# A new species of *Gemmula*, Weinkauff 1875; Evidence of two clades of Philippine species in the genus *Gemmula*

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A new species of Philippine turrid, *Gemmula ambara* (the amber gem turrid), is described. The type locality is Balicasag Island, Bohol, Phlippines; the holotype and several paratypes were collected at the type locality using tangle nets at depths of 70-120 fathoms. The species occurs offshore across a number of central Philippines localities; it has also been collected off Panglao Island using tangle nets and off Aliguay Island using small trawls. *Gemmula ambara* had been mistakenly regarded by collectors and dealers as a small variety of *Gemmula diomedea*, but a more careful examination of the shell morphology easily separates the two species. A preliminary analysis of the phylogeny of *Gemmula ambara* using standard molecular markers has revealed that in fact, this species is not closely related to *Gemmula diomedea* but instead, is more closely related to *Gemmula rosario* and *Gemmula lisajoni*. It appears that the species of *Gemmula* from the Philippines can be divided into two distinct branches, with *Gemmula ambara*, *Gemmula rosario* and *Gemmula lisajoni* comprising one branch, and *Gemmula diomedea*, *Gemmula speciosa*, *Gemmula kieneri*, and *Gemmula sogodensis* comprising a second branch.

## Introduction

Venomous marine snails belonging to the superfamily *Conoidea* are a significant component of the marine biodiversity of the Philippines and their venoms are proving to be a promising resource for novel biologically active compounds. The conoidean snails have traditionally been divided into three groups: cone snails (family Conidae), auger snails (family Terebridae) and turrids (family Turridae) — for reviews of these groups see Rockel *et al.* 1995; Bratcher and Cernohorsky 1987; Terryn 2007; Powell 1964, 1966. In numbers of species, the turrids are the largest group of conoideans, but also the most poorly characterized. Recently, it has become clear that the family *Turridae* is not monophyletic (Taylor *et al.* 1993; Puillandre *et al.* 2008); however, a generally accepted taxonomic treatment for the diverse groups formerly in the family *Turridae* has not yet been firmly established.

The type species of the family *Turridae* is *Turris babylonia* (Linnaeus, 1758); this taxon also defines the subfamily *Turrinae*. Included in the subfamily *Turrinae* are several genera, *e.g.*, *Turris*, Roding 1798, *Gemmula*, Weinkauff, 1875, *Lophiotoma*, Casey, 1904, *Polystira*, Woodring, 1928, and *Turridrupa*, Hedley, 1922. The taxonomy of the *Turridae* requires considerable taxonomic attention, with many of the recently collected Philippine turrid species remaining undescribed. This is primarily a deep-water group, with most species not accessible to the classical shallow-water collecting techniques that provided most marine gastropods described since the work of Linnaeus. In addition, the morphological differences between many closely related species are subtle, making it easy to confuse closely related forms.

**Figure 1.** *Gemmula ambara*, new species. Shown are three views of the holotype, which is 39.6 mm in length.

In this work we describe a new species in the *Turrinae*, in the genus *Gemmula*, Weinkauff, 1875 (the "gem turrids"). We define the species based on its distinctive morphological characteristics, but also establish its phylogenetic affinities through the use of standard molecular markers. The new species, the amber gem turrid, *Gemmula ambara*, is only the first of many undescribed Philippine turrids that we hope

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**Table 1.** *Gemmula ambara*, Types examined.

| Type | Length | Diameter | Specific Locality (All Philippines) |
|---|---|---|---|
| Holotype | 39.6 | 13.4 | Balicasag Island, Bohol |
| Paratype #1 | 38.1 | 12.6 | Balicasag Island, Bohol |
| Paratype#2 | 36.4 | 12.5 | Balicasag Island, Bohol |
| Paratype#3 | 36 | 12.4 | Balicasag Island, Bohol |
| Paratype#4 | 55.3 | 17.2 | Balicasag Island, Bohol |
| Paratype#5 | 55.6 | 17.2 | Balicasag Island, Bohol |
| Paratype #6 | 45.3 | 15.4 | Balicasag Island, Bohol |
| Paratype #7 | 43.1 | 14.4 | Balicasag Island, Bohol |
| Paratype #8 | 45.1 | 15.2 | Balicasag Island, Bohol |
| Paratype #9 | 44.8 | 14.6 | Balicasag Island, Bohol |
| Paratype #10 | 38.3 | 13.4 | Balicasag Island, Bohol |
| Paratype #11 | 34.1 | 11.8 | Balicasag Island, Bohol |
| Paratype #12 | 32.9 | 12 | Balicasag Island, Bohol |
| Paratype #13 | 41.5 | 13.6 | Balicasag Island, Bohol |
| Paratype #14 | 38.7 | 13.6 | Balicasag Island, Bohol |
| Paratype #15 | 52.6 | 16.7 | Balicasag Island, Bohol |
| Paratype #16 | 53.7 | 16.3 | Balicasag Island, Bohol |
| Paratype #17 | 36.3 | 12.7 | Balicasag Island, Bohol |
| Paratype #18 | 41.1 | 12.4 | Balicasag Island, Bohol |
| Paratype #19 | 45.4 | 15.3 | Balicasag Island, Bohol |
| Paratype #20 | 48.3 | 15.3 | Balicasag Island, Bohol |
| Paratype #21 | 47.8 | 16.1 | Balicasag Island, Bohol |
| Paratype #22 | 52.1 | 15.8 | Balicasag Island, Bohol |
| Paratype #23 | 42.5 | 14.2 | Balicasag Island, Bohol |
| Paratype #24 | 40.1 | 13.5 | Balicasag Island, Bohol |
| Paratype #25 | 53.6 | 18 | Punta Engaño, Mactan, Cebu |
| Paratype #26 | 32.8 | 11.3 | Panglao Island, Bohol |
| Paratype #27 | 33.3 | 11.7 | Aliguay Island |
| Paratype #28 | 23.3 | 7.1 | Aliguay Island |
| Paratype #29 | 24.5 | 8.7 | Aliguay Island |
| Paratype #30 | 50.1 | 16.7 | Aliguay Island |

(Paratypes 31-50: Aliguay Island; 51-55: Philippines (specific locality unknown))

**Figure 2.** *Gemmula ambara*, new species, compared to *Gemmula diomedea*, Powell, 1964. The three bottom specimens are *Gemmula ambara*, and the top specimen is *Gemmula diomedea*. Note the difference in the subsutural region.

will be characterized in the near future using a combination of both a shell morphology based taxonomic description, as well as molecular phylogeny.

## Methods

***Specimen collection.*** The holotype and paratypes from Balicasag Island were collected by fishermen using tangle nets offshore. Most of the other specimens examined were purchased through commercial sources in Mactan, Cebu.

***Preparation of genomic DNA.*** A specimen dissected by Yuri Kantor on July 1, 2004, (a male, 34 mm in length) in Balicasag Island, Philippines was used for the molecular analysis. Genomic DNA was prepared from 5 mg *Gemmula ambara* proboscis tissue using the Gentra Puregene DNA Isolation Kit (Gentra Systems, Minneapolis, MN) according to the manufacturer's standard protocol.

***Cloning and Sequencing of COI mtDNA.*** 10 ng of *Gemmula ambara* genomic DNA was used as a template for polymerase chain reaction (PCR) with oligonucleotides corresponding to COI forward primer (5' GGT CAA CAA ATC ATA AAG AYA TGY G 3') and COI reverse primer (5' TAA ACT TCA GGG TGA CCA AAR AAY CA 3'). The PCR cycling profiles are as follows: Initial denaturation (95°C, 60s); followed by 40 cycles of denaturation (95°C, 20s); annealing (55°C, 20s) and extension (72°C, 30s). The resulting PCR product was purified by gel electropheresis, recovered from agarose using High Pure PCR Product Purification Kit (Roche Diagnostics, Indianapolis, IN). The eluted DNA fragment was annealed to pNEB206A vector using the USER Friendly Cloning Kit (New England BioLabs, Inc., Beverly, MA) following manufacturer's suggested protocol and the resulting product transformed into DH5α competent cells.

The nucleic acid sequences of the resulting COI-encoding clones were determined according to the standard protocol for Automated Results.

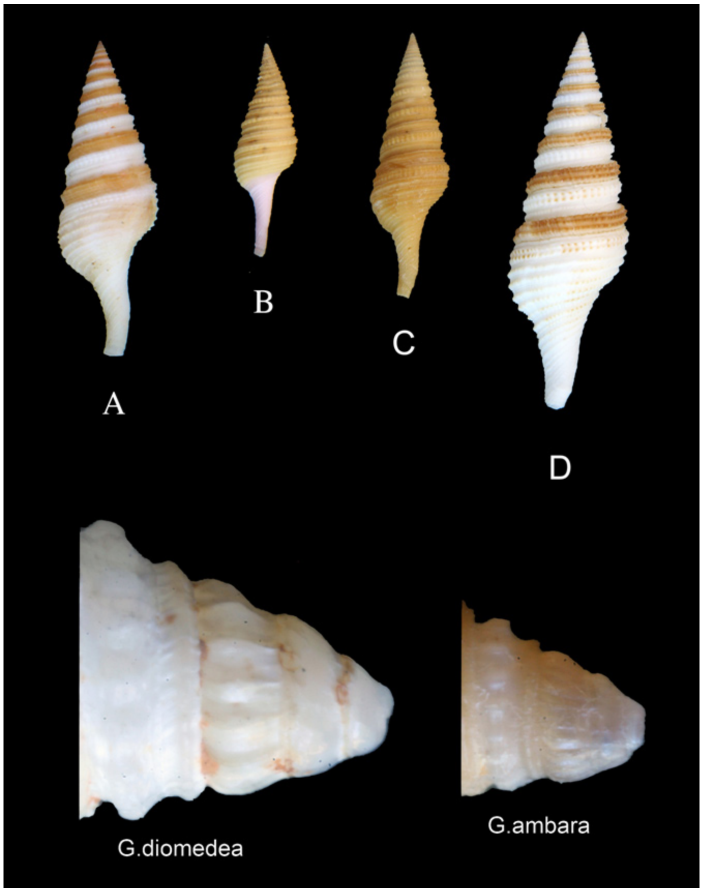


**Figure 3.** A comparison between *Gemmula ambara* (A) with *Gemmula lisajoni* (B), *Gemmula rosario* (C) and *Gemmula diomedea* variety (D). On the bottom are two of the protoconchs; note the differences in size and color between the protoconch of *Gemmula ambara* and *Gemmula diomedea*. The specimen of *Gemmula ambara* shown (A) is Paratype 19; although it is a dead-collected shell with a bore-hole, it had the best-preserved protoconch of all the specimens examined. The specimen in D is the form from Aliguay Island generally assigned to *G. diomedea*, but similar in shape to *G. congener*. The *G. diomedea/congener* complex needs to be more carefully analyzed, but all specimens in this group can be reliably separated from *G. ambara*.

***Molecular phylogeny using 12S mitochondrial ribosomal RNA.*** The nucleotide sequences were obtained using PCR and the phylogenetic tree constructed as described in Heralde et al. (2007).

## Results

### Morphological characterization of a new *Gemmula* species

***Range.*** Collected in the central Philippines, primarily from Balicasag Island, Bohol, using tangle nets, 70-150 fathoms, and from Aliguay Island, using small trawls, 30-100 fathoms.

***Remarks.*** This medium-sized *Gemmula* (the holotype is shown in Fig. 1) has the same general color pattern as *Gemmula diomedea*, but can be separated from that species by its distinctively different subsutural region (see Fig. 2). The molecular phylogeny (see next section) suggests that this species is most closely related to *Gemmula rosario* and *Gemmula lisajoni*.

***Description.*** Shell moderately broad fusiform, length 30-55 mm. Overall color of the shell is white, flushed with a distinct violet or purplish tone in some specimens (particularly when freshly collected), except for the subsutural area, which is always light amber to dark amber brown in color. The protoconch has 3-4 translucent yellowish-brown to purplish brown whorls, axially costate over the last two protoconch whorls. Post-nuclear whorls 9-10.

The spire whorls are sculptured with a prominent subsutural fold comprising two strong, closely-spaced spiral ribs, immediately adjacent to the suture, with the region in between moderately elevated, such that the two ribs comprise a prominent amber keel that borders the suture. In earlier spire whorls, there are two additional ribs in the anterior subsutural region and as the shell increases in size, the subsutural region can have as many as 6 to 7 spiral ribs, with the two posterior ones comprising a subsutural keel always the strongest. There are axial columns between the spiral ribs, quite strong in some specimens. A diagnostic feature of this species is that the entire subsutural region up to the peripheral sinus rib is amber in color, and the rest of the shell (except the colored protoconch) is white.

In the first few post-nuclear spire whorls, the most prominent features are the amber brown subsutural region and the strongly gemmulate sinus rib. By the fourth post-nuclear whorl, an additional spiral rib anterior to the gemmulate peripheral sinus rib becomes emergent and in mature specimens, two strong additional spiral whorls are found anterior to the sinus rib in the larger spire whorls.

In the body whorl, there are six principal spiral ribs and at the base, numerous weaker spiral ribs surrounding one major, somewhat crenulate rib. There are 16 spiral ribs with a varying number of weak ribs between the principal raised spirals along the canal

***Comparisons.*** *Gemmula ambara* looks superficially similar to smaller specimens of *Gemmula diomedea*; it can readily be distinguished from members of the *G. diomedea* complex from the Philippines because the entire subsutural region until the sinus rib at the periphery is amber brown. In all of the *G. diomedea*-like specimens (there are likely to be multiple species in this complex — compare the specimens tentatively assigned to *G. diomedea* in Figs. 2 and 3), the one or two raised subsutural ribs are brown but the remainder of the subsutural region has the same white background color as the rest of the shell (including the region between the two brown subsutural spiral ribs — white in *G. diomedea*, amber in *G. ambara*). When preserved, the protoconch is also diagnostic (see Fig 3): brown in *G. ambara*, generally white and much larger in *G. diomedea*, (although some varieties of *G. diomedea*, which may be different species, have a protoconch that is light yellowish). In addition, most Philippine specimens of *Gemmula diomedea* have a higher and narrower spire, and a proportionately shorter canal than *Gemmula ambara*. Most specimens of *G. diomedea* have diameter:length ratio = 0.30-0.32 while for *G. ambara* it is 0.33-0.35.

As noted in the molecular phylogeny section that follows, *Gemmula ambara* is most closely related to *G. rosario* and *G. lisajoni*. *G. rosario* is a pure brown shell, uniformly straw brown rather than amber and white, while *G. lisajoni* has a distinctive deeply purplish-violet canal, with a brown body (see Fig. 3). In contrast to *G. ambara*, there is no white coloration in either of these species. Other Philippine forms of *Gemmula* such as *G. speciosa*, *G. kieneri* and *G. sogodensis* are both morphologically and genetically even more distant and would not be mistaken for *Gemmula ambara*.

***Etymology.*** The amber colored subsutural region, characteristic of this species, is the origin of the name ambara.

***Types.*** A summary of type specimens is given in Table 1. The holotype

**Table 2.** Barcode sequences (CO1)

```
Gemmula ambara

GGTCAACAAATCATAAAGATATCGGAACATTATATATTTTATTTGGTATA
   S  T  N  H  K  D  I  G  T  L  Y  I  L  F  G  I
                                                         50
Gemmula ambara          GGTCAACAAATCATAAAGATATCGGAACATTATATATTTTATTTGGTATA
Gemmula lisajonii       GGTCAACAAATCATAAAGATATCGGGACATTATACATTTTATTTGGTATA
Gemmula diomedea        GGTCAACAAATCATAAAGATATCGGGACATTATATATTTTATTTGGTATA
Gemmula speciosa        GGTCAACAAATCATAAAGATATTGGAACATTATATATTTTATTTGGTATA
Turris spectabilis      GGTCAACAAATCATAAAGATATCGGAACTCTATACATCTTATTTGGTATA

TGATCCGGTCTAGTAGGAACTGCTTTAAGTCTTCTTATTCGAGCTGAATT
 W  S  G  L  V  G  T  A  L  S  L  L  I  R  A  E  L
                                                        100
Gemmula ambara          TGATCCGGTCTAGTAGGAACTGCTTTAAGTCTTCTTATTCGAGCTGAATT
Gemmula lisajonii       TGATCTGGTCTAGTAGGAACTGCTTTAAGTCTTCTTATTCGAGCTGAGTT
Gemmula diomedea        TGGTCCGGTCTAGTGGGAACTGCTTTAAGTCTTCTTATTCGAGCTGAATT
Gemmula speciosa        TGGTCCGGTCTAGTGGGAACTGCTTTAAGTCTTCTTATTCGAGCCGAATT
Turris spectabilis      TGATCCGGTCTAGTAGGGACTGCTTTAAGTCTTCTTATTCGAGCCGAATT

AGGGCAACCCGGAGCGTTACTTGGTGATGATCAATTATATAATGTCATTG
  G  Q  P  G  A  L  L  G  D  D  Q  L  Y  N  V  I
                                                        150
Gemmula ambara          AGGGCAACCCGGAGCGTTACTTGGTGATGATCAATTATATAATGTCATTG
Gemmula lisajonii       AGGGCAACCTGGAGCGTTACTTGGTGATGATCAATTATATAATGTCATTG
Gemmula diomedea        AGGGCAACCTGGAGCCCTGCTTGGTGACGATCAATTATATAATGTTATTG
Gemmula speciosa        AGGGCAACCTGGGGCGCTGCTTGGTGACGATCAATTATATAATGTAATTG
Turris spectabilis      AGGGCAACCTGGGGCGTTACTTGGTGATGACCAGCTGTATAATGTTATTG

TAACAGCTCATGCTTTTGTTATAATTTTCTTTTTAGTCATGCCAATAATA
V  T  A  H  A  F  V  I  I  F  F  L  V  M  P  I  I
                                                        200
Gemmula ambara          TAACAGCTCATGCTTTTGTTATAATTTTCTTTTTAGTCATGCCAATAATA
Gemmula lisajonii       TAACAGCTCATGCTTTTGTTATAATTTTTTTCTTAGTTATACCTATGATA
Gemmula diomedea        TAACGGCTCATGCTTTCGTTATAATTTTCTTCTTAGTTATGCCAATGATA
Gemmula speciosa        TAACGGCTCATGCTTTTGTTATAATTTTTTTTTTAGTTATACCAATGATA
Turris spectabilis      TAACAGCTCATGCTTTTGTTATAATCTTTTTTCTAGTAATGCCAATGATA

ATTGGTGGGTTTGGTAATTGATTAGTTCCATTAATGTTAGGAGCTCCTGA
 I  G  G  F  G  N  W  L  V  P  L  M  L  G  A  P  D
                                                        250
Gemmula ambara          ATTGGTGGGTTTGGTAATTGATTAGTTCCATTAATGTTAGGAGCTCCTGA
Gemmula lisajonii       ATTGGTGGATTTGGTAATTGATTGGTTCCATTAATATTAGGAGCTCCTGA
Gemmula monilifera      ATTGGGGGATTTGGTAATTGATTGGTTCCTTTAATGTTAGGGGCTCCTGA
Gemmula speciosa        ATTGGTGGATTTGGTAATTGATTGGTTCCTTTAATATTAGGAGCTCCTGA
Turris spectabilis      ATTGGTGGGTTTGGCAATTGATTGGTTCCTCTTATATTAGGGGCTCCTGA

CATAGCATTTCCTCGATTAAATAATATAAGTTTTTGATTACTTCCTCCTT
  I  A  F  P  R  L  N  N  I  S  F  W  L  L  P  P
                                                        300
Gemmula ambara          CATAGCATTTCCTCGATTAAATAATATAAGTTTTTGATTACTTCCTCCTT
Gemmula lisajoni        TATAGCATTCCCTCGATTAAATAATATAAGTTTTTGATTGCTTCCTCCTT
Gemmula diomedea        TATAGCGTTTCCTCGATTAAATAATATAAGTTTTTGGTTACTTCCTCCTT
Gemmula speciosa        TATAGCATTTCCTCGATTAAATAATATAAGTTTCTGGTTACTTCCTCCTT
Turris spectabilis      TATAGCATTCCCTCGATTAAACAATATAAGTTTCTGGCTGCTTCCTCCTT

CATTATTATTACTTTTGTCGTCGGCTGCAGTAGAAAGAGGGGCTGGTACT
S  L  L  L  L  L  S  S  A  A  V  E  R  G  A  G  T
                                                        350
Gemmula ambara          CATTATTATTACTTTTGTCGTCGGCTGCAGTAGAAAGAGGGGCTGGTACT
Gemmula lisajoni        CATTATTGTTACTTTTATCATCAGCTGCGGTAGAAAGAGGAGCTGGTACT
Gemmula diomedea        CGTTATTATTACTATTATCATCAGCTGCAGTAGAAAGCGGGGCTGGTACT
Gemmula speciosa        CATTATTGTTGCTATTATCATCAGCTGCAGTAGAAAGAGGGGCTGGTACA
Turris spectabilis      CTTTATTATTATTACTATCGTCAGCTGCAGTAGAAAGGGGAGCTGGTACT

GGATGAACTGTTTATCCCCCATTAGCTGGAAATTTAGCTCATGCTGGTGG
 G  W  T  V  Y  P  P  L  A  G  N  L  A  H  A  G  G
                                                        400
Gemmula ambara          GGATGAACTGTTTATCCCCCATTAGCTGGAAATTTAGCTCATGCTGGTGG
Gemmula lisajoni        GGATGAACTGTTTATCCCCCATTAGCTGGAAATTTAGCTCATGCTGGTGG
Gemmula diomedea        GGGTGAACCGTTTATCCTCCTTTAGCTGGAAACCTGGCTCACGCCGGTGG
Gemmula speciosa        GGATGAACTGTTTATCCTCCTTTAGCTGGAAATCTGGCTCACGCCGGTGG
Turris spectabilis      GGGTGAACTGTTTATCCTCCTCTGGCTGGAAATTTAGCGCACGCTGGAGG

TTCAGTAGATTTAGCTATTTTTTCTTTACACTTAGCTGGGGCATCTTCTA
  S  V  D  L  A  I  F  S  L  H  L  A  G  A  S  S
                                                        450
Gemmula ambara          TTCAGTAGATTTAGCTATTTTTTCTTTACACTTAGCTGGGGCATCTTCTA
Gemmula lisajoni        TTCGGTGGATTTGGCTATTTTTTCTTTACACTTAGCTGGGGCATCTTCTA
Gemmula diomedea        TTCAGTAGATTTAGCTATTTTTTCTTTGCACTTAGCTGGTGCATCTTCTA
Gemmula speciosa        TTCAGTAGATCTGGCTATTTTTTCTTTACACTTAGCTGGGGCATCTTCTA
Turris spectabilis      TTCGGTGGATTTAGCTATTTTTTCATTACATTTAGCCGGGGCATCTTCTA

TTTTAGGGGCAGTTAATTTTATTACTACTATTATTAATATACGATGAAAA
I  L  G  A  V  N  F  I  T  T  I  I  N  I  R  W  K
                                                        500
Gemmula ambara          TTTTAGGGGCAGTTAATTTTATTACTACTATTATTAATATACGATGAAAA
Gemmula lisajoni        TTTTAGGGGCAGTTAATTTTATTACTACTATTATTAATATACGATGAAAA
Gemmula diomedea        TTTTAGGGGCAGTTAACTTTATTACTACTATTATTAATATACGGTGAAAA
Gemmula speciosa        TTTTAGGGGCAGTTAATTTTATTACTACTATTATTAATATGCGATGAAAA
Turris spectabilis      TTTTAGGTGCAGTAAATTTTATTACTACTATTATTAATATACGATGAAAA

GGGATACAATTTGAACGTCTTTCTCTATTTGTATGATCAGTAAAAATTAC
 G  I  Q  F  E  R  L  S  L  F  V  W  S  V  K  I  T
                                                        550
Gemmula ambara          GGGATACAATTTGAACGTCTTTCTCTATTTGTATGATCAGTAAAAATTAC
Gemmula lisajoni        GGAATACAATTTGAGCGTCTTTCTTTATTTGTATGATCAGTAAAAATTAC
Gemmula diomedea        GGAATACAATTTGAACGTCTTTCTTTATTTGTATGGTCAGTAAAAATTAC
Gemmula speciosa        GGAATGAAATTTGAACGTCTTTCTTTATTTGTATGATCAGTAAAGATTAC
Turris spectabilis      GGAATACAATTTGAACGCCTTTCTTTATTTGTATGATCTGTAAAAATTAC

TGCTATTTTACTTTTACTTTCTTTACCAGTACTTGCAGGAGCTATTACAA
  A  I  L  L  L  L  S  L  P  V  L  A  G  A  I  T
                                                        600
Gemmula ambara          TGCTATTTTACTTTTACTTTCTTTACCAGTACTTGCAGGAGCTATTACAA
Gemmula lisajoni        TGCTATTTTACTTTTACTTTCTTTACCAGTACTTGCAGGAGCTATTACAA
Gemmula diomedea        TGCTATTTTACTTTTGCTTTCTTTACCGGTACTTGCAGGGGCTATTACGA
Gemmula speciosa        TGCTATTTTATTACTGCTTTCTTTACCAGTACTTGCAGGAGCTATTACAA
Turris spectabilis      TGCTATTTGCTTTTACTTTCCTTACCAGTACTTGCAGGGGCCATTACGA

TGCTTTTAACTGATCGAAATCTTAATACAGCTTTTTTTGATCCAGCAGGG
M  L  L  T  D  R  N  L  N  T  A  F  F  D  P  A  G
                                                        650
Gemmula ambara          TGCTTTTAACTGATCGAAATCTTAATACAGCTTTTTTTGATCCAGCAGGG
Gemmula lisajoni        TACTTTTAACTGATCGAAATCTTAATACGGCTTTTTTCGATCCAGCAGGA
Gemmula diomedea        TACTTTTAACTGATCGAAATCTTAATACCGCTTTTTTTGACCCAGCCGGA
Gemmula speciosa        TACTTTTAACTGATCGAAATCTTAATACCGCTTTTTTTGACCCAGCCGGA
Turris spectabilis      TACTTTTAACTGATCGAAATCTTAATACAGCTTTTTTTGACCCAGCCGGA

GGTGGAGATCCAATTTTATATCAACATTTGTTTTGGTTTTTTGGTCACCC
G  G  D  P  I  L  Y  Q  H  L  F  W  F  F  G  H  P
                                                        700
Gemmula ambara          GGTGGAGATCCAATTTTATATCAACATTTGTTTTGGTTTTTTGGTCACCC
Gemmula lisajoni        GGTGGAGATCCAATTTTATATCAACATTTGTTCTGATTCTTTGGTCACCC
Gemmula diomedea        GGTGGGGATCCAATTTTATACCAACATTTATTTTGGTTTTTTGGTCACCC
Gemmula speciosa        GGTGGAGATCCAATTTTATATCAACATCTGTTTTGGTTTTTTGGTCACCC
Turris spectabilis      GGCGGGGACCCAATCCTTATCAACACTTGTTCTGGTTTTTTGGTCACCC

TGAAGTTTA
  E  V
             709
Gemmula ambara          TGAAGTTTA
Gemmula lisajoni        TGAAGTTTA
Gemmula diomedea        TGAAGTTTA
Gemmula speciosa        TGAAGTTTA
Turris spectabilis      TGAAGTTTA
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**Legend:** Top row shows the nucleotide sequence of the *G. ambara* "barcode" interval in the cytochrome oxidase I gene; it has been translated. Below are the aligned nucleotide sequences for the five species indicated

will be deposited in the Philippine National Museum. Paratypes will be deposited in the Museum National d'Histoire Naturelle in Paris; the Academy of Natural Sciences in Philadelphia, Pennsylvania; the Harvard Museum of Comparative Zoology, in Cambridge, Massachusetts, the Natural History Museum, Geneva, Switzerland. All of these museums will also be provided with a printed version of this manuscript.

#### Molecular phylogeny of *Gemmula ambara*

A molecular phylogeny of *Gemmula ambara* was carried out; the results are shown in Figure 4 and Table 2. Phylogenetic trees based on 12S ribosomal mitochondrial DNA sequences were constructed using several standard methods (see Methods); the data reveal that of the species of *Gemmula* analyzed, *Gemmula ambara* is more closely related to *Gemmula rosario* and *Gemmula lisajoni* than to the other *Gemmula* species for which sequences were available (*Gemmula sogodensis*, *Gemmula speciosa*, *Gemmula diomedea* and *Gemmula kieneri*). This result was rather surprising, since shells of *Gemmula ambara* have generally been confused with smaller specimens of *Gem-*

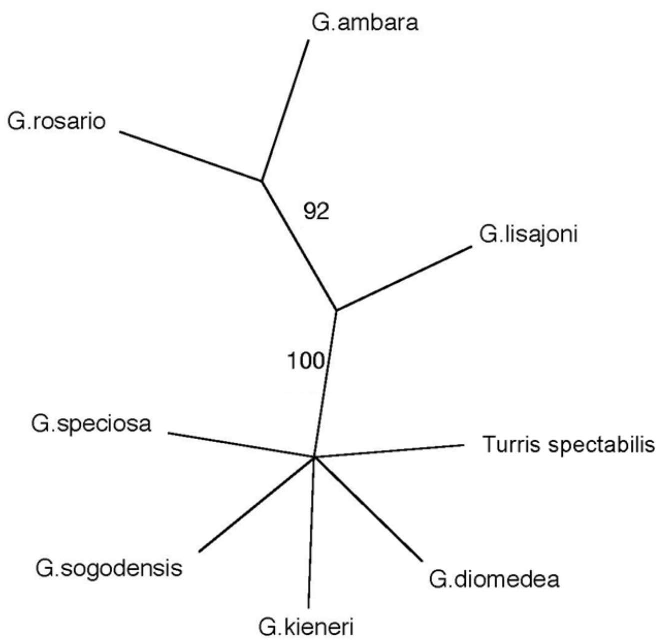


**Figure 4.** Phylogenetic analysis using 12S mitochondrial DNA sequences. Phylogenetic tree based on a small number of 12S rRNA sequences for the genus *Gemmula* showing strong support for placement of *Gemmula ambara* in a clade with *G. lisajoni* and *G.rosario* to the exclusion of other *Gemmula* species and the outgroup Turris spectabilis. Identical trees with similar support values were generated using Bayesian methods with posterior probabilities (indicated by the numbers) and maximum likelihood analysis with bootstrap analysis.

*mula diomedea*, as indicated above, since these forms share a white and brown color pattern.

The COI barcode sequence for *Gemmula ambara* was obtained and compared to other COI *Gemmula* sequences previously determined. The results for the barcode of all Philippine *Gemmula* species analyzed so far are in Table 2. As would be expected for distinct species, the barcode sequences diverge significantly from each other within the homologous COI interval.

## Discussion

In this paper, a new turrine species from the Philippines, the amber gem turrid, *Gemmula ambara*, is characterized. Most specimens examined have come from the Balicasag/Aliguay region of the Central Philippines where specimens have either been caught by tangle nets or using a small trawl at depths >30 fathoms. At these localities, it appears that the species is not uncommon, but has apparently been confused with similarly colored forms in the genus *Gemmula* that belong to the *Gemmula diomedea* complex. As is described above, there are a number of distinctive morphological features that reliably differentiate the shells of *Gemmula ambara* from the smaller *Gemmula diomedea* specimens of the same size as *G. ambara*.

The molecular phylogeny reveals that contrary to expectations from the similarities in color and shell morphology of *Gemmula ambara* to *G. diomedea*, it is most closely related to *Gemmula lisajoni* and *Gemmula rosario*, although the color pattern of *G. ambara* is distinct and easily separable from those species. With a more careful examination of morphological characters of these species, certain more subtle shell morphological characteristics appear to be better correlated with the true relationships than is color pattern. For example, the sculpture of the subsutural region and the size of the protoconch may prove to be a better indicator of genetic affinities, with color pattern potentially more sensitive to environmental factors.

Of the species for which molecular data are available in the Philippines that are conventionally assigned to the genus *Gemmula*, there now appear to be two very distinctive subgroups. One includes *Gemmula speciosa*, *Gemmula sogodensis*, *Gemmula kieneri* and *Gemmula diomedea*. The other includes *Gemmula rosario*, *Gemmula lisajoni* and the species we characterized in this work, *Gemmula ambara*. Other forms in *Gemmula* need to be analyzed and added to the phylogenetic tree. Recent evidence to be described elsewhere (M. Astilla and G. Concepcion, unpublished), suggests that some *Gemmula* species prey on terebellid polychaetes. Whether this prey preference is shared by *G. ambara* remains to be established.

Clearly, the first step to obtain a comprehensive framework for understanding the gem turrids is to elucidate the taxonomy and phylogenetic relationships within the group. A number of new species of *Gemmula* have been described from the Philippines (Olivera 1999; Olivera 2004). Recently, a molecular characterization of venom components of some of the Turrinae has been initiated (Heralde et al. 2008; Watkins et al. 2006) and therefore, it is now possible to systematically investigate pharmacologically-active compounds in this group of species, including *Gemmula ambara*. However, the taxonomic and phylogenetic definition of the undescribed turrine species from the Philippines clearly needs to be addressed in a timely manner, to keep pace with the molecular characterization of their venom components.

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