



Comparative biology of *Conus* in the light of phylogeny: a preliminary report

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ABSTRACT In this paper I summarize past interspecific comparative studies of *Conus*, and indicate how interpretation of the results differs in the absence of phylogenetic information from that now possible with a recently available phylogenetic hypothesis based on molecular genetic data. I first briefly review the origin of *Conus*, including when, where and from what ancestors the genus arose, and its subsequent evolutionary history, then summarize the results of comparative analyses of food and habitat resource use, and I examine whether these patterns, as well as those of larval development reflect a phylogenetic signal.

RIASSUNTO In questo articolo riporto una revisione dei passati studi di comparazione interspecifica su *Conus*, ed indico come l'interpretazione dei risultati differisce in assenza di informazioni filogenetiche. Tali informazioni sono ora disponibili anche grazie alle ipotesi filogenetiche recentemente proposte sulla base di dati molecolari. Esamino inizialmente l'origine del genere *Conus*, includendo quando, dove e da quale antenato il genere è sorto, e la sua successiva storia evolutiva. Quindi passo in rassegna i risultati delle analisi comparative su alimentazione e uso delle risorse di habitat, ed esamino se questi pattern, così come quelli sullo sviluppo larvale, riflettono un segnale filogenetico.

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INTRODUCTION

The Neogastropoda that this symposium celebrates contains the largest and most widely distributed genus of marine molluscs and probably of marine invertebrates, *Conus*, a taxon with several unusual attributes: 1) Its radular structure and function differ from those of other molluscs in that each radular tooth functions independently as a hollow hypodermic needle that is injected into prey organisms. 2) Conotoxins, small powerfully neurotoxic peptides, are injected through the radular tooth to overpower the prey. 3) The shape and structure of the shell differs markedly from those of its closest relatives; it is conical or biconic with a thick, strong outer whorl but with inner walls nearly completely dissolved.

Conus is a particularly important genus, especially in tropical regions, for several reasons: 1) It is the most diverse genus of marine molluscs, with more than 500 extant species, and very large numbers of species often co-occur in the same habitat, enhancing marine biodiversity in these communities. 2) The genus is very widely distributed, and some species occupy the entire Indo-West Pacific region, an area comprising one-fourth of the world ocean. 3) It can be very abundant, up to 40/m² but usually two orders of magnitude less dense. 4) Because of its carnivorous habits and abundance, *Conus* is ecologically important, especially in coral reef-associated communities. 5) The high specificity of conotoxins for particular receptor proteins and their small size and ease of characterization and synthesis makes them particularly useful for neurobiological and medical applications. 6) The shells of *Conus* are durable and well preserved in the fossil record, since its first appearance in the Lower Eocene, about 55 million years ago. Its large number of species combined with its geologic youth indicate a higher rate of evolutionary diversification than for any other marine gastropod group (STANLEY, 1979).

In this report I briefly review three topics, the origin and

subsequent evolutionary history of *Conus* to set the historical context, and two aspects of its comparative biology, use of habitat and food resources. All previous of comparative studies of *Conus* (e.g. KOHN, 1959, 1968, 1981, 1997, 2001; KOHN and ALMASI, 1993; KOHN & NYBAKKEN, 1975; NYBAKKEN, 1979) were constrained by having to treat all species as independent entities, because their phylogenetic relationships were unknown. Now for the first time, an objective, species-level phylogenetic hypothesis is available, based on the pioneering work of Thomas Duda, who has elucidated the nucleotide sequences of two genes in about 70 species (DUDA & PALUMBI, 1999; DUDA, KOHN & PALUMBI, 2001). We can thus begin to investigate how the similarity of species along ecological gradients relates to their phylogenetic affinities. In this preliminary account, I evaluate whether species that are more closely related according to the molecular phylogenetic hypothesis are more similar ecologically, that is with respect to their use of micro-habitat types and prey species.

ORIGIN AND SUBSEQUENT EVOLUTIONARY HISTORY OF CONUS

The oldest known species is *Conus concinnus*, described by James de Carle Sowerby in 1821 from the Lower Eocene of England. *C. rouaulti* D'Archiac, 1850, described from France, is very similar and probably contemporaneous. An uncritical compilation of the paleontological literature of *Conus* (KOHN, 1990; from which most of the following account is summarized) indicated that the first real radiation of the genus occurred in the Middle Eocene and that by Late Eocene it had spread widely around the world. More than 100 *Conus* species are known from Eocene strata. Some persisted from early in the epoch, but 75% of Late Eocene species originated then. The prevailing warm ocean



temperatures and open Tethys Sea probably played roles in this diversification in species richness and enhanced geographic range. During the cooler Oligocene epoch, both the numbers of species and the geographic range of the genus contracted. Only about 70 species are known, and the northernmost representatives of the genus, in present Denmark and Washington State, became extinct. In the Miocene, *Conus* blossomed with a wealth

of new forms. More than 300 Miocene species are known, and there were one or more major radiations. According to the fossil record, Europe continued to support the highest species richness of *Conus* in the Miocene. Then the seas cooled again, species richness dropped again, and the Indo-Pacific region became the center of again burgeoning diversity in the Pleistocene and Recent (Kohn, 1985). Overall, the temporal pattern of *Conus* diversity in the Cenozoic is a general increase, with times of rapid radiation punctuated by periods of reduced diversity. Extrinsic physical factors are the most likely causes of these patterns, because they closely parallel those shown by other gastropods and other invertebrate groups during Cenozoic time (e.g. RAUP, 1976).

COMPARATIVE HABITAT RESOURCE USE

Conus species occupy many different marine environments from the intertidal zone to depths of nearly 600m, but most comparative data derive from studies of coral reef-associated habitats, different types of which support different numbers of co-occurring species. The extensive sandy bottoms of atoll and barrier reef lagoons typically harbor the fewest species (1-8; mean=3). Topographically smooth, environmentally harsh intertidal benches support 6-9 (mean 8), typically support a set of small (maximum shell length 20-30mm) species that is quite constant in species composition throughout the Indo-Pacific region. As has long been known (Kohn, 1967) topographically complex, subtidal coral reef platforms, with many different microhabitats, have the most diverse assemblages (9-36 species;

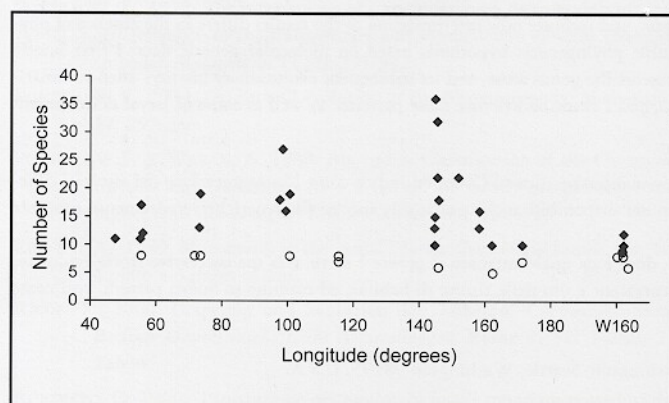


Fig. 1. Number of species of *Conus* on subtidal complex reef platforms (solid diamonds) and intertidal smooth limestone benches (open circles) in the Indo-West Pacific region, plotted against longitude. For reef platforms (N=26), each point represents a census of 22-650 (mean=197) individuals. For benches (N=13), each point represents a census of 38-414 (mean=133) individuals. Data from KOHN (1967, 1971, 2001).

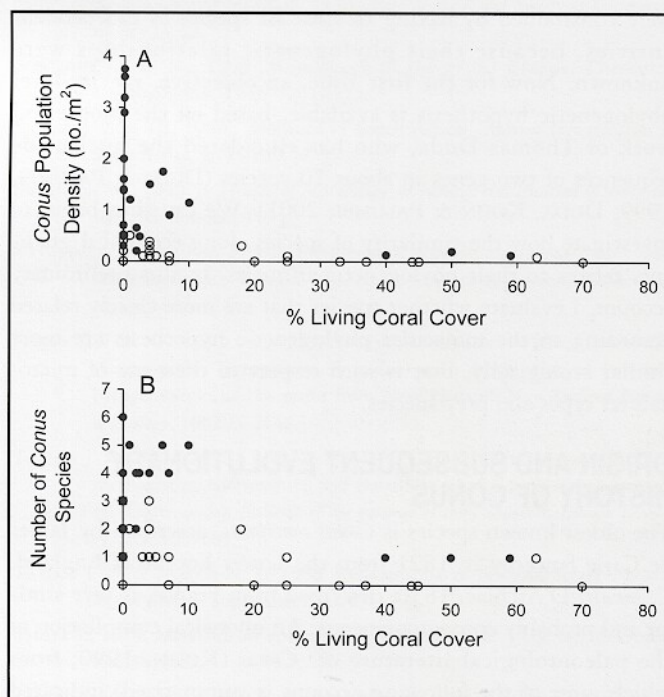


Fig. 2. Total *Conus* population density (A) and species richness (B) plotted against per cent cover of living coral. Both are negatively and highly significantly ($P < 0.001$) correlated with living coral cover. A: $r_s = -0.70$; B: $r_s = -0.59$; $N = 54$. Solid circles indicate data from Micronesia; open circles indicate data from Australian Great Barrier Reefs. Modified from KOHN (1983).

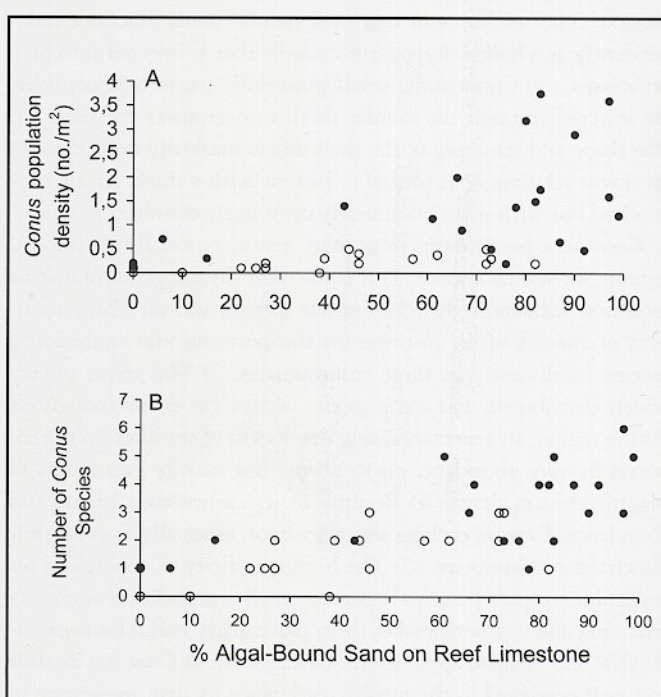


Fig. 3. Total *Conus* population density (A) and species richness (B) plotted against per cent cover of algal-bound sand on reef limestone. Both are positively and highly significantly ($P < 0.001$) correlated with cover of algal-bound sand on reef limestone. A: $r_s = 0.83$; B: $r_s = 0.84$; $N = 54$. Solid circles indicate data from Micronesia; open circles indicate data from Australian Great Barrier Reefs. Modified from KOHN (1983).



mean=16). Species richness is thus much more variable on reef platforms, and while species composition differs increasingly with distance in both, mean species turnover on reefs is 55% but only 39% on benches throughout the Indo-Pacific region (Kohn, 1997). There is also a strong longitudinal pattern of species richness on reefs, with maximum species richness in Papua New Guinea, declining to both the east and west, but no such pattern on benches (Fig. 1).

These striking between-habitat differences in *Conus* species richness also extend to within-habitat use of different microhabitats on coral reef platforms. These are complex, biogenic mosaics of different microhabitats, where both species richness and abundance reflect the availability of specific substrate types. Based on a series of transect studies that measured abundance and diversity of all *Conus* species collectively relative to available microhabitats (Kohn, 1983), the substrate on the reef favored least by *Conus* is living coral (Fig. 2). Its nematocysts sting the bare feet of the gastropods and thus deter treading on it, and it is an unfavorable microhabitat for prey organisms of *Conus* as well. The most favored substrate is a layer of sand bound by filamentous algae on

reef limestone, favorable both for shelter and food (Fig. 3).

Also within habitats, different *Conus* species vary both with respect to specific microhabitat types occupied and along the gradient between specialized and generalized use of these types. Fig. 4 illustrates how frequently different *Conus* species are found on different substrates, using the 12 species with the largest samples as examples. The most commonly used substrate types are listed at the right, arranged from the softest, in the foreground, to the hardest. Species are arranged from specialists on soft substrates at the left, through generalists toward the center, to specialists on hard substrates at the right. The histograms clearly show gradients of substrate type use: for example, *C. litteratus* and *C. leopardus* are specialists found almost exclusively on large patches of sand on reef platforms, *C. coronatus*, also on sand but typically in small pockets in reef limestone, *C. pennaceus*, on sand under coral rocks, and *C. rutilus* on bare reef limestone. *C. miliaris*, *C. lividus* and *C. flavidus* are much more generalized in their use of substrate types, and the other species shown are intermediate between the specialist and generalist categories.

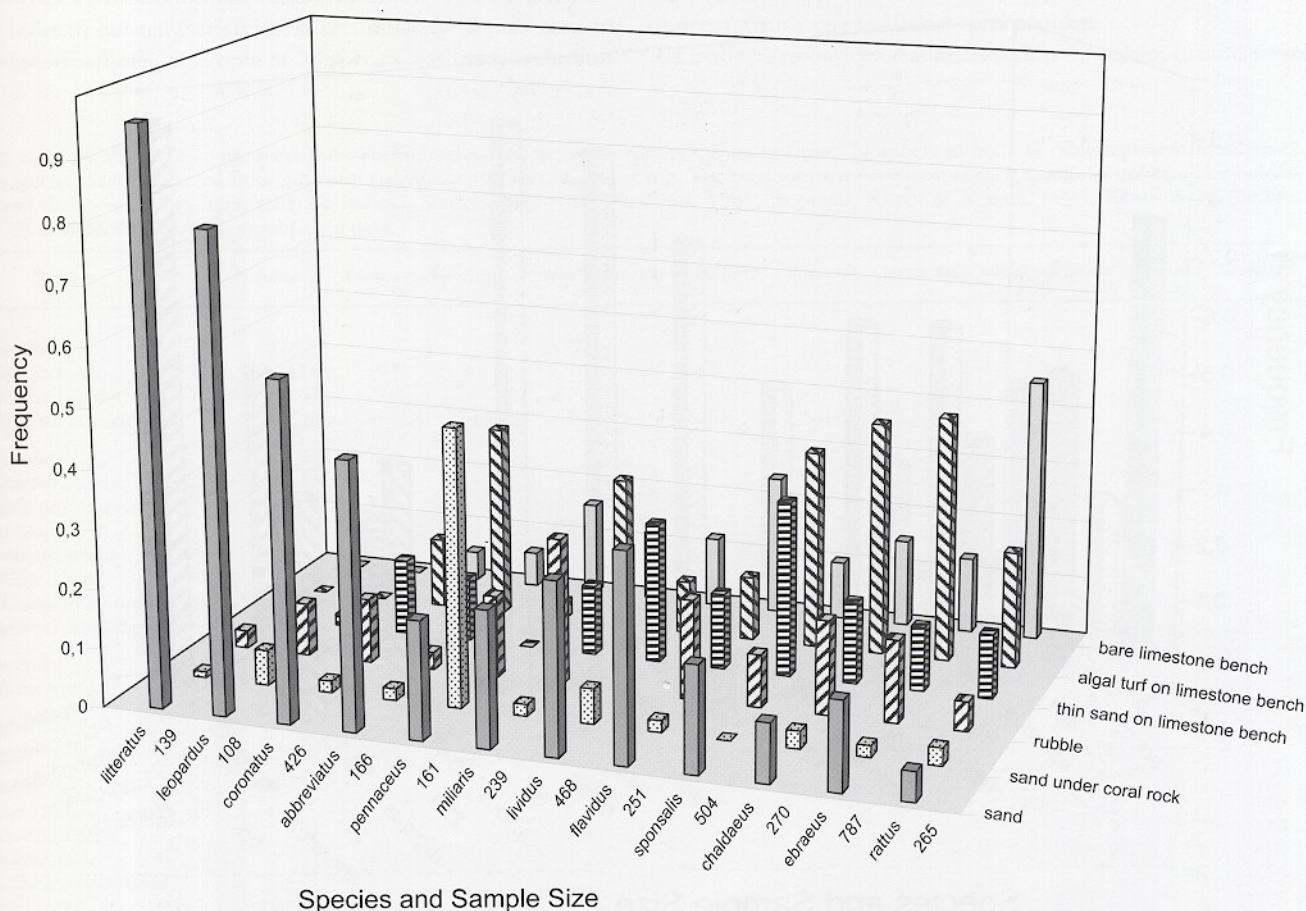


Fig. 4. Proportions of different substrate types used by 12 common *Conus* species in Indo-West Pacific coral reef-associated habitats. Sample sizes are to left of each species name. Bars represent the proportions of each substrate indicated at right used by each species. Species are arranged from those that occupy mainly soft substrates at left to those that occupy mainly hard substrates at right. Data are compiled from sources given in Table 1.



COMPARATIVE FEEDING BIOLOGY

Most *Conus* species prey exclusively or nearly so on members of only one major prey taxon: polychaete annelids, other gastropods, or fishes. Most species are in the first group, but a few of them also take unsegmented worms, particularly enteropneusts and echiurans. One species, *C. leopardus*, appears to prey only on the enteropneust *Ptychodera flava*. Within the predominant group of vermivorous species, co-occurring congeners have long been known to specialize on different taxa of polychaetes (Kohn, 1959, 1968; Kohn & Nybakken, 1975). Table 1 summarizes the diets of the 12 commonest vermivorous species from Indo-West Pacific reef-associated habitats, and Fig. 5 shows the high degree of specialization at the prey family level of the 11 species with the largest samples of identified prey organisms ($N > 50$).

Of the species shown in Fig. 5, some of the specialists on errant polychaetes prey more specifically on members of the family Eunicidae (*C. rattus*, *C. miliaris*, *C. miles*), one eats primarily nereids (*C. musicus*), and others eat both in similar proportions (*C. sponsalis*, *C. ebraeus*). Not shown because of smaller sample sizes are specialist predators of Amphinomididae (*C. imperialis*, *C. zonatus*). The commonest specialists on sedentary polychaetes are

typically those *Conus* species that occupy soft substrates (compare Figs. 4 and 5). They prey mainly on members of the family Terebellidae (*C. frigidus*, *C. lividus*) and Capitellidae (*C. frigidus*, *C. litteratus*). Only *C. lividus* eats a substantial number of enteropneusts, and *C. leopardus* (not shown because of smaller sample size) feeds exclusively on them.

COMPARATIVE BIOLOGY IN THE LIGHT OF PHYLOGENY

The first species-level phylogenetic hypothesis of *Conus*, based on cladistic analysis of nucleotide sequences of the mitochondrial 16S rRNA gene and an intron located in a nuclear calmodulin locus, is now available for about 70 species (Duda & Palumbi, 1999; Duda, Kohn & Palumbi, 2001). The latter paper provides a tree based on both genes that is less well resolved than that for either alone but includes 13 clades comprising more than one (2-9) species, onto which feeding groups, based on Fig. 5, Table 1, and data for smaller samples, were mapped. These results may be summarized as follows:

In seven clades the *Conus* species whose diets are known feed primarily on errant polychaetes (designated as "E" clades). Five of these comprise specialists on eunicids: E1: (*C. coronatus*, ((*C. miliaris*, *C. abbreviatus*) (*C. chaldaeus* (*C. ebraeus*, *C. dorreensis*))));

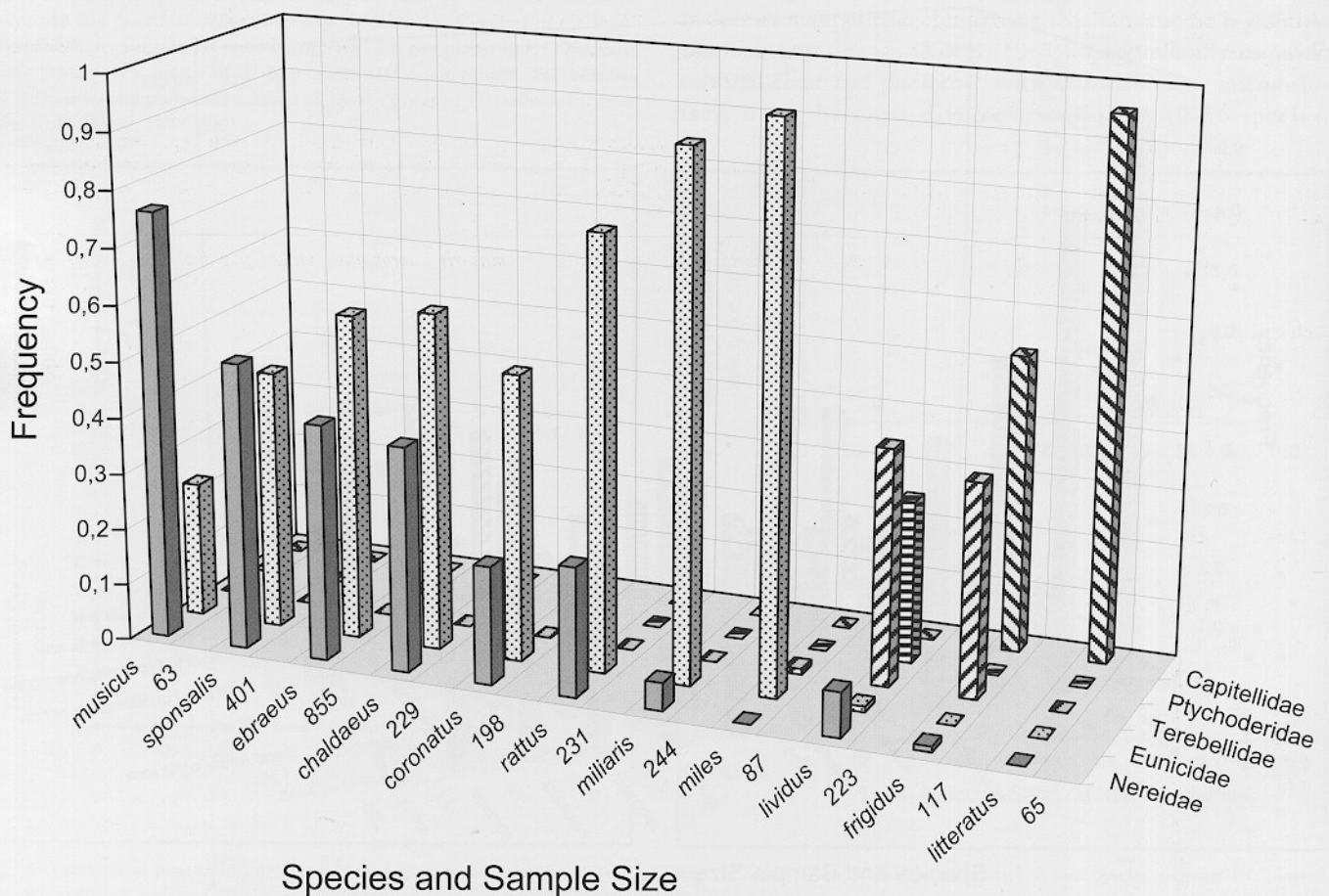


Fig. 5. Proportions of the most important prey families used by 11 common vermivorous *Conus* species in Indo-West Pacific coral reef-associated habitats. Sample sizes are to left of each species name. Bars represent the proportions of each prey family indicated at right consumed by each species. Species are arranged from those that prey mainly on errant polychaetes at left to those that prey mainly on sedentary polychaetes and a hemichordate at right. Data are compiled from sources given in Table 1.



E3: *C. eburneus*; E4: *C. biliosus*, (*C. balteatus*, *C. anemone*) *C. princeps*, and *C. glans*; E5: *C. vitulinus*; E6: *C. vexillum*, *C. rattus* (*C. mustelinus*, *C. miles*). Clade E2 comprises specialists on nereids: (*C. musicus* (*C. nux*, *C. sponsalis*)), and Clade E7, specialists on amphinomids: (*C. imperialis* (*C. regius*, *C. brunneus*)).

One clade (S1) comprises species that specialize on sedentary polychaetes of the family Terebellidae (*C. virgo* (*C. terebra* (*C. moreleti* (*C. flavidus*, *C. emaciatu*))))). Clade S2, (*C. quercinus* (*C. sanguinolentus*, *C. lividus*)) includes species that prey, respectively, on several polychaete families and *Ptychodera*, Capitellidae, and Terebellidae and *Ptychodera*.

Three clades comprise species that prey on fishes, F1: (*C. ster-cusmuscarum* (*C. striatus*, (*C. catus* (*C. striolatus* (*C. magus*, *C. consors*))))); F2: (*C. obscurus* (*C. geographus*, *C. tulipa*)); and F3: (*C. proximus* (*C. circumcissus*, *C. cinereus*)). Finally, all of the molluscivorous species tested comprise one clade (M1): (*C. marmoreus*, *C. araneosus* (*C. episcopus*, *C. pennaceus*, *C. omaria* (*C. textile*, *C. canonicus*) (*C. legatus*, *C. aureus*))). Molluscivorous *Conus* all prey exclusively on other gastropods, primarily other prosobranchs, but as in the case of the vermivores, they tend to specialize on members of different prey families (e.g. KOHN & NYBAKKEN, 1975). The diets of the four molluscivorous species with the largest identified prey samples (N=12-79), overlapped by only 0-29% (mean=10%) with respect to prey families.

In order to quantify the relation of similarity of prey taxa to phylogenetic affinity, a sample of 28 species was used, including

those represented by smaller sample sizes (N=10-49) than those shown in Table 1 and Fig. 5. The euclidean distances of all pairwise comparisons of the proportions of each prey family in the diet were determined in a proximity matrix. Clade members are much more similar to each other with respect to the proportions of each prey family in the diet (mean distance = 0.49; median = 0.48) than are non-clade members (mean distance = median distance = 0.90) (U test: $z = 5.2$, $P < 0.0001$). Species in the same clade are thus significantly more likely to have similar dietary requirements or preferences at the family level than are species in different clades.

As in the analysis of prey taxa used, similarity of microhabitat use was related to phylogenetic affinity. The sample comprised 36 species, including those represented by smaller sample sizes (N=10-64) and thus not shown in Fig. 5. The euclidean distances of all pairwise comparisons were again determined in a proximity matrix, and the values of clade members (mean = 0.55; median = 0.47) and non-clade members (mean = 0.79; median = 0.75) differed at the 0.0001 level (U test: $z = 5.1$). Species in the same clade are thus significantly more likely to occupy similar substrates than are species in different clades. For example, four of the six species in clade E1 primarily utilize algal turf on limestone benches, both members of Clade E3 occur on broad expanses of sand, and five of the nine members of Clade M1 and three of the six in Clade F1 occur mainly on sand under coral boulders.

Finally, I reconsider the relationship of developmental mode,

Tab. 1 - Prey of the 12 commonest Indo-Pacific reef-associated vermivorous species of *Conus*. Numbers in body of table represent numbers of prey species at left recovered from guts of the predator species of *Conus* at top. The predominant prey item of each species is indicated by boldface, and the second most important, by italics. Sources: KOHN (1959, 1968, 1981, in press), KOHN & ALMASI, 1993; KOHN & NYBAKKEN (1975), LEVITEN (1976), and unpublished data.

Species	<i>Conus</i>	<i>sponsalis</i>	<i>ebraeus</i>	<i>chaldaeus</i>	<i>rattus</i>	<i>distans</i>	<i>vexillum</i>	<i>miliaris</i>	<i>coronatus</i>	<i>miles</i>	<i>frigidus</i>	<i>flavidus</i>	<i>lividus</i>
Nereidae													
<i>Nereis jacksoni</i>		116	93		6					4			
<i>Perinereis singaporiensis</i>		67	272			21							
<i>Platynereis dumerilii</i>		26			154	1				1			16
Eunicidae													
<i>Lumbrineris sarsi</i>		38											
<i>Palola siciliensis</i>		1	344		92					4	45		
<i>Eunice afra</i>		83	6			101	48	34	18				1
<i>Eunice antennata</i>		3				31	2	20	5	1			
<i>Lysidice collaris</i>		41	8			1		64	30	22			
<i>Nematoneis unicornis</i>		5	9					42	23	1			
<i>Marphysa sanguinea</i>													
Glyceridae													
<i>Glycera tessellata</i>		7					10		30				
Capitellidae													
<i>Dasybranchus caducus</i>		1							27		62	25	
Terebellidae													
<i>Loimia medusa</i>										1	23		40
<i>Polycirrus medius</i>											9	22	20
<i>Tbelepus setosus</i>											2	11	18
<i>Nicolea gracilibranchia</i>												36	15
Maldanidae													
<i>Axiobella australis</i>											7	1	30
Hemichordata													
<i>Ptychodera flava</i>												5	65



particularly the loss of a planktonic larva, in *Conus* to phylogenetic affinity. DUDA & PALUMBI (1999) mapped this trait on their initial phylogenetic tree, based only on the calmodulin intron sequence, and concluded that all eight species on the tree that lack a planktonic stage in the life history evolved this trait independently from ancestors with planktonic larvae. Optimizing this trait to the combined 16S rRNA and calmodulin tree (DUDA, KOHN & PALUMBI, 2001) indicates a different pattern. The seven species with sequence data for both genes now belong to four clades, equivalent to E4 (*C. anemone*, *C. boeticus*), F1 (*C. magus*), F3 (*C. cinereus*, *C. proximus*), and M1 (*C. araneosus*, *C. penanceus*). Thus while the planktonic larva still appears to have been lost on several occasions during the evolution of *Conus*, all such cases may not have been independent. The small sample size (N=8) makes statistical analysis difficult. However, the distribution of species with non-planktonic development among clades does not differ significantly from the expected Poisson distribution ($\chi^2=4.8$; $0.1 < P < 0.05$).

DISCUSSION AND CONCLUSIONS

Conus, with more than 500 extant species the largest genus of marine molluscs, is also characterized by very large numbers of co-occurring species, particularly in coral reef-associated habitats of the Indo-Pacific region. For the past half century, studies of comparative biology of these assemblages have provided insights into geographic patterns of species diversity, use of food and substrate resources, life history and its relationship to biogeography, and patterns of evolutionary diversification. In all prior studies, for statistical purposes each *Conus* species had to be treated as an independent entity. This is clearly an oversimplification that is likely to lead to erroneous interpretations, because surely some species are more and others less closely related phylogenetically to each other. And similarity between species with respect to patterns of the attributes listed above results from intrinsic genetic or genealogic history as well as from adaptive responses to extrinsic ecological factors. However, it was not possible to incorporate this information into prior comparative studies. The reason of course is that although systematists since Linnaeus had hypothesized the existence of groups of related species within *Conus*, no phylogeny had ever been proposed. By providing the first objective, species-level phylogenetic hypothesis, the recent molecular phylogenetic study of more than 70 mainly Indo-Pacific species by Thomas Duda is an important advance toward removing this long-standing roadblock to understanding the comparative biology of *Conus*. Data sets on habitat and food resource use are shown to have a strong phylogenetic signal. That is, species within clades identified in the molecular study use significantly more similar substrate and prey types than do species that belong to different clades. This alone provides strong evidence that species are not independent entities for statistical purposes, and they should not be so treated. Future studies that address the problem of non-independence quantitatively, using methods of independent comparisons (e.g. FELSENSTEIN, 1985; PAGEL, 1992), as well as extending sequence analyses to additional genes, species, and populations

will lead to a much fuller understanding of comparative biology of the most diverse genus in the sea.

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