

# Evolution of the Toxoglossa Venom Apparatus as Inferred by Molecular Phylogeny of the Terebridae

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Toxoglossate marine gastropods, traditionally assigned to the families Conidae, Terebridae, and Turridae, are one of the most populous animal groups that use venom to capture their prey. These marine animals are generally characterized by a venom apparatus that consists of a muscular venom bulb and a tubular venom gland. The toxoglossan radula, often compared with a hypodermic needle for its use as a conduit to inject toxins into prey, is considered a major anatomical breakthrough that assisted in the successful initial radiation of these animals in the Cretaceous and early Tertiary. The pharmacological success of toxins from cone snails has made this group a star among biochemists and neuroscientists, but very little is known about toxins from the other Toxoglossa, and the phylogeny of these families is largely in doubt. Here we report the first molecular phylogeny for the Terebridae and use the results to infer the evolution of the venom apparatus for this group. Our findings indicate that most of the genera of terebrids are polyphyletic, and one species (“*Terebra*” (s.l.) *jungi*) is the sister group to all other terebrids. Molecular analyses combined with mapping of venom apparatus morphology indicate that the Terebridae have lost the venom apparatus at least twice during their evolution. Species in the genera *Terebra* and *Hastula* have the typical venom apparatus found in most toxoglossate gastropods, but all other terebrid species do not. For venomous organisms, the dual analysis of molecular phylogeny and toxin function is an instructive combination for unraveling the larger questions of phylogeny and speciation. The results presented here suggest a paradigm shift in the current understanding of terebrid evolution, while presenting a road map for discovering novel terebrid toxins, a largely unexplored resource for biomedical research and potential therapeutic drug development.

## Introduction

Marine snails are not what initially come to mind when discussing venomous animals, but the toxoglossan gastropods that use venom to capture their prey are among the most highly populous groups of marine invertebrates. Toxoglossate (“poison tongue”) gastropods include cone snails (*Conus*, within the family Conidae), auger snails or Terebridae, and “Turridae” (a complex of families) (Taylor et al. 1993; Puillandre et al. 2008). A venom apparatus made up of a muscular venom bulb and a tubular venom gland generally characterizes these marine mollusks. Toxins are injected into target animals via hollow disposable radular teeth and act to immobilize prey or defend against predators (Olivera 2002). The toxoglossan radula, often compared with a hypodermic needle, is considered a major anatomical breakthrough that assisted in the successful initial radiation of these animals in the Cretaceous and lower Tertiary (Shimek and Kohn 1981). There are believed to be >10,000 species of toxoglossate mollusks (Bouchet 1990); Toxoglossa are the most diverse and abundant group of predatory snails in species numbers. The pharmacological success of toxins from cone snails has led to their extensive use in characterizing cellular communication in the nervous system, but very little is known about toxins from terebrids and other Toxoglossa, and the phylogeny of these families is largely in doubt.

The Terebridae are a distinctive example of modular anatomical development within marine gastropods, with their foregut anatomy possessing various interchangeable

parts. All Terebridae are carnivorous and hunt prey; however, a significant number of terebrid species have lost the venom apparatus. Based on key structural differences in their foregut anatomy, Miller (1970, 1971) identified three distinct terebrid feeding types: Type I terebrids, of which there are two groups, IA and IB, have salivary glands, a long eversible labial tube, and a short and slightly retractable buccal tube (fig. 1A). Type I terebrids lack a radular and venom apparatus and therefore may not use toxins to subdue and immobilize their prey. These terebrids feed by grasping the prey with the anterior part of the labial tube and engulfing the prey whole. On the other hand, Type II terebrids exhibit characteristic toxoglossan feeding in that they impale the prey with a hypodermic radular tooth through which they deliver immobilizing toxins expressed in the venom gland, then grasp and engulf their catch with the sphincter of the labial tube. In addition to the hypodermic radular teeth and venom apparatus, these terebrids have salivary glands, an eversible labial tube of moderate length, and a long retractile buccal tube (fig. 1B). The final terebrid type, Type III, is similar to Type I in that they lack a venom apparatus but possess an unusual accessory feeding organ, the accessory proboscis structure, that is mostly uncharacteristic in other terebrids (fig. 1C). Type III terebrids use the accessory feeding organ to grasp and ingest tentacles of cirratulid polychaetes. To understand how the three strategies evolved, it is imperative to establish a molecular phylogeny for the Terebridae.

Similar to the majority of shelled mollusks, the taxonomy of the Terebridae is not very well characterized; their taxonomy is primarily based on shell morphology (Oyama 1961; Bratcher and Cernohorsky 1987; Terryn 2007) and relatively few studies describe their anatomy (Rudman 1969; Miller 1971; Taylor 1990; Taylor et al. 1993). This is surprising, given the relative abundance of terebrids and their global tropical distribution and shallow water

Key words: Toxoglossa evolution, Terebridae phylogeny, venomous marine snails, peptide toxins, venom apparatus.

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*Mol. Biol. Evol.* 26(1):15–25. 2009

doi:10.1093/molbev/msn211

Advance Access publication October 6, 2008

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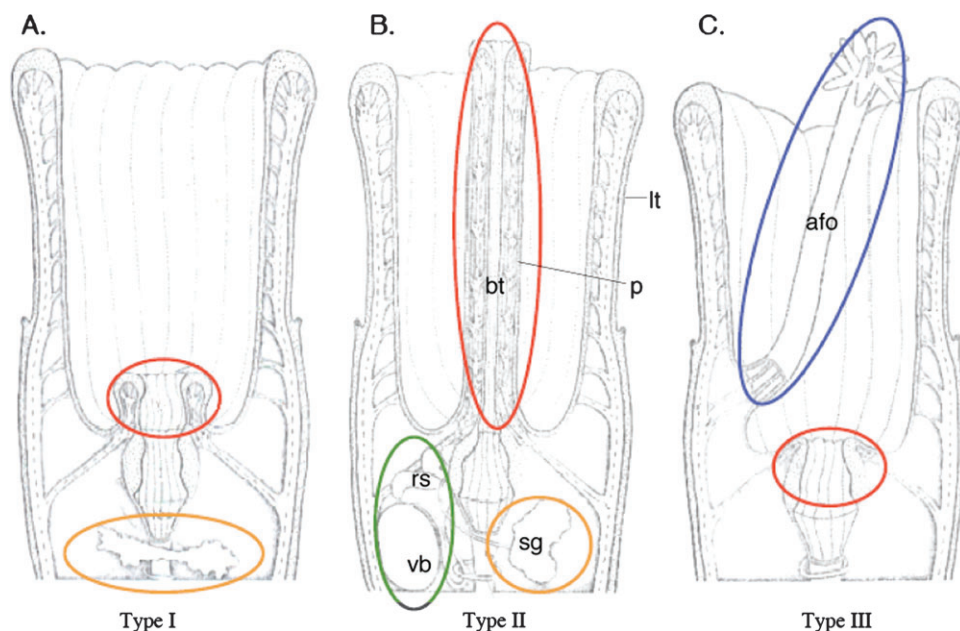


FIG. 1.—Schematic representation of Miller's foregut anatomy of the Terebridae. The three types of foregut anatomy Miller used to define feeding strategies within the Terebridae are depicted (Types I, II, and III). Anatomical features are labeled and highlighted in color: yellow = salivary glands (sg), red = buccal tube (bt), green = venom bulb (vb) and radular sac (rs), and blue = accessory feeding organ (afo). Also labeled are the proboscis (p), found only in Type II terebrids, and the labial tube (lt) (after Miller 1970).

occurrence. The last classification (Bouchet and Rocroi 2005) of the Gastropoda recognizes two subfamilies, Terebridae and Pervicaciinae, within the family Terebridae, which comprises >300 known species (Bratcher and Cernohorsky 1987; Terryn 2007) and may be another 100 unnamed taxa, especially in deeper waters (100–500 m).

We used a three-gene data matrix consisting of mitochondrial 12S and 16S rRNA and cytochrome oxidase subunit I (COI) sequences from 67 species to reconstruct the first molecular phylogeny of the Terebridae (fig. 2). We also assessed the evolution of terebrid feeding strategies by mapping the presence or absence of the venom apparatus on the phylogeny. The results presented here suggest a new hypothesis for understanding terebrid evolution: Our findings indicate that the Terebridae have independently lost the venom apparatus twice during their evolution.

The molecular phylogeny of the Terebridae additionally provides a road map for discovering novel terebrid toxins from those species with a venom apparatus. Given the similarities with cone snails, it is expected that the toxins from terebrids may be a rich source of neuroactive peptides to investigate cellular communication in the nervous system, but this is largely unexplored at present. Reconstructing a reliable phylogeny for the Terebridae is the first step toward characterizing their toxins using the emerging biodiversity/exoge-

nome combination strategy that has been developed for *Conus* venom peptides (Olivera and Teichert 2007).

## Materials and Methods

### Material

The collection details for the nonterebrid samples used in this study are shown in Table 1. All specimens used were collected during several expeditions to the West Pacific since 2004 and specifically fixed for molecular analysis. Living specimens were anesthetized by placing them in a solution with a concentration of 1 volume of  $MgCl_2$  for 13 volume of water, for 1 or 2 h. A piece of tissue (usually foot) was cut and fixed in 95% ethanol. Table 2 lists all terebrid specimens used in this study and the expedition sources. Vouchers are kept in the collection of the Muséum National d'Histoire Naturelle (MNHN). The species included in taxon sampling represent 12 of the 15 identified genera as defined in a recent shell morphology based revision of the Terebridae (Terryn 2007). Outgroups were chosen to form a nonmonophyletic group as recommended by Darlu and Tassy (1993). Representative species of Conidae and Turridae (s.l.) were chosen as closely related outgroups, and one species from the neogastropod family Harpidae was chosen as distant outgroup.

**Table 1**  
**Outgroups Used in the Study**

| MNHN N° | Cruise     | Coordinates, Depth              | Genus           | Species                     |
|---------|------------|---------------------------------|-----------------|-----------------------------|
| 40568   | EBISCO     | 21°10'S, 158°39'E, 650–723 m    | <i>Turridae</i> | <i>Cochlespira</i> sp.      |
| 17922   | Panglao 05 | 9°32.5'N, 123°41.8'E, 111–115 m | <i>Conidae</i>  | <i>Conus nereis</i>         |
| 40569   | Santo 06   | 15°37.3'S, 167°05.8'E, 78–91 m  | <i>Harpidae</i> | <i>Harpa</i> sp.            |
| 17685   | Santo 06   | 15°33.6'S, 167°16.6'E, 8–9 m    | <i>Turridae</i> | <i>Iotyrrix cingulifera</i> |

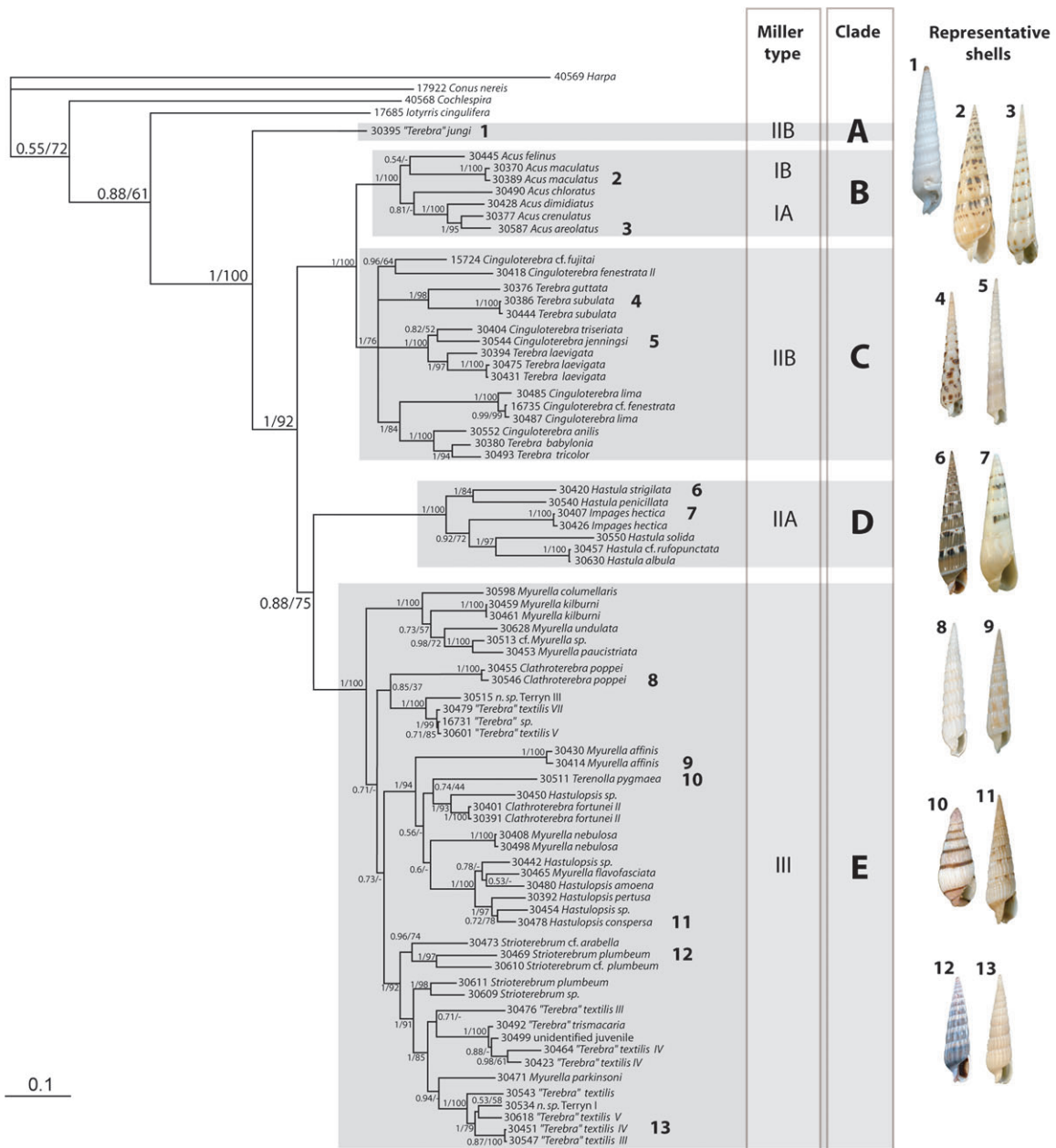


FIG. 2.—Combined phylogenetic tree. Consensus tree of ML analysis and BA using COI, 16S, and 12S data sets. PP and B are specified for each node. Miller types, IA, IB, IIA, IIB, and III, as described in the text are highlighted in the tree. Molecular analyses divide the Terebridae into five distinct clades, Clades A–E, indicated by the shaded gray areas. Representative shells, numbered 1–13, are shown for each clade. For clarity, multiple samples of the same species are shown only when there is a geographic difference, for example, 30370 and 30389 *Acus maculatus* from Panglao 2004 and Santo 2006 expeditions, respectively.

### Sequencing

DNA was extracted from foot or other tissue using Qiagen QIAamp Dneasy Tissue kit. Fragments of mitochondrial genes 12S, 16S, and COI were amplified using universal primers 12S1/12S3 (Simon et al. 1991), 16Sar/16Sbr (Palumbi 1996), and LCO1490/HCO2198 (Folmer et al. 1994), respectively. All polymerase chain reactions (PCRs) were performed in 25  $\mu$ l, containing 3 ng of DNA, 1 $\times$  reaction buffer, 2.5 mM MgCl<sub>2</sub>, 0.26 mM deoxynucleoside triphosphate, 0.3 mM each primer, 5% dimethyl sulfoxide, and 1.5 units of Qbiogene Q-Bio Taq. Amplification consisted

of an initial denaturation step at 94 °C for 4 min, followed by 37 cycles of denaturation at 94 °C for 30 s, annealing at 54 °C for 12S gene and 52 °C for 16S, followed by extension at 72 °C for 1 min. The final extension was at 72 °C for 5 min. The amplification of COI genes was performed similarly, except there were two annealing cycles: the first repeated five times at an annealing temperature of 45 °C and the second repeated 30 times at 50 °C as described by Herbert et al. (2003). PCR products were purified using exonuclease I and phosphatase and sequenced using the Applied Biosystem BigDyeTerminator V3.1 kit and the ABI3730XL

**Table 2**  
**List of Terebrid Samples Used in Molecular Phylogeny**

| MNHN N° | Cruise     | Coordinates, Depth                 | Genus                 | Species (Author)  | COI | 16S | 12S | Miller Type | Clade |
|---------|------------|------------------------------------|-----------------------|---|-----|-----|-----|-------------|-------|
| 30482   | BOA1       | 15°41.6'S, 167°02.1'E, 268–445 m   | <i>Cinguloterebra</i> | <i>fujitai</i> (Kuroda and Habe 1952)                           | x   | x   |     |             | C     |
| 30530   | EBISCO     | 20°29'S, 158°42'E, 197–230 m       |                       | <i>n. sp. Terryn III</i>  | x   | x   | x   |             | E     |
| 30515   | EBISCO     | 20°06'S, 160°23'E, 280–304 m       |                       | <i>n. sp. Terryn III</i>  | x   | x   | x   |             | E     |
| 30535   | Panglao 04 | 9°33.0'N, 123°46.5'E, 8–14 m       | <i>Hastula</i>        | <i>lanceata</i> (Linnaeus 1767)                                 | x   | x   |     |             | D     |
| 30370   | Panglao 04 | 9°37.4'N, 123°46.9'E, 3–20 m       | <i>Acus</i>           | <i>maculatus</i> (Linnaeus 1758)                                | x   | x   | x   | IB          | B     |
| 30404   | Panglao 04 | 9°35.3'N, 123°52.2'E, 84–87 m      | <i>Cinguloterebra</i> | <i>triseriata</i> (JE Gray 1824)                                | x   | x   | x   |             | C     |
| 30464   | Panglao 04 | 9°35.3'N, 123°52.2'E, 84–87 m      |                       | <i>“Terebra” textilis-group</i><br><i>textilis type IV</i>      | x   | x   | x   |             | E     |
| 30442   | Panglao 04 | 9°33.5'N, 123°48.6'E, 80–120 m     | <i>Hastulopsis</i>    | sp.   | x   | x   | x   |             | E     |
| 30443   | Panglao 04 | 9°37.4'N, 123°54.5E, 6–8 m         | <i>Acus</i>           | <i>felinus</i> (Dillwyn 1817)                                   | x   | x   | x   | IB          | B     |
| 30444   | Panglao 04 | 9°37.4'N, 123°54.5E, 6–8 m         | <i>Terebra</i>        | <i>subulata</i> (Linnaeus 1767)                                 | x   | x   | x   | IIB         | C     |
| 30445   | Panglao 04 | 9°37.4'N, 123°54.5E, 6–8 m         | <i>Acus</i>           | <i>felinus</i> (Dillwyn 1817)                                   | x   | x   | x   | IB          | B     |
| 30446   | Panglao 04 | 9°37.4'N, 123°54.5'E, 4–5 m        | <i>Terenolla</i>      | <i>pygmaea</i> (Hinds 1844)                                     | x   | x   |     | III         | E     |
| 30448   | Panglao 04 | 9°37.4'N, 123°54.5'E, 4–5 m        | <i>Terenolla</i>      | <i>pygmaea</i> (Hinds 1844)                                     | x   | x   | x   | III         | E     |
| 30449   | Panglao 04 | 9°37.4'N, 123°54.5'E, 4–5 m        | <i>Terenolla</i>      | <i>pygmaea</i> (Hinds 1844)                                     | x   | x   | x   | III         | E     |
| 30430   | Panglao 04 | 9°37.4'N, 123°54.5E, 6–8 m         | <i>Myurella</i>       | <i>affinis</i> (JE Gray 1834)                                   | x   | x   | x   | III         | E     |
| 30452   | Panglao 04 | 9°37.4'N, 123°54.5E, 6–8 m         | <i>Myurella</i>       | <i>affinis</i> (JE Gray 1834)                                   | x   | x   | x   | III         | E     |
| 30439   | Panglao 04 | 9°33.4'N, 123°48.4'E, 3 m          | <i>Myurella</i>       | <i>affinis</i> (JE Gray 1834)                                   | x   | x   | x   | III         | E     |
| 30511   | Panglao 04 | 9°35.7'N, 123°44.4'E, 0–2 m        | <i>Terenolla</i>      | <i>pygmaea</i> (Hinds 1844)                                     | x   | x   | x   | III         | E     |
| 30510   | Panglao 04 | 9°35.7'N, 123°44.4'E, 0–2 m        | <i>Myurella</i>       | <i>columellaris</i> (Hinds 1844)                                | x   | x   |     | III         | E     |
| 30459   | Panglao 04 | 9°35.7'N, 123°44.4'E, 0–2 m        | <i>Myurella</i>       | <i>kilburni</i> (RD Burch 1965)                                 | x   | x   | x   |             | E     |
| 30460   | Panglao 04 | 9°35.7'N, 123°44.4'E, 0–2 m        | <i>Myurella</i>       | <i>affinis</i> (JE Gray 1834)                                   | x   | x   | x   | III         | E     |
| 30587   | Panglao 04 | 9°37.4'N, 123°46.9'E, 3–20 m       | <i>Acus</i>           | <i>areolatus</i> (Link 1807)                                    | x   | x   | x   | IA          | B     |
| 30472   | Panglao 04 | 9°41.8'N, 123°51.1'E, 10 m         | <i>Cinguloterebra</i> | <i>fenestrata type I</i>  |     | x   | x   | IIB         | C     |
| 30534   | Panglao 04 | 9°42.1'N, 123°51.4'E, 3–4 m        |                       | <i>n. sp. Terryn I</i>  | x   | x   | x   |             | E     |
| 30465   | Panglao 04 | 9°29.4'N, 123°56.0'E, 15–20 m      | <i>Myurella</i>       | <i>flavofasciata</i> (Pilsbry 1921)                             | x   | x   | x   | III         | E     |
| 30408   | Panglao 04 | 9°29.4'N, 123°56.0'E, 15–20 m      | <i>Myurella</i>       | <i>nebulosa</i> (GB Sowerby I 1825)                             | x   | x   | x   |             | E     |
| 30450   | Panglao 04 | 9°43.3'N, 123°48.8'E, 123–135 m    | <i>Hastulopsis</i>    | sp.   | x   | x   | x   |             | E     |
| 30407   | Panglao 04 | 9°32.8'N, 123°45.9'E, 0–2 m        | <i>Impages</i>        | <i>hectica</i> (Linnaeus 1758)                                  | x   | x   | x   | IIA         | D     |
| 30451   | Panglao 04 | 9°36.8'N, 123°52.2'E, intertidal   |                       | <i>“Terebra” textilis-group</i><br><i>textilis type IV</i>      | x   | x   | x   |             | E     |
| 30431   | Panglao 04 | 9°36.8'N, 123°52.2'E, intertidal   | <i>Terebra</i>        | <i>laevigata</i> (JE Gray 1834)                                 | x   | x   | x   | IIB         | C     |
| 30454   | Panglao 04 | 9°36.4'N, 123°53.8'E, 60–62 m      | <i>Hastulopsis</i>    | sp.   | x   | x   | x   |             | E     |
| 30513   | Panglao 04 | 9°36.4'N, 123°53.8'E, 60–62 m      | cf. <i>Myurella</i>   | sp.   | x   | x   | x   |             | E     |
| 30455   | Panglao 04 | 9°36.4'N, 123°53.8'E, 60–62 m      | <i>Clathroterebra</i> | <i>poppei</i> (Terryn 2003)                                     | x   | x   | x   |             | E     |
| 30483   | Panglao 04 | 9°32.8'N, 123°42.1'E, 3–35 m       | <i>Terebra</i>        | <i>subulata</i> (Linnaeus 1767)                                 | x   | x   | x   | IIB         | E     |
| 30481   | Panglao 04 | 9°35.7'N, 123°44.4'E, 0–2 m        | <i>Myurella</i>       | <i>affinis</i> (JE Gray 1834)                                   | x   | x   | x   | III         | E     |
| 30401   | Panglao 05 | 9°39.2'N, 123°47.5'E, 255–268 m    | <i>Clathroterebra</i> | <i>fortunei type II</i>   | x   | x   | x   |             | E     |
| 30582   | Panglao 05 | 9°39.2'N, 123°47.5'E, 255–268 m    |                       | <i>“Terebra” textilis-group</i><br><i>textilis</i> (Hinds 1844) | x   |     | x   |             | E     |
| 30418   | Panglao 05 | 9°39.2'N, 123°47.5'E, 255–268 m    | <i>Cinguloterebra</i> | <i>fenestrata type II</i>                                       | x   | x   | x   | IIB         | C     |
| 30410   | Panglao 05 | 9°39.2'N, 123°47.5'E, 255–268 m    | <i>Cinguloterebra</i> | <i>fenestrata type I</i>  | x   | x   | x   | IIB         | C     |
| 30395   | Panglao 05 | 9°37.5'N, 123°40.2'E, 606–631 m    |                       | <i>“Terebra” bathyrhapse-group</i><br><i>jungi</i> (Lai 2001)   | x   | x   | x   | IIB         | A     |
| 30390   | Panglao 05 | 9°29.4'N, 123°44.4'E, 271–318 m    | <i>Cinguloterebra</i> | cf. <i>fenestrata</i> (Hinds 1844)                              | x   | x   | x   | IIB         | C     |
| 15724   | Panglao 05 | 9°27.4'N, 123°49.4'E, 273–356 m    | <i>Cinguloterebra</i> | cf. <i>fujitai</i> (Kuroda and Habe 1952)                       | x   | x   | x   |             | C     |
| 16731   | Panglao 05 | 9°30.1'N, 123°41.6'E, 356–396 m    |                       | <i>“Terebra”</i><br>sp.   | x   | x   | x   |             | E     |
| 16735   | Panglao 05 | 9°36.2'N, 123°43.8'E, 382–434 m    | <i>Cinguloterebra</i> | cf. <i>fenestrata</i> (Hinds 1844)                              | x   | x   | x   | IIB         | C     |
| 30584   | Panglao 05 | 9°34.3'N, 123°37.8'E, 729–733 m    |                       | <i>“Terebra” bathyrhapse-group</i><br><i>jungi</i> (Lai 2001)   | x   | x   | x   | IIB         | A     |
| 30406   | Panglao 05 | 6°24.1'S, 156°20.4'E, 1045–1207 m  | <i>Cinguloterebra</i> | sp.   | x   |     |     |             | C     |
| 30479   | Salomon 2  | 7°13.8' S, 158° 29.4' E, 286–423 m |                       | <i>“Terebra” textilis-group</i><br><i>textilis type VII</i>     | x   | x   | x   |             | E     |
| 30528   | Salomon 2  | 7°43.5'S, 158°29.7'E, 336–341 m    |                       | <i>“Terebra” textilis-group</i><br><i>textilis type VII</i>     |     | x   | x   |             | E     |

**Table 2**  
**Continued**

| MNHN N° | Cruise    | Coordinates, Depth                | Genus                              | Species (Author)                                  | COI | 16S | 12S | Miller Type | Clade |
|---------|-----------|-----------------------------------|------------------------------------|---|-----|-----|-----|-------------|-------|
| 30424   | Salomon 2 | 8°36.7'S, 157°21.0'E, 150–160 m   | <i>"Terebra" textilis-group</i>    | <i>textilis type VI</i>                           |     | x   | x   |             | E     |
| 30391   | Salomon 2 | 7°59.3'S, 157°33.3'E, 260 m       | <i>Clathroterebra</i>              | <i>fortunei type II</i>                           | x   | x   | x   |             | E     |
| 30501   | Salomon 2 | 8°25.5'S, 159°26.4'E, 543–593 m   | <i>"Terebra" bathyrhaphe-group</i> | <i>jungi</i> (Lai 2001)                           |     | x   | x   | IIB         | A     |
| 30487   | Salomon 2 | 8°39.5'S, 157°23.0'E, 214–243 m   | <i>Cinguloterebra</i>              | <i>lima</i> (Deshayes 1857)                       | x   | x   | x   |             | C     |
| 30579   | Salomon 2 | 8°39.5'S, 157°23.0'E, 214–243 m   | <i>"Terebra" textilis-group</i>    | <i>trismacaria</i> (Melvill 1917)                 | x   | x   | x   |             | E     |
| 30492   | Salomon 2 | 8°39.5'S, 157°23.0'E, 214–243 m   | <i>"Terebra" textilis-group</i>    | <i>trismacaria</i> (Melvill 1917)                 | x   | x   | x   |             | E     |
| 30499   | Salomon 2 | 8°39.5'S, 157°23.0'E, 214–243 m   |                                    | Unidentified juvenile                             | x   | x   | x   |             | E     |
| 30409   | Santo 06  | 15°33.1'S, 167°17.8'E, 15–25 m    | <i>Terebra</i>                     | <i>tricolor</i> (GB Sowerby I 1825)               | x   | x   | x   | IIB         | C     |
| 30389   | Santo 06  | 15°28.7'S, 167°15.2'E, 19 m       | <i>Acus</i>                        | <i>maculatus</i> (Linnaeus 1758)                  | x   | x   | x   | IB          | B     |
| 30378   | Santo 06  | 15°33.1'S, 167°12.2'E, 3–40 m     | <i>Myurella</i>                    | <i>nebulosa</i> (GB Sowerby I 1825)               |     | x   | x   |             | E     |
| 30629   | Santo 06  | 15°33.1'S, 167°12.2'E, 3–40 m     | <i>Myurella</i>                    | <i>undulata</i> (JE Gray 1834)                    | x   | x   | x   | III         | E     |
| 30628   | Santo 06  | 15°33.1'S, 167°12.2'E, 3–40 m     | <i>Myurella</i>                    | <i>undulata</i> (JE Gray 1834)                    | x   | x   | x   | III         | E     |
| 30626   | Santo 06  | 15°31.7'S, 167°09.7'E, 18–21 m    | <i>"Terebra" textilis-group</i>    | <i>textilis type V</i>                            | x   | x   | x   |             | E     |
| 30440   | Santo 06  | 15°31.7'S, 167°09.7'E, 18–21 m    | <i>"Terebra" textilis-group</i>    | <i>textilis type V</i>                            | x   | x   | x   |             | E     |
| 30456   | Santo 06  | 15°31.7'S, 167°09.7'E, 18–21 m    | <i>"Terebra" textilis-group</i>    | <i>textilis type V</i>                            | x   | x   | x   |             | E     |
| 30458   | Santo 06  | 15°31.7'S, 167°09.7'E, 18–21 m    | <i>"Terebra" textilis-group</i>    | <i>textilis type V</i>                            | x   | x   | x   |             | E     |
| 30634   | Santo 06  | 15°31.7'S, 167°09.7'E, 18–21 m    | <i>"Terebra" textilis-group</i>    | <i>textilis type V</i>                            | x   | x   | x   |             | E     |
| 30617   | Santo 06  | 15°31.7'S, 167°09.7'E, 18–21 m    | <i>"Terebra" textilis-group</i>    | <i>textilis type V</i>                            | x   | x   | x   |             | E     |
| 30618   | Santo 06  | 15°31.7'S, 167°09.7'E, 18–21 m    | <i>"Terebra" textilis-group</i>    | <i>textilis type V</i>                            | x   | x   | x   |             | E     |
| 30386   | Santo 06  | 15°36.6'S, 167°10.1'E, 8–20 m     | <i>Terebra</i>                     | <i>subulata</i> (Linnaeus 1767)                   | x   | x   | x   | IIB         | C     |
| 30619   | Santo 06  | 15°33.1'S, 167°12.2'E, 3–40 m     | <i>Hastulopsis</i>                 | <i>conspersa</i> (Hinds 1844)                     | x   | x   | x   | III         | E     |
| 30387   | Santo 06  | 15°33.1'S, 167°12.2'E, 3–40 m     | <i>Terebra</i>                     | <i>guttata</i> (Röding 1798)                      | x   | x   | x   | IIB         | C     |
| 30376   | Santo 06  | 15°33.1'S, 167°12.2'E, 3–40 m     | <i>Terebra</i>                     | <i>guttata</i> (Röding 1798)                      | x   | x   | x   | IIB         | C     |
| 30620   | Santo 06  | 15°31.3'S, 167°10.4'E, 3–18 m     | <i>Myurella</i>                    | <i>undulata</i> (JE Gray 1834)                    | x   | x   | x   | III         | E     |
| 30494   | Santo 06  | 15°34.4'S, 167°13.1'E, 9 m        | <i>Acus</i>                        | <i>crenulatus</i> (Linnaeus 1758)                 | x   | x   | x   | IA          | B     |
| 30377   | Santo 06  | 15°34.4'S, 167°13.1'E, 9 m        | <i>Acus</i>                        | <i>crenulatus</i> (Linnaeus 1758)                 | x   | x   | x   | IA          | B     |
| 30623   | Santo 06  | 15°36.9'S, 167° 10.5'E, 6–33 m    | <i>Hastulopsis</i>                 | <i>conspersa</i> (Hinds 1844)                     | x   | x   | x   | III         | E     |
| 30480   | Santo 06  | 15°36.9'S, 167° 10.5'E, 6–33 m    | <i>Hastulopsis</i>                 | <i>amoena</i> (Deshayes 1859)                     | x   | x   | x   |             | E     |
| 30463   | Santo 06  | 15°36.9'S, 167° 10.5'E, 6–33 m    | <i>Hastulopsis</i>                 | <i>conspersa</i> (Hinds 1844)                     | x   | x   | x   | III         | E     |
| 30478   | Santo 06  | 15°36.9'S, 167° 10.5'E, 6–33 m    | <i>Hastulopsis</i>                 | <i>conspersa</i> (Hinds 1844)                     | x   | x   | x   | III         | E     |
| 30388   | Santo 06  | 15°36.9'S, 167° 10.5'E, 6–33 m    | <i>Hastulopsis</i>                 | <i>pertusa</i> (Born 1778)                        | x   | x   | x   | III         | E     |
| 30392   | Santo 06  | 15°36.9'S, 167° 10.5'E, 6–33 m    | <i>Hastulopsis</i>                 | <i>pertusa</i> (Born 1778)                        | x   | x   | x   | III         | E     |
| 30373   | Santo 06  | 15°36.9'S, 167° 10.5'E, 6–33 m    | <i>Acus</i>                        | <i>dimidiatus</i> (Linnaeus 1758)                 | x   | x   | x   | IA          | B     |
| 30394   | Santo 06  | 15°36.9'S, 167° 10.5'E, 6–33 m    | <i>Terebra</i>                     | <i>laevigata</i> (JE Gray 1834)                   | x   | x   | x   | IIB         | C     |
| 30475   | Santo 06  | 15°36.9'S, 167° 10.5'E, 6–33 m    | <i>Terebra</i>                     | <i>laevigata</i>                                  | x   | x   | x   |             | E     |
| 30476   | Santo 06  | 15°36.9'S, 167° 10.5'E, 6–33 m    | <i>"Terebra" textilis-group</i>    | <i>textilis type III</i>                          | x   | x   | x   |             | E     |
| 30621   | Santo 06  | 15°36.9'S, 167° 10.5'E, 6–33 m    | <i>"Terebra" textilis-group</i>    | <i>textilis type IV</i>                           | x   | x   | x   |             | E     |
| 30616   | Santo 06  | 15°36.9'S, 167° 10.5'E, 6–33 m    | <i>"Terebra" textilis-group</i>    | <i>textilis type IV</i>                           | x   | x   | x   |             | E     |
| 30558   | Santo 06  | 15°31'S, 167°09'E, intertidal     | <i>Strioterebrum</i>               | <i>plumbeum</i> (Quoy and Gaimard 1833)           | x   | x   | x   |             | E     |
| 30469   | Santo 06  | 15°31'S, 167°09'E, intertidal     | <i>Strioterebrum</i>               | <i>plumbeum</i> (Quoy and Gaimard 1833)           | x   | x   | x   |             | E     |
| 30632   | Santo 06  | 15°31'S, 167°09'E, intertidal     | <i>Terebra</i>                     | <i>laevigata</i> (JE Gray 1834)                   | x   | x   | x   | IIB         | C     |
| 30614   | Santo 06  | 15°31'S, 167°09'E, intertidal     | <i>Strioterebrum</i>               | cf. <i>arabella</i> (Thiele 1925)                 |     | x   | x   |             | E     |
| 30613   | Santo 06  | 15°31'S, 167°09'E, intertidal     | <i>Terebra</i>                     | <i>laevigata</i> (JE Gray 1834)                   | x   | x   | x   | IIB         | C     |
| 30567   | Santo 06  | 15°34.7'S, 167°13.8'E, 14–25 m    | <i>Myurella</i>                    | <i>nebulosa</i> (GB Sowerby I 1825)               | x   | x   | x   |             | E     |
| 30374   | Santo 06  | 15°31.4'S, 167°09.7'E, intertidal | <i>Terebra</i>                     | <i>punctatostrata</i> (JE Gray 1834)              |     | x   | x   |             | C     |
| 30612   | Santo 06  | 15°29'S, 167°14.94'E, 2–4 m       | <i>Myurella</i>                    | <i>parkinsoni</i> (Bratcher and Cernohorsky 1976) | x   | x   | x   | III         | E     |
| 30471   | Santo 06  | 15°29'S, 167°14.94'E, 2–4 m       | <i>Myurella</i>                    | <i>parkinsoni</i> (Bratcher and Cernohorsky 1976) | x   | x   | x   | III         | E     |
| 30611   | Santo 06  | 15°35.2'S, 167°59.4'E, intertidal | <i>Strioterebrum</i>               | <i>plumbeum</i> (Quoy and Gaimard 1833)           | x   | x   | x   |             | E     |

Table 2  
Continued

| MNHN N° | Cruise   | Coordinates, Depth                   | Genus                                    | Species (Author)                            | COI | 16S | 12S | Miller Type | Clade |
|---------|----------|--------------------------------------|--|---|-----|-----|-----|-------------|-------|
| 30610   | Santo 06 | 15°35.2' S, 167°59.4' E, intertidal  | <i>Strioterebrum</i>                     | cf. <i>Plumbeum</i> (Quoy and Gaimard 1833) | x   | x   | x   |             | E     |
| 30609   | Santo 06 | 15°35.2' S, 167°59.4' E, intertidal  | <i>Strioterebrum</i>                     | sp.   | x   | x   | x   |             | E     |
| 30608   | Santo 06 | 15°35.2' S, 167°59.4' E, intertidal  | <i>Hastula</i>                           | <i>strigilata</i> (Linnaeus 1758)           | x   | x   |     | IIA         | D     |
| 30607   | Santo 06 | 15°35.2' S, 167°59.4' E, intertidal  | <i>Hastula</i>                           | <i>strigilata</i> (Linnaeus 1758)           |     | x   | x   | IIA         | D     |
| 30420   | Santo 06 | 15°35.2' S, 167°59.4' E, intertidal  | <i>Hastula</i>                           | <i>strigilata</i> (Linnaeus 1758)           | x   | x   | x   | IIA         | D     |
| 30416   | Santo 06 | 15°35.2' S, 167°59.4' E, intertidal  | <i>Hastula</i>                           | <i>strigilata</i> (Linnaeus 1758)           |     | x   | x   | IIA         | D     |
| 30414   | Santo 06 | 15°26.6' S, 167°15.2' E, intertidal  | <i>Myurella</i>                          | <i>affinis</i> (JE Gray 1834)               | x   | x   | x   | IIA         | E     |
| 30412   | Santo 06 | 15°26.6' S, 167° 15.2' E, intertidal | <i>Myurella</i>                          | <i>affinis</i> (JE Gray 1834)               | x   | x   | x   | III         | E     |
| 30598   | Santo 06 | 15°26.6' S, 167° 15.2' E, intertidal | <i>Myurella</i>                          | <i>columellaris</i> (Hinds 1844)            | x   | x   | x   | III         | E     |
| 30385   | Santo 06 | 15°26.6' S, 167° 15.2' E, intertidal | " <i>Terebra</i> " <i>textilis-group</i> | <i>textilis</i> (Hinds 1844)                | x   | x   | x   |             | E     |
| 30597   | Santo 06 | 15°26.6' S, 167° 15.2' E, intertidal | <i>Terebra</i>                           | <i>laevigata</i> (JE Gray 1834)             | x   | x   | x   | IIb         | C     |
| 30384   | Santo 06 | 15°26.6' S, 167° 15.2' E, intertidal | <i>Myurella</i>                          | <i>undulata</i> (JE Gray 1834)              |     | x   | x   | III         | E     |
| 30383   | Santo 06 | 15°26.6' S, 167° 15.2' E, intertidal | <i>Terebra</i>                           | <i>argus</i> (Hinds 1844)                   |     | x   | x   | IIb         | C     |
| 30382   | Santo 06 | 15°26.6' S, 167° 15.2' E, intertidal | <i>Terebra</i>                           | <i>cingulifera</i> (Lamarck. 1822)          | x   | x   | x   | IIb         | C     |
| 30552   | Santo 06 | 15°35.2' S, 167°59.4' E, intertidal  | <i>Cinguloterebra</i>                    | <i>anilis</i> (Röding 1798)                 |     | x   | x   | IIb         | C     |
| 30375   | Santo 06 | 15°31.1' S, 167°10.5' E, 7 m         | <i>Terebra</i>                           | <i>babylonia</i> (Lamarck 1822)             | x   | x   | x   | IIb         | C     |
| 30380   | Santo 06 | 15°31.1' S, 167°10.5' E, 7 m         | <i>Terebra</i>                           | <i>babylonia</i> (Lamarck 1822)             | x   | x   | x   | IIb         | C     |
| 30594   | Santo 06 | 15°31.1' S, 167°10.5' E, 7 m         | <i>Myurella</i>                          | <i>affinis</i> (JE Gray 1834)               | x   |     |     | III         | E     |
| 30550   | Santo 06 | 15°26.6' S, 167° 15.2' E, intertidal | <i>Hastula</i>                           | <i>solida</i> (Deshayes 1857)               |     | x   | x   | IIA         | D     |
| 30549   | Santo 06 | 15°26.6' S, 167° 15.2' E, intertidal | <i>Hastula</i>                           | <i>solida</i> (Deshayes 1857)               |     | x   | x   | IIA         | D     |
| 30417   | Santo 06 | 15°26.6' S, 167° 15.2' E, intertidal | <i>Hastula</i>                           | <i>solida</i> (Deshayes 1857)               | x   | x   | x   | IIA         | D     |
| 30437   | Santo 06 | 15°26.6' S, 167° 15.2' E, intertidal | <i>Hastula</i>                           | <i>albula</i> (Menke 1843)                  | x   | x   |     | IIA         | D     |
| 30438   | Santo 06 | 15°26.6' S, 167° 15.2' E, intertidal | <i>Hastula</i>                           | <i>albula</i> (Menke 1843)                  |     | x   | x   | IIA         | D     |
| 30571   | Santo 06 | 15°35.9' S, 167°01.3.1' E, 83–90 m   | <i>Clathroterebra</i>                    | <i>fortunei</i> type I?                     | x   | x   | x   |             | E     |
| 30498   | Santo 06 | 15°27.6' S, 167°14.3' E, 6–35 m      | <i>Myurella</i>                          | <i>nebulosa</i> (GB Sowerby I 1825)         | x   | x   | x   |             | E     |
| 30453   | Santo 06 | 15°29.6' S, 167°14.9' E, 2–5 m       | <i>Myurella</i>                          | <i>paucistriata</i> (EA Smith 1873)         | x   | x   | x   | III         | E     |
| 30546   | Santo 06 | 15°35.5' S, 167°02.7' E, 86–118 m    | <i>Clathroterebra</i>                    | <i>poppei</i> (Terry 2003)                  | x   | x   |     |             | E     |
| 30544   | Santo 06 | 15°28.6' S, 167°15.1' E, 3–31 m      | <i>Cinguloterebra</i>                    | <i>jenningsi</i> (RD Burch 1965)            | x   | x   | x   | IIb         | C     |
| 30603   | Santo 06 | 15°43.4' S, 167°15.0' E, 6 m         | <i>Terebra</i>                           | <i>laevigata</i> (JE Gray 1834)             | x   | x   | x   | IIb         | C     |
| 30541   | Santo 06 | 15°36.8' S, 167°08.5' E, 1–42 m      | <i>Myurella</i>                          | <i>affinis</i> (JE Gray 1834)               | x   | x   | x   | III         | E     |
| 30379   | Santo 06 | 15°32.5' S, 167°10.5' E, 1–10 m      | <i>Acus</i>                              | <i>dimidiatus</i> (Linnaeus 1758)           | x   | x   |     | IA          | B     |
| 30372   | Santo 06 | 15°32.5' S, 167°10.5' E, 5–10 m      | <i>Acus</i>                              | <i>dimidiatus</i> (Linnaeus 1758)           | x   | x   | x   | IA          | B     |
| 30493   | Santo 06 | 15°38.5' S, 167°15.1' E, 13 m        | <i>Terebra</i>                           | <i>tricolor</i> (GB Sowerby I 1825)         | x   | x   | x   | IIb         | C     |
| 30485   | Santo 06 | 15°32.5' S, 167°10.5' E, 5–10 m      | <i>Cinguloterebra</i>                    | <i>lima</i> (Deshayes 1857)                 | x   | x   | x   |             | C     |
| 30461   | Santo 06 | 15°42.7' S, 167°09.6' E, 2–3 m       | <i>Myurella</i>                          | <i>kilburni</i> (RD Burch 1965)             | x   | x   | x   |             | E     |
| 30601   | Santo 06 | 15°40.7' S, 167°0.5' E, 517–614 m    | " <i>Terebra</i> " <i>textilis-group</i> | <i>textilis</i> type V                      | x   | x   | x   |             | E     |
| 30570   | Santo 06 | 15°38.1' S, 167°05.9' E, intertidal  | <i>Myurella</i>                          | <i>undulata</i> (JE Gray 1834)              | x   | x   | x   | III         | E     |
| 30428   | Santo 06 | 15°38.1' S, 167°05.9' E, intertidal  | <i>Acus</i>                              | <i>dimidiatus</i> (Linnaeus 1758)           | x   | x   | x   | IA          | B     |
| 30547   | Santo 06 | 15°31.3' S, 167°09.91' E, 1–6 m      | " <i>Terebra</i> " <i>textilis-group</i> | <i>textilis</i> type III                    | x   | x   |     |             | E     |
| 30545   | Santo 06 | 15°31.3' S, 167°09.91' E, 1–6 m      | " <i>Terebra</i> " <i>textilis-group</i> | <i>textilis</i> type III                    | x   | x   | x   |             | E     |
| 30426   | Santo 06 | 15°35.4' S, 166°58.7' E, 3–8 m       | <i>Impages</i>                           | <i>hectica</i> (Linnaeus 1758)              | x   | x   | x   | IIA         | D     |
| 30543   | Santo 06 | 15°35.4' S, 166°58.7' E, 3–8 m       | " <i>Terebra</i> " <i>textilis-group</i> | <i>textilis</i> (Hinds 1844)                | x   | x   | x   |             | E     |
| 30457   | Santo 06 | 15°22.6' S, 167° 11.6' E, intertidal | <i>Hastula</i>                           | cf. <i>rufopunctata</i> (EA Smith 1877)     | x   | x   |     | IIA         | D     |
| 30542   | Santo 06 | 15°22.6' S, 167° 11.6' E, intertidal | <i>Hastula</i>                           | <i>penicillata</i> (Hinds 1844)             | x   | x   | x   | IIA         | D     |
| 30540   | Santo 06 | 15°22.6' S, 167° 11.6' E, intertidal | <i>Hastula</i>                           | <i>penicillata</i> (Hinds 1844)             | x   | x   | x   | IIA         | D     |
| 30490   | Santo 06 | 15°22.6' S, 167° 11.6' E, intertidal | <i>Acus</i>                              | <i>chloratus</i> (Lamarck 1822)             | x   | x   | x   | IB          | B     |
| 30425   | Santo 06 | 15°35.4' S, 166°58.7' E, 3–8 m       | <i>Strioterebrum</i>                     | cf. <i>arabella</i> (Thiele 1925)           | x   | x   | x   |             | E     |
| 30473   | Santo 06 | 15°35.4' S, 166°58.7' E, 3–8 m       | <i>Strioterebrum</i>                     | cf. <i>arabella</i> (Thiele 1925)           | x   | x   | x   |             | E     |

Table 2  
Continued

| MNHN N° | Cruise   | Coordinates, Depth             | Genus                            | Species (Author)                                  | COI | 16S | 12S | Miller Type | Clade |
|---------|----------|--------------------------------|----------------------------------|---|-----|-----|-----|-------------|-------|
| 30474   | Santo 06 | 15°35.4'S, 166°58.7'E, 3–8 m   | "Terebra" <i>textilis</i> -group | <i>textilis</i> type III                          | x   | x   | x   |             | E     |
| 17938   | Santo 06 | 15°35.4'S, 166°58.7'E, 3–8 m   | "Terebra" <i>textilis</i> -group | <i>textilis</i> (Hinds 1844)                      | x   | x   | x   |             | E     |
| 30381   | Santo 06 | 15°35.4'S, 166°59.7'E, 3–37 m  | <i>Acus</i>                      | <i>dimidiatus</i> (Linnaeus 1758)                 | x   | x   | x   | IA          | B     |
| 30630   | Santo 06 | 15°35.7'S, 166°59.3'E, 12 m    | <i>Hastula</i>                   | <i>albula</i> (Menke 1843)                        | x   | x   | x   | IIA         | D     |
| 30433   | Santo 06 | 15°33.4'S, 167°12.4'E, 2–6 m   | "Terebra" <i>textilis</i> -group | <i>textilis</i> type V                            | x   | x   | x   |             | E     |
| 30434   | Santo 06 | 15°33.4'S, 167°12.4'E, 2–6 m   | "Terebra" <i>textilis</i> -group | <i>textilis</i> type V                            | x   | x   | x   |             | E     |
| 30624   | Santo 06 | 15°33.4'S, 167°12.4'E, 2–6 m   | <i>Hastulopsis</i>               | <i>conspersa</i> (Hinds 1844)                     | x   | x   | x   | III         | E     |
| 30635   | Santo 06 | 15°33.4'S, 167°12.4'E, 2–6 m   | <i>Myurella</i>                  | <i>parkinsoni</i> (Bratcher and Cernohorsky 1976) | x   | x   | x   | III         | E     |
| 30423   | Santo 06 | 15°33'S, 167°16.7'E, 92 m      | "Terebra" <i>textilis</i> -group | <i>textilis</i> type IV                           | x   | x   | x   |             | E     |
| 30403   | Suva 4   | 15°26.4'S, 178°02.4'E, 50–51 m | <i>Cinguloterebra</i>            | <i>cumingii</i> (Deshayes 1857)                   | x   | x   | x   |             | C     |
| 30409   | Suva 4   | 15°26.4'S, 178°02.4'E, 50–51 m | <i>Cinguloterebra</i>            | <i>cumingii</i> (Deshayes 1857)                   |     | x   | x   |             | C     |

sequencer. All genes were sequenced in both directions for increased accuracy of each sequence. Sequences were deposited in GenBank (accession numbers: EU127881, EU015734, and EU685339–EU685783). COI sequences were also deposited in Barcode of Life Data Systems (<http://www.barcodinglife.org/views/taxbrowser.php?taxid=897>).

### Phylogenetic Analyses

COI sequences were manually aligned, and 12S and 16S were automatically aligned using ClustalW multiple alignment implemented using default parameters in BioEdit version 7.0.5.3 (Hall 1999). Visual inspection was used to search the automatic alignments for any obvious anomalies. Hypervariable regions of 12S and 16S genes were excluded from further analyses due to ambiguities in the alignments. Nucleotide substitution models were selected for each gene separately using the program Modeltest (Posada and Crandall 2001) in conjunction with PAUP 4.0b10 (Swofford 2002). The best model of evolution and parameters estimated for each gene are Tamura-Nei (TrN) + I (0.537) + G (0.885) for COI gene, transversion model (TVM) + I (0.310) + G (0.581) for 12S gene, and general time reversible (GTR) + I (0.402) + G (0.483) for 16S gene. Phylogenetic analyses were based on reconstructions using maximum likelihood (ML) and Bayesian analysis (BA). The ML heuristic search was conducted with 100 replicates with tree\_bisection and reconnection (TBR) branch swapping using PhyML 2.4.4 (Guindon and Gascuel 2003), and robustness of the nodes was assessed using nonparametric bootstrapping (Felsenstein 2004) with 1,000 bootstrap replicates for ML analysis.

BA consisted of six Markov chains (10,000,000 generations each with a sampling frequency of one tree each hundred generations) run in two parallel analyses using MrBayes (Huelsenbeck et al. 2001). The number of swaps that are tried each time the chain stops for swapping was four, and the chain temperature was set at 0.08. When the log-likelihood scores were found to stabilize, a consensus tree was calculated after omitting the first 0.25% of trees as burn-in.

For the combined analyses of the three genes, the same parameters were used for the ML analysis. For the BA, one different model was applied for each gene, each with six substitution categories. For the COI gene, as saturation was found on the third base of the codon, different models were applied for the two partitions (bases 1 and 2 vs. base 3). Finally, we have four unlinked partitions (COI bases 1 and 2, COI base 3, 12S, and 16S).

### Venom Apparatus Mapping

Data from cited literature and personal communications from A. Sysoev and J. Taylor helped to determine the presence or absence of a venom apparatus in the species used for the phylogenetic analysis. The absence or presence of the venom apparatus was then mapped on the tree using Mesquite V. 2.01 (Maddison W and Maddison DR 2007), using the option "tracing character history." The parsimony ancestral reconstruction method was used.

## Results

### Phylogenetic Analyses

One hundred and fifty six samples of terebrids were used to reconstruct the molecular phylogeny of the Terebridae. For the COI gene, 658 bp were sequenced. After alignments, we obtained a fragment of 534 and 455 bp for 12S and 16S genes, respectively. Trees obtained independently with COI, 12S, and 16S genes were only partly resolved, but no contradictions were found (results not showed). Consequently, 12S, 16S, and COI mitochondrial genes, including 131 taxa in the ingroup, were used to produce a combined data set for phylogenetic analyses (fig. 2).

Phylogenetic analyses strongly indicate that the Terebridae is monophyletic (posterior probabilities [PP] = 1, bootstraps [B] = 100) (fig. 2). *Terebra* s.l. "*Terebra*" *jungi* appears to be the sister group to all other terebrids (PP = 1, B = 92). Apart from "*T.*" *jungi* (Clade A), there are four major clades within the tree, designated in figure 2 as Clades B–E. Clade B is comprised primarily of the genus *Acus* plus one species currently placed in *Terebra*, *Terebra areolata*, which because of its placement in our tree, we tentatively define as *Acus areolatus* (PP = 1, B = 100). Clade C includes *Cinguloterebra* and additional species currently placed in *Terebra*, including the type species of *Terebra*, *Terebra subulata* (PP = 1, B = 76). Clade D includes species currently placed in the genera *Hastula* and *Impages* (PP = 1, B = 100).

Clade E is the largest clade and includes the genera *Myurella*, *Clathroterebra*, *Terenolla*, *Hastulopsis*, *Strioterebrum*, and the "*Terebra*" *textilis*-group (Terry 2007). *Myurella* itself is polyphyletic, with several species placed as sister groups of other genera. For example, *Myurella affinis*, the type species, is the sister group of *Terenolla*, *Hastulopsis*, some *Clathroterebra* species, and two other *Myurella* species (PP = 1, B = 94). *Clathroterebra* is also polyphyletic, with the two representative species used in our analyses, *Clathroterebra poppei* and *Clathroterebra fortunei*, type species of *Clathroterebra*, appearing in separate well-supported clades (PP = 1, B = 100) for both. The distinctiveness of the monotypic genus *Terenolla* appears to be confirmed in our analysis. *Hastulopsis* and *Strioterebrum* are paraphyletic. The "*Terebra*" *textilis*-group is dispersed within Clade E and constitutes a group that includes a large amount of undescribed diversity, both at the genus and species levels, hence the various types (*textilis* III, IV, V, and VII) listed in the tree.

### Evolution of Venom Apparatus

Miller's type classifications are indicated in the tree in figure 2 and table 2. "*Terebra*" *jungi* has a venom apparatus and is identified as a Type IIB feeder (Sysoev A, personal communication). Clade B corresponds to Miller's Type I feeders, which were further subdivided between Type IA and Type IB feeders. Type IA feeders are *Acus crenulatus* and *Acus dimidiatus* (Miller 1970, 1975). *Acus areolatus*, not hitherto recognized as a species of *Acus*, is however similar to the other species in Clade B in not having a venom apparatus and was identified as a Type IA feeder by Miller

(1975). The Type IB feeders in Clade B are *Acus felinus*, *Acus maculatus*, and *Acus chloratus*. The taxa that make up Clade C are all Type IIB feeders: *Terebra guttata*, *T. subulata*, *Terebra babylonica*, *Terebra tricolor*, *Cinguloterebra fenestrata*, *Cinguloterebra anilis*, and *Cinguloterebra jenningsi* (Taylor J, personal communication); (Miller 1970; Taylor 1990). Clade D made up exclusively of members of the genera *Hastula* (*Hastula strigilata*, *Hastula penicillata*, *Hastula solida*, *Hastula albula*, *Hastula* cf. *rufopunctata*) and *Impages* (*Impages hectica*) are Type IIA feeders (Miller 1979; Taylor 1990). Type III feeders are represented in the phylogenetic tree by Clade E, which includes species of *Myurella* (*Myurella columellaris*, *Myurella undulata*, *Myurella paucistriata*, *M. affinis*, *Myurella flavofasciata*, and *Myurella parkinsoni*), *Hastulopsis* (*Hastulopsis conspersa* and *Hastulopsis pertusa*), and *Terenolla* (*Terenolla pygmaea*) (Taylor J, personal communication) (Miller 1970; Taylor 1990).

In order to efficiently characterize toxins from various terebrid species, it is essential to first identify those species that have a venom apparatus. Shown in figure 3 is the mapping of the presence or absence of a venom apparatus in the terebrid species used to construct the molecular phylogeny in figure 2. The map clearly indicates that terebrids have lost the venom apparatus twice during their evolution, see Clades B and E. "*T.*" *jungi*, and the members of Clades C and D, use the typical toxoglossate venom apparatus to hunt prey.

## Discussion

The first molecular phylogeny of the Terebridae, presented in this report, significantly updates current hypotheses about the evolution of this group and will facilitate the efforts to characterize terebrid toxins. Although covering approximately 20% of the species-level diversity of the family, representing 12 of the 15 currently accepted genera, our data set does not include representatives of *Duplicaria* or *Pervicacia*, the latter made the type of a distinct family Pervicaciidae by Rudman (1969). However, our results highlight several problematic propositions about the Terebridae, while confirming the established hypotheses: 1) Terebridae family is monophyletic. 2) Shell shape was intuitively an appropriate character to group all terebrids in one taxon. Another substantiated hypothesis is that the monotypic genus *Terenolla* belongs in the Terebridae and is not a columbellid as suggested earlier (Bratcher and Cernohorsky 1987). A distinctive discovery revealed in figure 2 is that "*T.*" *jungi* is a sister group to all other terebrid species. This is an original finding unappreciated until the present molecular analysis.

This phylogeny implies that there is little congruence between the former genus-level classification based on shell morphology and clades recognized by molecular characters. The molecular phylogeny indicates that most of the genera proposed in the family Terebridae will have to be revised. This is evidenced by, for example, species earlier assigned to *Terebra* now appearing throughout the tree in Clades B ("*T.*" *areolata*, here classified in *Acus*), C (*T. subulata*, type species of *Terebra*, and several others), and E ("*Terebra*" *textilis*-group). In addition, "*T.*" *jungi* will require the



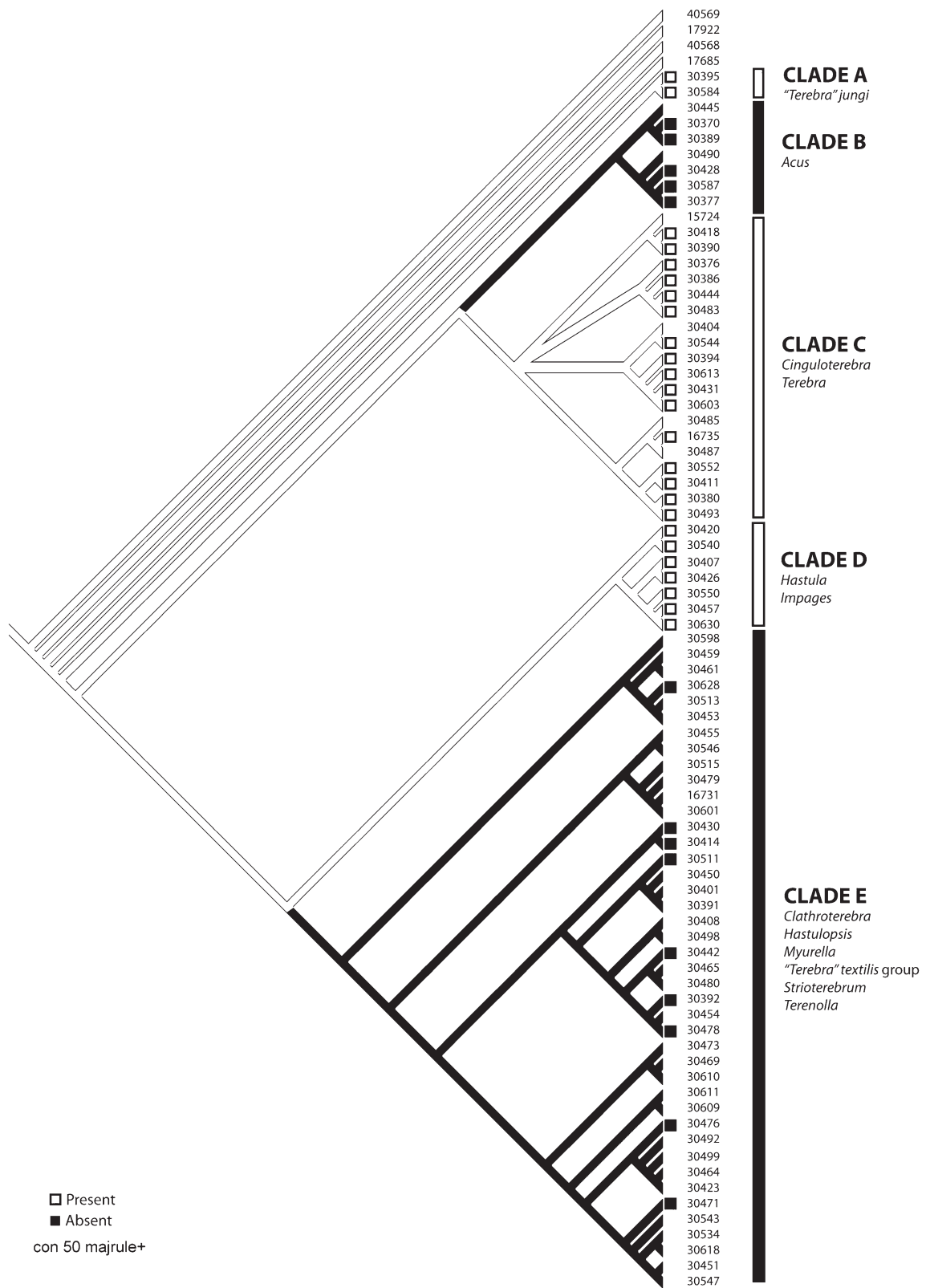


FIG. 3.—Possession of venom apparatus mapped onto Terebrid phylogeny. The presence or absence of a venom apparatus was mapped onto the molecular phylogeny of the Terebridae shown in figure 1. Terebrid species with a venom apparatus are indicated by a white box (□), whereas terebrid species without a venom apparatus are indicated by a black box (■). The map indicates that terebrids have independently lost the venom apparatus twice during their evolution.

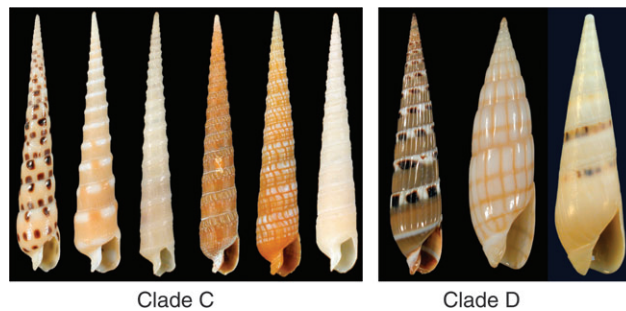


FIG. 4.—Terebrids with venom apparatus: representative shell images of the terebrid species in Clades C and D that have a venom apparatus. The species from left to right are Clade C: *Terebra subulata*, *Terebra guttata*, *Cinguloterebra jenningsi*, *Cinguloterebra anilis*, *Terebra babylonia*, *Terebra laevigata* and Clade D: *Hastula strigilata*, *Hastula solida*, and *Hastula hectica*.

establishment of a new genus (Terryn Y, Holford M, unpublished data). Despite this nonmonophyly of shell-defined genera, but based on the inclusion of their type species (except for *Acus*) in the analysis, we tentatively ascribe existing generic names to the four major clades: Clade B is thus *Acus* H. & A. Adams 1853, Clade C is *Terebra* Bruguière 1789, Clade D is *Hastula* H. & A. Adams 1853, and Clade E is *Myurella* Hinds 1844.

Evolutionary trends in the Toxoglossa have been reconstructed primarily through studies involving radular formation and anatomy of the digestive system (Mills 1979; Shimek and Kohn 1981; Kantor and Sysoev 1989; Taylor 1990; Simone 1999; Kantor and Taylor 2000). The molecular phylogeny presented here paints a plausible picture of how terebrids evolved such a diversity of feeding strategies. Our findings suggest that all terebrids appear to be derived from a common ancestor with a venom apparatus of the Miller Type IIB (fig. 3). Furthermore, mapping of the venom apparatus indicates that two lineages of terebrids independently lost their ability to hunt prey using toxins, Clades B and E.

There is a considerable correlation between our molecular phylogeny, Miller's anatomical groupings, and the ecological distribution of the terebrid species used in this study. Miller separated terebrids with a venom apparatus, Type II feeders, into two distinct groups, IIA and IIB. Type IIA and IIB terebrids differ in the shape of the buccal tube and shell morphology (Miller 1970). Type IIA terebrids have a long and slender buccal tube and small shiny shells, with 7–10 whorls and a flared aperture. Terebrids of Type IIB have a short, thick buccal tube, and the shells are large, long, and slender, with 15 or more whorls and a constricted aperture. Our phylogenetic analysis supports this separation. The species in Clade C, *Terebra*, have slender and multi-whorled shells, whereas those of Clade D, *Hastula*, are shiny with fewer whorls. The separation of the two clades is further supported by the ecological differences in their habitats. *Terebra* species of Clade C live buried in sandy or muddy subtidal flats, whereas *Hastula* species of Clade D live predominantly on surf beaches or in sand in reef pockets (Miller 1970, 1979). Similarly, terebrids that feed without the use of a venom apparatus, Types I and III feeders, are represented by two different clades in the molecular phylogeny, Clades B and E, respectively. Our analysis clarifies that the two clades without venom apparatus, Clades B and E, are not sister groups.

The Terebridae phylogeny in figure 2 sets the stage for efficient characterization of terebrid toxins and identification of the gene superfamilies that encode their toxins using the biodiversity first, exogenomic strategy recently applied to cone snails (Olivera 2006; Olivera and Teichert 2007). The exogenomic strategy was used to characterize cone snail toxins that target nicotinic receptors. In this strategy, phylogeny and molecular biology techniques are used to identify “exogenes,” genes of the toxins expressed in the venom duct. Exogenes rapidly evolve to respond to cues in their biotic environment and are thus a powerful marker for differentiating ecological or evolutionarily distinct organisms.

Clades C and D are the two major terebrid groups most suitable to investigate toxins for biochemical characterization (fig. 4). Furthermore, as Clades C and D are not sister clades, they may produce divergent toxins that could result in varied functional activity upon further characterization.

Initial reports on the toxins from terebrids (Imperial et al. 2003, 2007) indicate that they have precursor structures organized similarly to those of conotoxin gene superfamilies, with a highly conserved signal sequence, followed by a propeptide region, then the mature toxin region. As has been demonstrated with cone snails, the conserved signal sequence and propeptide region in terebrids are an exploitable feature that can be used to facilitate their characterization using molecular biology techniques, for example, as the basis for designing PCR primers. Preliminary results from the toxins characterized from *T. subulata* (Imperial et al. 2003) and *Hastula hectica* (Imperial et al. 2007) indicate that terebrid toxins are divergent from *Conus* toxins in several ways: 1) Terebrid toxins are not posttranslationally modified; facilitating the ability to chemically synthesize these peptides for testing of their function. 2) There is no homology between the gene superfamilies found in *Conus* and terebrid venom. These findings would indicate investigation of terebrid venom may reveal toxins with novel pharmacological specificity not found in *Conus* venom.

The biochemical and genetic characterization of terebrid toxins, while identifying novel compounds useful for investigating cell communication in the nervous system, will also provide additional characters to further clarify the phylogeny and evolutionary biology of these organisms. For toxoglossate gastropods, the dual analysis of

molecular phylogeny and venom function is an instructive combination for unraveling the bigger question of evolutionary diversification. This work is a first attempt to address these issues for the Terebridae.

### Acknowledgments

The authors thank Yuri Kantor for processing many of the terebrid specimens in the field, Virginie Heros and Philippe Maestrati for assistance throughout the field to curating samples at MNHN, the staff of MNHN's "Service de Systématique Moléculaire" for technical facilities, and John Taylor and Alexander Sysoev for providing unpublished anatomical information on terebrid taxa used in this study. The Panglao 2004 and Santo 2006 expeditions, which were the source of many specimens, were supported among others by the Total Foundation. Joint funding from National Science Foundation Chemistry Division and Office of International Science and Engineering postdoctoral fellowship (0610202) for M.H. also supported this work.

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Accepted September 10, 2008