991) and then transformed the ligations into competent *E. coli* cells. Colonies were screened through amplifications with M13 (CATTTTGCTGCCGGTCA, biotinylated for use in solid phase sequencing) and T3L (ATTAACCCCTCACTAAAGGGAAC) vector primers directly on colonies. The amplification products from colonies with properly sized inserts were then sequenced directly with solid phase sequencing as above.

SEQUENCE ALIGNMENT AND PHYLOGENETIC RECONSTRUCTION

Sequences were aligned by eye with the XESEE program (Cabot & Beckenbach, 1989). We reconstructed phylogenies from each data set and combined data sets with distance [MEGA (Kumar, Tamura & Nei, 1993) and PHYLIP (Felsenstein, 1993)] and/or maximum parsimony methods [PAUP* (Swofford, 2000)]. *Strombus luhuanus* and *Terebra subulata* were used as outgroups in the 16S phylogenetic reconstruction. *Conus distans*, the most basal species from the 16S phylogeny, was used as the outgroup for phylogenetic reconstruction from the calmodulin intron sequences and combined data. Gaps or missing data were only ignored among pairwise comparisons. Several algorithms were employed to estimate genetic distances including Kimura 2-parameter and Jukes-Cantor models. Levels of support for clades were estimated with bootstrap methods. Clades with bootstrap values greater than 50% were accepted as well supported; clades with bootstrap values less than 50% were collapsed.

We mapped feeding modes of species onto the phylogenies and interpreted the patterns of diet evolution among *Conus* species. Diets of species were classified

according to the major component of their diet – fish, molluscs, amphinomid polychaetes, other errant polychaetes, or sedentary polychaetes – based on feeding ecology studies by Kohn and others (see Appendix).

TIME SCALE DERIVATIONS

In order to estimate the times of origination of clades with distinct feeding modes, we applied time scales estimated from fossil and biogeographic data to trees constructed with an enforced molecular clock. We estimated maximum likelihood scores of the neighbor-joining trees constructed from 16S and calmodulin with the Treescor function in PAUP* (Swofford, 2000) and compared the likelihood scores estimated with and without an enforced molecular clock to determine if rates of divergence are not significantly 'unclocklike' among the data sets. The parameters used in the calculation of these scores were derived with Modeltest 3.0 (Posada & Crandall, 1998).

Several of the taxa in our data set have fossil records extending into the Miocene (Appendix). Another species, *Conus regius*, occurs in the western Atlantic and may be related to species in the Pacific. We used the ages of the oldest pairs of closely related species, based on deepest fossil records occurring in the Miocene, and species divergences across the Isthmus of Panama, to design time scales for the trees constructed with a molecular clock if the data used to construct the trees were not significantly unclocklike. We assume that if two lineages coexisted at a particular time, then the divergence of these lineages had to have taken place prior to that time. Because the time scales derived from fossil and isthmian divergence data are based on the times at which two lineages existed independently and not the actual date of divergence (which could be much earlier), they reflect the minimum time estimates of species originations and divergences. The branch-lengths from the maximum likelihood trees built with enforced molecular clocks were estimated with PAUP* (Swofford, 2000) and converted into time with calibrations from the fossil and transisthmian divergence data.

RESULTS

16S DATA

Approximately 450 bp of the 16S gene were obtained for 72 *Conus* species and the two outgroup species (Genbank accession numbers AF174140 through AF174213). In some cases, sequences were collected from more than one individual of a species; however, intraspecific sequence diversity was very low, even across huge geographic ranges (e.g. no sequence variation detected among individuals of *C. ebraeus* from Papua New Guinea to Hawai'i).

The average pairwise Kimura 2-parameter distance among species within the *Conus* ingroup, excluding *C. californicus*, was 12.4% and the range was 1.0–21.4%. The 16S sequence of *C. californicus* was very distant from that of all other *Conus* species, with individual pairwise distances ranging from 17.0 to 24.0% and an average genetic distance of 20.3% from all other *Conus* species – this latter value is just less than the average distance of the rest of the *Conus* ingroup to the outgroups (28.1% to *Strombus luhuanus* and 25.1% to *Terebra subulata*).

CALMODULIN DATA

Calmodulin intron sequences ranging in length from 261 to 300 bp were obtained from 73 species—68 *Conus* species which overlap with the 16S data set and one outgroup, *Terebra subulata* (Genbank accession numbers AF113252 through AF113321). Sequences from more than one specimen per species were only obtained for a few species. As was the case for 16S sequences, very little intraspecific sequence diversity was found among calmodulin intron sequences.

We were unable to align calmodulin intron sequences from *Conus californicus* and the outgroup species, *Terebra subulata*, with the sequences obtained from the rest of the *Conus* species. Based on the estimated dissimilarity of these species to the main *Conus* ingroup from the 16S data, inability to align the calmodulin intron sequences is probably due to the degree of genetic divergence between these species and lack of conservation within intron sequences compared to within 16S sequences. The unalignable sequences were excluded from further analyses.

Pairwise Kimura 2-parameter distances ranged from a minimum of 0.4% to a maximum of 17.5% for the calmodulin intron sequences and the average distance among species was 9.7%. On average, the distances estimated from calmodulin intron sequences were 78% less than the distances estimated from 16S sequences.

PHYLOGENETIC RECONSTRUCTION

Neighbor-joining was used to construct 16S, calmodulin intron, and combined data trees from Kimura 2-parameter distances among sequences; branches with bootstrap support less than 50% were collapsed (Figs 1–3). Other distance methods and tree building algorithms gave similar results (trees not shown). The phylogenetic reconstruction from combined data included only taxa in which sequences from both loci were obtained.

Many distinct clades were identified in each of the 50% majority rule bootstrap trees (Figs 1–3, see also Table 1): 15 in the 16S phylogeny, 14 in the calmodulin phylogeny, and 13 in the combined data phylogeny. Within these phylogenies, all clades supported by

bootstrap values greater than 50% were labelled on the branch leading to the deepest node of the clade according to the known feeding mode of its members (Figs 1–3); A = amphinomid-eaters, E = errant worm-eaters, F = fish-eaters, H = hemichordate-eaters, M = mollusc-eaters, and S = sedentary polychaete-eaters (diets of individual species are listed in the Appendix). Roman numerals are used to identify particular clades and these labels are conserved among all phylogenies to identify the same group of species; numbers with decimals denote the occurrence of a clade that contains less or different species in other phylogenetic reconstructions (e.g. clade F1.1 in Fig. 1 does not contain all species that occur in clade F1 in Figs 2 and 3).

Four clades (E1–E3, E5, and E6) contain the same sets of species in all phylogenies. The other clades are strongly supported in one data set, but only weakly supported in the other (A1, E4, F1, F2, F3, H1, M1, and S1). Two are unique to a single phylogeny (S2 and S3): clade S2 in the 16S phylogeny comprises *Conus arenatus* and *C. pulicarius*; calmodulin sequences were not obtained from *C. arenatus*. Clade S3 in the calmodulin phylogeny comprises *C. furvus* and *C. litteratus* and 16S sequences were not obtained from *C. furvus*. In all cases except clades A1, F3, and S1, the partially identified clades were found in the 16S phylogeny.

MAPPING OF DIETS

All of the mollusc-eating species cluster together in the calmodulin and combined data phylogenies (clade M1, Figs 2 and 3), although their relationships are much less resolved in the 16S phylogeny (clades M1.1–M1.3, Fig. 1). The fish-eating species cluster in three clades in the calmodulin and combined data phylogenies (F1–F3, Figs 2 and 3), but these clades are less resolved in the 16S phylogeny (F1.1, Fig. 1).

Half of the clades in our phylogenies comprised errant polychaete-eating species (clades A1 and E1–E6; Figs 1–3). Their broad distribution throughout the calmodulin phylogeny, especially those whose members specialize on Eunicidae, suggests that errant polychaetes are the ancestral diet of *Conus*. The species that prey on amphinomid polychaetes, a group of errant polychaetes known as fireworms, *Conus brunneus*, *C. imperialis*, and *C. regius* cluster together in the 16S and combined data phylogenies (clade E7; Figs 1, 3), although this clade is only partially resolved in the calmodulin phylogeny (clade E7.1, Fig. 2).

In all but a few cases, the clades of errant and sedentary polychaete-eating *Conus* species comprise species that tend to specialize on the same prey family (Table 1, Fig. 3). To compare diet composition more quantitatively, we calculated pairwise proportional similarity (PS_i ; Kohn & Riggs, 1982; also known as



Figure 1. Phylogenetic hypothesis for 72 *Conus* species reconstructed from Kimura 2-parameter distances among mitochondrial 16S rDNA sequences. *Strombus luhuanus* and *Terebra subulata* were used as outgroups. Bootstrap values greater than 50% are indicated on branches. Clades with bootstrap values less than 50% were collapsed. Clades are labelled according to the known feeding mode of its members: E=errant polychaete-eaters; F=fish-eaters, dashed branches; H=hemichordate-eaters; M=mollusc-eaters, fine branches; S=sedentary polychaete-eaters. Diets of individual species are listed in the Appendix. Numbers identify particular clades; numbers with decimals denote the occurrence of a clade that contains more or different species in other phylogenetic reconstructions. Species with undescribed diets are in italics.



Figure 2. Phylogenetic hypothesis for 70 *Conus* species reconstructed from Kimura 2-parameter distances among calmodulin intron sequences. *C. distans*, the most basal species in the 16S phylogeny, was used as the outgroup. Bootstrap values greater than 50% are indicated on branches. Clades with bootstrap values less than 50% were collapsed. Clades are identified as in Figure 1.

percentage similarity: Krebs, 1999) at the prey family level for all vermivorous species with adequate diet data ($N = 31$ species; diet samples with range of 11–847

and mean of 124 prey items). We then determined whether members of the vermivorous clades identified in Table 1 had more similar diets than expected from

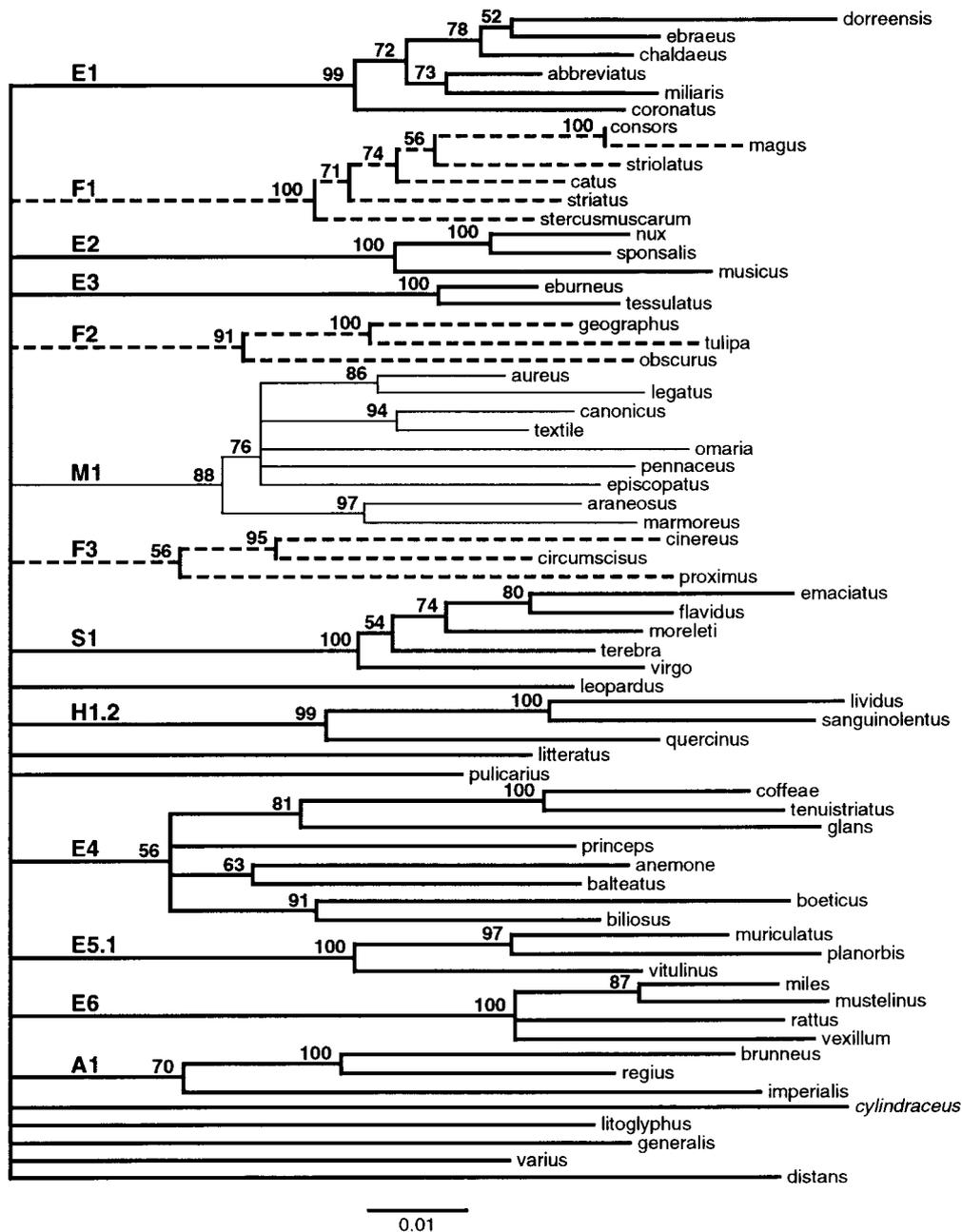


Figure 3. Phylogenetic hypothesis for 66 *Conus* species reconstructed from Kimura 2-parameter distances among combined mitochondrial 16S and calmodulin intron sequences. *C. distans*, the most basal species in the 16S phylogeny, was used as the outgroup. Bootstrap values greater than 50% are indicated on branches. Clades with bootstrap values less than 50% were collapsed. Clades are identified as in Figure 1.

the overall distribution of PS_1 values in the entire sample (Fig. 4). Of the 465 pairwise comparisons, those between non-clade members are much lower (mean $PS_1=0.29$; median $PS_1=0.17$, $N=428$), than those between clade members (mean $PS_1=0.66$; median $PS_1=0.72$, $N=37$). The mean values for each clade are

indicated in Figure 4. The diets of clade members are far more similar to each other than those of non-clade species pairs (Mann-Whitney U test: $U=3547$; $P<0.0001$).

The six species in clade E1 all prey primarily on errant polychaetes, predominantly (51–93%, mean =

Table 1. Bootstrap support for clades identified in phylogenetic reconstructions from 16S rRNA and calmodulin sequences and combined data and the diets of the species in these clades (see Figs 1–3 and Appendix)

Clade	Support, %			Diet
	16S	Calmodulin	Both	
E1	99	76	99	Errant polychaetes, mainly Eunicidae
E2	99	80	100	Errant polychaetes, mainly Nereidae
E3	100	80	100	Errant polychaetes
E4	100 ¹	69	56	Errant polychaetes, mainly Eunicidae
E5	95 ¹	98	100	Errant polychaetes, mainly Eunicidae
E6	100	97	100	Errant polychaetes, mainly Eunicidae
A1	67	78 ¹	70	Errant polychaetes, only Amphinomidae
S1	96	58 ¹	100	Sedentary polychaetes, mainly Terebellidae
S2	95	–	–	Sedentary polychaetes, mainly Capitellidae
S3	–	97	–	Sedentary polychaetes, mainly Maldanidae
H1	100 ¹	97 ²	99 ²	Hemichordates (<i>Ptychodera</i>)
F1	80 ¹	98	100	Fishes
F2	88 ¹	85	91	Fishes
F3	<50	100 ¹	56	Fishes
M1	<50	68	88	Gastropods

¹Not all species included.

²Sequences not available for all species in clade.

73%) of the family Eunicidae and secondarily (5–42%, mean = 21%) the Nereidae. Prey family similarity (PS_I) between species in this clade averaged 0.74 (range 0.57–0.92; *N* = 15 pairwise comparisons) (Fig. 4).

The three species in clade E2 specialize more on nereids (49–76% of diet, mean = 62%) and eunicids (23–49%, mean = 32%); like members of E1, they very rarely consume sedentary polychaetes. At one locality the errant polychaete *Lepidonotus* sp. (Family Polynoidae) comprised 35% of the diet of *C. sponsalis* (Kohn & Almasi, 1993). Prey family PS_I between pairs of species in this clade averaged 0.77 (range 0.73–0.85; *N* = 3) (Fig. 4).

The diets of the two species comprising clade E3 are known only from very small samples but appear quite diverse. *Conus eburneus* is known to prey on members of at least three errant (Eunicidae, Phyllodocidae, Glyceridae) and sedentary (Capitellidae, Orbiniidae,

Chaetopteridae) polychaete families. The only polychaetes recorded from *C. tessulatus* food samples were one nereid, one eunicid and one sigalionid, and both species are known to eat fishes occasionally as well (Kohn, 1968; Kohn & Nybakken, 1975; Reichelt & Kohn, 1985).

Eunicidae constitute the predominant (80–100%; mean = 90%) prey of four of the five species in clade E4 whose diets are known. The fifth, the southern Australian species *Conus anemone*, consumes similar numbers of eunicids and nereids. Pairwise prey family PS_I values among the three species in this clade for which data were adequate (i.e. samples of at least 11 identified food items) averaged 0.94 (range 0.91–0.97; *N* = 3) (Fig. 4).

Species in clades E5 and E6 also prey primarily or exclusively on eunicids. Diet data for only two members of E5, *Conus biliosus* and *C. vitulinus*, were adequate to include in the quantitative analysis; their family-level diet PS_I = 0.80. Clade E6 resembles E1 and E4 in that its members specialize on Eunicidae (64–100%, mean = 80%). Pairwise prey family PS_I among the three species in this clade for which data were adequate averaged 0.85 (range 0.77–1.00; *N* = 3) (Fig. 4).

Finally among the specialists on errant polychaetes, clade E7 comprises three species, each from a different geographic region, that probably prey exclusively on Amphinomidae, a family whose members are very rarely consumed by any other *Conus*. However, we had too few data for *C. brunneus* (Eastern Pacific) and *C. regius* (Caribbean) to include them in the matrix of PS_I values with *C. imperialis* (Indo-Pacific). *C. brunneus* and *C. imperialis* cluster together in the 16S and combined data phylogenies with *C. regius*, but only *C. brunneus* and *C. regius* cluster together in the calmodulin phylogeny.

Most of the specialists on sedentary polychaetes cluster together in a well supported clade in all phylogenies (S1, S1.1; Figs 1–3); all five of its members prey mainly on terebellids, as does *Conus frigidus*, in clade H1.1. In the smaller clades, the species whose diets are known prey mainly on Capitellidae (*C. pulicarius*, clade S2) and Maldanidae (*C. litteratus*, clade S3). Capitellids are also the main prey (33%) of *C. sanguinolentus*, a member of clade H1. Clade S2 in the 16S phylogeny (Fig. 1), contains a species, *C. arenatus*, that preys on both errant (Nereidae and Eunicidae) and sedentary (Maldanidae and Capitellidae) polychaetes. The latter two families each comprise about a third of its diet.

Three of the five species that include hemichordates in their diets cluster together in all phylogenies (clade H1 and H1.1, Figs 1–3). Of the other two, *Conus leopardus*, the only complete specialist on hemichordates, does not show close affinities to any other species in our data set. *Ptychodera* predominates in

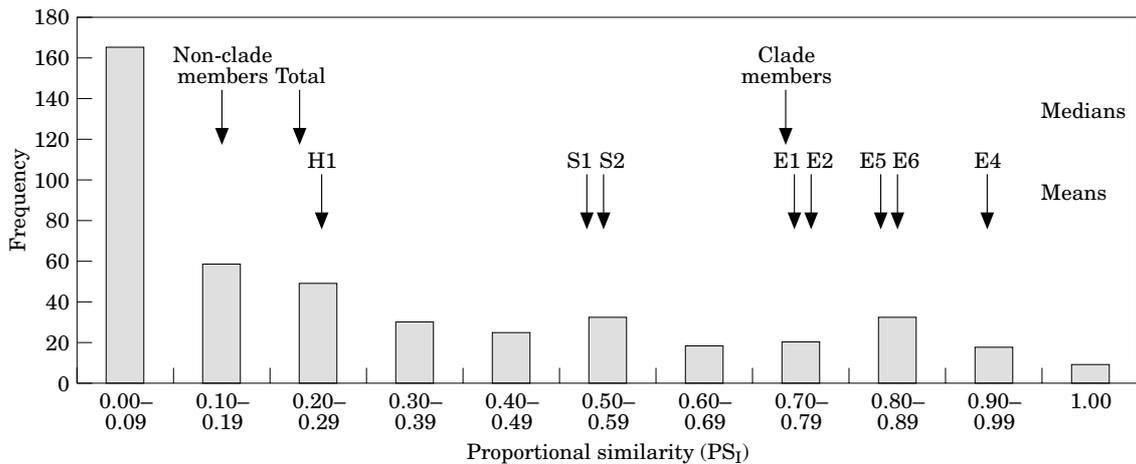


Figure 4. Frequency distribution of proportional similarity values (PS_1 of Kohn & Riggs, 1982) of composition of diets in nature at the prey family level, for all pairwise combinations of 31 vermivorous species of *Conus*. Arrows indicate median values of all data ($N=465$), non-clade members ($N=428$), and clade members ($N=37$), and mean values for the clades indicated in Figures 1–3. In clades E5, S1 and S2, $N=1$ and arrows indicate the single PS_1 values. Diets of species within clades are much more similar to each other than diets of species in different clades (U test: $P<0.0001$).

the diet of *C. quercinus*, is about one-third in *C. lividus*, and 7% in *C. sanguinolentus*. *C. flavidus*, whose diet includes 3% hemichordates, is firmly in clade S1, consistent with its preference for hemichordates. *C. frigidus*, not in the calmodulin data set, is a member of clade H1 as mentioned above, but it preys only on sedentary polychaetes, mainly capitellids (60%) and terebellids (30%). PS_1 values within this clade are thus lower (mean=0.26, range 0–0.87) than in the other vermivorous clades. Albeit with low bootstrap support (<50%) all sedentary polychaete and hemichordate-eating species cluster together in the uncollapsed calmodulin phylogeny (tree not shown) although this clade is not present in the uncollapsed 16S phylogeny and it includes clade E2 in the uncollapsed combined data phylogeny. Dietary data for predators of sedentary polychaetes were adequate to determine similarity values for only one species pair in each clade. In S1, whose members prey mainly on the family Terebellidae, $PS_1=0.50$ for *Conus emaciatus* and *C. flavidus*. In S2, whose members prey mainly on Capitellidae, $PS_1=0.52$ for *C. pulicarius* and *C. arenatus* (Fig. 4).

In the large single clade of *Conus* species that prey on other gastropods (M1), quantitative dietary data were adequate to calculate similarity values for only four of the nine species. *C. canonicus* feeds mainly on Nassariidae and Muricidae, *C. textile* on Vermetidae, *C. episcopatus* on Cypraeidae, and *C. pennaceus* on cephalaspidean opisthobranchs. Mean PS_1 was 0.10 and range 0–0.29, with the first two species listed overlapping the most. Unfortunately, information on the identity of the fishes eaten by piscivorous *Conus*

species in clades F1–F3 is too sparse for quantitative comparisons of any of their diets.

TIME SCALES

The likelihood scores of the trees constructed with and without an enforced molecular clock from the 16S data set were significantly different ($-\ln L=7698.32$ and 7113.61 for the trees constructed with and without an enforced molecular clock respectively, $df=72$, $P<0.0001$). The likelihood scores of the trees constructed with and without an enforced molecular clock from the calmodulin data were also significantly different ($-\ln L=3518.53$ and 3471.74 respectively; $df=68$, $P=0.03$), but the probability was much greater than that from the 16S data. In order to determine whether this significant result was due to the inclusion of a single taxon whose rate of evolution is different than those of the other taxa analysed, we individually excluded single taxa from our data set and estimated the likelihood scores of trees constructed with and without an enforced molecular clock. Likelihood scores from these trees were not significantly different only with the exclusion of *Conus varius* ($-\ln L=3489.36$ and 3449.58 for the trees constructed with and without an enforced molecular clock respectively, $df=67$, $P=0.14$), apparently due to a slower rate of evolution in this species (see Fig. 2). In the other cases of taxon exclusion, the likelihood scores from the trees constructed with and without an enforced molecular clock remained significantly different, with P -values less than 0.05. We therefore applied time scales to the calmodulin maximum likelihood tree constructed with

an enforced molecular clock and which excluded *C. varius* (Fig. 5).

Nine of the species used in these analyses have fossil records dating back to the Miocene: *Conus californicus*, *C. canonicus*, *C. eburneus*, *C. litteratus*, *C. lividus*, *C. magus*, *C. quercinus*, and *C. virgo*. The remaining species are confined to the Pliocene or Pleistocene or have no fossil record (see Appendix). Two of the Recent species with Miocene records are closely related, *C. lividus* and *C. quercinus* (Figs 2, 3); the Kimura 2-parameter distance between these taxa is 6.1%. The oldest known specimens of *C. quercinus* are from the Tjilalang Beds 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). On the other hand, the oldest record of *C. lividus* is from the Vigo Shale, Philippines (Dickerson, 1921), also probably Middle or Upper Miocene (Shuto, 1975), spanning an age of 5.2 to 12 Mya (Odin *et al.*, 1997). Thus the lineages that gave rise to these species coexisted at latest 11 Mya. Therefore, 6.1% is the minimum distance reflecting divergence either previous to or during the Middle Miocene at approximately 11 Mya and the rate of divergence over this period is less than or equal to 0.6%/Myr.

Conus brunneus (eastern Pacific) and *C. regius* (western Atlantic) are sister species (see Figs 1–3) that likely diverged with the emergence of the Isthmus of Panama (completed about 3 Mya) or somewhat earlier due to oceanographic changes associated with isthmus (Coates *et al.*, 1996; Knowlton *et al.*, 1993; Collins *et al.*, 1996). For example, divergence of alpheid shrimp across the Isthmus is thought to have spanned the time range of 3 to 7 Myr (Knowlton *et al.*, 1993). The genetic distance between these species is 4.7%. Assuming a time of divergence for these taxa of 3–7 Myr, the rate of calmodulin sequence divergence is 0.67–1.6%/Myr. Although more precise dates are needed to estimate time scales, we take the estimate of 0.6%/Myr as consistent with both calibration points.

ORIGINATION AND DIVERGENCE ESTIMATES OF CLADES

All of the mollusc-eating species cluster together in one well-defined clade in the calmodulin and combined data phylogenies (clade M1, Figs 2, 3, 5), indicating that mollusc-eating arose once in *Conus*. The time scale in Figure 5 indicates that this clade originated about 16 Mya (see also Table 2), in the Middle Miocene.

The fish-eating species cluster in three distinct clades in the calmodulin and combined data phylogenies (clades F1, F2, F3; Figs 2, 3, 5), but these clades are also only partially resolved in the 16S phylogeny (Fig. 1). Due to lack of resolution at deep nodes in the calmodulin and combined data phylogenies, it is

unclear how many times fish-eating arose; this should be examined with data from other molecular loci. If the species in clades F1 and F2 originated from a common ancestor, the time scale suggests that these lineages arose during the Lower Miocene, 21 Mya (Fig. 5, Table 2).

If piscivory arose three times during the evolutionary history of *Conus*, giving rise to three distinct clades of fish-eating species, then the time scale suggests that piscivory may have arisen as early as 5.7, 13, and 19 Mya (Upper to Lower Miocene) in clades F1, F2, and F3, respectively (Fig. 5, Table 2).

The worm-eating species cluster together by diet in all but a few cases as described above. The dates of originations of errant polychaete-eating clades are 11–24 Mya (Fig. 5, Table 2).

Assuming a common origin of species with diets of sedentary polychaetes and hemichordates, our time scale suggest that the sedentary worm-eating feeding mode may have arisen 22 Mya (Lower Miocene) (Fig. 5, Table 2). Within this group, the hemichordate-eating clade arose 11 Mya (Middle Miocene).

DISCUSSION

PHYLOGENY OF *CONUS*

The phylogenies reconstructed from the 16S, calmodulin, and combined data are generally congruent (Figs 1–3, Table 1). The main difference in the phylogenies is that the 16S phylogeny is much less resolved than the calmodulin phylogeny, and some branching patterns among closely related species differ. Because the calmodulin sequences diverge less among species than the 16S sequences, it is not surprising that the former is more robust. Many more sites among the 16S sequences are likely saturated for substitutions than in the calmodulin sequences, possibly as a result of slower rates of evolution within nuclear versus mitochondrial DNA. Because the combined data set and calmodulin phylogenies (Figs 2, 3) are nearly identical, lack of resolution in the 16S data does not conflict with the calmodulin data. Further phylogenetic resolution may be obtained from other loci, possibly longer introns (e.g. the 700 bp intron at the other calmodulin locus in *Conus*—see Material and Methods).

EVOLUTION OF DIET

The phylogenetic hypotheses presented here include numerous well supported clades of species with similar diets (Table 1), and our results thus indicate that the type of prey consumed is a rather conservative trait among *Conus* species. Because it is the most widespread diet phylogenetically, the data suggest that feeding on errant polychaetes is the ancestral feeding

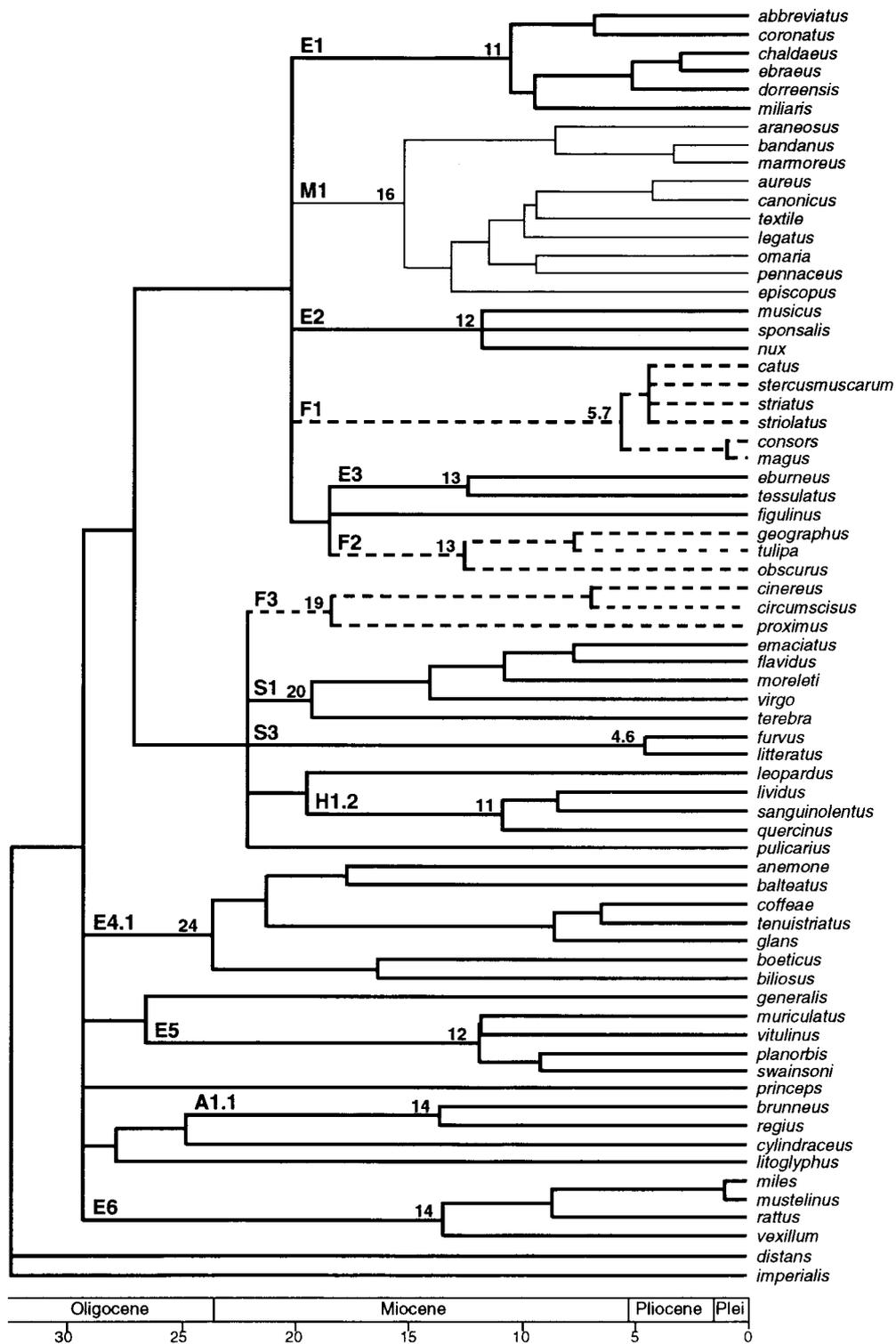


Figure 5. Phylogenetic hypothesis for 69 *Conus* species reconstructed from the topology in Figure 3 with maximum likelihood under an enforced molecular clock. Parameters used: transition-transversion ratio = 1.4336; base frequencies: A = 0.2447, C = 0.2143, G = 0.2181, T = 0.3229; proportion of invariable sites = 0; gamma distribution shape parameter = 1.3704. Time scale derived from the divergence of *C. lividus* and *C. quercinus* at 11 Mya. Clades are identified as in Figure 1. Values above branches leading to clades are estimates of the ages of the clades and were derived from the maximum likelihood branchlengths from the base of a clade to its terminal tips and the time scale.

Table 2. Age estimates of clades of *Conus* species with distinct feeding modes identified in the calmodulin phylogeny (Fig. 5). Geological age = earliest date at which two members of the clade coexisted, from fossil data (Appendix) or biogeographic data (dates from Harland *et al.*, 1990). Origination estimates given for ages for the time scale calculated from maximum likelihood branchlengths (see Results and Fig. 5). Mya = millions of years ago

Clade	Geological age, Mya	Origination estimate
E1	0.0–1.64	10.7
E2	0.0–1.64	11.9
E3	0.0–1.64	12.6
E4	1.64–5.2	23.9
E5	0.0–1.64	12.1
E6	0.0–1.64	13.7
A1.1	3.0	13.8
F1	1.64–3.40	5.74
F2	0.0–1.64	12.7
F3	0.0–1.64	18.7
H1*	11	11.0
M1	1.64–5.2	15.5
S1.1	0.0–1.64	19.6
S3	Recent	4.62
(F1 and F2)	1.64–3.40	20.5
(S) [H1.2, S1 and S3]	11	22.4

* Values used in derivation of the time scale.

mode of *Conus*. Feeding specialists on other gastropods (clade M1) and on several polychaete families, Terebellidae (S1), Nereidae (E2), and Amphinomidae (A1), probably arose only once, while fish-eating may have arisen two or three times. Specialization on the errant polychaete family Eunicidae may be plesiomorphic or may have arisen several times. The sequence divergence estimates suggest that specialization on gastropods, fishes, and some polychaete families arose within the Miocene and that all of this diversity in feeding ecology survived to the present.

If we consider sedentary polychaetes and hemichordates to be members of a functional group of sedentary worms, then all known sedentary worm-eating species cluster together in a single, though not well supported, clade in the uncollapsed calmodulin phylogeny and together with clade E2 in the uncollapsed combined data phylogeny (uncollapsed trees not shown). Bootstrap support for this clade is low in both trees, but its presence in the calmodulin phylogeny suggests that sedentary polychaete-eating only arose once. This hypothesis could be tested with data from other loci. The relationships among these clades

also suggest that hemichordate-eating species arose from sedentary polychaete-eating lineages.

Two of the clades of fish-eating species, the clade of mollusc-eating species, several clades of errant polychaete-eating species, and *Conus figulinus* cluster together in the calmodulin phylogeny (Figs 3, 5). The absence of this group in both the 16S and combined data phylogenies might be due to the lack of resolution provided by the 16S data, though data from other loci could confirm the validity of this cluster. This pattern of relationships suggests that both molluscivores and two of the three clades of piscivores shared a common ancestor that was likely a predator on errant polychaetes. The third clade of piscivores, clade F3, may have arisen from a sedentary worm-eating lineage (see Fig. 5), but the resolution of our data at this level is quite low. Again, data from other molecular loci could help determine the number of origins of piscivory within the genus.

Some species in our data set feed on both errant and sedentary polychaetes, although only in *C. arenatus* does the minority functional group comprise a substantial proportion of the diet (33%). In the others, *C. eburneus*, *C. lividus*, *C. miliaris*, and *C. sponsalis*, it accounts for less than 5% of the diet. These species fall out in several clades of vermivores (Figs 1–3), suggesting that the broader vermivorous diets of these species arose from errant polychaete-eating lineages.

Conus californicus, with its broad diet of fishes, gastropods, bivalves, crustaceans, and worms, is quite distant from all other *Conus* species (Fig. 1). Although its catholic diet and phylogenetic position might suggest that the ancestral diet of *Conus* was broad, *C. californicus* likely evolved its generalist diet separately from the origins of piscivory and molluscivory among other lineages, perhaps as a result of the absence of congeners in its geographic range (Kohn, 1966).

DIETS, RADULAE AND PHYLOGENY

The possession of a particular radula type is correlated with phylogeny in several cases, particularly among molluscivores, within clades of piscivores, and among amphinomid-eating species. The distinctness of the radular tooth and the evolutionary relationships among the fish-eating clades F1 and F2 indicate that the lineages that gave rise to these groups have had separate evolutionary histories and that possession of a particular radula type in these clades is more strongly correlated with phylogeny than diet. Moreover, the possession of similar radula types among species in the mollusc and amphinomid-eating clades likely results from the common ancestry of the species within these clades rather than from the convergence of radula types for preying on molluscs and amphinomids.

Endean & Rudkin (1965) distinguished two types of

radular teeth in piscivorous species, congruent with our clades F1 and F2. All species in clade F3 share the same radula type with F1 (Rolán & Raybaudi Massilia, 1994). Thus radula type among piscivores is probably a good indicator of phylogeny, particularly whether a species is related to species in clade F1 or F3 *vs* F2 in our calmodulin and combined data phylogenies (Figs 2, 3). For example, while *C. monachus* and *C. purpurascens* are either related to the species of clade F1 or F3, *C. cuvieri* is likely a member of clade F2 based on the radula types of these species (Endean & Rudkin, 1965) and the apparent robustness of radular characters in these clades. Moreover, we predict that other species that possess the amphinomid-eating type of radular tooth, *C. archon*, *C. bartschi*, *C. genuanus*, and *C. zonatus* (Nybakken, 1990), will prove to be most closely related to the species of clades A1 and A1.1 in our phylogenies and also to feed on amphinomids.

In a comparative morphometric analysis of the radular teeth of eleven molluscivorous *Conus* species, Nishi & Kohn (1999) differentiated three species groups based on a suite of four discrete and six continuous characters. Seven of these species are among the nine molluscivores in our phylogenetic trees, and in all cases the independent molecular and morphometric data sets are completely congruent. Of the two pairs of sister species in the trees, *C. araneosus* and *C. marmoreus* belong to Nishi and Kohn's Group A, and *C. canonicus* and *C. textile* to Group B. *C. omaria*, *C. pennaceus* and *C. episcopatus* are each other's closest relatives in the tree and comprise Nishi and Kohn's morphometric group C.

Among the other *Conus* clades, radula type does not appear to be closely correlated with diet or phylogeny. For example, *C. abbreviatus*, *C. litteratus*, and *C. leopardus* all possess the same radula type in the classification of Nybakken (1990), even though these species respectively prey on errant polychaetes, sedentary polychaetes, and hemichordates, respectively, and are all distantly related to each other (Fig. 2). Moreover, although most species in several vermivorous clades share similar radular types (e.g. S2, E2, and E6), many of the vermivorous clades are composed of species with different radular teeth (e.g. E1, E4, H1, S1, and S3). These results show that although in some cases radular tooth form is a good indicator of phylogeny, this is not always the case.

EVOLUTIONARY HISTORY

The evolutionary history of *Conus* revealed by the fossil record is highlighted by its origin in the Lower Eocene and major radiations in the Miocene and Pleistocene (Kohn, 1990). Application of the time scale derived from the divergence of *C. lividus* and *C. quercinus* shows that clades with distinct feeding modes

arose prior to the Lower Pliocene at the latest, in contrast to what was expected from the fossil records of the species in these clades (Table 2). In particular, all clades of errant polychaete-eating species, the mollusc-eating clade, two of the fish-eating clades, and most clades of sedentary polychaete-eating species arose at least 10–20 Mya (Table 2), during the Middle to Lower Miocene. The average origination date of all clades in our data set is 13.3 Mya.

For example, origination estimates imply that molluscivory arose prior to 15.5 Mya (Table 2), during the Middle Miocene. The fossil record supports this interpretation: two extant molluscivorous species, *C. canonicus* and *C. textile*, have been reported from Miocene deposits (Martin, 1879–1880; Van der Vlerk, 1931).

Piscivory may have originated 1–3 times in *Conus* and our results show that the three clades identified in our phylogenies appeared at latest during the Upper Miocene, 5.7 Mya (Table 2). Two extant piscivorous species, *C. achatinus* and *C. magus*, have been found in Middle Miocene deposits (Beets, 1941; Shuto, 1975). Thus our estimate of the origin of piscivory is also reasonable.

Although we have not included all *Conus* species in our phylogenetic reconstructions, our results also show that the current diversity of *Conus* is a result of major radiations in the Miocene and that the vast extinction of *Conus* in the Pliocene and subsequent radiation in the Pleistocene (Kohn, 1990) were not as dramatic as the fossil data suggest. It is likely that the evolutionary history of *Conus* as interpreted from the fossil record is affected by sampling biases, particularly in terms of the lack of information from Pliocene deposits in the Indo-Pacific region.

RAPID ECOLOGICAL EVOLUTION

Feeding mode appears to be an evolutionary conservative trait and diets have only rarely changed since the first major radiation of *Conus* in the Miocene. This pattern is similar to patterns of trophic diversifications among other organisms in which specializations occur shortly after the origination of the group or following a 'macroevolutionary lag' (*sensu* Jablonski & Bottjer, 1991). For example, palaeontological and phylogenetic studies show that most of the contemporary angiosperm (Crane, Friis & Pedersen, 1995) and avian (Chiappe, 1995) ecological and morphological diversity developed very early during the evolutionary histories of these groups. Richman & Price (1992) and Richman (1996) also found that feeding habits diversified only very early during the evolutionary history of warblers of the genus *Phylloscopus*. These patterns suggest that early radiations of species are coupled with rapid ecological diversification.

The phylogenetically related patterns of feeding biology in *Conus* revealed by this study suggest important aspects of the evolution of food habits in this genus. Because feeding mode is so conserved, one or several factors might constrain diet within lineages. These may be related to behavioural and physiological activities during prey searches, including chemosensory acuity and search image related to the detection and recognition of prey, and structural attributes, including shell gape, radula, and venom related to attack, capture, and consumption of prey. Genes that encode peptide neurotoxins that are expressed in the venoms of *Conus* have been shown to evolve very rapidly (Duda & Palumbi, 1999b); thus, it is unclear how venom components could limit dietary shifts. Also, because changes in feeding mode seem to have been confined to the Miocene radiations of this group, the origins of new diets may have been promoted by the evolution of key behavioural, physiological, morphological, and biochemical innovations.

ACKNOWLEDGEMENTS

We thank the individuals and institutions that have contributed or facilitated in the collection of specimens for this study, especially H. Conley and G.C. Fiedler, and also P.S. Armstrong, R. Cowie, K.A. del Carmen, A. Kay, C. Meyer, F. Moritzohn, G. Paulay, S. Romano, B. Smith, D. Strang, the Bernice P. Bishop Museum (Honolulu, Hawai'i), the Christensen Research Institute (Madang, Papua Niugini), and the University of Guam. We also thank D. Baum and D. Hartl for reviewing earlier manuscripts and F. Cipriano, L. Geyer, K.K. Ingram, S. Lavery, M. Hare, E. O'Brien, D. Plouffe and N. Singh for discussions. This work was supported by the Research Corporation of the University of Hawai'i and Organismic and Evolutionary Biology Department of Harvard University and grants from the Conchologists of America, Hawaiian Malacological Society, Lerner-Gray fund for marine research, Sigma-Xi, and the Western Society of Malacologists, and grants to Stephen R. Palumbi from the National Science Foundation.

REFERENCES

- Abrard R. 1946.** Fossiles néogènes et quaternaires des Nouvelles-Hébrides. *Annales de Paléontologie* **32**: 3–113.
- Beets C. 1941.** Eine jungmiocäne Mollusken-Fauna von der Halbinsel Mangkalihat, Ost-Borneo. *Overdruk uit de Verhandelingen van het Geologisch-Mijnbouwkundig Genootschap voor Nederland en Koloniën, Geologische Serie* **13**: 1–219.
- Beets C. 1950.** On Quaternary Mollusca from the islands of Boenjoe and Tarakan, E. Borneo. *Leidsche Geologische Mededeeling* **15**: 241–264.
- Beets C. 1986.** Molluscan fauna of the Lower Gelingseh Beds, Sangkulirang area, Kalimantan Timur (East Borneo). *Scripta Geologica* **82**: 1–82.
- Bouchet P, Warén A. 1980.** Revision of the north-east Atlantic bathyal and abyssal Turridae (Mollusca, Gastropoda). *Journal of Molluscan Studies, Supplement* **8**: 1–119.
- Bullen RA. 1901.** Pleistocene Mollusca from the raised beach deposits of Perim Island. *Proceedings of the Malacological Society of London* **4**: 254–255.
- Cabot EL, Beckenbach AT. 1989.** Simultaneous editing of multiple nucleic acid sequences with ESEE. *Computer Applications in the Biosciences* **5**: 233–234.
- Chiappe LM. 1995.** The first 85 million years of avian evolution. *Nature* **378**: 349–355.
- Coates AG, Jackson JBC, Collins LS, Cronin TM, Dowsett HJ, Bybell LM, Jung PM, Obando JA. 1996.** Closure of the Isthmus of Panama: the near-shore marine record of Costa Rica and western Panama. *Geological Society of America Bulletin* **104**: 814–828.
- Collins TM, Frazer K, Palmer AR, Vermeij GJ, Brown WM. 1996.** Evolutionary history of northern hemisphere *Nucella* (Gastropoda, Muricidae): Molecular, morphological, ecological, and paleontological evidence. *Evolution* **50**: 2287–2304.
- Cossmann M. 1900.** Faune Pliocénique de Karikal. *Journal de Conchyliologie* **48**: 14–66.
- Cox LR. 1927.** Neogene and Quaternary Mollusca from the Zanzibar Protectorate. In: *Report on the Palaeontology of the Zanzibar Protectorate*. Government of Zanzibar, 13–102.
- Cox LR. 1931.** The geology of the Farsan Islands, Oizan and Kamaran Island, Red Sea. Part 2. Molluscan palaeontology. *Geological Magazine* **68**: 1–13.
- Crane PR, Friis EM, Pederse KR. 1995.** The origin and early diversification of angiosperms. *Nature* **374**: 27–33.
- Cruz LJ, Corpuz G, Olivera BM. 1978.** Mating, spawning, development and feeding habits of *Conus geographus* in captivity. *Nautilus* **92**: 150–153.
- Dennant J, Kitson AE. 1902.** Catalogue of the described species of fossils (except Bryozoa and Foraminifera) in the Cainozoic fauna of Victoria, South Australia and Tasmania. *Records of the Geological Survey of Victoria* **1**: 89–147.
- Dickerson RE. 1921.** Notes on a fauna of the Vigo Group and its bearing on the evolution of marine molluscan faunas. *Proceedings of the California Academy of Sciences* **11**: 1–26.
- Duda TF Jr, Palumbi SR. 1999a.** Developmental shifts and species selection in gastropods. *Proceedings of the National Academy of Sciences USA* **96**: 10272–10277.
- Duda TF Jr, Palumbi SR. 1999b.** Molecular genetics of ecological diversification: Duplication and rapid evolution of toxin genes of the venomous gastropod *Conus*. *Proceedings of the National Academy of Sciences USA* **96**: 6820–6823.
- Durham JW. 1950.** 1940 E.W. Scripps Cruise to the Gulf of California. Part II. Megascopic paleontology and marine stratigraphy. *Geological Society of America Memoirs* **43**: 1–177.
- Eames FE. 1950.** On the ages of certain Upper Tertiary beds

- of peninsular India and Ceylon. *Geological Magazine* **87**: 233–252.
- Endean R, Rudkin C. 1965.** Further studies of the venoms of Conidae. *Toxicon* **2**: 225–249.
- Felsenstein J. 1993.** *Phylogeny inference package (PHYLIP)*, version 5.3c. Seattle: Dept. of Genetics, University of Washington.
- Glibert M. 1960.** Les Conacea fossiles du Cénozoïque étranger. *Institut Royal des Sciences Naturelles de Belgique, Mémoires, 2nd Ser.* **64**: 1–132.
- Harland WB, Armstrong RL, Cox AV, Craig LE, Smith AG, Smith DG. 1990.** *A geologic time scale 1989*. Cambridge: Cambridge University Press.
- Harris GF. 1897.** *Catalogue of the Tertiary Mollusca in the Department of Geology, British Museum (Natural History). Part I. The Australasian Tertiary Mollusca*. London: British Museum (Natural History).
- Jablonski D, Bottjer DJ. 1991.** The origin and diversification of major groups: environmental patterns and macroevolutionary lags. In: Taylor PD, Larson GP, eds. *Major Evolutionary Radiations*. Oxford: Clarendon Press, 17–57.
- Johnson CR, Stablum W. 1971.** Observations on the feeding behavior of *Conus geographus* (Gastropoda: Toxoglossa). *Pacific Science* **25**: 109–111.
- King L. 1953.** A Miocene marine fauna from Zululand. *Transactions of the Geological Society of South Africa* **56**: 59–91.
- Knowlton N, Weight LA, Solorzano LA, Mills DK, Bermingham E. 1993.** Divergence in proteins, mitochondrial DNA, and reproductive compatibility across the Isthmus of Panama. *Science* **260**: 1629–1632.
- Kohn AJ. 1959.** The ecology of *Conus* in Hawaii. *Ecological Monographs* **29**: 47–90.
- Kohn AJ. 1960.** Ecological notes on *Conus* (Mollusca: Gastropoda) in the Trincomalee region of Ceylon. *Annual Magazine of Natural History, Series 13* **2**: 309–320.
- Kohn AJ. 1963.** Venomous marine snails of the genus *Conus*. In: Keegan HL, MacFarlane WV, eds. *Venomous and poisonous animals and noxious plants of the Pacific area*. London: Pergamon Press, 83–96.
- Kohn AJ. 1966.** Food specialization in *Conus* in Hawaii and California. *Ecology* **47**: 1041–1043.
- Kohn AJ. 1968.** Microhabitats, abundance and food of *Conus* on atoll reefs in the Maldive and Chagos Islands. *Ecology* **49**: 1046–1062.
- Kohn AJ. 1978a.** The Conidae (Mollusca: Gastropoda) of India. *Journal of Natural History* **12**: 295–335.
- Kohn AJ. 1978b.** Ecological shift and release in an isolated population: *Conus miliaris* at Easter Island. *Ecological Monographs* **48**: 323–336.
- Kohn AJ. 1980.** *Conus kahiko*, a new Pleistocene gastropod from Oahu, Hawaii. *Journal of Paleontology* **54**: 534–541.
- Kohn AJ. 1981.** Abundance, diversity, and resource use in an assemblage of *Conus* species in Enewetak Lagoon. *Pacific Science* **34**: 359–369.
- Kohn AJ. 1987.** Intertidal ecology of Enewetak Atoll. In: Devaney DM, Reese ES, Burch BL, Helfrich PL, eds. *The Natural History of Enewetak Atoll, Vol. I, The Ecosystem: Environments, Biotas, and Processes*. Washington: US Dept. of Energy, 139–157.
- Kohn AJ. 1990.** Tempo and mode of evolution in Conidae. *Malacologia* **32**: 55–67.
- Kohn AJ. 1997.** Ecological and biogeographic attributes of *Conus* on the Indian Ocean's southernmost coral reefs. In: Wells FE, ed. *The marine flora and fauna of the Houtman Abrolhos Islands, Western Australia*. Perth: Western Australian Museum, 113–131.
- Kohn AJ. 1998.** Family Conidae. In: Beesly PL, Ross GJB, Wells A, eds. *The southern synthesis. Fauna of Australia, Vol. 5*. Melbourne: CSIRO Publishing.
- Kohn AJ, Almasi KN. 1993.** Comparative ecology of a biogeographically heterogeneous *Conus* assemblage. In: Wells FE, Walker DI, Kirkman H, Lethbridge R, eds. *The marine flora and fauna of Rottnest Island, Western Australia*. Perth: Western Australian Museum, 523–538.
- Kohn AJ, Arua I. 1999.** An early Pleistocene molluscan assemblage from Fiji: Gastropod faunal composition, paleoecology and biogeography. *Palaeogeography, Palaeoclimatology, Palaeoecology* **146**: 99–145.
- Kohn AJ, Hunter C (2001).** The feeding process in *Conus imperialis*. *Veliger* **44**: 232–234.
- Kohn AJ, Nishi M, Pernet B. 1999.** Snail spears and scimitars: a character analysis of *Conus* radular teeth. *Journal of Molluscan Studies* **65**: 461–481.
- Kohn AJ, Nybakken JW. 1975.** Ecology of *Conus* on eastern Indian Ocean fringing reefs: Diversity of species and resource utilization. *Marine Biology* **29**: 211–234.
- Kohn AJ, Perron FE. 1994.** *Life history and biogeography: Patterns in Conus*. Oxford: Oxford University Press.
- Kohn AJ, Riggs AC. 1982.** Sample size dependence in measures of proportional similarity. *Marine Ecology Progress Series* **9**: 147–151.
- Kosuge S. 1969.** Fossil mollusks of Oahu, Hawaii Islands. *Bulletin of the National Science Museum Tokyo* **12**: 783–794.
- Krebs CJ. 1999.** *Ecological methodology, 2nd ed.* Menlo Park: Addison Wesley Longman.
- Kumar S, Tamura K, Nei M. 1993.** *MEGA: molecular evolutionary genetics analysis, version 1.01*. University Park: Pennsylvania State University.
- Ladd HS. 1982.** Cenozoic fossil molluscs from western Pacific islands; Gastropods (Eulimidae and Volutidae through Terebridae). *United States Geological Survey Professional Papers* **1171**: 1–100.
- Leviton PJ. 1978.** Resource partitioning by predatory gastropods of the genus *Conus* on subtidal Indo-Pacific coral reefs: The significance of prey size. *Ecology* **59**: 614–631.
- Linnaeus C. 1758.** *Systema Naturae per Regna triae Naturae*, 10th ed. Stockholm.
- MacNeil FS. 1960.** Tertiary and Quaternary Gastropoda of Okinawa. *United States Geological Survey Professional Papers* **339**: 1–148.
- Marchuk D, Drumm M, Saulino A, Collins FS. 1991.** Construction of T-vectors, a rapid and general system for direct cloning of unmodified PCR products. *Nucleic Acids Research* **19**: 1154.

- Marsh H. 1971.** Observations on the food and feeding of some vermivorous *Conus* on the Great Barrier Reef. *Veliger* **14**: 45–53.
- Martin K. 1879–1880.** *Die Tertiärchichten auf Java, nach den Entdeckungen von F. Junghuhn, 28 Tafeln.* Leiden.
- McDowall J. 1974.** Cone predation. *Hawaiian Shell News* **22**: 3.
- Miller JA. 1989.** The toxoglossan proboscis: structure and function. *Journal of Molluscan Studies* **55**: 167–181.
- Nishi M, Kohn AJ. 1999.** Radular teeth of Indo-Pacific molluscivorous species of *Conus*: a comparative analysis. *Journal of Molluscan Studies* **65**: 483–497.
- Nomura S. 1935.** Catalogue of the Tertiary and Quaternary Mollusca from the Island of Taiwan (Formosa) in the Institute of Geology and Palaeontology, Tohoku Imperial University, Sendai, Japan. *Scientific Reports of the Tohoku Imperial University, Sendai, Japan, 2nd Series (Geology)* **18**: 53–228.
- Nomura S, Hatai K. 1935–1937.** A note of the fossil marine fauna from Okinawa-Zima, Ryukyu Group. *Transactions and Proceedings of the Palaeontological Society of Japan* No. 1–9.
- Nybakken J. 1970.** Correlation of radula tooth structure and food habits of three vermivorous species of *Conus*. *Veliger* **12**: 316–318.
- Nybakken J. 1978.** Population characteristics and food resource utilization of *Conus* in the Galapagos islands. *Pacific Science* **32**: 271–280.
- Nybakken J. 1979.** Population characteristics and food resource utilization of *Conus* in the Sea of Cortez and West Mexico. *Journal of Molluscan Studies* **45**: 82–97.
- Nybakken J. 1990.** Ontogenetic change in the *Conus* radula, its form, distribution among the radula types, and significance in systematics and ecology. *Malacologia* **32**(1): 35–54.
- Nybakken J, Perron FE. 1988.** Ontogenetic change in the radula of *Conus magus* (Gastropoda). *Marine Biology* **98**: 239–242.
- Odin GS, Montanari A, Coccioni R. 1997.** Chronostratigraphy of Miocene stages: A proposal for the definition of precise boundaries. In: Montanari A, Odin GS, Coccioni R, eds. *Miocene stratigraphy: An integrated approach.* Amsterdam: Elsevier, 597–629.
- Oostingh CH. 1938.** Die Mollusken des Pliocäns von Süd-Bantam in Java. *Ingenieur Nederl.-Indië* **5**: 17–33.
- Ostergaard JM. 1939.** Reports on fossil Mollusca of Molokai and Maui. *Occasional Papers of the Bernice P. Bishop Museum* **15**: 67–77.
- Palumbi SR. 1996a.** Nucleic acids II: the polymerase chain reaction. In: Hillis DM, Moritz C, Mable BK, eds. *Molecular Systematics, 2nd Edition.* Sunderland, Massachusetts: Sinauer Associates, 205–247.
- Palumbi SR. 1996b.** Macrospatial genetic structure and speciation in marine taxa with high dispersal abilities. In: Ferraris JR, Palumbi SR, eds. *Molecular Zoology: Advances, strategies, and protocols.* New York: Wiley-Liss, 101–117.
- Posada D, Crandall KA. 1998.** Modeltest: testing the model of DNA substitution. *Bioinformatics* **14**: 817–818.
- Reichert RE, Kohn AJ. 1985.** Feeding and distribution of predatory gastropods on some Great Barrier reef platforms. *Proceedings of the Fifth International Coral Reef Congress* **5**: 191–196.
- Richman AD. 1996.** Ecological diversification and community structure in the Old World leaf warblers (Genus *Phylloscopus*): a phylogenetic perspective. *Evolution* **50**: 2461–2470.
- Richman AD, Price T. 1992.** Evolution of ecological differences in the Old World leaf warblers. *Nature* **355**: 817–821.
- Röckel D, Korn W, Kohn AJ. 1995.** *Manual of the living Conidae. Vol. I, Indo-Pacific.* Wiesbaden: Christa Hemmen Verlag.
- Rolán E, Raybaudi Massilia G. 1994.** New investigation on the radular teeth of *Conus*. *Argonauta* **8**(1–6): 6–59; **8**(7–12): 7–68.
- Shuto T. 1975.** Preliminary correlation of the Neogene molluscan faunas in Southeast Asia. *Geology and Paleontology of Southeast Asia* **15**: 289–301.
- Stanley SM. 1979.** *Macroevolution.* San Francisco: W.H. Freeman.
- Stanton RJ Jr. 1966.** Megafauna of the Upper Miocene Castaic Formation, Los Angeles County, California. *Journal of Paleontology* **40**: 21–40.
- Stuart M. 1912.** The geology of the Henzada District, Burma. *Records of the Geological Society of India* **41**: 240–253.
- Swofford DL. 2000.** *Paup*. Phylogenetic analysis using parsimony (*and other methods). Version 4.* Sunderland, Massachusetts: Sinauer Associates.
- Tanaka T, Nobuhara T, Ozawa T. 1995.** First record of fossil *Cleobula betulina* (Gastropoda: Conidae) from the Upper Pliocene Dainichi Sand of the Kakegawa Group, Shizuoka Prefecture, central Japan and its paleoenvironmental significance. *Venus* **54**: 49–56.
- Taylor JD. 1978.** Faunal response to the instability of reef habitats: Pleistocene molluscan assemblages of Aldabra Atoll. *Palaeontology* **21**: 1–30.
- Taylor JD. 1985.** The anterior alimentary system and diet of *Turriculanellia spuria* (Gastropoda: Turridae). In: Morton B, Dudgeon D, eds. *Proceedings of the Second International Workshop on the Malacofauna of Hong Kong and Southern China.* Hong Kong: Hong Kong University Press, 175–190.
- Taylor JD, Kantor YI, Sysoev AV. 1993.** Foregut anatomy, feeding mechanisms, relationships and classification of the Conoidea (=Toxoglossa) (Gastropoda). *Bulletin of the Natural History Museum London (Zoology)* **59**: 125–170.
- Troschel FH. 1866.** *Das Gebiss der Schnecken Part 2.* Berlin: Nikolai.
- Van der Vlerk IM. 1931.** Caenozoic Amphineura, Gastropoda, Lamellibranchiata, Scaphopoda. *Leidsche Geologische Mededeelingen* **5**: 206–296.
- Walls JG. [1979].** *Cone shells: A synopsis of the living Conidae.* Neptune City: New Jersey: T.F.H. Publications.
- Winnepenninckx B, Backeljau T, de Wachter R. 1993.** HPLC purification of PCR products for direct sequencing. Extraction of high molecular weight DNA from molluscs. *Trends in Genetics* **9**: 407.

APPENDIX

Diet and paleontological records for the *Conus* species investigated in this study. For species determinations see Röckel *et al.* (1995). Diet type: M, molluscivorous; P, piscivorous; V, vermivorous. For vermivorous species, 'Main diet taxa' indicates the most common polychaete families or unsegmented worm group (e.g. enteropneusts) found in analyses of gut contents or faecal matter. Main diet taxon categories in parentheses are suspected based on radular tooth morphology (see Kohn *et al.*, 1999).

Species	Diet type	Main diet taxa	Earliest fossil
<i>C. arenatus</i> Hwass, 1792	V	eunicids, nereids, capitellids ⁶⁻⁹	Pleistocene ^{28,29}
<i>C. aulicus</i> Linné, 1758	M	gastropods ⁹	Pleistocene ³⁰
<i>C. aureus</i> Hwass, 1792	M	(gastropods)	Upper Pliocene ^{31,32}
<i>C. balteatus</i> Sowerby I, 1833	V	eunicids, nereids ^{2,6,8}	Lower Pleistocene ³³
<i>C. bandanus</i> Hwass, 1792	M	gastropods ^{1a}	–
<i>C. betulinus</i> Linné, 1758	V	capitellids ⁵	Upper Pliocene ³⁴
<i>C. biliosus</i> (Röding, 1798)	V	eunicids ^{5,6b}	–
<i>C. boeticus</i> Reeve, 1843	V	(polychaetes)	–
<i>C. brunneus</i> Wood, 1828	V	amphinomids ¹⁰	Lower Pliocene ³⁵
<i>C. bullatus</i> Linné, 1758	P	fishes ⁸	–
<i>C. californicus</i> Reeve, 1844	V,M	gastropods, bivalves, polychaetes ¹¹	Upper Miocene ³⁶
<i>C. canonicus</i> Hwass, 1792	M	gastropods ⁶	Miocene ³⁰
<i>C. catus</i> Hwass, 1792	P	fishes ^{1,6}	Pliocene ³⁷
<i>C. chaldaeus</i> (Röding, 1798)	V	eunicids, nereids ^{1-3,6-8,13,14}	Pleistocene ³⁸
<i>C. cinereus</i> Hwass, 1792	P	fishes ¹⁵	Pliocene ³⁰
<i>C. circumcissus</i> Born, 1778	P	(fishes)	Pleistocene ³⁰
<i>C. coffeae</i> Gmelin, 1791	V	eunicids ^{6c}	–
<i>C. consors</i> Sowerby I, 1833	P	fishes ¹⁶	–
<i>C. coronatus</i> Gmelin, 1791	V	eunicids, capitellids ^{5,6,8,12-14}	Pleistocene ^{38,39}
<i>C. cylindraceus</i> Broderip & Sowerby, 1833	?	–	Pleistocene ³⁹
<i>C. distans</i> Hwass, 1792	V	eunicids ^{1,2,6,7}	Pleistocene ⁴⁰
<i>C. dorreensis</i> Péron, 1807	V	eunicids ^{3,4}	Pleistocene ⁴¹
<i>C. ebraeus</i> Linné, 1758	V	eunicids, nereids ^{1,2,6-8,13,14}	Pleistocene ^{25,37,41,42}
<i>C. eburneus</i> Hwass, 1792	V,P	eunicids, capitellids ^{6,8,16}	Middle Miocene ³⁹
<i>C. emaciatus</i> Reeve, 1849	V	terebellids ⁶⁻⁸	Pleistocene ⁴³
<i>C. episcopatus</i> Hwass, 1792	M	gastropods ⁶	Pliocene ³⁰
<i>C. figulinus</i> Linné, 1758	V	polychaetes ¹²	Pliocene ^{44,45}
<i>C. flavidus</i> Lamarck, 1810	V	capitellids, terebellids ^{1,7,8,13}	Pleistocene ^{25,30,38,43,46,47}
<i>C. frigidus</i> Reeve, 1848	V	capitellids, terebellids ^{6-8,14}	–
<i>C. furvus</i> Reeve, 1843	?	–	–
<i>C. generalis</i> Linné, 1767	V	eunicids ¹⁵	Lower Pleistocene ³³
<i>C. geographus</i> Linné, 1758	P	fishes ^{17,18}	Pleistocene ^{25,39,48}
<i>C. glans</i> Hwass, 1792	V	eunicids ^{6,12}	Upper Pliocene ³¹
<i>C. imperialis</i> Linné, 1758	V	amphinomids ¹	Lower Pliocene ⁴⁹
<i>C. legatus</i> Lamarck, 1810	M	(molluscs)	–
<i>C. leopardus</i> (Röding, 1798)	V	enteropneusts ^{1,6,7,16}	Pleistocene ^{25,38}
<i>C. litoglyphus</i> Hwass, 1792	V	eunicids ⁶	Pleistocene ³⁸
<i>C. litteratus</i> Linné, 1758	V	capitellids ^{6,8,16}	Lower Miocene ^{45,50,51}
<i>C. abbreviatus</i> Reeve, 1883	V	eunicids, nereids ^{1,2}	Pleistocene ^{25,26}
<i>C. anemone</i> Lamarck, 1810	V	eunicids, nereids ^{3,4}	Upper Pliocene ²⁷
<i>C. araneosus</i> [Lightfoot], 1786	M	gastropods ⁵	–

continued

APPENDIX – continued

Species	Diet type	Main diet taxa	Earliest fossil
<i>C. lividus</i> Hwass, 1792	V	enteropneusts, terebellids ^{1,6-8,12,13,16}	Middle to Upper Miocene ^{32,52}
<i>C. magus</i> Linné, 1758	P	fishes ¹⁹	Middle Miocene ⁵³
<i>C. marmoreus</i> Linné, 1758	M	gastropods ^{7,8,16}	Pleistocene ³⁰
<i>C. miles</i> Linné, 1758	V	eunicids ^{1,2,4,6-8}	–
<i>C. miliaris</i> Hwass, 1792	V	eunicids, nereids ^{2,3,6-8,13,14,20}	Pleistocene ^{38,39}
<i>C. moreleti</i> Crosse, 1858	V	terebellids ¹⁵	Pleistocene ⁴⁰
<i>C. muriculatus</i> Sowerby I, 1833	V	eunicids, nereids ^{16d}	Pliocene ^{54d}
<i>C. musicus</i> Hwass, 1792	V	nereids ⁶⁻⁸	–
<i>C. mustelinus</i> Hwass, 1792	V	eunicids, nereids ^{6,8}	Pleistocene ³⁰
<i>C. nux</i> Broderip, 1833	V	nereids ^{21,22}	–
<i>C. obscurus</i> Sowerby I, 1833	P	fishes ²³	Pleistocene ³⁸
<i>C. omaria</i> Hwass, 1792	M	(gastropods)	Pleistocene ³⁸
<i>C. pennaceus</i> Born, 1778	M	gastropods ^{1,5,6}	Pleistocene ^{25,28}
<i>C. planorbis</i> Born, 1778	V	(polychaetes)	Pleistocene ³⁰
<i>C. princeps</i> Linné, 1758	V	eunicids ²¹	Upper Pliocene ³⁵
<i>C. proximus</i> Sowerby II, 1859	P	fishes ¹⁵	–
<i>C. pulicarius</i> Hwass, 1792	V	capitellids ^{1,16}	Pleistocene ²⁵
<i>C. quercinus</i> [Lightfoot], 1786	V	enteropneusts, sabellids ¹	Upper Miocene ³⁰
<i>C. rattus</i> Hwass, 1792	V	eunicids ^{1,2,6-8,12-14}	Pleistocene ^{25,38}
<i>C. regius</i> Gmelin, 1791	V	amphinomids ²⁴	–
<i>C. sanguinolentus</i> Quoy & Gaimard, 1834	V	cirratulids, other polychaetes ^{8,13}	–
<i>C. sponsalis</i> Hwass, 1792	V	nereids, eunicids ^{1-4,6-8,14}	Pleistocene ³⁸
<i>C. stercusmuscarum</i> Linné, 1758	P	(fishes)	–
<i>C. striatus</i> Linné, 1758	P	fishes ^{1,6}	Upper Pliocene ^{30,39}
<i>C. striolatus</i> Kiener, 1845	P	fishes ^{6e}	–
<i>C. swainsoni</i> Estival & von Cosel, 1986	V	(polychaetes)	–
<i>C. tenuistriatus</i> Sowerby II, 1858	V	(polychaetes)	Pleistocene ³³
<i>C. terebra</i> Born, 1778	V	terebellids ^{6,12f}	Pleistocene ^{38,39}
<i>C. tessulatus</i> Born, 1778	V	nereids, eunicids ^{7,8}	Pleistocene ^{29,38}
<i>C. textile</i> Linné, 1758	M	gastropods ^{1,6-8,12}	Pleistocene ³⁰
<i>C. tulipa</i> Linné, 1758	P	fishes ⁷	Pleistocene ²⁵
<i>C. varius</i> Linné, 1758	V	(polychaetes)	Pleistocene ³⁸
<i>C. vexillum</i> Gmelin, 1791	V	eunicids ^{1,2,6}	Pleistocene ^{28,29,38}
<i>C. virgo</i> Linné, 1758	V	terebellids ^{6,12}	Middle Miocene ⁵⁵
<i>C. vitulinus</i> Hwass, 1792	V	eunicids ^{1,2,8}	Pleistocene ^{25,48,56}

References to main diet taxa

¹Kohn (1959); ²Leviton (1978); ³Kohn & Almasi (1993); ⁴Kohn (1997); ⁵Kohn (1978a); ⁶Kohn & Nybakken (1975); ⁷Kohn (1968); ⁸Reichel & Kohn (1985); ⁹Röckel *et al.* (1995); ¹⁰Nybakken (1970); ¹¹Kohn (1966); ¹²Kohn (1960); ¹³Marsh (1971); ¹⁴Kohn (1987); ¹⁵Kohn (in prep.); ¹⁶Kohn (1981); ¹⁷Johnson & Stablum (1971); ¹⁸Cruz, Corpuz & Olivera (1978); ¹⁹Nybakken & Perron (1988); ²⁰Kohn (1978b); ²¹Nybakken (1978); ²²Nybakken (1979); ²³Kohn (1963); ²⁴V.O. Maes, pers. comm.

^alisted as *C. marmoreus*; ^blisted as *C. parvulus*; ^clisted as *C. scabriusculus*; ^dlisted as *C. sugillatus*; ^elisted as *C. ranunculus*; ^flisted as *C. clavus*.

References to earliest fossil appearance

²⁵Kosuge (1969); ²⁶Kohn (1980); ²⁷Dennant & Kitson (1902); ²⁸Cox (1931); ²⁹Glibert (1960); ³⁰Van der Vlerk (1931); ³¹Oostingh (1938); ³²Shuto (1975); ³³Kohn & Arua (1999); ³⁴Tanaka, Nobuhara & Ozawa (1995); ³⁵Durham (1950); ³⁶Stanton (1966); ³⁷Harris (1897); ³⁸Taylor (1978) + pers. comm.; ³⁹Ladd (1982); ⁴⁰Kohn (unpub. data); ⁴¹Kohn (1997); ⁴²Nomura & Hatai (1935–1937); ⁴³Bullen (1901); ⁴⁴Cossmann (1900); ⁴⁵Eames (1950); ⁴⁶Nomura (1935); ⁴⁷Ostergaard (1939); ⁴⁸MacNeil (1960); ⁴⁹Cox (1927); ⁵⁰Stuart (1912); ⁵¹King (1953); ⁵²Dickerson (1921); ⁵³Beets (1941); ⁵⁴Abroad (1946); ⁵⁵Beets (1986); ⁵⁶Beets (1950).