

**THE PHYLOGENETIC UNDERPINNINGS FOR SPATIAL
PATTERNS OF MORPHOLOGICAL DISPARITY: ANALYSES
USING STROMBID GASTROPODS**

A Thesis

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TABLE OF CONTENTS

ABSTRACT.....	iii
CHAPTER	
1 INTRODUCTION.....	1
2 REVIEW OF LITERATURE.....	2
3 MATERIALS AND METHODS.....	6
DNA Extraction, Amplification, and Sequencing.....	6
Phylogenetic Analyses.....	8
Reanalysis of Morphospace Data.....	9
4 RESULTS.....	11
H3.....	11
COI.....	11
Combined H3 and COI.....	14
Reanalysis of Morphospace Data	14
5 DISCUSSION.....	25
Phylogenetic Relationships Within <i>Strombus s.l.</i> and Taxonomy.....	25
Filling Space Quickly (Neotropical Variance).....	26
Out of the Blue	26
Why Were <i>Tricornis</i> and <i>Lentigo</i> Misdesignated.....	27
6 CONCLUSION.....	29
BIBLIOGRAPHY.....	30
VITA.....	34

ABSTRACT

Attempting to understand the relationship between morphological and taxonomic diversification has become a central concern of both paleobiology and ecology. Ordinating species in morphospace provides a visual representation of trends in form. In morphospace analysis, each axis of a graph represents a quantitative measure of a morphological trait or combination of traits found in a group of closely related organisms. How species fill open morphospace provide some insight into the history of adaptive radiations. Furthermore, if we map species' positions in morphospace onto an independently derived phylogenetic hypothesis, we should be able to detect trends in morphological divergence along different lineages. The Strombidae, a family of shelled marine snails, shows a wide variety of forms among its 70 or so extant species, making it an ideal group for such analysis. A previous study has developed a well-defined morphospace for *Strombus* and *Lambis*, the two most species-rich and morphologically diverse genera in the Strombidae. Here I use portions of one nuclear (histone H3) and one mitochondrial (cytochrome oxidase I) genes to reconstruct the phylogeny of these two genera. I included 32 of the 50 extant species of *Strombus*, representing 10 of 11 extant subgenera and three of the nine species of *Lambis* representing two of three extant subgenera, in my phylogenetic analysis. Maximum likelihood bootstrap and Bayesian majority consensus phylogenetic analyses were performed on each gene individually and on both combined. The resultant phylogenetic tree suggests *Lambis* as a monophyletic radiation nested within the genus *Strombus*. This *Lambis* radiation seems to be driving exploration and colonization of morphospace along the first principal component axis. In addition, all New World species of *Strombus* are more closely related to each other than to congeners from the Indo-West Pacific, causing polyphyletic subgenera (*Tricornis* and *Lentigo*). Lastly, while the total volume of morphospace filled by the New World radiation was equivalent

to that seen among Old World species, the placement of that volume differed, being shifted to more flared forms that may reflect its shared ancestry with the strombid subgenus *Euprotomus*.

CHAPTER 1 INTRODUCTION

Attempting to understand the relationship between morphological and taxonomic diversification has become a central concern of both paleobiology (Gould 1991; Foote, 1995; and see below) and ecology (Norton 1995; Bickel and Losos 2002; Harmon et al. 2003). The concept of morphospace, defined as a multidimensional geometric representation of morphological characters used to study the distribution of observed and potentially unobserved forms, is central to these efforts (Bookstein 1985). In a universal morphospace it would, in theory, be possible to ordinate all realized and imaginable forms. In practice, however, we are usually interested in quantifying differences among relatively small groups of similar forms, and so limit the scope of morphospace examined. In these quantitative morphospaces, each axis represents a quantitative measure of a morphological trait or combination of traits. As more taxa are added to the morphospace, character variance from the centroid, or mean, state and morphospace volume typically increase (Roy and Foote 1997). The patterns in which clades fill open morphospace provide insight into the history of adaptive radiations (Harmon et al. 2003). By comparing phylogenetic distributions of species to the morphospace they occupy, measures of both developmental constraints and ecological saturation can be generated (Raup 1965).

CHAPTER 2 REVIEW OF LITERATURE

Raup and Michelson (1965) created the conceptual framework for morphospace analysis by defining four variables that described coiled shell growth. They proposed that differences in the values for: 1) shape of the generating curve, 2) rate of increase in the size of the generating curve per revolution, 3) distance between the generating curve and the coiling axis, and 4) rate of movement of the generating curve along the axis per revolution, could explain all possible coiled shell morphotypes. McGhee (1980) followed with a geometric analysis of articulate brachiopods, which used the variables from Raup and Michelson (1965), with the addition of a parameter for specific whorl modification rate, to recreate possible variations in shell shape. McGhee also used the log of the whorl expansion rate to define the position of biconvex brachiopods in morphospace, and in doing so was able to describe constraints in morphology related to limitations of articulation and surface to volume ratios.

Some years later, in a series of papers contrasting patterns in morphospace with patterns of taxonomic diversity, Foote (1991, 1992, 1993, 1994, 1995, 1996) set the stage for elucidating underlying patterns of morphospace occupation. Foote defined morphospace occupation for a number of different taxa, using a host of methods including Elliptical Fourier Analysis, Cartesian coordinates of homologous landmarks, and discrete morphological characters (see Foote 1997 for a full description of taxa and methodology). Subsequent studies have used morphospace to interpret large scale developmental, ecological, and evolutionary trends within species groups (Wills et al. 1994; Eble 2000; Roy et al. 2001; Hulsey and Wainwright 2002). For example, in an attempt to analyze changes observed in ammonites across the Lias Dogger stratigraphic boundary, Neige et al. (2001) found that two ammonite families, Graphoceratidae and Hammatoceratidae, accounted for most morphological disparity [terminology of Gould 1991 and

Foote 1995 referring to measures of morphological diversity based on the squared Euclidean distance between species]. However, without a well resolved phylogeny, these authors could not discern the roles of internal factors (heterochrony) and external constraints (temporal variation in sea level) in this diversification.

Several studies of fossil taxa have since combined morphospace studies with cladistic analyses (e.g. Willis et al. 1994, Wagner 2001; Stone 2003). Morphospaces are generated based on overall morphology, whereas cladistic analyses are based on a subset of the total characters, and the two metrics are not strictly independent. If, however, we map a morphospace onto an independently derived phylogenetic hypothesis, for instance one based on molecular data (as can be done with extant taxa), we should be confident that morphospace patterns are not an artifact of phylogenetic results, or vice versa (Bookstein 1985; Wagner 2000).

Species richness and morphological disparity within strombid gastropods (conchs and their kin) make them excellent candidates for a study of constraints versus lability. Previous work has detailed strombid taxonomy (Abbott 1960; Abbott 1961; Stone 2001), biogeography (Abbott 1960; Briggs 1998), and morphological disparity (Roy et al. 2001; Stone 2001). All strombids possess a second notch just above the siphonal canal known as the strombid notch, from which the left highly developed eye protrudes. The strombid notch allows for easy identification and the fossil record for the family is well documented (Piccoli et al. 1991; Roy 1996). The family is divided into five genera (*Strombus*, *Lambis*, *Terebellum*, *Tibia*, and *Rimella*) exhibiting marked differences in morphology (Abbott 1960). There are around 70 extant strombid species, all living in tropical and subtropical waters. Their veliger larvae are photopositive over their 2-4 week planktonic development, potentially resulting in broad

dispersal (Stoner and Davis 1997; Stoner et al. 1997). All members of the family exhibit determinate shell growth (Abbott 1960), providing an unambiguous gauge of adult size.

The two most species-rich genera of strombids are *Lambis* and *Strombus*. Both are algavores associated with shallow-water rocky and sandy surfaces and are similar in their soft tissue anatomies, egg masses, and radulae (Abbott 1961). Their shells, however, show striking differences. *Lambis* characters distinguishing them from *Strombus* include the development of long digitations on the apertural borders of the shell and the absence or reduction of the posterior mantle filament (Abbott 1960).

Roy et al. (2001) examined spatial patterns of morphological disparity for these two genera across the Indo-Pacific. The family exhibits a strong longitudinal gradient in taxonomic diversity, with the maximum number of species occurring in Indonesia and the Philippines. Strombids, in fact, are often cited as the classic example of the Indo-West Pacific diversity gradient (Briggs 1999). Roy et al. (2001), however, found that morphological disparity was highest in areas peripheral to the Indo-West Pacific (IWP) center of taxonomic diversity. This pattern lead them to conclude that morphological disparity in this group is largely decoupled from patterns of species richness. A lack of concordance between morphological and taxonomic patterns raises important questions as to how taxa are added to morphospace and the effect their addition has on the group's morphological disparity. For instance, morphospace that expands gradually may indicate speciation by expansion into new niches. Alternately, uneven filling of a morphospace (as in Neige et al. 2001) may indicate that niches have been filled by an adaptive radiation, with further morphological diversification halted by developmental constraints. More fundamentally, mapping morphospace filling onto a phylogeny can determine if bursts in disparity are caused by adaptive innovation within a clade or are simply the result of lumping

disparate taxa. To address these questions, assessing phylogenetic relationships among taxa is essential (Schluter, 2000; Harmon et al. 2003).

In this thesis, I create a molecular phylogeny to assess the relationship of *Lambis* to *Strombus* (as well as the interrelationship of individual *Strombus* subgenera), so as to provide a basis for interpreting patterns of occupation in the Roy et al. (2001) morphospace. I use DNA sequences from two protein-coding gene regions, one mitochondrial (cytochrome oxidase subunit I, COI) and one nuclear (histone subunit 3, H3), to infer phylogenetic relationships among representatives of all subgenera of *Strombus* and *Lambis*. These results suggest that 1) the currently defined genus *Strombus* is paraphyletic with respect to *Lambis*, whose morphology shows greater disparity, and 2) Neotropical strombids are monophyletic, and have filled most of the morphospace occupied by the far older (and paraphyletic) Indo-West Pacific strombids in a relatively short amount of time.

CHAPTER 3 MATERIALS AND METHODS

DNA Extraction, Amplification, and Sequencing

Genomic DNA was extracted using protocols that varied with the quality and age of samples (see Table 1 for complete collection information). For well preserved museum samples and for fresh tissue, I used a modified cetyltrimethylammonium bromide (CTAB) extraction, followed by phenol/chloroform extraction and alcohol precipitation protocol (Toonen 1997). When this approach failed (usually for older museum samples), a modified version (Chase et al., 1998) of the QIAmp DNA extraction kit (QIAGEN, Chatsworth, CA, USA) was used.

Table 1 A list of the individuals sequenced in the phylogenetic analyses. COI and H3 indicate how many base pairs were recovered by PCR for each individual respectively. NMNH - Smithsonian National Museum of Natural History, FLMNH = University of Florida Museum of Natural History, DE = Delaware Museum of Natural History, PA = The Academy of Natural Sciences in Philadelphia.

Genus	species	locality	collector
<i>Aporrhais</i>	<i>pespelicani</i>	Western Mediterranean	K. Roy
<i>Lambis</i>	<i>chiagra</i>	Cocos (Keeling), Australia	L. Kirkendale
<i>Lambis</i>	<i>truncata</i>	Diamond Is., Coral Sea	K. Roy
<i>Lambis</i>	<i>lambis</i>	Amani-U-Shima, Japan	K. Roy
<i>Strombus</i>	<i>alatus</i>	Cedar Key, Florida, USA	NMNH 286816
<i>Strombus</i>	<i>aurisdianae</i>	Panglao Is., Phillipines	F. Costura
<i>Strombus</i>	<i>bulla</i>	Ngeruktabel Is., Palau	NMNH 241835
<i>Strombus</i>	<i>canarium</i>	Phillipines, Cebu, Bantayan	ANS A6074
<i>Strombus</i>	<i>costatus</i>	Exuma Sound, Bahamas	M. Taylor
<i>Strombus</i>	<i>dentatus</i>	Orote Peninsula, Guam	NMNH 282386
<i>Strombus</i>	<i>epidromis</i>	Coral Sea	K. Roy
<i>Strombus</i>	<i>fragilis</i>	Guam	FLMNH 292199
<i>Strombus</i>	<i>fusiformis</i>	Zanzibar, Tanzania	FLMNH 286487
<i>Strombus</i>	<i>galeatus</i>	Pacific Panama	E. Garcia
<i>Strombus</i>	<i>gallus</i>	Little Stirrup Cay, Bahamas	M. Taylor
<i>Strombus</i>	<i>gibberulus</i>	Guam	K. Roy
<i>Strombus</i>	<i>gigas</i>	Berry Islands, Bahamas	NMNH
<i>Strombus</i>	<i>gracilior</i>	Pacific Panama	E. Garcia
<i>Strombus</i>	<i>granulatus</i>	Pacific Panama	E. Garcia
<i>Strombus</i>	<i>haemastoma</i>	Orote Peninsula, Guam	NMNH 282389
<i>Strombus</i>	<i>labiatus</i>	E. Diamond Is., Coral Sea	K. Roy

(table continued)

<i>Strombus</i>	<i>luhuanus</i>	Amani-U-Shima, Japan	Smith
<i>Strombus</i>	<i>lentiginosus</i>	Mulinu'u Peninsula, Samoa	E. Garcia
<i>Strombus</i>	<i>maculatus</i>	Rangiroa, Tuamotu	FLMNH 291895
<i>Strombus</i>	<i>microurceus</i>	Orote Peninsula, Guam	FLMNH 282387
<i>Strombus</i>	<i>mutabilis</i>	Amani-U-Shima, Japan	DE 053597
<i>Strombus</i>	<i>peruvianus</i>	Pacific Panama	P. Williams
<i>Strombus</i>	<i>pugilis</i>	Panama, Bocas Del Toro	P. Marko
<i>Strombus</i>	<i>raninus</i>	Exuma Sound, Bahamas	M. Taylor
<i>Strombus</i>	<i>sinuatus</i>	Selinog Is., Mindanao, Phillipines	F. Costura
<i>Strombus</i>	<i>taurus</i>	Hospital Pt., Guam	FLMNH 282384
<i>Strombus</i>	<i>thersites</i>	Ryukyu Is., Okinawa	DE A6075
<i>Strombus</i>	<i>urceus</i>	Cleaverville, W. Australia	E. Garcia
<i>Strombus</i>	<i>vittatus</i>	Broome, W. Australia	E. Garcia
<i>Strombus</i>	<i>vomer</i>	Cleaverville, W. Australia	E. Garcia
<i>Strombus</i>	<i>wilsoni</i>	Orote Peninsula, Guam	FLMNH 282390

1-2 μ L of DNA from each genomic extraction was used as the template for amplification using the polymerase chain reactions (PCR). All reactions were carried out at 50 μ L volumes in a PTC-100 or PTC-200 thermal cycler (MJ Research, Inc, Watertown, MA) under the following conditions: an initial hotstart to 94°C, a first cycle of 94°C for 3 min, 50°C for 2 min, 72°C for 2 min followed by 35 cycles of 94°C for 35 s, 50°C for 1 min., and 72°C for 1.25 min.

A 710 bp region of the mitochondrial cytochrome oxidase c subunit I (COI), was amplified using the primers of Folmer et al. (1994) (LCO1 = 5' – CGTCAACAAATCAT AAAGATATTGG-3', HCO1 = 5'-TAAACTTCAGGGTGACCAAAAAATCA-3'). In reactions where sequencing across the full length of the COI fragment proved problematic, two internal primers were designed from aligned sequence obtained using the above primers (LCO1731 = 5'-AGCT-CCTGATATRGCYTTYCC-3', HCO2004 = 5'-CTCAAACGTATDCCYCGYCAYC-3'). A 350 bp region of the nuclear Histone-3 gene (H3) was amplified using primers from Colgan et al. (1998) (H3A = 5'-ATGGCTCGTACCAAGCA-GACVGC-3', H3B = 5'-ATATCCTTRGGCATRATRGTGAC-3').

PCR reactions were visualized on 1.1% agarose gels. In those reactions producing a single band of the expected size, two or three amplicons per individual were pooled and cleaned using the Strataprep PCR Purification Kit (Stratagene, La Jolla, CA). Reactions producing multiple bands, or reactions that proved difficult to sequence directly, were gel excised, polished using Stratagene's PCR Polishing Kit, and then cloned using Invitrogen's Zero Blunt-II TOPO Cloning Kit (Invitrogen, Carlsbad, CA). Plasmids from colonies were isolated using Wizard Plus SV Minipreps (Promega, Madison, WI), digested with Eco-RI enzyme (New England Biolabs, Beverly, MA) and visualized on agarose gels. Plasmids containing inserts of the desired size were directly sequenced using M13f and M13r primers provided with the TOPO Cloning Kit. All products were fluorescently labeled with dye-terminators (ABI, Foster City, CA) and directly sequenced in both directions using the ABI 377 Automated DNA Sequencer at LSU's Museum of Natural Sciences.

Phylogenetic Analyses

640 bp of COI and 325 bp of H3 were aligned by eye. No insertions or deletions were present in either of the markers, and both fragments remained in open reading frames over their total respective lengths. *Aporrhais pespelecani* was used as the outgroup in our analysis because it proved closer to the ingroup taxa than other species (*Terebellum terebellum*, *Tibia fusus*) analyzed in preliminary sequencing work.

In order to determine the DNA substitution models that most closely fit our data, each of the character sets (COI, H3, and combined) were analyzed in Model Test (Posada and Crandall 1998) and Mr. Model Test (J. A. A. Nylander, www.ebc.uu.se/systzoo/staff/nylander) using hierarchical likelihood ratio tests (HLRTs, Huelsenbeck and Crandall 1997) for several different truncated variations of the data matrices. Both full data sets and alternatives shortened so that no

missing characters were present consistently yielded the same evolutionary models, so the full set (including missing characters for some taxa) was used in the analysis.

Phylogenetic trees were created in PAUP 4.0 (Swofford 2001) for H3 (TrN+I+G) and COI (TvM+I+G) separately, using a heuristic Maximum Likelihood (ML) search with five random additions of taxa under the TBR branch swapping option and bootstrapped using 100 replicates. An Incongruence Length Difference Test (implemented in PAUP under the title partition homogeneity test) was performed on the resulting COI and H3 trees, which were found to be congruent (p value = 0.96), justifying analysis of a combined data set (Cunningham 1997). A tree was created for the combined data set under the HLRT model (TvM+I+G) using a heuristic ML search with five random additions of taxa and the TBR branch swapping option. The results from this search were bootstrapped using 100 replicates. For an alternative measure of topological support, a Bayesian analysis was performed on the COI (GTR+I+G) and H3 (HKY+I+G) data sets, as well as the combined data set (GTR+I+G), using Mr. Bayes (Huelsenbeck & Ronquist, 2001). Each data set was run independently three times for 1.2 million generations per run. The .p output files for each run were then imported into EXCEL where scatter plots were graphed to assess parameter stationarity. The .t output files were imported into PAUP where Majority Rule Consensus Trees were created excluding the first 200,000 generations required for all parameters to reach stationarity (Leache and Reader 2002).

Reanalysis of Morphospace Data

The principal component analysis used in Roy et al. (2001) was based on Elliptical Fourier Analyses (EFA) of shell outline data. Briefly, they imported a number of digitized shell images for each species into Elliptical Fourier Analysis (EFA) software (Rohlf and Ferson 1992; Isaev 1995; Isaev and Denisova 1995), then visually fit harmonics to each shell outline. A

principal component analysis (PCA) of the standardized EFAs using a covariance matrix was then used to define the axes of a shape morphospace. The study focused on interspecific trends, therefore intraspecific variation in both size and shape were standardized so that species appear as points in morphospace without a measure of error. I plotted a subset (36 species) of PCA scores from the Roy et al. data (82 species), corresponding to the species analyzed genetically. To show that trends described here were not biased by plotting only those species present in my phylogenetic analysis, the entire set of scores was also plotted in each case.

In addition to replotting the morphospace of Roy et al. (2001), I also calculated morphospace volume. The geometric mean of the ranges along the first six principal component axes were computed to provide a reference of large scale trends in amounts of morphospace volume occupied by the geographic provinces. Morphospace volume in the Indo-Pacific assemblage without *Lambis* was also calculated to determine the degree to which *Lambis* affects the volume occupied by the Indo-Pacific group.

CHAPTER 4 RESULTS

H3

Of the 325 base pairs of H3 sequenced and aligned for all 36 species, 66 sites were variable and 47 sites were parsimony informative. Although H3 occurs in multiple copy histone clusters, these appear to undergo rapid concerted evolution (DeBry and Marzluff 1994; Thatcher and Gorovsky 1994; Rooney 2002); I detected no signs of heterozygosity in any of the chromatographs for H3 sequences. The deepest well-supported node in the H3 tree (fig. 1), with a Maximum Likelihood Bootstrap (MLB) value of 71 and Bayesian Posterior Probability (BPP) of 99, unites a clade including all Neotropical species and some Indo-West Pacific (IWP) species (including all *Lambis*). Two species, *S. lentiginosus* (subgenus *Lentigo*) and *S. thersites* (*Tricornis*), for which COI could not be recovered, also fall into this Neotropical/IWP clade. The subgenera *Canarium*, *Labiostrombus*, *Laevistrombus*, and *Doxander* fall into an unresolved paraphyletic IWP grade. *Lambis* is monophyletic, with the two IWP *Tricornis* species sampled (themselves monophyletic) possibly their sister group. The New World species are monophyletic (MLB 74, BPP 100), indicating that the subgenera *Tricornis* and *Lentigo* defined by Abbott (1961), both including Neotropical and IWP species, are polyphyletic.

COI

COI sequences were recovered from 34 of the 36 species from table 1. Of the total 640 aligned base pairs of COI, 260 sites were variable and 227 were parsimony informative. Both Bayesian and Maximum Likelihood trees created using the COI data recovered similar tree topologies (fig. 2). The rapid evolution of COI provides resolution at terminal nodes. *Strombus* (*sensu stricto*) (MLB 96, BPP 94) and *Euprotomus* (MLB 100, BPP 93) are well supported as monophyletic groups. *Tricornis* again appears polyphyletic, as different species fall into both

Fig 1. Maximum Likelihood tree constructed from 325 bp of nuclear histone H3. ML bootstrap support (100 replicates) is given above corresponding branches, with Bayesian posterior probabilities shown beneath.

Neotropical (MLB 98) and IWP (MLB 92) clades. The genus *Lambis* is again well supported as a monophyletic clade (MLB 94, BPP 87).

Combined H3 and COI

The congruency of the H3 and COI trees (p value = 0.96) allowed these markers to be combined into a single analysis (Cunningham 1997). The combined tree provides better resolution of overall topology (fig. 3). As in the H3 tree and, to a lesser extent the COI tree, a deep node (MLB 83, BPP 96) splits the tree into a large clade containing all Neotropical and some Indo-West Pacific subgenera, and an unresolved paraphyletic group with subgenera found only in the Indo-West Pacific. The monophyletic Old World subgenus *Euprotomus* (MLB 100, BPP 100) has strong Bayesian support (BPP 96) as the sister to a monophyletic (MLB 96, BPP 100) New World radiation, although ML bootstrap support for this node is weak. The monophyly of *Strombus* (*s.s.*) in the New World clade is strongly supported (MLB 99, BPP 100).

Lambis nests as a monophyletic group (MLB 98, BPP 100) inside *Strombus*, with IWP *Tricornis* as a possible sister clade. Abbott's (1960) subgenus *Tricornis* is polyphyletic, with Neotropical species more closely related to other Neotropical forms than their IWP congeners.

Reanalysis of Morphospace Data

The results of the reanalysis of strombid morphospace are shown in figure 4. Principal Component 1 (PC1) appears to measure outer apertural lip digitation, with digitate species possessing negative PC1 values. Principal Component 2 (PC2) correlates with shell fusiformity, with whorl diameter increasing with negative values.

Looking at the relationship of *Strombus* to *Lambis* in the morphospace analysis (figs. 4a, based on species used in this phylogenetic study, and fig. 4c, using all species as in Roy et al.

2001), two trends are readily apparent. *Lambis* explains most ($4a = 68.8\%$; $4c = 76\%$) of the disparity ($4a = -2.0318$ to $.03464$; $4b = -3.7$ to $.0701$) in relation to the first principal component (PC1 – digitation from apertural lip). *Strombus* accounts for the majority of the disparity (both $4a$ & $4c = -2.40911$ to $.9404$) in relation to the second principal component (PC2 – body whorl fusiformity), spanning the entire range of values for this component.

More subtle trends emerge when we examine the placement of Neotropical versus Indo-West Pacific *Strombus* species in this morphospace (figs. 4b & 4d). Although species from these two geographic regions share the same range of values along PC2, there is some separation along PC1, with IWP species accounting for a slightly larger percent (84% vs. 68% respectively) of the disparity along this axis. In general, IWP species tend to be more fusiform, with the majority of species showing positive PC2 values, although a number of IWP species show negative PC2 values as well. In contrast, Neotropical species tend to have a larger flare in the outer apertural lip (negative PC2 values), with only two species possessing positive PC2 values. *Euprotomus*, an IWP subgenera more closely related to Neotropical subgenera than to its IWP congeners (fig. 3), shows negative PC2 values, indicating the more flared body form typical of the Neotropical species. When morphospace positions of the proposed polyphyletic subgenera *Tricornis* and *Lentigo* (two of five extant species analyzed) are examined, we see IWP and Neotropical forms occupying the same general area of the morphospace for each.

Comparing volumes of morphospace (the mean of the variance along the first six principal component axes) occupied by Neotropical and IWP forms present in this study, it is clear that New World species occupy a much smaller (1.99) volume of morphospace than IWP *Strombus* species (3.09). Adding three *Lambis* species to the IWP group produces a rise in morphospace volume occupation (4.38) above that expected (3.31) if we extrapolate a linear

relationship from *Strombus* (slope = .073). Analysis of the full data set, which increased the number of both *Strombus* and *Lambis* included, further supported this trend.

Fig 2. Maximum Likelihood tree constructed from 640bp of mitochondrial COI. ML bootstrap support (100 replicates) is given above corresponding branches, with Bayesian posterior probabilities shown beneath.

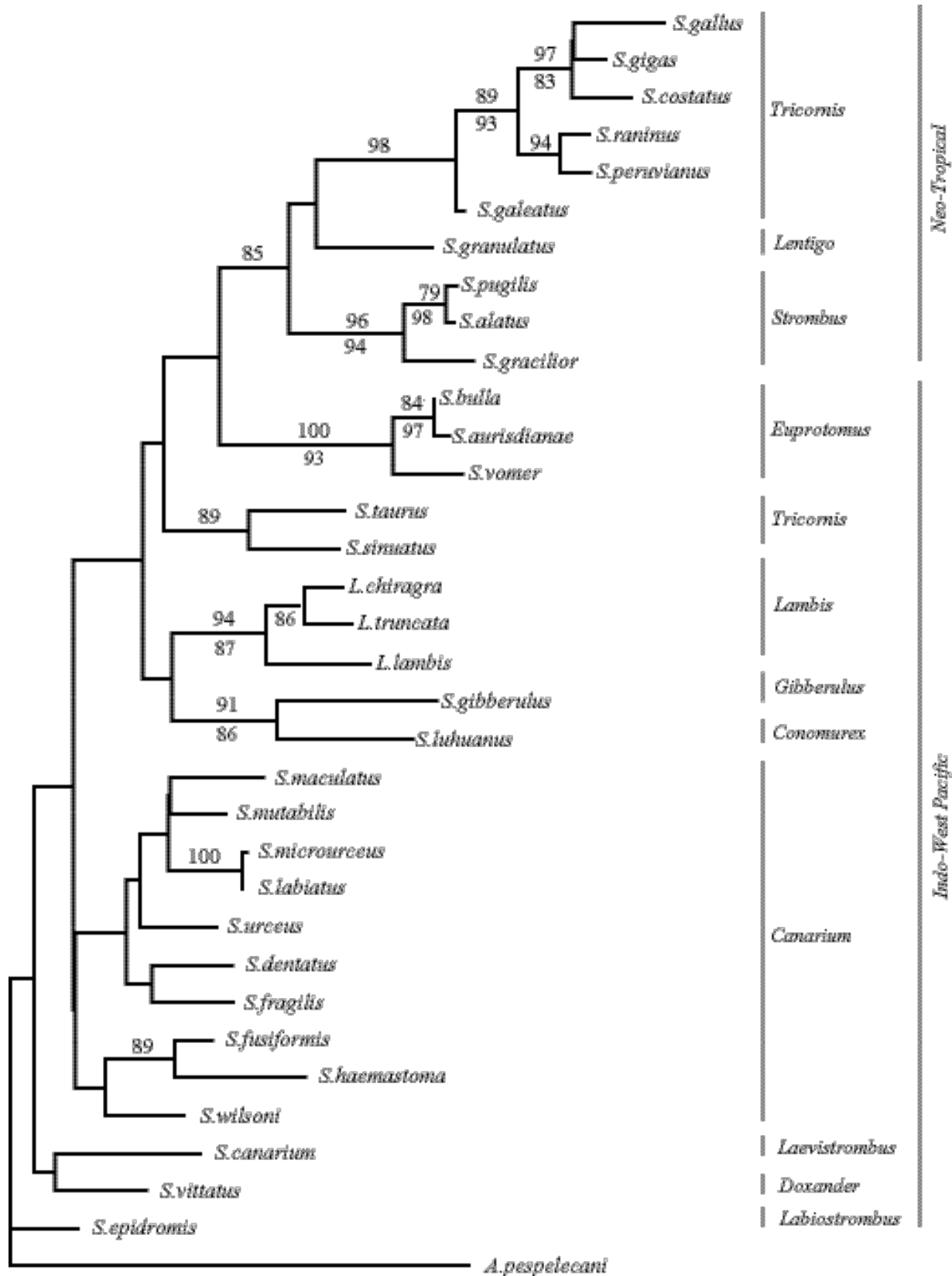


Fig 2.

Fig 3 Tree bisection and reconnection maximum likelihood bootstrap tree of 640bp COI and 325bp H3 with both Bootstrap support (100 replicates, above) and Bayesian Posterior Probabilities (below) represented on corresponding branches

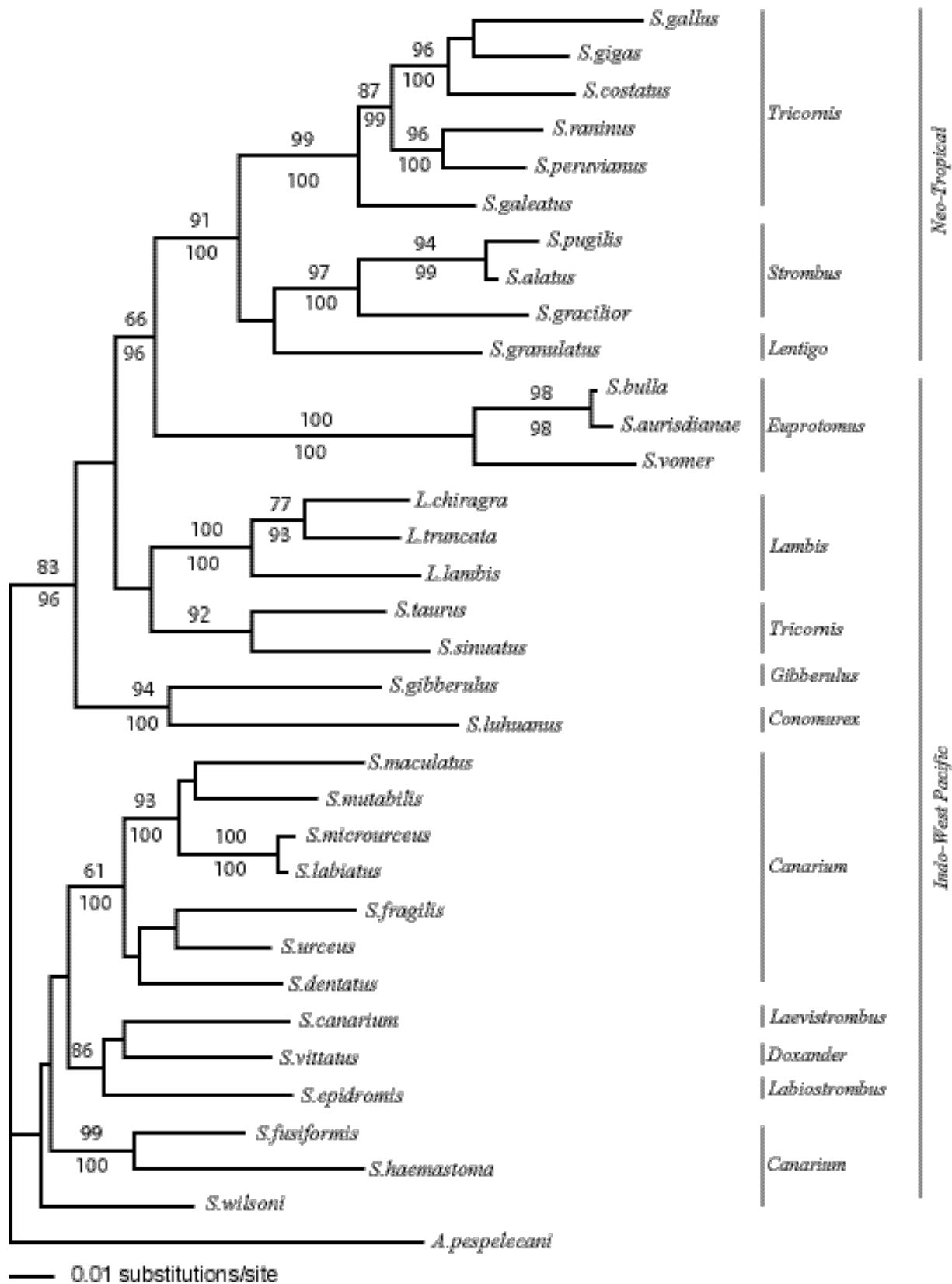


Fig 3.

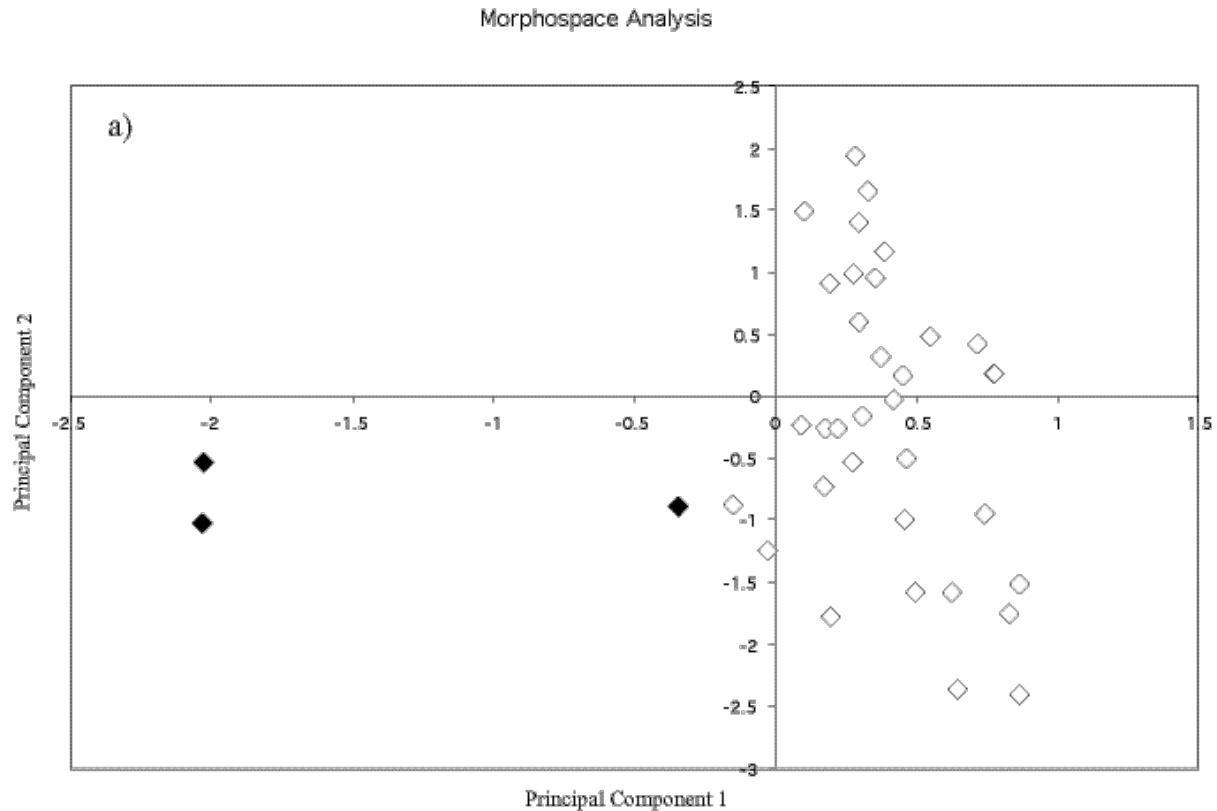


Fig 4a.

Fig 4. Principal Component Morphospace Analysis of the species represented in table 1. Principal Component 1 (PC1) is a measure of outer apertural lip digitation, with digitate individuals expressing negative PC1 numbers. Principal Component 2 (PC2) is a measure of shell fusiformity, with whorl diameter increasing with negative values. (a) Individuals of *Strombus s. l.* are represented with open diamonds and *Lambis* with filled. (b) Morphospace analysis of *Strombus s. l.* coded to demarcate Old World (filled in) from New World (un-filled), polyphyletic New World strombid subgenera (circles – *Tricornis*; square – *Lentigo*), and *Euprotomus* (triangles). (c) Analysis of all complete data set used in Roy et al. 2001, *Strombus s.l.* represented with open diamonds and *Lambis s.l.* with filled. (d) Analysis of all *Strombus s.l.* species present in the complete data set used in Roy et al. 2001, New World (unfilled circles) and Old World (filled) are both represented. Boxed numbers on (d) represent overlapping points on graph.

Morphospace Analysis

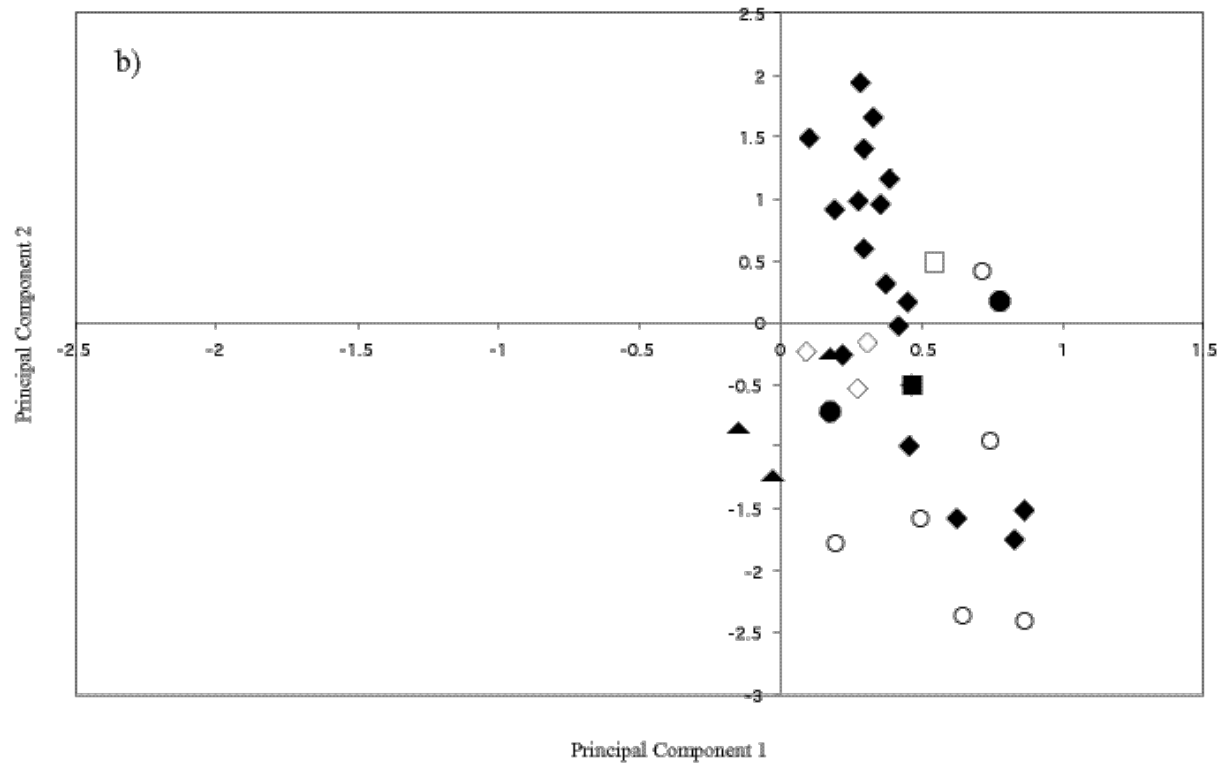


Fig 4b.

Morphospace Analysis

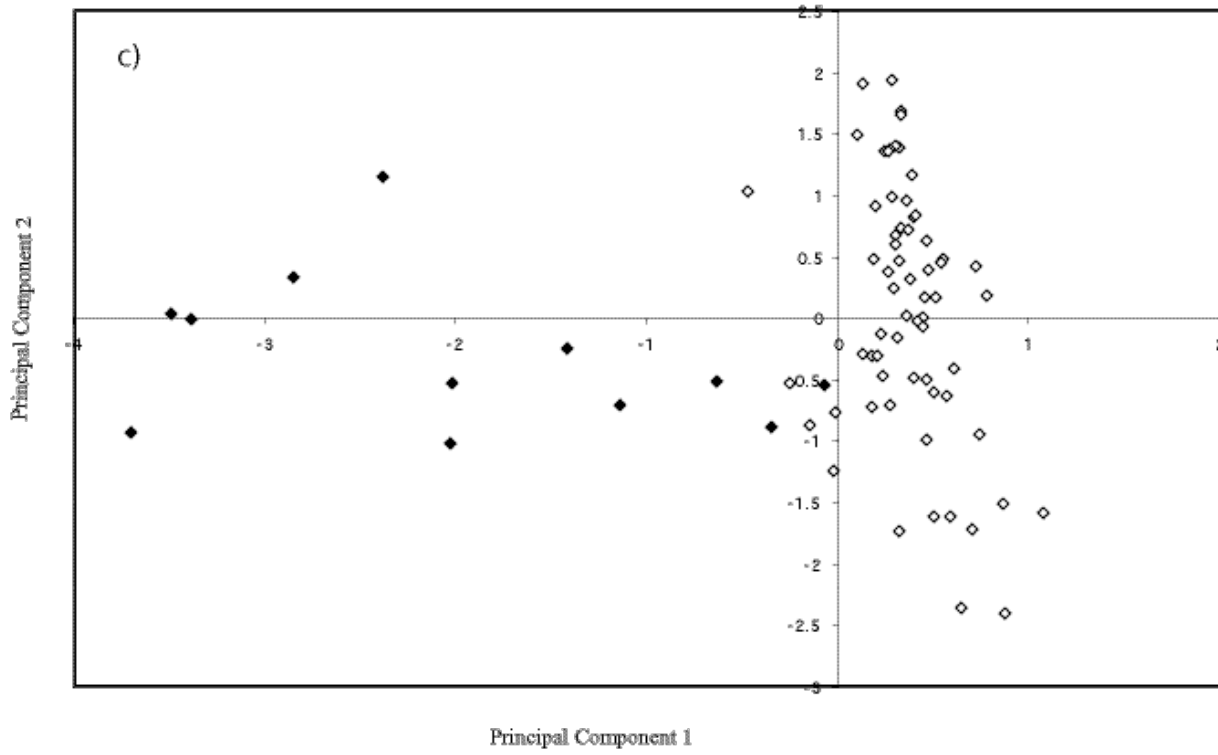


Fig 4c.

Morphospace Analysis

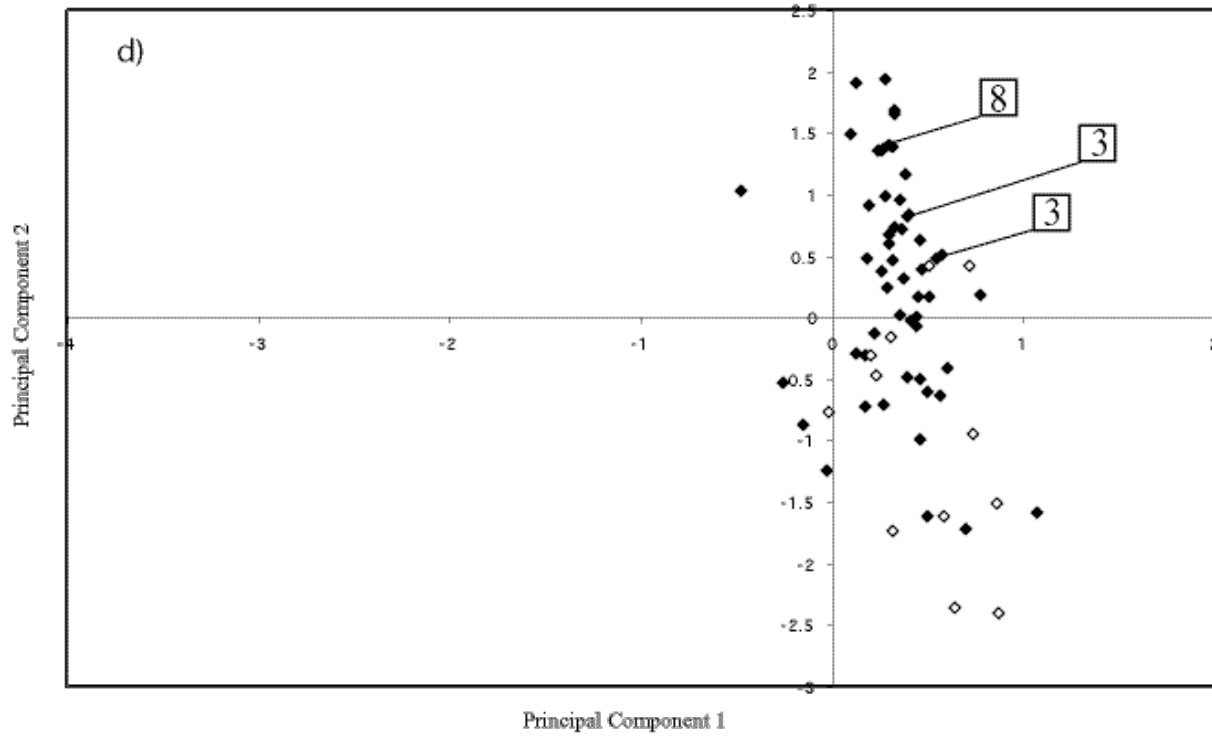


Fig 4d.

CHAPTER 5 DISCUSSION

Phylogenetic and Taxonomic Relationships within *Strombus s.l.*

Previous descriptive (Abbott 1960, 1961) and morphological character-based (Stone 2001) analyses conflict in their taxonomic designations of subgenera in the family Strombidae. My molecular data provide the first estimate of relationships within this group that is independent of morphological data. The slowly evolving nuclear gene (histone H3) recovers a tree with some resolution at deeper nodes and little resolution at the terminal branches. The more rapidly evolving mitochondrial COI showed complementary strengths, recovering a tree with little internal node resolution but better support toward branch tips. Substitution saturation at COI has been noted previously in mollusks (Marko 2002). While the H3 and COI trees both have problems resolving relationships within *Strombus* and *Lambis* by themselves, they are statistically congruent (p value = 0.96) and combine to provide a reasonable account of the phylogeny of these two genera.

My findings largely refute Stone's 2001 cladistic analysis proposing *Lambis* as polyphyletic and *Strombus* as paraphyletic. It should be noted that Stone's two most parsimonious trees (both constructed from 9 *Lambis* and 3 *Strombus*) were supported by bootstrap values well less than 50%. My phylogenetic analyses supports the inclusion of *Lambis* within *Strombus*; therefore, *Strombus* as defined by Abbott (1961) is paraphyletic. Generally, support for the monophyly of subgenera within *Strombus* as designated by Abbott (1961) is good or, at least, support for polyphyly of these taxa is lacking. However, two subgenera that include both New World and Indo-West Pacific species are notable exceptions. *Tricornis* and *Lentigo* (based on H3 tree) have Neotropical species more closely related to other Neotropical species than to their Indo-West Pacific congeners (fig. 3), suggesting both are polyphyletic.

Euprotomus, a subgenus found only in the Old World, exhibits the opposite of this trend, being more closely related to New World subgenera than to other IWP subgenera (fig. 3). When viewed in conjunction with morphospace occupation, we can begin to tease apart underlying patterns of morphological evolution.

Filling Space Quickly (Neotropical Variance)

The total volumes of morphospace filled by Neotropical and IWP species of *Strombus s. l.* (figs 4b & 4d), do not differ much between the two geographical regions, despite the greater genetic disparity of the paraphyletic IWP species assemblage and the youth of the New World radiation relative to the Old World radiation (relative ages based on ML tree branch lengths - Fig 3.). Thus, this crown clade of Neotropical strombids have filled out the flared portion of the shape morphospace relatively quickly.

Also of note is that *Euprotomus*, a monophyletic subgenus supported as the sister taxa to the Neotropical clade of strombids (fig. 3), exhibits negative PC2 values similar to those of the Neotropical species. Thus the Old World subgenus that is phylogenetically most closely related to the monophyletic Neotropical radiation is also more similar in form to its New World relatives than to its Old World neighbors. This gives two independent indications that *Euprotomus* gave rise to the Neotropical radiation.

Out of the Blue

evolutionary radiation is defined as cladogenesis accompanied by ecological and morphological differentiation (Harmon et al. 2003). My data suggest such a cladogenetic event (fig. 3) in *Lambis*' past. Adding *Lambis* to the morphospace creates a sizeable jump in morphological disparity. The strong support for *Lambis* as a monophyletic clade within *Strombus* (figs. 1-3) suggests that *Lambis* is a relatively young evolutionary radiation originating

within *Strombus* s. l. The fossil record also supports the relative youth of *Lambis* (Abbott 1961), with a first appearance in the late Miocene or early Pliocene. Figures 4a and 4b show that *Lambis* spans most of the morphospace defined by PC1 (68% of total variation), whereas *Strombus* has filled the entire range of values along the PC2 axis.

The only species other than members of *Lambis* to possess apertural lip digits are found in *S.taurus* (fig 5) and to a lesser extent, *S.sinuatus*, which form a sister clade to *Lambis*. The small amount of digitation on the outer lip, however, apparently causes *S.taurus* not to fall out within *Lambis* in the morphospace analysis. This may be due to *S.taurus* retaining the *Strombus* outline with only a small amount of apertural digitation.

Why Were *Tricornis* and *Lentigo* Misdesignated?

Examining the placement of the two polyphyletic subgenera in *Strombus*, *Tricornis* and *Lentigo*, in morphospace suggests why multiple independent analyses are often needed to decipher evolutionary histories. Neotropical members of *Tricornis* show the same morphometric pattern as other New World forms, having lower PC2 values as a whole than those exhibited by their IWP counterparts. *Lentigo*, however, exhibits the opposite pattern, with its Neotropical species possessing a positive PC2 value and fusiform body shape. It should be noted that support for the polyphyletic grouping of *Lentigo* is based solely on H3, and as such, should be looked upon with caution.

Wiens et al. (2003) suggested that phylogenies based on morphological analyses are often misled by convergence in morphocharacters with adaptational significance (Hennig 1966, Wiley 1981, Hedges and Sibley 1994, McCracken et al. 1999). If strombid shells have evolved to confer protection against predation (Vermeij 1989), then shell outlines, such as those studied here evolved, at least in part, due to adaptational changes. Convergence results when disparate

evolutionary lineages independently evolve the same character states. The filling out of *Strombus* along PC1, with the forms intermingling around the zero value seems to indicate a case where convergence might confound morphological studies. Both morphological and molecular data are prone to adaptive convergence, but molecular data normally include many more characters allowing for less weight to be placed on convergent state changes.

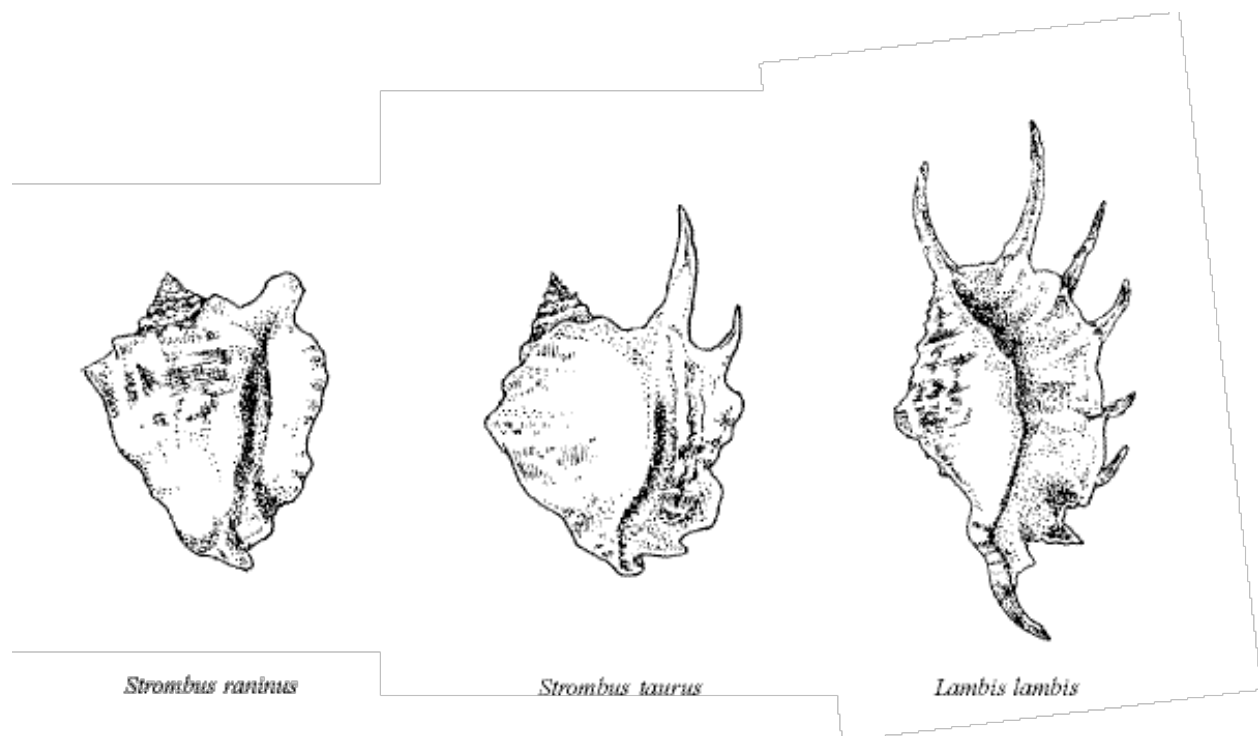


Fig 5.

Fig 5. Shell morphologies for three *Strombus* species. *S.raninus* is a New World species presently placed in the subgenus *Tricornis*. *S.taurus*, also in *Tricornis*, is found in the Marshall and Marianas Ids. *L.lambis* ranges from eastern Africa to the Indo-Pacific. Illustrations by Ben Anders

CHAPTER 6 CONCLUSION

Despite an inability to demonstrate the monophyly of all *Strombus* subgenera, phylogenetic relationships inferred here using molecular data generally support the taxonomic classifications proposed by Abbott (1960). The major conflict between his groupings and mine (for the subgenera *Tricornis* and *Lentigo*), apparently result from parallelisms between the Indo-West Pacific and Neotropical forms, and the designation of *Lambis* as a separate genus. It should be noted that without benefit of independently derived phylogenetic hypotheses, processes such as the convergent evolution and evolutionary radiation, seen within families such as Strombidae, can be difficult to recognize and account for.

The evolutionary context provided by my molecular phylogeny of the genera *Strombus* and *Lambis* can reveal how morphospace fills. Evolutionary radiations may in some cases fill the same total volume of morphospace as clades with a much longer history, as seen here for the Neotropical strombids. Interestingly, while the total volume of morphospace filled by the New World radiation was equivalent to that seen among Old World species, the placement of that volume differed, being shifted to more flared forms that may reflect its shared ancestry with *Euprotomus*. *Euprotomus* is a relatively flared Old World subgenus that is the sister to the New World radiation. *Lambis*, on the other hand, shows us that the origin of morphological innovation in a lineage allows the rapid accumulation of forms in previously uninhabited areas of morphospace.

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