

Defining a clade by morphological, molecular, and toxinological criteria: distinctive forms related to *Conus praezellens* A. Adams, 1854 (Gastropoda: Conidae)[†]

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ABSTRACT

We carried out a definition of the *Conus praezellens* A. Adams, 1854, species group using a combination of comparative morphological data, molecular phylogeny based on standard genetic markers, and toxinological markers. Prior to this work, *Conus praezellens* was generally postulated to belong to a clade of similarly high-spined, smaller species such as *Conus pagodus* Kiener, 1845, *Conus memiae* (Hahe and Kosuge, 1970) and *Conus arcuatus* Broderip and Sowerby, 1829. The molecular phylogeny and toxinological data demonstrate that these earlier hypotheses are incorrect, and that instead, *Conus praezellens* is in a branch of *Conus* that includes *Conus stupa* (Kuroda, 1956), *Conus stupella* (Kuroda, 1956), *Conus acutangulus* Lamarck, 1810, and surprisingly, some species that are morphologically strikingly different, *Conus mitratus* Sowerby, 1870, and *Conus cylindraceus* Broderip and Sowerby, 1830. A more careful analysis of the morphologically diverse forms assigned to *Conus praezellens* suggests that from the Philippine material alone, there are at least three additional species new to science, *Conus andremenezi*, *Conus miniexcelsus*, and *Conus rizali*. A reevaluation of protoconch/early teleoconch morphology also strongly suggests that *Conus excelsus* Sowerby III, 1908, is related to these species. Together, the different data suggest a clade including the 10 species above that we designate the *Turriconus* (Shikama and Habe, 1968) clade; there are additional distinctive forms within the clade that may be separable at the species level. The phylogenetic definition using the multidisciplinary approach described herein provides a framework for comprehensively investigating biodiverse lineages of animals, such as the cone snails.

Additional keywords: Neogastropoda, *Turriconus*, 12SrRNA sequences, phylogenetic analysis, exogenes

INTRODUCTION

The evolutionary histories of biodiverse *Conus* lineages are a challenge to elucidate. In part this is because the genus is so speciose (about 700 species) but also because most prior data in the literature is morphological. The usual approach is to characterize each species in the lineage based on its shell morphology and to evaluate phylogenetic relationships using additional anatomical data, when available.

Prior attempts to divide *Conus* into subgeneric groups have been based largely on shell morphology. In this work, we focus on one particular branch of *Conus* that includes the species known as *Conus praezellens*. Several previous attempts to determine which species are most closely related to *Conus praezellens* have grouped *C. praezellens* with other high-spined forms (Figure 1) that are collected in deep offshore locations. Some of the specific prior hypotheses that have been proposed are summarized in Table 1.

In the most comprehensive modern treatise on Indo-Pacific *Conus* species (Röckel et al., 1995), *Conus praezellens* is regarded as most closely related to *Conus acutangulus*. In most of the schemes shown in Table 1 (Marsh and Ripplingale, 1964; Okutani, 2000), *Conus praezellens* is grouped together with *Conus acutangulus* in the subgenus *Conasprella* Thiele, 1929. The designated type of *Conasprella* is "*C. cancellatus*" (= *C. pagodus*). Another species generally thought to belong to this subgenus is the Eastern Pacific *Conus arcuatus*. In one of the proposals (da Motta, 1991), *Conus praezellens* and *Conus acutangulus* are in two different subgeneric groups: *Conus praezellens* in *Conasprella* and *Conus acutangulus* in *Kermasprella* Powell, 1958 (which this author regards as a subgenus of the genus *Profundiconus* Kuroda, 1956). Among the species included with *Conus acutangulus* in *Kermasprella* are forms such as *Conus memiae* and *Conus nereis* (Petuch, 1979), the latter regarded by Röckel et al., 1995 as a form of *Conus wakayamaensis* (Kuroda, 1956). Thus, the high-spined

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Figure 1. High-spired *Conus* species previously postulated to be related to *Conus praecellens*: Top from left: *Conus acutangulus*, "typical form"; *Conus acutangulus*, "deep-water form"; *Conus nereis*. Middle from left: *Conus praecellens*, "Aliguay form"; *Conus praecellens*, "sowerbii form"; *Conus andremenezi* new species (Holotype, MSI); *Conus pagodus*. Bottom from left: *Conus miniexcelsus*, new species (Holotype, deposited at MSI); *Conus rizali* new species (Paratype 2, MSI); *Conus arcuatus*. All of the specimens shown are from the Philippines, except for *Conus arcuatus*. Measurements provided in Appendix I.

Table 1. Previous taxonomic assignments of *Conus praececellens* and *Conus acutangulus*.

Marsh and Rippingale (1964):	DaMotta (1991):	Okutani (2000):
<i>Conasprella praececellens pagodus arcuatus acutangulus wakayamaensis</i>	<i>Conasprella praececellens pagodus arcuatus</i>	<i>Conasprella praececellens pagodu gracatapi acutangulus</i>
	<i>Kernasprella acutangulus memiae wakayamaensis nereis jaspideus</i>	<i>Endemnoconus memiae wakayamaensis ione</i>

Conus species including *Conus praececellens* were either all grouped together in *Conasprella*, or were divided into a *praececellens/pagodus* group (*Conasprella*) and an *acutangulus/memiae* group (*Kernasprella*).

These shell morphology-based suggestions can be independently evaluated using molecular data. If only morphological analyses are used, the resulting systematics may not reflect evolutionary trends as the traits may be subject to selection forces that do not reflect common descent. Distinguishing similarity by descent (reflecting the phylogeny) from similarity directed by selection (convergence or parallelism) is problematic without independent data corroborating the morphological evidence. Hence a widespread attempt to define biodiversity using molecular markers, notably a segment of the COI gene, has led to the “barcode initiative”. Although this initiative has been widely implemented, workers who need to identify field specimens require a more seamless integration of the molecular with the morphological data.

Our first goal is to define a phylogenetic tree with clades that reflect the branching pattern and in turn the evolutionary history of the species. Molecular data provide independent evidence for such a phylogeny and a useful organizational framework for in-depth studies of species-rich groups. The morphological traits mapped onto such a tree distinguish the respective roles of common descent and selection in the evolutionary process.

In this work, we focus on the definition of the putative clade that includes *Conus praececellens*. Using 12SrRNA sequences, we specifically evaluate which *Conus* species are most closely related to *Conus praececellens*. In addition to using a molecular phylogeny to assess morphology-based taxonomy, we have gathered molecular data in the form of the genes that encode toxins expressed in the venom ducts of cone snails. As will be shown, these highly specialized “exogenes” (Olivera, 2006) are useful in defining discrete branches of a large biodiverse lineage such as the cone snails. This three-pronged approach defines a more complete picture of the evolutionary history of these biodiverse cone snails with the result that previous morphologically informed hypotheses may be more objectively assessed.

MATERIALS AND METHODS

SPECIMEN COLLECTION. Most forms in the *Conus praececellens* complex are collected offshore from 30–250 meters in depth. The bulk of Philippine specimens in collections assigned to this species were collected (together with such species as *Tibia fusus* (Linnaeus, 1758) and *Xenophora solaris* (Kosuge and Nomoto, 1972) around 1960, primarily from a few classical fish trawler localities (Maqueda Bay in Samar Is, Tayabas Bay in Luzon); at the time, these were mostly identified as *Conus sowerbii* (see Reeve, 1849; Springsteen and Loebrcera, 1986). Because other forms in the *praececellens* complex are mostly from even deeper water, these were less well represented in collections; most specimens available in museums are poorly preserved and/or dead-collected. However, the combination of gill net and hookah collections in the Cebu/Bohol area of the Central Philippines and intensive small trawl collections around the Island of Aliguay has increased accessibility to several forms in the *Conus praececellens* complex. Some of these are smaller specimens that were sparsely represented in earlier collections. A range of live-collected specimens with preserved protoconchs has become available, which has facilitated the reevaluation of the *Conus praececellens* species complex.

PHYLOGENETIC ANALYSIS. We aligned sequences using Clustal X (Larkin et al., 2007) and refined by eye using MacClade (Maddison and Maddison, 2005). The tree was inferred using MrBayes (Huelsenbeck et al., 2001; Ronquist and Huelsenbeck, 2003). The run comprised 1,000,000 generations with the first 25% of the sampled generations discarded as burn-in trees. Two MCMCMC runs (metropolis-coupled Monte-Carlo markov-chain), using four chains each, were used to thoroughly explore tree space. Convergence of the likelihoods was determined by comparing the average standard error of the difference (ASED) in split frequencies between the two runs and by comparing plots of the log-likelihood after the burnin to the end of the runs. Optimality was also judged adequate when the PSRF (Potential scale reduction factor) for the total tree length and for each model parameter reached 1.00.

IDENTIFICATION AND SEQUENCING OF GENOMIC CLONES ENCODING O-SUPERFAMILY PEPTIDES. Genomic DNA was prepared from 50 mg each of tissues of *Conus acutangulus*, *Conus mitratus*, *Conus praececellens*, and *Conus stupa* using the Genra PUREGENE DNA Isolation Kit Kit (Genra Systems, Minneapolis, MN) according to the manufacturer’s standard protocol. These genomic DNAs were used as templates for polymerase chain reaction (PCR) with oligonucleotides corresponding to conserved 5’ intron and 3’ UTR sequences of omega and delta prepropeptides. The resulting PCR products were purified using the High Pure PCR Product Purification Kit (Roche Diagnostics, Indianapolis, Indiana) following the manufacturer’s suggested protocol.

The eluted DNA fragments were annealed to pNEB206A vector and the resulting products transformed into competent DH5a cells, using the USER Friendly Cloning Kit (New England BioLabs, Beverly, Massachusetts) following manufacturer's suggested protocol. The nucleic acid sequences of the resulting omega and delta toxin-encoding clones were determined according to the standard protocol for automated sequencing.

MORPHOMETRIC ANALYSIS. Using dial calipers, we measured maximum diameter (mm) and total length (mm; including spire height) of species within the *praecellens* complex. Relative diameter was calculated as the ratio of maximum diameter to total length. All species were represented by multiple samples. In view of the low between-sample variation, we calculated a single mean relative diameter for each species.

RESULTS

SYSTEMATIC DESCRIPTIONS OF THREE NEW SPECIES OF *CONUS*

BY BALDOMERO M. OLIVERA AND JASON BIGGS

Superfamily Conoidea Fleming, 1822

Family Conidae Fleming, 1822

Subfamily Coninae Rafinesque, 1815

Genus *Conus* Linnaeus, 1758

Conus andremenezi Olivera and Biggs, new species.
(Figures 1, 2, 6)

Description: Biconical in shape, mature specimens from 25–53mm. Moderately solid, and with a relatively high spire, and generally broader than most related forms ($D/L \approx 0.47$). Last whorl is broadly conical, with raised spiral ribs that are not smooth but always undulating (and in some specimens, the ribs seem to have arch-like protuberances, instead of a continuous smooth rib). Raised ribs on the body whorl are well separated from each other, with interstices that have axial scales between them.

The body whorl has an off-white ground color with characteristic purplish-brown spots that occur in zones; in the two darker zones, the spots generally cover more spiral ribs and extend into the interspaces (although there is considerable variation). The protoconch is decolled in most specimens, but when preserved it is a rounded conical shape, translucent, very light yellowish brown or off-white; the protoconch is followed by two white early teleoconch whorls that are lightly nodulose and angled at the periphery. The spots begin to appear on the periphery of the third or fourth teleoconch whorl, and typically these are more closely spaced to each other than are the larger spots in the later spire whorls.

Type Material: The Holotype is deposited in the Marine Science Institute (MSI) at the University of the Philippines; Paratypes are deposited at the Academy of Natural Sciences of Philadelphia, Philadelphia, Pennsyl-

vania (ANSP 421619); the Muséum national d'Histoire naturelle, Paris, France (MNHN 21131); the Field Museum of Chicago, Chicago (FMHN 312461); the Harvard Museum of Comparative Zoology, Cambridge Mass (MCZ 361611); Zoological Museum of Moscow State University, Moscow, Russia (Lc-37964) and The Bailey-Matthews Museum, Sanibel, Florida (BMSM 38672) (see Appendix or a complete listing of paratypes).

Type Locality: The type locality for *Conus andremenezi* is Aliguay Island, Philippines, where most specimens in the type series have been collected by commercial fishermen using small trawls at depths around 150 m. Another established locality is off Panglao, Bohol, from Balicasag Island to Momo Beach where the species has been collected by tangle nets in deeper water (~200–300m).

Geographical Distribution: From the Central to Northern Philippines, probably to Viet Nam and possibly much further West (see discussion below). In the recent book of Thach (2005), the specimen figured as *Conus praecellens* (Plate 61, Fig. 34) is likely to be a specimen of *Conus andremenezi*.

Etymology: This species honors the memory of Andre Menez, one of the giants of the field of toxinology.

Remarks: The sculpture on the spire whorls is diagnostic: the spiral ribbons on the larger spire whorls are raised, relatively narrow to very narrow, and always far apart. The wide spacing on the spire whorls between narrow raised spiral ribs is a diagnostic trait of this species; in most similar forms, the spiral ribbons or ridges are much closer together and are more like flattened ribbons, broad and shallow. The broad shape, purplish-brown spots, undulating spiral ribs, and widely spaced ribs on the spire are characteristic features that separate the species from similar forms.

When the protoconch is decolled, this species is difficult to separate from some closely related forms that are potentially variants of *Conus praecellens*. Most specimens can generally be differentiated by the distinctive purple-brown color, the broader shell, the widely spaced spiral ribbons on the spire whorls, and when preserved, the conical protoconch. Most specimens in the type series come from Aliguay.

There is a group of Philippine specimens, not from the type locality, which we tentatively assign to this species. These were collected by the Musorstom expeditions promoted by the Muséum national d'Histoire naturelle, Paris, to Lubang Island/Mindoro. Several large mature specimens of *Conus andremenezi*, all dead collected, were examined. All of these were collected at depths between 160–198 meters; at more shallow collection stations, this form was absent and a narrower *Conus praecellens* variety was present. This provides a more accurate estimate of the depth at which this species occurs.

Finally, there is a small specimen figured by Röckel et al. (Plate 54, Fig. 14) that appears to be a juvenile of *Conus andremenezi*; if the identity of this specimen can

Figure 2. Two morphospecies with non-“*praecellens*-like” protoconchs from Aliguay. All of the specimens shown are from Aliguay Island, Philippines, except the lower left specimen which was collected from southern Japan. Top row: *Conus andremenezi*; Bottom row: *Conus miniexcelsus*. For *Conus andremenezi*, the Holotype (left), Paratype 7 (second from right), and Paratype 11 (right) are shown. For *Conus miniexcelsus*, the Holotype (second from right), Paratype 2 (third from right), and Paratype 15 (rightmost specimen) are shown. All of the types figured are deposited at the Marine Science Institute (MSI), University of the Philippines. Measurements provided in Appendix 1.

be verified, it extends the range of this species across the entire Indian Ocean since the specimen is reported to be from Somalia. Thus, although almost all of specimens examined were from the Central Philippines, there is strong evidence for the occurrence of the species in the Northern Philippines, and the possibility that it may have a geographic distribution that is much wider is raised by the Somali specimen in the Raybaudi-Massila collection.

Conus miniexcelsus Olivera and Biggs, new species
(Figures 1, 2, 4, 6 and 8)

Conus praecellens f. subaequalis.—Robin, 2005: 424,
fig. 14 (non *Conus subaequalis*, Sowerby III, 1870)

Description: A moderately small shell; adult size range,
25–37 mm. High-spined, with both spire and body whorl

having a straight outline, making the shell narrowly biconical. The larval shell has 3.0–3.5 whorls, translucent brownish or purplish. There are 9–11 teleoconch whorls, the first three being ivory-white, without spots, providing a notable contrast to the translucent-colored protoconch. At around the fourth teleoconch whorl, broad brownish spots appear, centered around the periphery. The ground color is white, with chestnut-brown spots. On the body whorl there are a series of flat spiral ribbons. The shell pattern on the body whorl can be divided into 3–5 zones. The most posterior, next to the suture, are a series of about 6 spiral ribbons with extremely fine chestnut brown spots. These are followed by a zone with 3 noticeably broader spiral ribbons that have deeper brown and larger spots. In most specimens, this is followed by three spiral ribbons that have a finer spotted pattern (but not as fine as in the spiral ribbons in the first zone, closest to the suture). The remainder of the shell towards the tip is covered by spiral ribbons that are darker in color and more heavily spotted; typically the first 3 to 4 are darker than those towards the anterior end of the shell, although there is considerable variation in this regard. In some specimens, the light zone continues to the anterior of the shell.

Type Material: The Holotype is deposited at the Marine Science Institute at the University of the Philippines, Paratypes are deposited at the Field Museum, Chicago, Illinois (FMNH 312462); the Museum National d'Histoire Naturelle, Paris, France (MNHN 21132); the Harvard Museum of Comparative Zoology, Cambridge, Massachusetts (MCZ 361609); the Academy of Natural Sciences of Philadelphia, Philadelphia Pennsylvania (ANSP 421620); Zoological Museum of Moscow State University, Moscow, Russia (Lc-37965) and The Bailey-Matthews Museum, Sanibel, Florida (BMSM 38673) (see Appendix or a complete listing of paratypes).

Type Locality: Aliquay Island, Philippines. Most specimens in the type series were collected by the commercial small-trawl operations off Aliquay Island, at depths of 30–150m.

Geographical Distribution: Presently known from the Central Philippines to Wakayama, Japan (Paratype #23).

Etymology: The specific epithet emphasizes some striking and unexpected similarities to *Conus excelsus* despite the considerable disparity in size.

Remarks: A distinguishing characteristic of this species are the spots on the whorls closest to the suture, which are generally extremely fine in pattern and greater in number than for any other similar species, followed by the thicker, darker brown spiral ribbons at the center of the body whorl. These features are clearly illustrated in the specimens shown in Figure 3. *Conus miniexcelsus* is a distinct species, most easily confused with *Conus praecellens*. However, as discussed above and shown in Figure 3, the differences in protoconch

and early teleoconch morphology between the two species are consistent distinguishing characters. This feature puts *Conus miniexcelsus* in the same group as *Conus acutangulus*, *Conus andremerenzi*, and *Conus excelsus* (except that the spire of *Conus acutangulus* has strong tubercles at the sutures). *Conus andremerenzi* is generally larger, with coarse spots that are purplish brown in color instead of chestnut. *Conus miniexcelsus* is probably most similar to *Conus excelsus*, although there is a striking difference in size at maturity. The two Japanese specimens examined are more solid and chunky than the Aliquay material. Figure 8 shows the different shape and color of the protoconchs and the characteristic switch in *Conus miniexcelsus* from a conical translucent purplish brown protoconch, to the ivory white first teleoconch whorls, and finally to the normal spotted pattern.

Conus rizali Olivera and Biggs, new species.
(Figures 1, 3, 6)

Description: The species is medium-sized; specimens examined are 26–39 mm in length. The shell is biconic, with an unusually tall, straight, and sharply pointed spire and a straight-sided body whorl, sharply angled at the shoulder. Outline narrow ($D/L = 0.397 \pm 0.011$); The larval shell has two whorls, and is *praecellens*-like but somewhat proportionally broader than for most specimens of *Conus praecellens*; this is followed by two teleoconch whorls that have a characteristic white-matte surface, somewhat crinkly; starting with the fourth teleoconch whorl, there are 8–9 spotted spire whorls.

The body whorl is characterized by shallow spiral ribbons with only a narrow interstitial space between them; these are broadly spotted in light yellow-brown. Characteristically, immediately below the periphery, the first spiral ribbon lacks spots, leaving a white zone. Although there is some variation, the spots are much lighter in color than in related forms (Paratype 2 almost completely lacks spots in the body whorl).

Type Material: The Holotype is deposited at the Marine Science Institute at the University of the Philippines; Paratypes are deposited at the Academy of Natural Sciences of Philadelphia, Philadelphia, Pennsylvania (ANSP 421621); the Harvard Museum of Comparative Zoology, Cambridge, Massachusetts (MCZ 361610) and in the Museum National d'Histoire Naturelle, Paris France (MNHN 21133) (see Appendix for a complete listing of paratypes).

Type Locality: All type specimens were obtained from commercial dealers in the Philippines, and the exact collection locality of the types could not be verified. Springsteen and Leobrara show a figure of *Conus rizali* (labeled *Conus subaequalis*) indicating Punta Engaño, Cebu, suggesting that these were probably collected by fishermen using tangle nets at depths of 100–200m.

Geographical Distribution: Philippines.

Figure 3. Three distinctive forms with “*praecellens*-like” protoconchs. Top row is a series of *Conus praecellens*, “sowerbii form”. Bottom row is *Conus praecellens*, “Aliguay form”. In the middle row are two specimens of *Conus rizali* new species, the Holotype (left) and Paratype 2 (right), both MSI. Measurements provided in Appendix 1.

Etymology: This species is named in honor of Jose Rizal, the National Hero of the Philippines. Dr. Rizal, who was executed by the Spanish Colonial Administration in 1898, collected shells as a hobby.

Discussion: Of all of the similar forms, *Conus rizali* has the most narrow outline ($D/L = 0.397 \pm 0.011$); specimens of *Conus praecellens* from Aliguay, which are generally narrower than the “Sowerbii form” have a $D/L = 0.44$, and for *Conus miniexcelsus*; ($D/L = 0.416$) these are both narrower than *Conus andremanezzi*.

Although Röckel et al., put this species in synonymy with *Conus praecellens*, we believe that it is a distinctive form that can readily be separated from specimens

assigned to *Conus praecellens*. The narrower outline, the shallow ribbons on the body whorl, and differences in protoconch/early teleoconch morphology separate *Conus rizali* from other related forms. *Conus rizali* was previously figured as *Conus subaequalis* Sowerby III, 1870, by authors. This name was used by Springsteen and Leobrera, and by Lim and Wee (1992); however, the specimen recently figured by Robin (2008) as *Conus subaequalis* is not *Conus rizali* but *Conus minexcelsus*. A specimen was also figured by Röckel et al. (Plate 54, Figure 6) but they refer this to *Conus praecellens*. *Conus rizali* is sufficiently distinct so that it can immediately be picked out from other related forms discussed elsewhere in this article: the narrow outline of the shell sets it

immediately apart, and in fact the form that is most similar in outline is *Conus gratacapi* from Japan, which is an unrelated species. This species has only been intermittently collected over the last four decades, and never in large numbers. As has been discussed in detail by Röckel et al., and is shown in the original figure of Sowerby (which Röckel et al., reproduced), the specimens that we assign to *Conus rizali* are clearly not conspecific with the figure of *Conus subaequalis*, which likely refers to a different form in the *Conus praecellens* complex.

MORPHOLOGICAL DEFINITION OF SPECIES IN THE *CONUS PRAECELLENS* COMPLEX: TWO "MINIEXCELSUS-LIKE" FORMS

In this section, we describe and define two distinctive forms in the *Conus praecellens* complex from the Philippines. As will be defined in the Discussion, the "*Conus praecellens* complex" can be divided into two broad groups on the basis of protoconch morphology, the "*praecellens*-like" forms and the "*miniexcelsus*-like" forms.

The two *miniexcelsus*-like forms from Aliguay Island (i.e., those with non-*praecellens*-like protoconchs), which we proposed to designate as new species, are discussed first. The Aliguay specimens of these two forms are easily distinguishable from each other (see Figure 4). Since both of these *miniexcelsus*-like forms were apparently unnamed, these are formally described in the section above. The appendix summarizes the individual type specimens on which the new taxa are based.

Conus andremenezi Olivera and Biggs, new species (Figure 2)

This form may be similar or identical to *Conus bicolor* Sowerby I, 1833, which is a preoccupied name. Sowerby then provided a new name in 1841, *Conus sinensis*. Röckel et al., (1995) stated that "taxonomic status of *Conus bicolor*/*Conus sinensis* (Sowerby II, 1841) remains disputable because the type specimen is lost and the type figure (Plate 54, fig. 3) does not match *C. praecellens* in a satisfying way: the pictured shell has a comparatively low spire...is somewhat bulbous below the shoulder and its color pattern consists of brown axial flames...we favor synonymy with *Conus praecellens*." The figure shown by Röckel et al. (originally drawn from "*Conus bicolor*") is similar to the species we describe above as *C. andremenezi*; we have not adopted the name *Conus sowerbii* for this species because the syntype in the British Museum does not appear to be conspecific with *C. andremenezi*.

We believe that *Conus andremenezi* is clearly distinguishable from typical Philippine specimens of *C. praecellens*; first, the protoconch is not "*praecellens*-like"; second, this form is generally broader and has a characteristic purplish-brown coloration. Furthermore, on the body whorl, there are raised but not flattened

spiral ridges that undulate, with a wide space between ribbons with axial scales between the spiral ridges. More consistently, the sculpture on the spiral whorls has narrow, raised ridges, widely spaced from each other. This suite of characteristics consistently distinguishes this species from *C. praecellens* of similar size (see Figure 1) and from *Conus miniexcelsus* (see next species); *Conus rizali* is even more distinctive from *C. andremenezi*. There are a group of small *Conus praecellens* that are most easily confused with *Conus andremenezi*; these are discussed under *Conus praecellens* below.

Conus miniexcelsus Olivera and Biggs, new species (Figure 2)

This very distinctive species is characterized by its relatively narrow shell outline ($D/L \approx 0.42$ vs. 0.47 for *C. andremenezi*), the multispiral protoconch of 2.5–3.0 whorls, which is translucent and distinctly brownish or purplish and contrasts in its color with the first 2.0–2.5 teleoconch whorls that are ivory white. In most specimens, these white whorls are smooth or have, at most, nearly obsolete tubercles. This is followed by 6–10 spotted teleoconch whorls that are grooved and have strong axial structure so that the upper part of each spire whorl has a distinctly tiled appearance. The body whorl has shallow spiral ribbons with regular, brown spots that have a characteristic pattern described in the Appendix in detail. The colored, translucent protoconch contrasting in color with the first two shiny-white teleoconch whorls, combined with the slender shape and the very fine spotted pattern are diagnostic of this distinctive species. There is considerable variation in how dark the spots are; a range of variations is shown in Figure 4. A full description of this new species was provided in the previous section; detailed measurements of all the types are provided in the appendix. Almost all specimens have been collected by small trawls in Aliguay from 60–130 m, but occasional specimens have also been collected using gill nets off Balicasag Island. One specimen assigned to this species from southern Japan is included in the type series (Figure 4). Figure 2 makes evident the generally finer pattern and narrower shell shape of *Conus miniexcelsus* compared to *Conus andremenezi*. In addition the spiral ribbons of the body whorl of *Conus miniexcelsus* are smooth, but are distinctly crenulated in *Conus andremenezi*.

OVERVIEW AND DESCRIPTION OF THE "PRAECELLENS-LIKE FORMS"

Conus praecellens remains a confusing taxon, and the scheme proposed below is not entirely satisfactory; multiple forms have been assigned to this species by various workers. Even after the two "*miniexcelsus*-like forms" are separated as new species, what remains still comprises a confusing set of specimens, most of which we are provisionally retaining in *C. praecellens*. We believe that the retention of diverse forms within *C. praecellens*

Figure 4. An illustration of the “*miniexcelsus*-like complex”. Shown are five specimens (left five) and close-ups of their respective protoconchs (right five). Left, top and bottom: *Conus miniexcelsus* (Paratypes 2 and 18, respectively), middle: *Conus excelsus*, and right top and bottom: *Conus acutangulus*. Measurements provided in Appendix 1.

will prove to be only an interim solution, and new morphologically similar species will be identified once a more extensive molecular and morphological analysis has been carried out over a wider suite of specimens from a greater geographic range.

There is a widely illustrated specimen designated as “a possible syntype” of *Conus praecellens* from the British Museum. This is atypical of specimens assigned to *C. praecellens* from the Philippines. This possible syntype from the China Sea is lighter in color and finer in sculpture on the body whorl than either of the two major Philippine varieties that we include in *C. praecellens*. The first group, “the Aliguay form,” which is small and light colored, has been extensively collected both by the small dredge operations in Aliguay Island, and is the form illustrated in Figure 3. A second more variable group that we refer to as the “sowerbii forms,” include larger specimens that vary considerably in shell pattern, shape, and size. These comprise most specimens collected by fish trawlers in the period from 1955–1965, particularly from two localities, Tavabas Bay and Maqueda Bay. A third group is that referred to by previous authors as *Conus subaequalis* and

is described above as *Conus subaequalis*. The three forms are shown in Figure 5.

Conus praecellens “Aliguay form”
(Figure 3)

The series of specimens that we assign to *C. praecellens*, “Aliguay form”, appears to be the closest to the type in the BMNH in shell pattern; these have mostly been collected in 30–80 fathoms off Aliguay Island, between Mindanao and Bohol in the Philippines. The Philippine specimens are smaller than the BMNH “syntype” (average size ≈ 24 mm); key features that distinguish this form are a blunt, paucispiral protoconch of two whorls, the relatively smaller size, and the chestnut color of the spots. Details of the spire sculpture and body whorl that are also diagnostic are delineated below.

The shells of this form (see Figure 5) typically have 2.0–2.5 protoconch whorls, the first being quite spherical and inflated, and the second narrower and more elongate. The first two teleoconch whorls are typically white and flattened compared to the protoconch whorls, and they are knobbed on the periphery, while the two

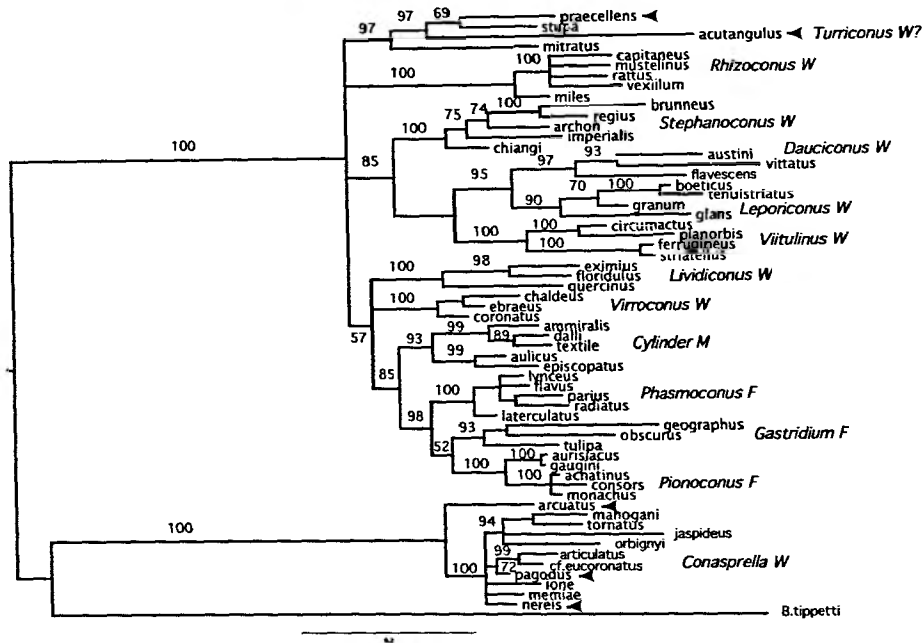


Figure 5. Phylogenetic tree of some *Conus* species based on 12SrRNA sequences. Species shown in Figure 1 are indicated with arrows. Branches are labeled with Bayesian confidence values (posterior probabilities expressed as percentages). These data clearly separate *Conus acutangulus* and *Conus praezellens* from the *Conasprella* species with 100% confidence and join them with *C. stupa* and *C. mitratus* with 97% confidence.

protoconch whorls are smooth. There are 8 to 9 whorls spotted with a chestnut brown color, with the first one or two spotted whorl(s) also knobbed. The early teleoconch whorls are characterized by a deep spiral groove on the upper section of the whorl; these spiral grooves gradually increase in number as the whorls get larger; these are narrow furrows that can be bisected by axial sculpture that varies considerably in strength; in specimens where the axial sculpture is strong, the area immediately adjacent to the suture looks as if it were tiled, since the combination of the spiral grooves and the axial sculpture divide the area between grooves into square sections. The broader part of the spiral whorl is smooth to the periphery; the lower suture is below the sharply angled periphery.

Conus praezellens "sowerbii forms" (Figure 3)

There are forms in the *C. praezellens* complex most commonly found in collections; most specimens were collected by trawlers around 1960 in great numbers in the Maqueda and Carigara bays of Samar Island, and in Tayabas Bay of Southwestern Luzon, from Jolo Island in the Sulu Sea. The "sowerbii forms" are larger and more densely spotted than the specimens of the "Aliguay form" described above. There is considerable variation in shell morphology: some specimens are slender and narrow with fine sculpture; others appear to be much broader at the shoulders with an overall coarser sculpture. However, when preserved, the protoconchs of all of these have the typical highly inflated first whorl, with only two,

pearly white protoconch whorls. A range of specimens collected from various Philippine localities, all with typical "praezellens-like" protoconchs that are well preserved are shown in Figure 5 (the contrast between these and the "Aliguay form" is also illustrated in that figure).

Given the distinctive (and mutually similar) protoconchs of both the Aliguay and the "sowerbii forms," we have provisionally assigned these in *C. praezellens*. However, further characterization of both the radular and gut morphology, as well as a molecular characterization, may prove that these are distinct from each other, and that there are additional separable species embedded in the "sowerbii forms", a possibility that clearly needs to be further evaluated.

Conus praezellens, other distinctive varieties.

A smaller form of *Conus praezellens* was recently collected by MNHN, off Aurora, Eastern Luzon, Philippines. These specimens were notable because they were very similar to *Conus andremanezzi*, but much smaller. They are easily separable from *Conus andremanezzi* because they have the typical "praezellens-type" protoconch. These look very different from the two *Conus praezellens* "forms" described above. We note that in general, *Conus andremanezzi* is a larger species; however, there have been juvenile *Conus andremanezzi* specimens collected using *lumum-lumum* nets in the Camotes Sea, along with specimens of the *Conus praezellens*, "Aliguay form," described above. It seems likely that only juvenile specimens of *Conus andremanezzi* are collected at this

locality because *lumun-lumun* nets were used, and it presumably takes longer than the three months *lumun-lumun* nets are laid out for *Conus andremerenzi* to reach full maturity. These small specimens of *Conus andremerenzi* do have the characteristic protoconchs of that species, though they are somewhat lighter in color than the Aliguay series. In contrast, the variety of *Conus praecellens* collected off Aurora (trawled at 83 m depth) have the *praecellens*-type protoconch. At the same site, the MNHN expedition collected two dead, somewhat eroded specimens, that were larger in size, in a trawl 189–307 meters in depth that are likely to be true *Conus andremerenzi*.

In addition, a species was recently described as *Conus beatrix*, Poppe and Tagaro, 2006. We have not had an opportunity to examine the Holotype of this species; it may be that these represent a series of unusually pale specimens, possibly continuous with the *Conus praecellens* “Aliguay form”, described above. If this were the case, and further molecular evidence shows that these are separable from *Conus praecellens*, then these authors would have provided a potentially valid species name for *Conus praecellens* “Aliguay form.”

Conus rizali Olivera and Biggs, new species (Figure 3)

This distinctive species was previously recognized by Springsteen and Leobrera (1986) and by Lim and Wee (1992) as morphologically separable from any of the other forms assigned to *C. praecellens* or *C. acutangulus*. In their treatment of this complex, Springsteen and Leobrera (1986) provided a figure of this form, to which the name *Conus subaequalis* Sowerby III, 1870, was assigned, with the locality Punta Engaño, Cebu, Philippines. This locality suggests that the specimens they examined were collected by gill nets in deep water. A similar specimen was illustrated by Röckel et al. (plate 54, figure 6) and labeled “*Conus praecellens*” from Davao, Philippines (likely collected by tangle nets off Balut Island, Davao). In our description of *Conus rizali* (see above), the name we propose for this form, we discuss a number of distinguishing morphological characteristics. The name assigned by both Springsteen and Leobrera and Lim and Wee, *Conus subaequalis* does not appear to refer to this form (Röckel et al. reproduce the original figure of Sowerby which refers to a smaller shell, broader in outline with seemingly more deeply colored spots than *Conus rizali*.)

The slender, high-spined, and biconical shape of *C. rizali* is similar to the Japanese *Conus gratacapai*, a poorly understood, rare, deep-water species. Several museum specimens of *C. gratacapai* were examined, including some Paratypes of the latter. Several striking morphological differences indicate that the two forms are not conspecific. Most notably, *C. gratacapai* does not have the sutural structure of *C. rizali*; there is a smooth transition between whorls in the former, but a peripheral overhang between spire whorls in the latter. Another obvious difference are the light brown spots of *C. rizali*, which are absent in

C. gratacapai Pilsbry, 1904. The spire sculpture of the two forms differs significantly as well.

Other “*miniexcelsus*-like” forms: *Conus excelsus* and *C. acutangulus*.

The presence of a translucent multispiral brownish or purplish protoconch, followed by several ivory white teleoconch whorls is a striking morphological feature of *Conus miniexcelsus*. In this respect, two well-known species are “*miniexcelsus*-like”: *C. excelsus* and *C. acutangulus*. Both have the same characteristic translucent protoconch and ivory white early teleoconch whorls (see Figure 6). All of these forms have the body whorl covered by spiral ribbons. The major difference is in the highly nodulose whorls of *C. acutangulus*, versus the smoother whorl of *C. miniexcelsus*. *Conus excelsus* is somewhat intermediate in this respect. There are also the striking and obvious differences in size, *C. excelsus* being by far the largest. Although the shells are different in pattern and size at maturity in these three species (Figure 4), note the similar purplish brown translucent protoconchs, followed by the ivory white early teleoconch whorls before the regular shell pattern is initiated. Typical *Conus acutangulus* (Figure 4, top right) and the “deep-water form” (Figure 4, bottom right) are both distinctly more nodulose while there is a striking similarity between *Conus excelsus* and *Conus miniexcelsus* in their protoconchs and early teleoconch whorls.

Conus excelsus Sowerby III, 1908 (Figure 4)

This is one of the largest species in the group (to over 100 mm). The protoconch consists of about 3.25 whorls, with a maximum diameter of about 1 mm. The protoconch whorls are grayish, and early teleoconch whorls are bright white that then begin to have brown radial blotches of varying size in the later whorls. Early teleoconch whorls have one deep spiral groove, increasing to up to 4 spiral grooves in the later whorls. Although body whorl can be almost smooth, some specimens have variably spaced, axially striate spiral groups separated by granulose ribbons. Most specimens in the Philippines are from Balut Island, Davao, collected by gill nets at depths of approximately 100–150 fathoms. Although *C. excelsus* is strikingly different in size, the body whorl sculpture, the protoconch and early teleoconch whorls show such strong similarities to *C. miniexcelsus* that a close genetic relationship between the two species seems highly likely.

Conus acutangulus Lamarck, 1810 (Figure 4, 6)

Among Philippine specimens, there appear to be two varieties of *Conus acutangulus*, the “typical form” (Figure 4, top row, right), which conforms to the neotype designated by Kohn (Kohn, 1951) and a variety that we will refer to as the “deep-water form” (Figure 4, bottom row, right). This is a well-known species, and the only

Figure 6. Distinctive forms proposed to belong to the *Turriconus* clade. Top row (showing the shell and, in the inset above it, a close-up of the corresponding protoconch), from left to right: *Conus excelsus*; *miniexcelsus* (Holotype); *acutangulus*, "typical form"; *acutangulus*, "deep-water form"; *andremenezi* (Holotype); *praecellens*, "sowerbii form"; *rizali* (Holotype); *praecellens*, "Aliguay form". Lower row, from left to right: *Conus stupa*; *stupella*; *mitratus*; *cylindraceus*. The protoconch of *Conus stupa* is not shown; it is extremely eroded in the figured specimen. Measurements provided in Appendix 1.

issue is whether the two distinct forms described below are conspecific or not

"Typical form" (description after Röckel et al., 1995): This is a small to medium sized shell; the larval shell is multispiral with 3 to 4 whorls. The teleoconch whorls are strongly tuberculate for at least the first eight post-nuclear whorls, a distinctive characteristic. The body whorl has strong spiral ribbons or ribs that are separated by grooves with strong axial threads. The shell is largely brown, except for small, scattered white blotches at the shoulder and center. The aperture is white. This form is collected in relatively shallow water, typically between 3–20 m. Divers in Batangas Bay, Luzon, collected most Philippine specimens; more recently divers in Nucnucan, Bohol, have collected the typical form. Fully mature specimens are 25–38 mm in length, D/L \approx 0.50–0.53. Typical fully mature non-Philippine specimens are also illustrated by Röckel et al. 1995 (plate 54, figs. 19 and 21).

"Deep-water forms:" The deep-water varieties typically occur between 30 to 150 meters, and are collected either by dredging or gill nets. These forms do not have the brown to dark brown color of the typical variety, but

are mostly white with sparse light orange-brown or grayish flecks. Generally smaller than the typical form, most specimens are 16–22 mm in length. This form has been collected in Aliguay and Pamilacan Islands. A comparison of available deeper-water specimens assigned to *C. acutangulus* reveals considerable variation that needs to be more carefully investigated and defined. These forms clearly occur outside the Philippines; Röckel et al., illustrate a specimen from the Solomon Islands (plate 54, fig. 17).

MOLECULAR PHYLOGENY: RELATIONSHIP OF *CONUS PRAECELLENS* TO OTHER HIGH-SPIRED *CONUS* SPECIES

Most of the forms investigated in this article were collected in the Central Philippines, primarily by gill nets or trawlers offshore. A number of smaller *Conus* species of similar shape, including several diverse forms assigned to *Conus praecellens*, are collected in this way (Figure 1). As described above, in most taxonomic work, *Conus praecellens* is either explicitly discussed or implicitly grouped with similarly shaped, high-spired, small,

deep-water *Conus* species even when authors do not endorse a specific phylogenetic scheme (for example see Robin, 2008; Röckel et al., 1995; Walls, 1979).

Table 1 summarizes previously proposed taxonomy based on shell morphology, which can be assessed using molecular data. For comparison, a phylogenetic tree based on 12S mitochondrial DNA includes *Conus praececellens* and *C. acutangulus*, along other vermivorous, molluscivorous, and piscivorous *Conus* species (see Figure 5).

This molecular phylogeny assigns *C. praececellens* and *C. acutangulus* to the same clade. The surprising, yet clear-cut, result is that most other species proposed to be included in *Conasprella* with *C. praececellens* by previous workers based on shell morphology actually cluster in a branch extremely divergent from most *Conus*. The type species of *Conasprella*, *C. pagodus*, is on this very distant branch, which we refer to as the “*Conasprella* clade”. Additional molecular data support these findings (Bandyopadhyay et al., 2008). These data, discussed below suggest that the degree of divergence makes it untenable to keep these species (*C. pagodus*, etc.) within the same genus as other *Conus* species.

Definition of a Clade Based on Morphological and Molecular Data: The combination of the morphological analysis of the various species and distinctive forms above combined with the available molecular data (Figure 5) provides a framework for defining the group of *Conus* spp. most related to *C. praececellens* and *C. acutangulus*. As discussed above, some of the superficially similar high spired *Conus* species are not at all closely related based on the molecular data. In the Philippines, there are ten species and 12 distinctive forms which we assign to this group that we designate the *Turriconus* clade (*Conus excelsus* = *Conus nakayasui*, type species). A comprehensive taxonomic revision of the genus *Conus* is currently being carried out by A. Kohn; we suggest that *Turriconus* is a distinct branch within the major group of species that together, comprise the genus *Conus*.

The molecular work definitively excludes a number of *Conus* spp. from *Turriconus*; clearly, species such as *Conus pagodus*, *Conus memiae*, *Conus nereis*, and other deep-water species with high spires such as *Conus bohollensis* (Petuch, 1979), *Conus eugrammatus* (Bartsch and Rheder, 1943), as well as non-Philippine species such as *Conus jaspideus* (Gmelin, 1791) and *Conus arcuatus*, though morphologically similar to *C. praececellens*, do not belong in the *Turriconus* clade. The 10 species and 12 distinctive forms that comprise the *Turriconus* clade in the Philippines are shown in Figure 6, and summarized in Table 2; as outlined in the table, these fall into four groups that will be discussed in turn.

The first group is the *excelsus/acutangulus* group (Group I), with four species and five distinctive forms. This group includes the type of *Turriconus*, *Conus excelsus*. The second is the *praececellens* group with two species and three distinctive forms. The third is the *mitratus* group of at least two species and fourth, the *stupa* group.

Table 2. Morphologically distinctive “forms” in the *Turriconus* clade (*Conus excelsus*, type species).

Group I	<i>Conus excelsus</i> <i>C. miniexcelsus</i> <i>C. andrenezezi</i> <i>C. acutangulus</i> “typical form” <i>C. acutangulus</i> “deep water form”
Group II	<i>C. praececellens</i> “sowerbii forms” <i>C. praececellens</i> “Aliquay form” <i>C. rizali</i>
Group III	<i>C. mitratus</i> * <i>C. cylindraceus</i>
Group IV	<i>C. stupa</i> <i>C. stupella</i> *

*Peptide toxins belonging to the O-superfamily have been determined for these “forms.”

The first group of species, the *excelsus/acutangulus* group, is characterized by a generally conical protoconch, without the spherical, inflated first protoconch whorl. The protoconch is translucent, brownish purplish or light yellowish in color; and followed by two or more, much whiter, teleoconch whorls. The four species in this group are *Conus excelsus*, *Conus miniexcelsus*, *Conus andrenezezi*, and the two forms of *Conus acutangulus* described above.

In the second group, the *praececellens* group (Group II), the characteristic feature is a white protoconch of two whorls, with the first protoconch whorl being inflated and spherical (i.e., “*praececellens*-like”). The characteristic shape of this protoconch is diagnostic of this group (as shown in Figure 6); there may be more species than are recognized here, since this is a rather variable assemblage of forms as is discussed above. *C. praececellens* and *C. rizali* are the two species in this group, with at least two distinctive forms assigned to *C. praececellens*.

The third group in *Turriconus*, based on molecular data and expanded using morphological similarities, is the *Conus mitratus* group (Group III). These species have much more elongated body whorls than are found in the two groups above. They seem to share the characteristic white early teleoconch whorls before the mature coloration is expressed with the first group. On morphological grounds alone, we assign two species to this group: *Conus mitratus* and *Conus cylindraceus* (Broderip and Sowerby, 1830). The molecular evidence in this paper is only provided for *Conus mitratus*; however, corroborative molecular evidence for *Conus cylindraceus* has been obtained by others (C. Meyers, personal communication).

The fourth group in the *Turriconus* clade is a subgenus (*Kurodaconus*) (Group IV) recognized as distinctive by some workers; the molecular evidence suggests these species should be included in *Turriconus*. There are two species in this group, *Conus stupa* and *Conus stupella*; it is the opinion of several workers on *Conus* that these may not be separable species, since they always appear to occur together. This suggestion needs to be further evaluated. These forms differ from Groups I and II by the smooth body whorl, while the species in these groups have the spiral ribbons or ribs.

The overall hypothesis, based on the combined molecular and morphological data, is presented in Table 2. We used this working hypothesis as a guide to analyze toxins in the putative species of the *Turriconus* clade, from which DNA samples were available (marked by asterisks in Table 2).

Using Toxinological Markers to Evaluate the *Turriconus* Clade: The hypothesis presented in Table 2 was experimentally tested using a toxinological analysis. Since cone snails are venomous animals, they use toxins in their venom to capture prey, defend against predators and for competitive interactions. Since each species presumably has a different spectrum of prey, predators and competitors, the genes encoding venom components are "exogenes," which diverge very rapidly as new species evolve. The peptide toxins that are present in *Conus* venoms are encoded by only a few gene superfamilies; these are predicted to undergo accelerated evolution.

Conus peptide genes are examples of exogenes (Olivera, 2006); their gene products act exogenously, targeting other animals (instead of acting endogenously within the cone snail itself). A considerable amount of prior work has demonstrated that each *Conus* species has its own distinct complement of venom peptides, with the same peptides not found in venoms of even closely related species. What would be predicted when a group of closely related species is analyzed is that the gene products encoded by a particular conopeptide superfamily will be highly related to each other, but not identical in sequence. This toxinological prediction was used to test if the species proposed to be in the *Turriconus* clade do indeed have closely related (but not identical) toxins, as would be expected for exogene products.

All species for which DNA was available were analyzed; since no complementary DNA (cDNA) samples were available, the analysis had to be carried out on genomic DNA. The gene superfamily used for the analysis was the O-superfamily; it is possible to determine the sequence of the mature toxins because there is a conserved intronic sequence that borders the mature toxin region (see Materials and Methods) thus, PCR primers can be used to determine peptide toxin sequences from each species. The O-superfamily has diverged into two branches (Olivera et al., 1999; Terlau and Olivera, 2004) one hydrophilic, which includes the ω - and κ -conotoxins from fish hunting cone snail venoms (the " ω -branch") and the second highly hydrophobic; in fish hunting cone snails this includes the δ and μ O-conotoxins (the " δ -branch"). PCR primers used to amplify genes in each branch are different and therefore toxin sequences can be separately obtained. This analysis was carried out and the results are shown in Figure 7.

It is clear from the figure that representatives of all four species groups, separated using our aforementioned morphological/molecular phylogenetic analyses, yielded homologous O-superfamily peptide sequences that fall into both the ω - and the δ -branches; all of which share a high degree of sequence identity. Moreover, as predicted

Homologous Peptide Toxins from the O-superfamily

ω - screen

C. acutangulus At6.1
CGGRPRAPCRQYIQCCSHS~~CNT~~FLGTCV*
C. praezellens Pr6.1
CGSPRARCRQYLOCCSRHCNKFLGMCV*
C. mitratus Mp6.1
CGGRCAPCRQYSQCCSRVCKE~~F~~NKCR*
C. stupa Sa6.1
CGPPRARCRQYLOCCSRRCIKFLNMCV*

δ - screen

C. acutangulus At6.7
DECLAGSEFCGFLKIGPPCCSGYCLFVCL*
C. mitratus Mp6.7
ECRANGATCGILKPGAKCCDGGWCFV~~C~~IG*
C. praezellens Ps6.7
RDDDCVAGGGCGEFPKIGGFCSSGWCFVCT*
C. stupa Sa6.7
ECFPKDTFCGFPSSEGAPCCSGWCFVCA*

Figure 7. Toxinological analysis. Predicted mature toxin sequences from two distinct branches of the O-superfamily of conopeptides for four members of *Turriconus*: *Conus acutangulus*, *Conus mitratus*, *Conus praezellens*, and *Conus stupa*. An independent comparison of the toxin sequences from the two branches, the hydrophilic ω and the hydrophobic δ , each demonstrate the close relationship between the different species of the *Turriconus* clade analyzed.

by the exogenomic hypothesis (Olivera, 2006), these peptide sequences have diverged from each other. Therefore, the postulated accelerated evolution of these exogenes, which, in turn, is an indicator of species divergence, is indeed observed.

DISCUSSION

This study has used three types of data: comparative morphology, molecular phylogeny based on standard gene markers, and toxinological markers (i.e., peptide toxin sequences). This three-pronged effort was aimed at branch definition leading to a specific phylogenetic hypothesis. As is typical, none of the individual data sets were as comprehensive or complete as might be desired; nevertheless, the combination made the phylogenetic framework proposed a compelling one.

MORPHOLOGICAL EVIDENCE: IMPORTANCE OF THE PROTOCONCH/EARLY TELEOCONCH. Springsteen and Leobrera (1986), separated two forms in the *C. praezellens* complex from the Philippines, the commonly trawled form (designated *Conus souerbyi*), and a second, much more slender form, with lighter yellowish brown spots, assigned to *Conus subaequalis* (*op. cit.*, pl. 71, figs. 1, 2). This treatment was subsequently adopted by Lim and Wee (1992). Thus, while Walls (1979) and Röckel et al., only recognized *C. praezellens* and *C. acutangulus*, these workers recognized three distinctive forms from the Philippines/Southeast Asia

("C. sowerbii" (= *C. praezellens*), "*C. subaequalis*," and *C. acutangulus*).

One reason why the definition of forms has been challenging is because most available specimens did not have good protoconch (or early teleoconch) whorl preservation. Philippine specimens in this group from many localities have a dark periostracum layer covering the spire of the shell, which is routinely removed by commercial shell dealers using acid, a treatment that destroys key features critical for morphological differentiation. In the analysis below, we used these "compromised" specimens in our morphometric analyses but focused primarily on the few specimens with well-preserved protoconch and early teleoconch whorls for discriminating between forms. Once consistent differences in protoconch and early teleoconch morphologies were established, additional morphological characters were used to help separate distinctive forms. This general approach was used for the morphological definition of forms described above.

The basic approach is illustrated in Figure 8, which show two forms collected off Aliguay Island; these have proven to be particularly illuminating. Both would have been assigned by Röckel et al. (1995) to *Conus praezellens*. In Figure 8, the specimen on the right is a specimen of what we refer to as the "Aliguay form" of *C. praezellens*; the other specimen (on the left) is *Conus miniexcelsus* new species. The top section of Figure 8 shows the shells of the two specimens of approximately same size. Although their shell patterns differ, the considerable variation observed in this complex led to a rather confused situation in the past. The major morphological observation that changed this situation is illustrated in the lower section of Figure 8, which shows a magnified view of the well-preserved protoconch and early teleoconch whorls of the two specimens; we believe that the differences depicted are sufficiently diagnostic to definitively assign the specimens illustrated into two distinct groups (i.e., *Conus praezellens* and *Conus miniexcelsus*). The multispiral brownish protoconch of *C. miniexcelsus* contrasts with the protoconch of the specimen assigned to *C. praezellens*; the early teleoconch whorls are also distinctively different. Using these as the major criteria for separating forms makes it simpler to identify other shell morphological characteristics that consistently differ, even though each individual character might have a considerable range of variation. This approach has provided a much more consistent suite of characters to allow a definition of different morphospecies.

Using these criteria, the Philippine forms previously assigned to *C. praezellens* fall into two separable groups: a group that has a characteristic protoconch of 2.0–2.5 whorls, with a rounded and somewhat inflated first whorl; the protoconchs of these forms are pearly-white; we have referred to these as the "*praezellens*-like forms." The specimens that do not have this very characteristic type of protoconch, herein collectively referred to as the "*miniexcelsus*-like forms", have the first protoconch whorl not rounded, nor inflated. The entire protoconch of the latter generally has a more triangular (conical)

Figure 8. Comparison of two morphospecies collected in Aliguay Island, Philippines. The specimen at the left is *Conus miniexcelsus* (Holotype), and on the right is *Conus praezellens*, "Aliguay form". Top: The whole shell. Bottom: Close-up of the protoconch and first few teleoconch whorls.

outline when compared to the "*praezellens*-like forms;" these protoconchs are typically translucent-brown, translucent-yellowish or off-white. Although this color can be subtle, the contrast to the pure-white early teleoconch whorls is usually diagnostic.

THE MOLECULAR EVIDENCE

A major conclusion from the molecular analysis is that *C. praezellens* and *C. acutangulus* do not belong in the *Conasprella* clade. Instead, they form a distinct branch among the major group of species in *Conus*. Another unexpected result from the molecular phylogenetic analysis is the other species, *Conus stupa* and *Conus mitratus*, branch within the same well-supported clade as *C. acutangulus* and *C. praezellens*. In particular, *Conus mitratus* has strikingly different shell morphology from *C. praezellens* and *C. acutangulus*. In all previous taxonomic work, *Conus mitratus* and *Conus stupa* have

been assigned to different subgenera from *Conus praecellens* and *Conus acutangulus*.

THE CONTRIBUTION OF TOXINOLOGICAL MARKERS. Our starting point for defining the clade of cone snails that includes *Conus praecellens* were the previous proposals in the literature for subgenera (or genera, when *Conus* was split into multiple genera) that included *Conus praecellens*. In most proposals, *Conus praecellens* was, in effect, proposed to be related to other high-spined, deep-water species such as *Conus pagodus*, *Conus memiae*, and *Conus arcuatus*, which are usually assigned to *Conasprella*. In some of the prior hypotheses, this group of species was split in two; e.g., *Conasprella* and *Kermasprella* (da Motta, 1991), or *Conasprella* and *Endemnoconus* (Okutani, 2000). Although these various proposals differ in detail, they all group *C. praecellens* and *C. acutangulus* with species such as *C. pagodus*, *C. memiae* and *C. arcuatus*. However, both the molecular phylogenetic results using standard molecular markers and the exogenomic data using toxinological markers are inconsistent with all of these hypotheses; only *Conus praecellens* and *Conus acutangulus* appear to be closely related to each other by the latter two criteria. All of the other species previously grouped with *Conus praecellens* in prior phylogenetic proposals based on shell morphology are now assigned to a distant and different branch of cone snails from *Conus praecellens/Conus acutangulus* (see phylogenetic tree in Figure 2).

Instead, the combined data led to a new and strikingly different phylogenetic framework for the *Conus* species comprising the branch that includes *Conus praecellens*; this proposal is summarized in Figure 7 and Table 2. An entirely unexpected set of species appears to be more closely related to *Conus praecellens* (in Groups 3 and 4 in Table 2). These *Conus* species (*C. stupa*, *C. stupella*, *C. mitratus*, and *C. cylindraceus*) were never previously proposed to be in the same clade/subgenus as *C. praecellens* and *C. acutangulus*.

The use of toxinological markers has buttressed the molecular phylogenetic analysis. The presence of highly similar peptide toxins that belong to the O-superfamily of conopeptides indicates that the various groups that branch together with *Conus praecellens* are indeed related, using an independent toxinological data set.

THE TURRICONUS CLADE: OVERVIEW

A more comprehensive morphological analysis reveals that various distinctive forms previously assigned to *Conus praecellens* are likely not conspecific; three new species were described and additional distinct forms defined. Thus, *Conus praecellens* and the newly described species, *Conus miniexcelsus*, *Conus rizali*, and *Conus andremenezzi* are now proposed to be species in the same clade.

Finally, the morphological analysis focused on the importance of protoconch/early teleoconch whorl morphology. These morphological characters strongly suggest

that Group I in Table 2 should include *Conus excelsus* given its strikingly similar protoconch/early teleoconch morphology to *C. acutangulus* and *C. miniexcelsus*. Clearly, there is a difference in size: *Conus excelsus* is much larger at maturity. We would predict that the molecular phylogeny and toxinology of *C. excelsus* will reveal a particularly close affinity to *Conus miniexcelsus* (which is, in part, the basis for the proposed name of the latter). Unfortunately, *Conus excelsus* is rare and we have been unable to obtain a live specimen to date. However, we propose to call the entire group the *Turriconus* clade, with *C. excelsus* as the type species. In terms of species diversity, it would appear that Groups 1 and 2 in Table 2 are the dominant species groups of this clade — except for the differences in protoconch morphology detailed above, they are all high-spined species with the body whorls characterized by spiral ribs or ribbons. *Conus excelsus* is the type species of the subgenus *Turriconus*.

All three types of data used for this investigation need to be expanded. A more extensive molecular phylogeny needs to be carried out on all of the forms indicated in Table 2, including, in particular, the designated “distinctive forms” so that an evaluation of whether these are separable species can be carried out. The new species we have proposed need to be rigorously evaluated both by the standard molecular markers as well as by their toxin genes. More refined molecular phylogeny should also allow a better resolution of how the various forms in the proposed *Turriconus* clade are related to each other and to other species of *Conus*. In addition, the exogenomic analysis, while in agreement with the molecular phylogeny, also needs to be extended to all of the species in the *Turriconus* clade, as well as to other gene superfamilies expressed in venom ducts (in addition to the O-gene superfamily that was shown in Figure 8). The determination of venom peptide sequences by this type of analysis is, in itself, of considerable intrinsic value, since it would allow the predicted gene products from each species to be chemically synthesized and directly tested for functional activity. Finally, the morphological analyses to date are based only on shell morphology. Clearly, other morphological features, particularly the internal anatomy, need to be evaluated; these will serve as an independent test of the phylogenetic hypothesis presented. Potentially, mutually shared and distinctive morphological features of species in this clade of *Conus* may be discovered.

The interaction between the three prongs that are the basis of the approach used in this manuscript has a potential synergy that goes beyond clade definition. The morphological analysis of distinctive forms within the *Conus praecellens* complex identifies candidates that may or may not be different species, separable from the ones already recognized. Using standard molecular phylogeny will help to define this; however, using exogene analysis should be even more definitive: if these were indeed separable species, then none of the toxin gene products should exactly overlap in their sequence,

because of the hypermutation in exogenes that accompanies speciation events.

If a distinctive form was truly a separable species, different toxin sequences would be predicted — if it were merely a variant of the same species, then identity in most venom peptide sequences should be found (except for allelic differences). In some of the well-known species (e.g., *Conus textile*, Linnaeus, 1758, and *Conus striatus* Linnaeus, 1758) that are distributed all over the Indo-Pacific, it has been shown that major venom peptides have the same sequence, even from variants whose shells may be distinguishable from each other because of the long period it may have taken for a species to spread across the entire Indo-Pacific, from the Red Sea to Hawaii.

The inclusion of exogenes in the investigation of biodiversity has a significance that goes beyond differentiating between morphologically closely related species. The divergence of exogenes from one species to the next is indicative of different biology, shaped by different selection pressures. This is essentially a molecular readout of the deeper biological/historical/ecological differences between species that might look morphologically similar. Thus, the characterization of toxin genes in the case of *Conus* not only serves as a tool for branch definition, but is a potentially important entry point toward a more profound understanding of the biological differences between species, a molecular readout that could provide insights into the complex changes that accompany the speciation events that give rise to a biodiverse lineage of animals such as the cone snails.

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LITERATURE CITED

Bandyopadhyay, P.K., B.J. Stevenson, J.P. Ownby, M.T. Cady, M. Watkins, and B.M. Olivera. 2005. The mitochondrial

- genome of *Conus textile*, *coxI-coxII* intergenic sequences and Conoidean evolution. *Molecular Phylogenetic Evolution* 46: 215–223.
- da Motta, A.J. 1991. A systematic classification of the gastropod family Conidae at the generic level. *La Conchiglia*, Rome, 48 pp.
- Huelsenbeck, J.P., F. Ronquist, R. Nielsen, and J.P. Bollback. 2001. Bayesian inference of phylogeny and its impact on evolutionary biology. *Science* 294: 2310–2314.
- Larkin, M.A., G. Blackshields, N.P. Brown, R. Chenna, P.A. McGettigan, H. McWilliam, F. Valentin, I.M. Wallace, A. Wilm, R. Lopez, J.D. Thompson, T.J. Gibson, and D.G. Higgins. 2007. Clustal W and Clustal X version 2.0. *Bioinformatics* 23: 2947–2948.
- Lim, C.F., and V.T.H. Wee. 1992. Southeast Asian *Conus*, a sea-shells book. *Seaconus Private Limited*, Singapore, 100 pp.
- Maddison, D.R. and W.P. Maddison. 2005. *MacClade* 4.08.
- Marsh, J.A. and O.H. Ripplingale. 1964. *Cone Shells of the World*. 3rd Edition. *Jacaranda Press*, Melbourne, 186 pp.
- Okutani, T. 2000. *Marine Mollusks in Japan*. Tokai University Press, Tokyo, 1221 pp.
- Olivera, B.M., 2006. *Conus* peptides: biodiversity-based discovery and exogenomics. *Journal of Biological Chemistry* 281: 31173–31177.
- Olivera, B.M., C. Walker, G.E. Cartier, D. Hooper, A.D. Santos, R. Schoenfeld, R. Shetty, M. Watkins, P. Bandyopadhyay, D.R., and Hillyard. 1999. Speciation of cone snails and interspecific hyperdivergence of their venom peptides. Potential evolutionary significance of introns. *Annals of New York Academy of Science* 870: 223–237.
- Reeve, L.A. 1849. *Monograph of the genus Conus*. L. Reeve and Co., London.
- Robin, A. 2008. *Encyclopedia of Marine Gastropods. XENOPHORA and ConchBooks*, Hackenheim, 480 pp., 461 pls.
- Röckel, D., W. Korn, and A.J. Kohn. 1995. *Manual of the Living Conidae (Vol. I: Indo-Pacific Region)* Verlag Christa Hemmen, Wiesbaden, 517 pp.
- Ronquist, F.J.P. and Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.
- Shikama, T. and T. Habe. 1968. A New Japanese Cone, *Turriconus nakayasui* with Reference to *Enbrikena stupa* group. *Venus* 26: 3–4.
- Springsteen, F.J. and F.M. Loebnera. 1956. *Shells of the Philippines*. *Carfel Seashell Museum*, Manila, 377 pp., 100 pls.
- Terlau, H. and B.M. Olivera. 2004. *Conus* venoms: a rich source of novel ion channel-targeted peptides. *Physiological Reviews* 84: 41–68.
- Thach, N.N. 2005. *Shells of Vietnam*. *ConchBooks*, Hackenheim, 338 pp., 91 pls.
- Walls, J.G., 1979. *Cone Shells. A synopsis of the living Conidae*. TFH Publications Inc. Ltd., Neptune City, 1011 pp.

APPENDIX

Baldomero M. Olivera and Jason S. Biggs

Conus andremenezi, summary of type specimens (Figures of types are cross-referenced)

	Length	Width	Locality (PI)	Depository (Cat#)
Holotype (Figs 1, 2, 6)	36.7	16.9	Aliguay	MSI
Paratype 1	52.9	21.7	Aliguay	*
Paratype 2	40.1	29.1	Aliguay	*
Paratype 3	40.6	20.1	Aliguay	*
Paratype 4	43.1	22.0	Aliguay	*
Paratype 5	42.7	20.4	Aliguay	MCZ 361611
Paratype 6	26.8	12.4	Aliguay	*
Paratype 7 (Fig 2)	29.4	14.0	Balicasag Is.	Lc-37964
Paratype 8	44.0	20.9	(Philippines)	*
Paratype 9	46.9	20.9	(Philippines)	*
Paratype 10	28.7	13.6	(Philippines)	*
Paratype 11 (Fig 2)	23.0	10.7	(Philippines)	MSI
Paratype 12	29.6	13.0	(Philippines)	FMHN 312461
Paratype 13	33.2	15.4	(Philippines)	BMSM 38672
Paratype 14	26.0	12.6	(Philippines)	*
Paratype 15	43.3	21.4	(Philippines)	*
Paratype 16	26.6	13.1	(Philippines)	*
Paratype 17	31.5	14.6	(Philippines)	*
Paratype 18	39.5	18.5	Aliguay	*
Paratype 19	35.8	17.1	Aliguay	ANSP 421619
Paratype 20	27.7	13.1	Aliguay	*
Paratype 21	30.5	15.4	Aliguay	*
Paratype 22	29.2	13.8	Panglao Is.	MNHN 21131
Paratype 23	46.9	21.8	Olango Is.	*
Paratype 24	37.4	16.7	Aliguay	*
Paratype 25	36.4	17.6	Aliguay	*
Paratype 26	34.6	15.4	Aliguay	*
Paratype 27	44.6	21.9	Aliguay	*
Paratype 28	48.7	22.3	Aliguay	*
Paratype 29	38.6	18.7	Aliguay	*
Paratype 30	33.9	15.3	Aliguay	*
Paratype 31	35.1	15.4	Aliguay	*
Paratype 32	42.5	20.1	Aliguay	*
Paratype 33	35.2	17.0	Aliguay	*
Paratype 34	37.3	17.6	Aliguay	*
Paratype 35	36.8	17.7	Aliguay	*
Paratype 36	21.6	9.3	Camotes Sea	*
Paratype 37	23.8	11.2	Camotes Sea	*

Conus miniexcelsus, summary of type specimens

	Length	Width	Locality (PI)	Depository (Cat#)
Holotype (Figs 1, 2, 6, 8)	22.0	19.6	Aliguay	MSI
Paratype 1	27.7	11.8	Aliguay	*
Paratype 2 (Fig 2)	27.3	11.6	Aliguay	MSI
Paratype 3	29.0	12.5	Aliguay	*
Paratype 4	27.4	10.8	Aliguay	*
Paratype 5	28.3	11.4	Aliguay	*
Paratype 6	25.5	10.5	Aliguay	*
Paratype 7	31.1	11.6	(Philippines)	*
Paratype 8	31.5	13.1	(Philippines)	Lc-37965
Paratype 9	28.9	12.3	(Philippines)	FMNH 312462
Paratype 10	35.1	13.7	(Philippines)	*
Paratype 11	27.6	11.6	(Philippines)	MCZ 361609

(Continued)

APPENDIX
(Continued.)

	Length	Width	Locality (PI)	Depository (Cat#)
Paratype 12	28.3	12.5	(Philippines)	ANSP 421620
Paratype 13	36.6	15.0	(Philippines)	*
Paratype 14	35.8	14.7	Panglao Is.	BMSM 38673
Paratype 15	35.5	13.2	(Philippines)	*
Paratype 16	27.2	11.5	Aliguay	MNHN 21132
Paratype 17	26.9	11.5	Aliguay	*
Paratype 18 (Fig 2)	18.5	7.4	Aliguay	MSI
Paratype 19	25.5	11.4	Aliguay	*
Paratype 20	33.7	13.5	Aliguay	*
Paratype 21	32.5	13.6	Balicasag Is.	*
Paratype 22	15.8	6.4	Panglao Is.	*
Paratype 23	33.3	14.2	Minabe, Wakayama, Japan	*
Paratype 24	28.8	12.4	Olango Is.	*

Conus rizali, summary of type specimens

	Length	Width	Locality (PI)	Depository (Cat#)
Holotype (Figs 3, 6)	26.6	12.4	Philippines	MSI
Paratype 1	36.3	14.0	Philippines	*
Paratype 2 (Figs 1, 3)	26.1	10.2	Philippines	MSI
Paratype 3	34.5	13.9	Philippines	*
Paratype 4	38.2	15.8	Philippines	ANSP 421621
Paratype 5	37.3	14.8	Philippines	MCZ 361610
Paratype 6	39.0	14.5	Philippines	MNHN 21133
Paratype 7	37.8	14.2	Philippines	*
Paratype 8	35.9	14.4	Balut Is.	*

Abbreviations: MSI, Marine Science Institute, University of the Philippines, Quezon City, Philippines; ANSP, Academy of Natural Sciences, Philadelphia, PA, USA; MNHN, Muséum national d'Histoire naturelle, Paris, France; MCZ, Museum of Comparative Zoology, Harvard University, Cambridge, MA, USA; BMSM, The Bailey-Matthews Shell Museum, Sanibel FL, USA; FMHN, Field Museum of Chicago, Chicago IL, USA; Lc, Zoological Museum of Moscow State University, Moscow, Russia; *, These specimens will be deposited in public museums, but have not yet been assigned.