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Venomous Gastropods: *Conus*, conoideans and other neogastropod families

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ABSTRACT: A review of the present undestanding of the mechanism of envenomation by cones is presented. The expanding applications of cone snail venom components in biomedical science are the degree to wich the envenomation strategy may be shared by other venomous gastropod groups is explored based on a preliminary molecular phylogenetic analasys. Finally, some perspectives for the future are discussed.

RIASSUNTO: In questo lavoro viene inizialmente presentata una revisione delle conoscenze attualmente disponibili sul meccanismo di uso dell'apparato velenifero da parte delle specie di *Conus*. Quindi viene introdotto il campo in crescente espansione dell'applicazione in medicina dei componenti del cocktail tossinico utilizzato dai coni. Si esplora inoltre, sulla base di un'analisi filogenetica indipendente su base molecolare, la questione del possibile livello di condivisione dei medesimi meccanismi veleniferi in altri gruppi di neogasteropodi. Infine vengono discusse alcune prospettive di studio per il futuro.

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INTRODUCTION

The remarkable biology of cone snails (*Conus*) can be encapsulated by two well-established, if improbable observations. Cone snails are the only known gastropods capable of killing humans, first documented by Rumphius nearly three centuries ago (Rumphius, 1705). In addition, some *Conus* are the only gastropods known to capture fish as major prey, a phenomenon first established by Alan Kohn (Kohn, 1956). Although most cone snail species are not capable of killing humans and do not hunt fish, the fact that some species have evolved these seemingly impossible capabilities highlight the unusual evolutionary directions of the genus.

One evolutionary breakthrough that makes such unusual biology possible is the presence of a complex venom with corresponding anatomical adaptations for venom delivery, including harpoon-like teeth which also serve as hypodermic needles. Recently, cone snail venoms have been the focus of considerable biochemical and physiological investigation. These studies have revealed that this large successful genus (500 species) has evolved a highly sophisticated neuropharmacology.

This paper comprises three sections: first is a review of our present understanding of the mechanism of envenomation by predatory cone snails. The second section introduces an emerging field, the expanding applications of cone snail venom components in medicine. A third section addresses a scientific question not yet incisively addressed: the degree to which the cone snail envenomation strategy may be shared by other venomous gastropod groups. Specifically, how much overlap in mechanism to *Conus* will be found in other toxoglossate gastropods such as the Terebridae and the Turridae? These are the most obvious groups that may have mechanisms similar to the cone snails (family Conidae) since they are conventionally placed either in the same superfamily (Conoidea) or suborder (Toxoglossa) by most taxonomists. As mechanisms underlying cone snail envenomation become increasingly well elucidated at a genetic and molecular level, a comparison between the three major groups of neogastropods that envenomate prey becomes more feasible. In this paper, we discuss the likelihood of overlap between *Conus* and other toxoglossate molluscs, an evaluation based not on a direct characterization of the venoms of the other gastropod groups, but rather on an assessment of relationships between various families within the Neogastropoda.

After the review of *Conus* envenomation, the overview of potential medical applications of *Conus* venom components and the assessment of relationships between the Conoideans and other family groups within the neogastropods, the brief Discussion section includes some perspectives for the future.

I. Overview of *Conus* Envenomation

Conotoxins. The initial biochemical characterization of venoms from several *Conus* species firmly established that the biologically-active principles are small, highly structured polypeptides called conotoxins (alternatively, *Conus* peptides or conopeptides), which potently affect nervous system function by binding to specific molecular targets, primarily ion channels or receptors on the surface of neurons (Olivera et al., 1985a). The majority of conotoxins are neurotoxins between 8-45 amino acids in length. Despite their small size, conotoxins are confor-

mationally relatively rigid - in most cases, the three-dimensional structure is stabilized by multiple intramolecular disulfide crosslinks within the polypeptide (for overviews, see (Olivera et al., 1990; Olivera, 1997)). As a class, conotoxins are the smallest neurotoxins from animal venoms directly encoded by genes.

Since the discovery and characterization of the first conotoxins, an intriguing juxtaposition has emerged. On the one hand, *Conus* venoms have proven to be exceedingly complex. On the average, every cone snail has a venom repertoire of over 100 diverse conopeptides, each encoded by a different gene. On the other hand, there is an underlying simplicity: the great majority of conotoxins found in the ca. 500 different species of cone snails belong to only a few gene superfamilies (Olivera et al., 1999). All conotoxins of a superfamily share conserved sequence features. Thus, although a *Conus* venom is a complex biochemical mixture, several generalizations apply.

The genes encoding conotoxins are expressed in the cells lining the lumen of venom ducts of cone snails. The initial translation products are polypeptide precursors between 80-120 amino acids in length (Woodward et al., 1990). For most conotoxins, multiple post-translational modifications occur, including covalent modification of some amino acids (Craig et al., 1999a) and trimming of the precursor into the mature, biologically-active *Conus* peptide (the majority of which are 12-30 amino acids). Thus, most of the polypeptide precursor is trimmed off as maturation of the biologically-active conotoxin occurs. Conotoxin precursors (with some post-translational modifications) are stored in the venom duct as granules. As the venom transits from the duct through the proboscis to the hollow, harpoon-like radular tooth, a processing cascade to mature conotoxins occurs, very probably involving proteolytic secretions from the proboscis (Olivera et al., 1985b).

After venom is injected by a cone snail, each individual conotoxin probably targets a single molecular component in the nervous system of the injected animal. However, groups of different conotoxins in the same venom may act together towards a common physiological end. Such a synergistic group of venom peptides is called a conotoxin "cabal" (Olivera and Cruz, 2001). One example is the "motor cabal," a group of toxins that inhibits neuromuscular transmission in prey animals. One component of the motor cabal might inhibit release of neurotransmitter, another blocks the neurotransmitter receptor on the muscle, and a third component might inhibit electrical signaling on the muscle membrane. Together, such a group of toxins would efficiently suppress locomotion of the prey. Most cone snails have a motor cabal of conotoxins that rapidly and efficiently cause paralysis in prey. However, there are other functional toxin cabals with different physiological endpoints. For example, a "lightning strike cabal" has been identified in certain fish-hunting cone snail venoms; these elicit a rapid, potent electrical shock-like syndrome from the site of injection, stunning the prey and causing immediate immobilization. Some species (such as the Panamic fish-hunting species, *Conus purpurascens*) have both a "lightning-strike" and a "motor" cabal of toxins (Terlau et al., 1996). It has been suggested that *Conus* species that capture schools of fish using a net strategy have a cabal of peptides in their venom that deadens sensory circuitry, so that the engulfed fish seem sedated; this has been termed the "nirvana cabal" (Olivera and Cruz, 2001).

Divergence of venoms between *Conus* species. A surprising insight arising from the characterization of different *Conus* venoms is the remarkable divergence of conotoxins between cone snail species. Since any cone snail venom can have >100 different components, it might have been expected that a significant fraction would be conserved across all *Conus* species. Instead, it appears that every *Conus* has its own distinct complement of peptides.

This has been established using both biochemical and molecular genetic methods. What has emerged from these studies is that the mature toxin region of conotoxin genes hypermutate rapidly as speciation occurs. In contrast, other sequence elements of conotoxin genes, in particular the exons encoding the signal sequences at the N-terminal end of every conotoxin precursor, are unusually conserved. Thus, at the genetic level there is a striking contrast: one part of a conotoxin gene, the mature toxin region (which is always at the C-terminal end) undergoes hypermutation, while the other end of the translation product (the N-terminal signal sequence) shows an unprecedented sequence conservation (Woodward et al., 1990; Olivera, 1997).

Over the time period relevant for evolution of new *Conus* species, hypermutation at the C-terminal, mature-toxin region provides a mechanism for cone snails to explore many peptide sequences. In essence, the snails have used what is now a state-of-the-art technology for drug development in pharmaceutical companies, the "combinatorial library strategy" for drug development (except that cone snails antedated the large pharmaceutical firms by over 50 million years!).

The highly conserved signal sequences within individual conotoxin gene superfamilies imply a correspondingly conserved cellular secretion and maturation pathway. We postulate that the signal sequences of conotoxin precursors may direct these to particular intracellular membrane loci associated with secretory pathways with appropriate accompanying accessory factors such as post-translational modification enzymes, and possibly, specific chaperone-type proteins for facilitating folding and disulfide bond formation of specific peptide superfamilies.

In essence, the N-terminal regions of conotoxin genes are conserved when two homologous sequences are compared from different species, but focal hypermutation results in a very rapid sequence divergence in the C-terminal mature toxin regions. It was postulated that the large introns characteristic of conotoxin genes may play a role in the differential rates of mutation observed (Olivera et al., 1999); recently, a specific mechanism for hypermutation has been proposed (Conticello et al., 2001).

Thus, the biologically active, mature venom components show an amazing sequence diversity from one species to the next. This appears to be one basis for the evolutionary success of this large group of marine neogastropods, arguably the largest living genus of marine animals (Röckel et al., 1995).

II. Medical and basic neurobiological research applications of *Conus* venom peptides

Although one impetus for investigating conotoxins was the mortality and morbidity caused by cone snail envenomation, an accelerating interest in these peptides stems from what seems to be another improbable juxtaposition: the potential of *Conus* venom components to serve as therapeutic agents. As we discuss below, conotoxins are already being used as diagnostic tools, and for basic biomedical investigations in understanding nervous systems. However, recent research on conotoxins has demonstrated some exciting therapeutic possibilities.

One conotoxin, ω-conotoxin MVIIA, was initially purified and characterized from the venom of the fish-hunting *Conus* species, *Conus* magus, approximately twenty years ago by Michael McIntosh, then an undergraduate at the University of Utah. This compound is now sufficiently far along in terms of drug development that it may be approved this year in the US as a commercial drug under the generic name "ziconotide" (Elan Pharmaceuticals, which will market the drug, has received an "approvable" letter from the U.S. Federal Drug Administration) (McIntosh et al., 1982; Olivera, 2000). The structure of this peptide, originally derived from the venom of *Conus magus* is shown in Table I. The commercial drug, which is being synthesized chemically, is identical in every respect to the natural product.

The proposed therapeutic application of Ziconotide is to alleviate intractable pain syndromes, in particular the malignant pain of cancer patients. The present therapy for intense pain involves opiate drugs such as morphine. Given this, why was it feasible to develop a more complex compound? Ziconotide has a number of disadvantages compared to morphine, most notably in terms of the requirements for drug delivery - Ziconotide cannot be taken orally, and even worse, has to be injected directly into the spinal cord.

Ziconotide targets a particular molecular form of voltage-gated calcium channel, found in all vertebrate nervous systems. In the human spinal cord, this calcium channel isoform is very restricted in its distribution: it is found in synapses between input pain fibers and spinal cord nerve cells which transmit pain signals to the brain. Blocking this synapse blocks transmission of a pain signal to the higher CNS centers (Olivera, 2000); the result is that the patient does not perceive the intense pain that would otherwise manifest itself.

Morphine also helps to block transmission of this signal; however, a major problem with morphine is that if it has to be used repeatedly, patients develop tolerance. This is because

Table I. Example of paralytic conotoxins

I.		
α-conotoxin GI (*Conus geographus*)	ECCNPACGRHYSC#	Physiological effect: cause paralysis of the fish prey of these piscivorous species by blocking synaptic transmission. Physiological mechanism similar to major neurotoxins found in Cobra and related snake venom
α-conotoxin EI (*Conus ermineus*)	RDOCCYHPTCNMSNPQIC#	Molecular mechanism: antagonists of nicotinic acetylcholine receptor, skeletal muscle subtype
II.		
ω-conotoxin GVIA (*Conus geographus*)	CKSOGSSCSOTSYNCCRSCNOYTKRCY#	Physiological effect: blocks neurotransmitter release which results in paralysis of the fish prey of these piscivorous species
ω-conotoxin MVIIA (*Conus magus*)	CKGKGAKCSRLMYDCCTGSCRSGKC#	Molecular mechanism: antagonists of calcium channels
III.		
δ-conotoxin PVIA (*Conus purpurascens*)	EACYAOGTFCGIKOGLCCSEFCLPGVCFG#	Physiological effect: cause paralysis of fish (*C. purpurascens*) and mollusc (*C. textile*) by blocking synaptic transmission
δ-conotoxin TxVIA (*Conus textile*)	WCKQSGEMCNLLDQNCCDGYCIVLVCT^	Molecular mechanism: delays inactivation of sodium channels

morphine activates a receptor in the spinal cord (the opioid receptor) which intrinsically becomes less sensitive as it is turned on (in pharmacological parlance, it is "down-regulated"). Thus, after repeated use, patients become tolerant to morphine and it becomes increasingly difficult to alleviate their pain. However, for Ziconotide the continual use of the conotoxin does not result in down-regulation of its targeted voltage-gated calcium channel, and patients do not become tolerant to the drug. Thus, cancer patients who have become tolerant to morphine are candidates for Ziconotide therapy. This conotoxin drug has already been through extensive clinical trials in human patients and final approval to market Ziconotide is anticipated in the year 2001.

In addition to Ziconotide, several other *Conus* peptides are being explored for their therapeutic potential. One that has entered clinical trials is a 17-amino acid peptide discovered by Craig Clark, another undergraduate at the University of Utah, which is now called conantokin-G (Olivera et al., 1985b). This peptide is being developed as a drug for cases of intractable epilepsy. The peptide acts as a specific inhibitor of an important central nervous system component known as the NMDA receptor; conantokin-G quiets down overactive neuronal circuitry by inhibiting NMDA receptors. In animal models, the efficacy of the drug compared to its behavioral toxicity seems much better than are existing therapies for epilepsy (White et al., 2000). This compound is being developed by a small biotech company, Cognetix Inc. of Salt Lake City, Utah, in collaboration with a drug-delivery company, Medtronic, Inc. of Minneapolis, Minn.

The two examples above are furthest along in terms of clinical development for therapeutic use. A number of other conotoxins have been tested in animal models and shown to have promise as therapeutic agents. One of the most novel of these peptides is contulakin-G, an O-glycosylated, 17-amino acid peptide from *Conus geographus* which is believed to be a possible agonist of a specific neurotensin receptor subtype in the central nervous system; this has shown promising analgesic properties (Craig et al., 1999b; Wagstaff et al., 2000). Other *Conus* peptides being explored by Cognetix have potential application as local anesthetics, muscle relaxants, and in demyelinating diseases such as multiple sclerosis. Among the other *Conus* peptides being developed is ω–conotoxin CVID as an analgesic (by Xenome, Inc. of Brisbane, Australia) (Lewis et al., 2000; Nielsen et al., 2000; Wright et al., 2000). So far, only a miniscule fraction of the total number of conotoxins have been explored for therapeutic applications; the activity in this area is clearly increasing exponentially as monitored by publications in pharmacological journals, number of patent applications being filed and patents which have issued in the last few years (Jones and Bulaj, 2000; Jones et al., 2001).

Conotoxins also have clear uses in diagnostic medicine; one of the applications which is already well established is the use of radiolabeled ω–conotoxins for evaluating potential patients with the Lambert-Eaton myasthenic syndrome, an autoimmune neurological disorder associated with small cell lung carcinomas (Lennon, 1996; Lang et al., 1998). The radiolabeled peptide is used to determine whether the patient has elevated levels of autoantibodies that may interfere with the proper functioning of voltage-gated calcium channels at the junction between motor nerves and muscle.

Finally, the application of individual conotoxins as basic research tools in neuroscience is now very well established. Many *Conus* peptides have proven to be useful in identifying molecular components in various functional circuits, and indeed in certain cases these peptides are the only agents available for assaying involvement of certain molecular targets. Particularly notable are the use of ω–conotoxins for inhibiting neurotransmitter release (Olivera et al., 1994) and the use of α–conotoxins for identifying nicotinic acetylcholine receptor subtypes (McIntosh et al., 1999). There are now over 2,200 publications in the primary research literature that describe experiments where conotoxins have been employed as basic research tools, In effect, cone snail venom components are being widely used by neuroscientists to understand our own brains.

III. Neogastropod families and the superfamily Conoidea

Background. Recently our laboratories carried out a phylogenetic reconstruction of a large group of *Conus* species (>70) (Espiritu et al., 2001) using mitochondrial 16S RNA sequences. A number of other gastropods were included in this analysis - the original intent was to have these serve as the outgroups for identifying clades of species in the genus *Conus*. As a consequence, sequence data from several different neogastropod families became available. We present the data and the analysis of the mitochondrial 16S ribosomal RNA from six neogastropod families: Conidae, Turridae, Terebridae, Costellaridae, Mitridae, and Olividae (see Fig. 1 for the species analyzed). One mesogastropod from the family Cerithidae, *Rhinoclavis aspera*, is also included here to serve as the outgroup for rooting phylogenetic trees.

The phylogeny of the neogastropods is in flux (for reviews, see (Ponder, 1973; Taylor and Morris, 1988; Kantor, 1996)), and therefore any new molecular data should contribute to the evaluation of the many alternative proposals regarding their phylogeny. Although a general revision of neogastropod phylogeny was not our goal, the preliminary analysis we carried out supports a surprising and unexpected phylogenetic hypothesis that should be examined further by a more comprehensive study.

It has been the general practice to organize families of Neogastropoda into superfamilies (or suborders), implying that the families within a particular superfamily are more closely related to each other than to other neogastropod groups. The neogastropod group of most direct concern to the authors is the venomous superfamily Conoidea (Conacea, or suborder Toxoglossa). Traditionally, three large recent neogastropod families - Conidae, Turridae and Terebridae - are included in the Conoidea. This is one grouping which remains a relatively constant feature of most taxonomic proposals made for the Neogastropoda.

In addition to the Conoidean species, our analysis included species in the families Costellaridae, Mitridae and Olividae (see Fig. 1D). In one of the more recent conventional phylogenies, these are grouped together in the superfamily Muricoidea with many other neogastropod families. In some other taxonomic schemes, these families are assigned to a smaller superfamily, Volutacea. One standard widely used taxonomy for the species analyzed here is shown in Table II.

All conventional phylogenies predict that all neogastropod groups would be more divergent from *Rhinoclavis aspera* than they would be from each other (since mesogastropod and neogastropod families are usually assigned to separate orders of the class Gastropoda). An additional prediction of most conventional phylogenies is that species in the Conidae, Turridae and Terebridae should cluster with each other more than with species in the other neogastropod families analyzed, i.e., the Costellaridae, Mitridae and Olividae. Thus, each proposed taxonomy makes clear predictions regarding molecular results. As we show below, the first prediction above is indeed fulfilled by our data. However, the separation of the families of toxoglossate molluscs into a presumably monophyletic superfamily or suborder within the order Neogastropoda is not supported by the data. The results are much more consistent with a "star phylogeny," i.e., all of the neogastropod families analyzed diverged from a common ancestor at approximately the same time.

Preliminary reconstruction of neogastropod phylogeny using mitochondrial ribosomal RNA. Sequences of mitochondrial 16S ribosomal RNA from the neogastropod species, and one mesogastropod species are shown in Table III. Three of the sequences included were part of the >70 sequences published in previous reports on the molecular phylogeny of the genus *Conus* (Monje et al., 1999; Espiritu et al., 2001). The *Conus* species analyzed include *Conus ermineus* (Born, 1778), a piscivorous species from the Atlantic, *Conus textile* ((Linnaeus, 1758), a molluscivorous species collected in the Philippines, and *Conus californicus* (Reeve, 1843), an Eastern Pacific generalist species that probably eats polychaete worms as its major class of prey. Two species conventionally assigned to the subfamily Turrinae were analyzed, *Turris spectabalis* (Reeve, 1843) and *Lophiotoma albina* (Lamarck, 1822). The third turrid analyzed was *Clavus unizonalis* (Lamarck, 1822), in the subfamily Drillinae. In a recent proposal for reclassifying the turrids, the Turrinae and Drillinae were assigned to different families (to be named Turridae and Drillidae (Taylor et al., 1993)). In addition to the turrid and

Table II
Suprageneric Taxonomy According to Vaught (Vaught, 1989)

Species analyzed	Subfamily	Family	Superfamily
Order Mesogastropoda			
Rhinoclavis aspera (Linné, 1758)	Cerithiinae	Cerithiidae	Cerithioidea
Order Neogastropoda			
Oliva miniacea (Röding, 1798)	Olivinae	Olividae	Muricoidea
Mitra mitra (Linné, 1758)	Mitrinae	Mitridae	Muricoidea
Mitra ustulata (Reeve, 1844)	Mitrinae	Mitridae	Muricoidea
Vexillum compressum (Sowerby, 1874)		Costellaridae	Muricoidea
Vexillum granosum (Gmelin, 1791)		Costellaridae	Muricoidea
Conus ermineus (Born, 1778)		Conidae	Conoidea
Conus textile (Linné, 1758)		Conidae	Conoidea
Conus californicus (Reeve, 1844)		Conidae	Conoidea
Turris spectabilis (Reeve, 1843)	Turrinae	Turridae	Conoidea
Lophiotoma albina (Lamarck, 1822)	Turrinae	Turridae	Conoidea
Clavus unizonalis (Lamarck, 1822)	Drillinae	Turridae	Conoidea
Terebra subulata (Linné, 1767)		Terebridae	Conoidea
Terebra crenulata (Linné, 1758)		Terebridae	Conoidea

Table III

	1				50
Conus ermineus	TACGAAGTCG	GA.CCTGCCC	AGTGAG....	TTTTAAACGG	CCGCGGTACT
Conus textile	TACGAAGTCG	GA.CCTGCCC	AGTGAG....	TTTTAAACGG	CCGCGGTACT
Conus californicus	TATGAAGTCG	GA.CCTGCCC	AGTGAA...T	TTTTCAACGG	CCGCGGTACT
Turris spectabilis	TAAGGAGTCG	GA.CCTGCCC	AGTGAA.TTA	TTTTTAACGG	CCGCGGTACT
Lophiotoma albina	TAGGGAGTCG	GA.CCTGCCC	AGTGAA..TT	TTTTTAACGG	CCGCGGTACT
Clavus unizonalis	TAAGGAGTCG	GA.CCTGCCC	AGTGAAG..T	TTTTTAACGG	CCGCGGTACT
Terebra crenulata	TGGGGAGTCG	GA.CCTGCCC	GGTGAAA..T	TTTTTAACGG	CCGCGGTACT
Terebra subulata	ATGGGAGTCG	GA.CCTGCCC	GGTGAA..TT	TTTTTAACGG	CCGCGGTACT
Vexillum compressum	.AAAGAGTCG	GATCCTGCCC	AGTGAAAATT	ATTTTAACGG	CCGCGGTACC
Vexillum granulosum	.AAAGAGTCG	GA.CCTGCCC	AGTGATAA.T	TTTTTAACGG	CCGCGGTACC
Mitra mitra	TAGGGAGTCG	GA.CCTGCCC	AGTGAA....	TTTTCAACGG	CCGCGGTACT
Mitra ustulata	TAGGGAGTCG	GA.CCTGCCC	GGTGAA....	TTTTTAACGG	CCGCGGTACT
Rhinoclavis aspera	TAGGGAGTCG	GA.CCTGCCC	GGTGAAAAA.	.TTTTAACGG	CCGCGGTACT
Oliva miniacea	TGGGGAGTCG	GA.CCTGCCC	GGTGAAAA..	TTTTTAACGG	CCGCGGTACT

	51				100
Conus ermineus	CTGACCGTGC	AAAGGTAGCA	TAATCATTTG	CCTTATAATT	GAAGGCTGGA
Conus textile	CTGACCGTGC	AAAGGTAGCA	TAATCATTTG	CCTTATAATT	GAAGGCTGGA
Conus californicus	CTGACCGTGC	AAAGGTAGCA	TAATCATTTG	CCTTATAATT	GAAGGCTGGA
Turris spectabilis	CTGACCGTGC	AAAGGTAGCA	TAATCATTTG	CCTTATAATT	GAAGGCTAGT
Lophiotoma albina	CTGACCGTGC	AAAGGTAGCA	TAATCATTTG	CCTTATAATT	GGAGGCTAGT
Clavus unizonalis	CTGACCGTGC	AAAGGTAGCA	TAATCATTTG	CCTTATAATT	GGAGGCTAGT
Terebra crenulata	CTGACCGTGC	AAAGGTAGAC	TAATCATTTG	CCTTATAATT	GAAGGCTAGT
Terebra subulata	CTGACCGTGC	AAAGGTAGCA	TAATCATTTG	CCTTATAATT	GAAGGCTAGT
Vexillum compressum	CTGACCGTGC	AAAGGTAGCA	TAATCATTTG	CCTTGTAATT	TAAGGCTAGT
Vexillum granulosum	CTGACCGTGC	AAAGGTAGCA	TAATCATTTG	CCTTGTAATT	TAAGGCTAGT
Mitra mitra	CTGACCGTGC	AAAGGTAGCA	TAATAATTTG	CCTTATAATT	GAAGGCTGGT
Mitra ustulata	CTGACCGTGC	AAAGGTAGCA	TAATAATTTG	CCTTATAATT	GAAGGCTAGA
Rhinoclavis aspera	CTGACCGTGC	AAAGGTAGCA	TAATCACTTG	CCTTATAATT	GAAGGCTGGT
Oliva miniacea	CTGACCGTGC	AAAGGTAGCA	TAATCATTTG	CCTTATAATT	GAAGGCTAGT

	101				150
Conus ermineus	ATGAATGGTT	TGACAAGAAT	ACACCTGTCT	CTTTTAGGCT	GCCTAGAATT
Conus textile	ATGAATGGTT	TGACAAGAAT	ACACCTGTCT	CTTTTAGATT	ACTTAGAATT
Conus californicus	ATGAATGGTT	TGACAAGAGT	GCAACTGTCT	CTTTATGATT	CAATAGAATT
Turris spectabilis	ATGAATGGTT	TGACAAGAAT	ATGGCTGTCT	CTTTATAATT	TTATAGAACT
Lophiotoma albina	ATGAATGGTT	TGACAAGAAT	ATAGCTGTCT	CTTTATAACT	TTATAGAATT
Clavus unizonalis	ATGAATGGTT	TGACAAGAAT	ATAGCTGTCT	CTTTTTGATT	TATTAGAACT
Terebra crenulata	ATGAATGGTT	TGACAAGAAT	GTAGCTGTCT	CTTCATAATT	TGGTAGAATT
Terebra subulata	ATGAATGGTT	TGACAAGAAT	ATGGCTGTCT	CTTTATAATT	TGATAGAATT
Vexillum compressum	ATGAAAGGTT	TGACAAGAAT	ATAACTGTCT	CCTGTTGGTT	TAATAGAACT
Vexillum granulosum	ATGAAAGGTT	TGACAAGAAT	ATAACTGTCT	CCTTTTGGTT	TAATAGAATT
Mitra mitra	ATGAATGGTT	TGACGAGAAT	AAAGCTGTCT	CTTTGCAACT	TTTTAGAAAT
Mitra ustulata	ATGAATGGTT	TGACGAGAAT	AAAGCTGTCT	CTTTACAACT	CTATAGAAAT
Rhinoclavis aspera	ATGAATGGTT	TGACGAAAGC	ACAGCTGTCT	CTCTCCCGTT	TTATAAAAAT
Oliva miniacea	ATGAATGGTT	TGACGAGAAT	ATTACTGTCT	CTACTTGATT	TACTAGAAAT

	151				200
Conus ermineus	TTATCTTTGG	ATGAAAAAGT	CCAAATATTA	TTAAAAGACA	AGAAGACCCT
Conus textile	TTATCTTTGG	ATGAAAAAGT	CCAGATTTAA	TTAAAAGACA	AGAAGACCCT
Conus californicus	TTATTTTGGG	ATGAAAAAGT	CCTAATATAA	TTAAAAGACA	AGAAGACCCT
Turris spectabilis	TTATTTTAAA	GTGCAGAAGC	TTTAATTAAA	TTAAAAGACA	AGAAGACCCT
Lophiotoma albina	TTATTTTAAA	GTGAAGAAGC	TTTAATGTAA	TTAAAAGACA	AGAAGACCCT
Clavus unizonalis	TTATTTTAAG	GTGAAGAAGC	CTTAATTTAA	TTAAAAGACA	AGAAGACCCT
Terebra crenulata	TTATTTTTAG	GTGAAAAAGC	CTTAATGTAA	TTAATAGACA	AGAAGACCCT
Terebra subulata	TTATTTTCAG	GTGAAAAAGC	CTTGATTAAA	TTAATAGACA	AGAAGACCCT
Vexillum compressum	TTTCTTATAA	GTGAAGAGAC	TTATATACAA	TTAATAGACA	AGAAGACCCT
Vexillum granulosum	TTACTTATAA	GTGAAGAGGC	TTATATACAA	TTAATAGACA	AGAAGACCCT
Mitra mitra	TTATTTTCGG	ATGAAAAAGT	CCGTATACCA	TTAAAAGACA	AGAAGACCCT
Mitra ustulata	TTATTTGCAG	ATGAAAAAGC	CTGCATAATA	TTAAAAGACA	AGAAGACCCT

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Rhinoclavis aspera      TAACATTTGG GTGAAGAGGC CCAAATTGAA TTAAAGGACG AGAAGACCCT
Oliva miniacea          TTATCTTCAG GTGAAGAAGC CTGAATAGAA TTAAAAGACA AGAAGACCCT

                        201                                                250
Conus ermineus          ATCGAGCTTT AGAAAAATTA GTAGAC.TTA A..TATAAAT CAATATAAGT
Conus textile           ATCGAGCTTT AGAGAAGTTA ATAGAC.TTA G..TTTAAAC CAATATAGAT
Conus californicus      ATCGAGCTTG AAATAAATTA ACAAAAGAAA AATTT..... .ATTAG..AC
Turris spectabilis      ATCGAGCTTA AAAAAAATTT TTAGAAACAA ATTAA..... .TAAAA.ATT
Lophiotoma albina       ATCGAGCTTT AAAAAAATCT TTAGAAATTA ATTAA..... .TAAATGATC
Clavus unizonalis       GTCGAGCTTT AAAAAAGTCA ATAGAAATTA CCTTA..... .ATTAA...A
Terebra crenulata       ATCGAGCTTA AAAGAATTTA GTGGATTTTT AAATAG.... .TTTATTGGT
Terebra subulata        ATCGAGCTTG AAGGAATTTG ATGGGTAAAA AATAAGCCTT ATTGGTGGAG
Vexillum compressum     ATCGAGCTTT AAATCAGTTA ATAGAAATAA ATGAAATAAA TG.......C
Vexillum granulosum     ATCGAGCTTG AAATCAATTA ATAGAAATTA AACTGAATAG ACA......T
Mitra mitra             ATCGAGCTTT AAAAAAATTC AGTAGACCAT GTAACTAATT AAA......T
Mitra ustulata          ATCGAGCTTT AAAAGATTTA ATGGGTTAAA AACTTATTAT GAG.....CT
Rhinoclavis aspera      GTCGAGCTTT AGGGGAAGGA GGGAAATTTT ATATTTAT.. ..........
Oliva miniacea          ATCGAGCTTG AAACAAATTA ATGGATTAAA AACAATAAG. .......TAA

                        251                                                300
Conus ermineus          AAAAGGAAAA ..CTACTAAA TACTTTGGTT GGGGCAACCG AGGAGTAAAT
Conus textile           AAAAGGAAAA ..CTATTAAA TACTTTGGTT GGGGCAACCG AGGAGCAAGT
Conus californicus      AAAAAAATTG TGTTGTTAAA CATTTTGGTT GGGGCAACTA AGGAGTAAAA
Turris spectabilis      TTAAAAAATT AGCTAAGAAA TATTTTAGTT GGGGCGACTA AGGAACAAAC
Lophiotoma albina       TTAAAAAATC AGCTATTGAA TATTTTGGTT GGGGCGACTA AGGAACAGAA
Clavus unizonalis       CTAAAAAATC ATCTATTAAA AATTTTGGTT GGGGCGACTA AGGAACAAAA
Terebra crenulata       TAAGAGGTAT AACTGCTGAG ATTTTTGGTT GGGGCGACTG AGGAACATTT
Terebra subulata        AAAAGGGGTA AACTGTTGAA TATTTTGGTT GGGGCGACTG AGGAACAGGA
Vexillum compressum     ATAAAATCAA ATCTATTAAA AATTTTGGTT GGGGCGACTA AGGAACAGCT
Vexillum granulosum     ATAAAACTAA GTCTATTAAA AATTTTGGTT GGGGCGACTA AGGAACAGCT
Mitra mitra             CTAGAAAGTC ATCTCCTGAA AATTTTAGTT GGGGCGACTA AGGAACAAAT
Mitra ustulata          TAAGACAGTT ATCCATTAAG AATTTTAGTT GGGGCGACTA AGGAACAAAC
Rhinoclavis aspera      .AAATACATG GCCTCACTCA CCCTTTAGTT GGGGCGACTA GGGAACAATA
Oliva miniacea          TAAAAGTGTT AGCCATTGAA CATTTTGGTT GGGGCGACTA AGGAACAAAC

                        301                                                350
Conus ermineus          AGAGCCTCCT T.....TGAA TTACAAAT.C CTA.CATGTA ATTGATCC.A
Conus textile           AAAGCCTCCT TT.....AAA TA.GTAAATC TTG.CTTGTG .TTGATCC.A
Conus californicus      AAAGCCTCCT T...TATGTT AAGATAAA.C TAA.CAAGTA C.TGATCC.A
Turris spectabilis      AAAGCTTCCT T...TG.ATA AA.AATAA.C ATTT.AAGTA .TGGATCC.A
Lophiotoma albina       AAAGCTTCCT T...AA.ATA ATTAATGA.C ATT.CATGTA .TTGATCC.A
Clavus unizonalis       AGAGCTTCCT T...T.TACA TAATAAAATC TAT..AAATT .TTGATCC.A
Terebra crenulata       AAAGCTTCCT T...TA.ATG TAGATATA.C GTA.CAAGTG .TTGATCC.A
Terebra subulata        AAAGCTTCCT T...TG.TGG T.GTGATA.C ATA.CAGGTG .TTGATCC.A
Vexillum compressum     AAAGCTTCCT ATTAAAA... ..TTTAAATC TTTTCAAGTA G.TGA.CCCA
Vexillum granulosum     AAAGCTTCCT ATTTAA.... ..TTTAAA.C CTT.CAAGTA G.TGATCC.A
Mitra mitra             AAAGCTTCTT TAAAACACGT CTTTTG...C TCATTAGCTT ..TGATCC.A
Mitra ustulata          AAAGCTTCCT CATTAGCA.. TTTTTA..CC T.AT.AAGTT C.CGATCC.A
Rhinoclavis aspera      AAAGCTTCCC TT......AT TTTCTAAA.T TTATATATTA ..GGATCC.A
Oliva miniacea          AAAGCTTCCC T.....ATAT AAAATAGGTT CACCGACAT. ..TGATCC.A

                        351                                                400
Conus ermineus          AAAA...TTT TGATCAAAGG AA...TTAGT TACC.GTAGG GATAACAGCA
Conus textile           AAA...TTTT TGATCAAGGG AA...TTACT TACC.GTAGG GATAACAGCA
Conus californicus      AAC...TTTT TGATCAAAGA AAA...TAGT TACC.GTAGG GATAACAGCA
Turris spectabilis      GAA...TGTT TGATTGAGAG AA...TTAGT TACC.GTAGG GATAACAGCA
Lophiotoma albina       AAA...TTTT TGATTAAAGA ATA...TAGT TACC.GTAGG GATAACAGCA
Clavus unizonalis       AAAA..TTTT TGATTAAAAG AA...TTAGT TACC.GCAGG GATAACAGCA
Terebra crenulata       AAA...TTAA TGATTAAAGG AA...TTAGT TACC.GTAGG GATAACAGCA
Terebra subulata        AAAG...TGT TGATTAAAGG AA...TTAGT TACC.GTAGG GATAACAGCA
Vexillum compressum     GAAAA..TTC TGGTTAAAGA AA...TTAGT TACC.GTAGG GATAACAGCA
Vexillum granulosum     GAAAA..TTC TGATTAAAGA AA...TTAGT TACC.GTAGG GATAACAGCA
Mitra mitra             GAAA...TTC TGATTAATAA AA...TTAGT TACC.GTAGG GATAACAGCA
```

Mitra ustulata	GAAGCA.TTC	TGATTAATAG	AA...TTAGT	TACC.GTAGG	GATAACAGCA
Rhinoclavis aspera	GCATTAAAGC	TGCTGATCAA	AAGAATTAGT	TACCCGCAGG	GATAACAGCA
Oliva miniacea	AAAGA..TTT	TGATTAATGA	AA...TTAGT	TACC.GTAGG	GATAACAGCA
	401				450
Conus ermineus	TTATCTTTTT	CAAGAGCCCA	TATCGAAAAA	AAGGTTTGTG	ACCTCGATGT
Conus textile	TTATCTTTTT	TAAGAGCCCA	TATCGAAAAA	AAGGTTTGTG	ACCTCGATGT
Conus californicus	TTATCTTTTT	TGAGAGTTCC	TATCGAAAAA	AAGGTTTGTG	ACCTCGATGT
Turris spectabilis	TTATCTTTTT	TGAGAGTTCT	TATCGAAAAA	AAGGTTTGTG	ACCTCGATGT
Lophiotoma albina	TTATCTTTTT	TGAGAGTTCT	TATCGAAAAA	AAGGTTTGTG	ACCTCGATGT
Clavus unizonalis	TTATCTTTTT	TGAGAGCTCT	TATCGAAAAA	AAGGTTTGTG	ACCTCGATGT
Terebra crenulata	TTATCTCCTT	TGAGAGTTCT	TATCGAAAAG	GGGGTTTGTG	ACCTCGATGT
Terebra subulata	TTATCCTTTT	TGAGAGTTCA	TATCGAAAAA	GGGGTTTGTG	ACCTCGATGT
Vexillum compressum	TTATCTTTTT	TGAGAGCTCT	TATCGAAAAA	AAGGTTTGTG	ACCTCGATGT
Vexillum granulosum	TTATCTTTTT	TGAGAGCTCA	TATCGAAAAA	AAGGTTTGTG	ACCTCGATGT
Mitra mitra	TTATCTTTTT	TGAGAGCTCT	TATCGAAAAA	AAGGTTTGTG	ACCTCGATGT
Mitra ustulata	TTATCTTTTT	TGAGAGTTCT	CATCGAAAAA	AAGGTTTGTG	ACCTCGATGT
Rhinoclavis aspera	TTATCCTTCT	TGAGAGACCA	TATCGAAAGA	AGGGGTTGTG	ACCTCGATGT
Oliva miniacea	TTATCTTTTT	TGAGAGCTCT	AATCGAAAAA	AAGGTTTGTG	ACCTCGATGT
	451				500
Conus ermineus	TGGACCAGAA	TATCCTGAAG	ATGCAGAAGT	CTTTAAGGG.	...TTGGTCT
Conus textile	TGGACCAGAA	TGTCCTAAAG	ATGCAGAAGT	CTTTAAGGG.	...TTGGTCT
Conus californicus	TGGACCAGAA	TATCCTGAAG	ATGCAGAAGT	CTTCAAGGG.	...TTGGTCT
Turris spectabilis	TGGACCAGAA	TATCCTAAAG	ATGCAGAAGT	CTTTAAGGG.	...TTGGTCT
Lophiotoma albina	TGGACCAGAA	TATCCTAAAG	ATGCAGAAGT	CTTTAAGGG.	...TTGGTCT
Clavus unizonalis	TGGACCAGAA	TATCCTAAAG	ATGCAGAAGT	CTTTAAGGG.	...TTGGTCT
Terebra crenulata	TGGACCAGAA	TGTCCTGAAG	ATGCAGAAGT	CTTTAAGGG.	...TTGGTCT
Terebra subulata	TGGACCAGAA	TGTCCTGAAG	ATGCAGAAGT	CTTCAAGGG.	...TTGGTCT
Vexillum compressum	TGGACCAGAA	TATCCCAAAG	ATGTAGCAGT	CTTTAAGGGA	GGGTTGGTCT
Vexillum granulosum	TGGACCAGAA	TATCCTAAAG	ATGCAGCAGT	CTTTAAAGG.	...TTGGTCT
Mitra mitra	TGGACCAGAA	TATCCTAAAG	ATGCAGCAGT	CTTTAAGGG.	...TTGGTCT
Mitra ustulata	TGGACCAGAA	TGTCCTAAAG	ATGCAGCAGT	CTTTAAGGG.	...TTGGTCT
Rhinoclavis aspera	TGGACTAGGA	TATCCGGATG	GTGCAGAAGC	CCTCAAAGG.	...TTGGTCT
Oliva miniacea	TGGACCAGAA	TATCCCAAAG	GTGTAGCAGC	CTTTAAAGG.	...TTGGTCT
	501	511			
Conus ermineus	GTTCGACCAT	T			
Conus textile	GTTCGACCAT	T			
Conus californicus	GTTCGACCAT	T			
Turris spectabilis	GTTCGACCAT	T			
Lophiotoma albina	GTTCGACCAT	T			
Clavus unizonalis	GTTCGACCAT	T			
Terebra crenulata	GTTCGACCAT	T			
Terebra subulata	GTTCGACCAT	T			
Vexillum compressum	GCTCGACCAT	T			
Vexillum granulosum	GTTCGACCAT	T			
Mitra mitra	GTTCGACCAT	T			
Mitra ustulata	GTTCGACCAT	T			
Rhinoclavis aspera	GTTCGACCAT	T			
Oliva miniacea	GTTCGACCAT	T			

Table III. The DNA sequences above were obtained from tissue collected directly from live gastropods. The live specimen was cooled down in an ice bath for 5-10 min, the shell was smashed with a mallet, and the specimen quickly dissected on an ice block. The fresh hepatopancreas of the dissected snail was either quickly placed in liquid nitrogen or immediately extracted with buffer. The method used for DNA extraction is basically the rapid one-step extraction (ROSE) method of Steiner et al. (Steiner et al., 1995). This technique eliminates the need for organic solvent extraction and enzyme digestion, and involves a rapid one-step process. The DNA extracted was analyzed by agarose gel-electrophoresis, and high molecular weight (>25kb) DNA was routinely obtained by these procedures. The initial extraction gave a 260:280 ratio that was considerably less than that for pure DNA (circa 1.7). Most samples were further purified using centrifugal dialysis (Milipore), concentrating the DNA (to ~7mg/ml) and removing lower molecular weight impurities. Thus, most samples analyzed had an A260:A280 ratio greater than 1.6. After one year of storage, agarose gel-electrophoresis suggested that the molecular weight of the DNA remained >30kb. The primers used for PCR as described in Monje et al. (Monje et al., 1999). All sequences above have been deposited in Genbank.

Conus species analyzed, two other Conoidean species in the genus *Terebra* (*T. crenulata* (Linnaeus, 1758) and *T. subulata*(Linnaeus, 1767)) are included in this survey. *Terebra subulata* is a venomous species, while *T. crenulata* is one of the larger *Terebra* species that do not have a venom duct.

The five other species from which mt 165 rRNA sequences were obtained include *Mitra mitra* (Linnaeus, 1758) and *Mitra ustulata* (Reeve, 1844) (in the Mitridae), *Vexillum compressum* (Sowerby, 1874) and *Vexillum granosum* (Gmelin, 1791) (in the Costellaridae) and *Oliva miniacea* (Röding, 1798) (in the Olividae). Both *Mitra* and *Vexillum* were originally included in the Mitridae. However, on the basis of differences in the radula, the Costellaridae have been recognized as a separate family in more recent taxonomic work.

The relevant mitochondrial sequences for the 14 species are shown in Table III, and these were aligned as described in the Table legend. A phylogenetic reconstruction was made using either parsimony or maximum distance (see Fig. 2). In addition, the divergence was quantitated using the Kimura two-parameter method; the pairwise divergence values for all species analyzed is shown in Table IV.

If we use the rate previously used for *Conus* of 0.33% per 10^6 years (range 0.24-0.40%), which was calibrated on the basis of the fossil record of the genus (Kohn, 1990), the time of divergence of the various species within each family can be estimated. Thus, some of the species appear to have diverged in the Miocene, including species in the same genus (i.e., *Vexillum compressum* and *Vexillum granosum* in the Costellaridae), as well as some species assigned to different genera (i.e., *Turris spectabilis* and *Lophiotoma albina* – however, these are both assigned to the same subfamily, the Turrinae). In contrast, some species in the same genus appear to have diverged significantly earlier, in the Eocene. Such early diverging taxa include *Terebra subulata* and *Terebra crenulata* (Terebridae) and *Mitra mitra* and *Mitra ustulata* (Mitridae).

The data generally support the conventional assignment of the species in Table II into the family groups indicated. The three species of Turridae, for example, exhibit a divergence range (7.2 - 11.3%) which is clearly smaller than their divergence from other neogastropods (14.4 - 20.8%) or from the mesogastropod outgroup species (29.1 - 30.1%).

Implications of the molecular data. The results described above, though preliminary, support some rather unconventional phylogenetic hypotheses regarding the neogastropod families analyzed. We summarize the major trends indicated by the data, and discuss each in turn:

1) All neogastropod groups included in this study are approximately equally divergent from the mesogastropod species used as the outgroup (the cerithid *Rhinoclavis aspera*).

2) In general, all neogastropod groups (which can be assigned to six different families by conventional taxonomy) are approximately equidistant from each other, with the pairwise divergences between neogastropod families being less than the divergence from the mesogastropod *Rhinoclavis*.

3) The Turridae exhibit an apparently smaller divergence distance from all other neogastropod groups.

4) The Costellaridae appear to be closer to the Turridae than to any other neogastropod group (and vice versa).

The % divergence from the mesogastropod *Rhinoclavis aspera* is approximately equal for all neogastropod groups analyzed. If we use the values for the rate of divergence within the genus *Conus* (derived from the analysis of seventy different *Conus* species and correlating the divergence distance values with the fossil record), the age of the last common ancestor between *Rhinoclavis* and the neogastropod families included in this study is

TABLE IV. Kimura Two - Parameter Divergence Distances (%)

	Conidae			Turridae			Terebridae		Costellaridae		Mitridae		Olividae	Mesogastropod
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. *Conus ermineus*	0.0	7.31	15.3	20.8	18.3	17.0	21.9	24.0	23.8	19.9	21.4	22.5	23.3	29.5
2. *Conus textile*		0.0	15.2	20.8	19.7	15.6	20.7	20.8	22.0	19.4	21.7	21.6	22.5	29.3
3. *Conus californicus*			0.0	18.9	17.1	16.3	20.3	19.3	21.7	19.0	20.1	19.6	19.1	29.2
4. *Turris spectabilis*				0.0	7.2	11.3	19.3	17.5	17.0	16.7	18.0	15.9	18.0	29.1
5. *Lophiotoma albina*					0.0	10.4	17.3	16.0	14.9	14.4	17.2	17.3	17.0	30.1
6. *Clavus unizonalis*						0.0	18.0	17.9	15.1	14.9	17.2	16.7	16.4	28.6
7. *Terebra crenulata*							0.0	12.7	22.5	20.4	23.6	18.1	19.6	31.4
8. *Terebra subulata*								0.0	22.6	20.9	22.7	18.1	18.7	31.4
9. *Vexillum compressum*									0.0	6.5	22.0	22.2	19.6	32.7
10. *Vexillum granulosum*										0.0	21.7	20.2	19.9	29.1
11. *Mitra mitra*											0.0	14.3	19.9	28.3
12. *Mitra ustulata*												0.0	17.1	29.6
13. *Oliva miniacea*													0.0	29.4
14. *Rhinoclavis aspera*														0.0

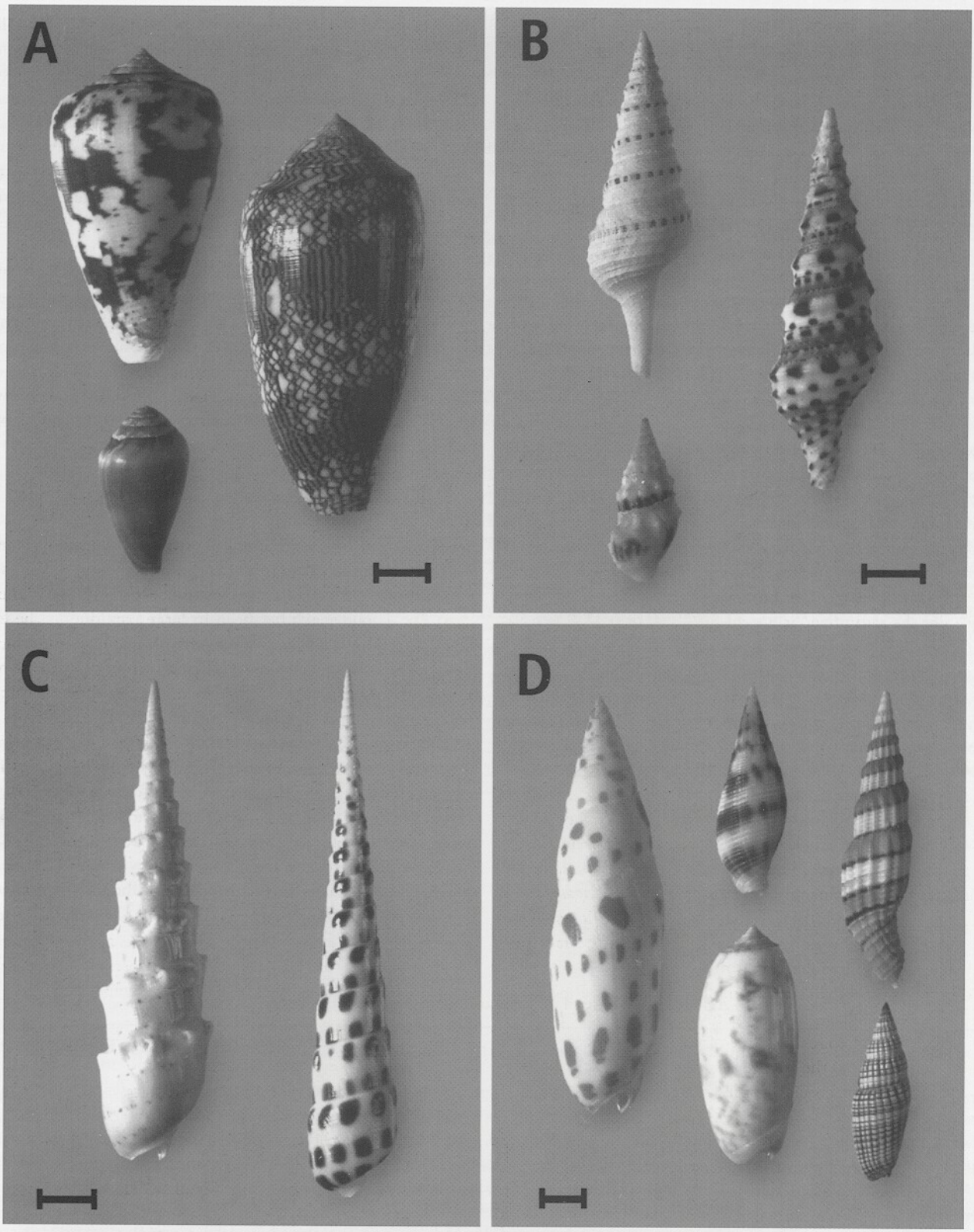

Figure 1. Neogastropod families analyzed in Tables III and IV: Conidae (A); Turridae (B); Terebridae (C) and three non-Conoidean families (D), Mitridae, Costellaridae and Olividae. The *Conus* species from top left, clockwise, are *Conus ermineus* (Bonaire), *Conus textile* (Philippines), *Conus californicus* (California, USA). Turridae analyzed, from top left, clockwise: *Lophiotoma albina* (Philippines), *Turris spectabilis* (Philippines), *Clavis unizonalis* (Philippines). The *Terebra* species analyzed: left, *Terebra crenulata* (Western Samoa); right, *Terebra subulata* (Australia). Other neogastropods not belonging to the superfamily Conoidea: from top left, clockwise, family Mitridae - *Mitra mitra* (Philippines), *Mitra ustulata* (Philippines); family Costellaridae - *Vexillum compressum* (Philippines); *Vexillum granosum* (Philippines), family Olividae - *Oliva miniacea* (Philippines). Color figure prepared by Kerry Matz.

The localities indicated above are of the actual specimens figured. For specimens actually discussed and mit.DNA analyzed, all were from the Philippines except for *Conus ermineus* and *Conus californicus*, which were from Bonaire and California, respectively.

estimated at ca. 84-100 mya. Whether the rate-of-divergence parameter can be extrapolated linearly to that extent is one reservation in this calculation.

The other major result from this study is that all six neogastropod families are essentially equidistant from each other with the exceptions noted below. If one applies the calculation of age of divergence of *Conus* from the other five neogastropod families, the best estimate is that this divergence of neogastropod families occurred close to the K-T boundary, during the late Cretaceous or the Paleocene. The data therefore strongly suggest that a single ancestral line diverged from the mesogastropod ancestor sometime during the Mesozoic, and gave rise to the six neogastropod families in this study sometime around the K-T boundary.

An anomaly in the data is that the divergence distance of the turrids from all other neogastropod groups is consistently less than calculated for any other pair of families. It should be noted that the Turridae are generally deeper water molluscs than the other groups analyzed, with some very deep-water forms. We observed in the previous study of *Conus* that there was a similar anomaly in calculating the divergence of the fish-hunting *Conus* species from mollusc-hunting *Conus* using *Conus textile*, a shallow water mollusc-hunting species, vs. *Conus gloriamaris*, which typically lives at depths of 100 meters. Whether a deep-water habitat (with lower temperatures and perhaps longer generation times) can account for the apparently less divergence seen between the Turridae and other neogastropod groups remains to be established. Other explanations for these data cannot be eliminated at this time.

The most surprising result was the lack of evidence for clustering of toxoglossate families, conventionally included in the superfamily Conoidea (Conacea, Toxoglossa). Thus, the Turridae, Conidae and Terebridae appear to be no more closely related to each other than they are to any of the other neogastropod families. Indeed, among the groups analyzed, the closest relationship between families appears to be between the families Costellaridae and Turridae. The molecular results raise the issue of whether the toxoglossate molluscs are a monophyletic group; a previous analysis also failed to group *Conus* and *Hastula* (in the Terebridae) together as a clade (Harasewych et al., 1997). The species in the Turridae analyzed appear to be less diverged from the two species in the Costellaridae than they are from *Conus* and *Terebra*.

Additionally, the two Costellarid species are significantly more distant from the Mitridae than from the Turridae, which provides strong molecular support for the separation of Costellaridae from Mitridae into distinct families. These two groups do not appear more closely related to each other than any other pair of neogastropod families analyzed.

If the Costellaridae and Turridae are indeed the most closely related families, the separation of Turridae, Terebridae and Conidae into a superfamily division separated from other neogastropod groups would not be tenable. Although the results are admittedly limited both with respect to the number of species analyzed and the number of genetic loci measured, they raise fundamental questions about the conventional taxonomic scheme presently used for Neogastropoda.

The neogastropod families included in this study seem like a classical star phylogeny. In many ways, the data have striking parallels in the evolution of mammalian orders. The molecular analysis of mammals shows a similar sudden diversification near the K-T boundary. It is tempting to hypothesize a common cause for these similar patterns: the geological catastrophe that led to the Cretaceous extinction. The parallel can be extended: just as the complete extinction of the dinosaurs on land provided an opportunity for the mammalian radiation, the total extinction of ammonites in marine habitats may have given a once in 10^8 year ecological opportunity for predatory gastropod lineages to undergo an unprecedented radiation.

IV. Discussion and Perspectives

Since the first biochemical study of *Conus* venoms, considerable progress has been made in understanding the molecular mechanisms underlying snail envenomation. It is clear that the success of the cone snails has been in large part due to the evolution of a remarkable array of conotoxins, as the pharmacological agents underlying the activity of their venoms. It is estimated that there are ca. 50,000 different molecular forms of conotoxins in the venoms of living cone snails. At the genetic level, this has involved an unprecedented diversification of a few gene superfamilies. It appears that the cone snails' success is due in part to the ability to mutate these genes as changes in the environment occur over a geological time period. What this mechanism of hypermutation is remains to be elucidated, but in effect, as an aggregate, the genus *Conus* has apparently evolved appropriate new conotoxins to meet the challenges of new ecological situations during the entire Tertiary period. The extraordinary pharmaceutical properties of *Conus* venom peptides makes them useful as basic tools in neuroscience, as diagnostic agents, and somewhat unexpectedly, as therapeutic drugs.

In the results presented above, we provide an indirect assessment of whether other groups included in the superfamily Conoidea might have underlying strategies of envenomation using conotoxin-like peptides, as has been established for *Conus*. The analysis of the pedigree of various neogastropod families discussed in the sections above suggest that instead of having various stem groups within Conoidea from which the cone snails evolved, the phylogeny fits a star phylogeny better than a branching tree phylogeny. This implies that around the K-T boundary, there was a radiation of all of the different neogastropod groups at approximately the same time. The predicted phylogenetic reconstruction, if confirmed, appears to us to make it less likely that groups such as the Turrinae, and the Drillinae within the family Turridae, or the venomous Terebridae (such as *Terebra subulata*) will overlap considerably with the molecular

and genetic strategy of the cone snails. The possibility that all of the major toxoglossate groups (Conidae, Turridae and Terebridae) arose at the same time as nonvenomous families in Neogastropoda (Mitridae, Olividae) increases the probability that the different Conoidean groups may each have evolved its own characteristic venom components. The probability that different genes may have been recruited for use in venom in the course of their divergence from a common ancestral form is increased by our results. A branching tree organization of Conoidean families would have been consistent with a stepwise evolution of venom genes. This becomes a less tenable alternative if all the neogastropod families diverged from each other at more or less the same time, as suggested by a star phylogeny. Clearly, the only way to settle this question definitively is to undertake the direct analysis of turrid and Terebrid venoms.

Finally, the molecular analysis presented above suggests that the standard taxonomic scheme for neogastropod phylogeny needs reevaluation.

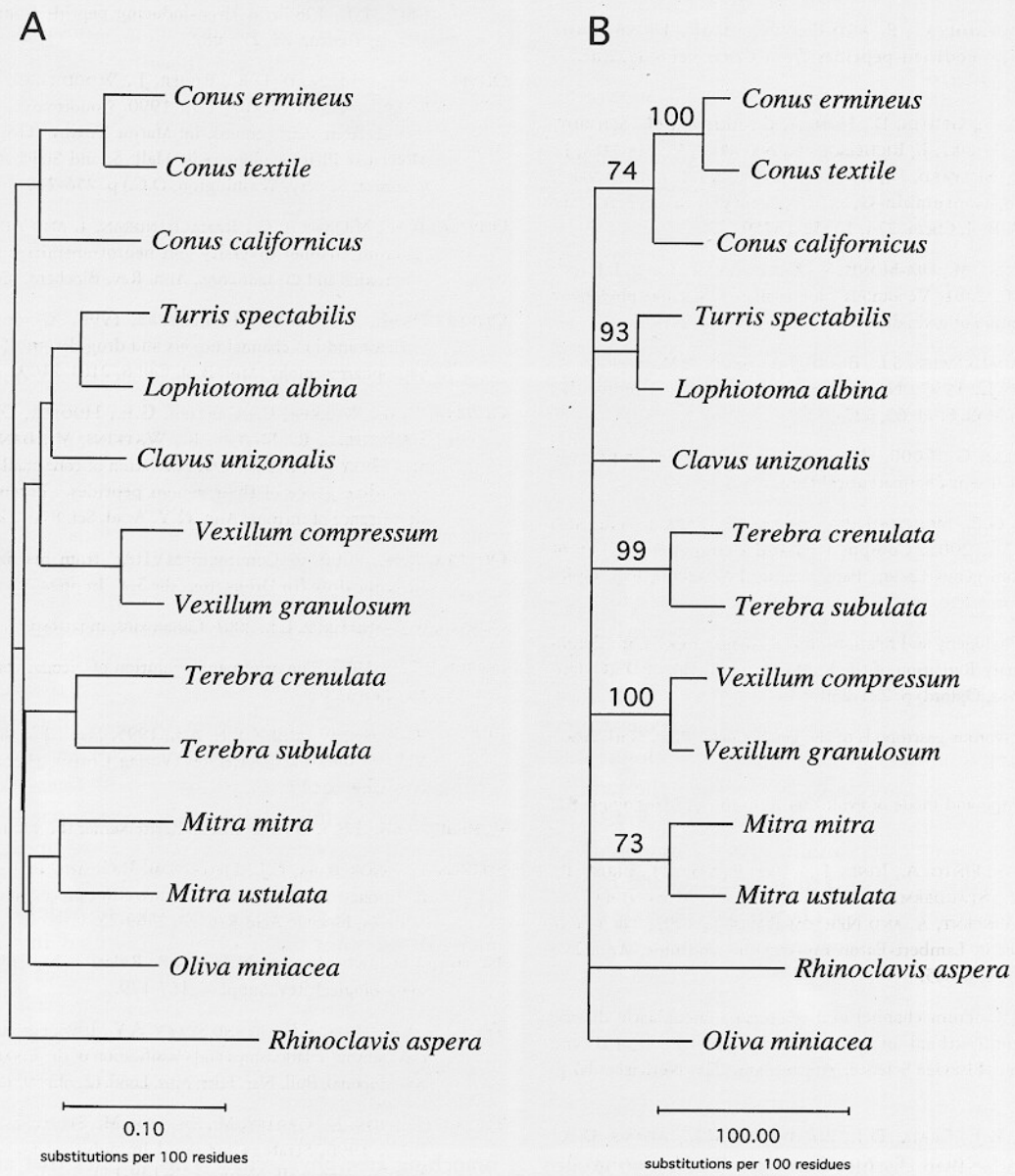

Figure 2. Two phylogenetic reconstructions of several gastropod families. The mitochondrial 16S ribosomal RNA sequence data were obtained as described in the legend to Table III. The sequence alignment shown in Table III was used to generate the phylogenetic trees. (A) A phylogenetic reconstruction using a heuristic search with a minimum evolution distance criterion. In this reconstruction, uncorrected distance parameters were calculated and used to search for optimal trees. (B) An alternative phylogenetic reconstruction using a heuristic search with parsimony as an optimality criterion. A bootstrap analysis was performed to assign confidence levels to groupings in the tree. Confidence levels are shown on each branch. Groupings with levels below 50% are not shown.

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