11-1-2006

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Recommended Citation

Latiolais, J., Taylor, M., Roy, K., & Hellberg, M. (2006). A molecular phylogenetic analysis of strombid gastropod morphological diversity. *Molecular Phylogenetics and Evolution, 41* (2), 436-444. https://doi.org/10.1016/j.ympev.2006.05.027

Available online at www.sciencedirect.com
ScienceDirect
Molecular Phylogenetics and Evolution 41 (2006) 436–444
MOLECULAR PHYLOGENETICS AND EVOLUTION
www.elsevier.com/locate/ympev

A molecular phylogenetic analysis of strombid gastropod morphological diversity

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Received 9 February 2006; revised 19 May 2006; accepted 24 May 2006
Available online 3 June 2006

Abstract

The shells of strombid gastropods show a wide variety of forms, ranging from small and fusiform to large and elaborately ornamented with a strongly flared outer lip. Here, we present the first species-level molecular phylogeny for strombids and use the resulting phylogenetic framework to explore relationships between species richness and morphological diversity. We use portions of one nuclear (325 bp of histone H3) and one mitochondrial (640 bp of cytochrome oxidase I, COI) gene to infer relationships within the two most species-rich genera in the Strombidae: *Strombus* and *Lambis*. We include 32 species of *Strombus*, representing 10 of 11 extant subgenera, and 3 of the 9 species of *Lambis*, representing 2 of 3 extant subgenera. Maximum likelihood and Bayesian analyses of COI and of H3 and COI combined suggest *Lambis* is nested within a paraphyletic *Strombus*. Eastern Pacific and western Atlantic species of *Strombus* form a relatively recent monophyletic radiation within an older, paraphyletic Indo-West Pacific grade. Morphological diversity of subclades scales positively with species richness but does not show evidence of strong phylogenetic constraints.

Keywords: Morphospace; Shell shape; Cytochrome oxidase I; Histone H3; *Strombus*; *Lambis*

1. Introduction

Biological diversity can be measured in ways ranging from simple counts of species or higher taxa to quantitative measures of morphological, functional or phylogenetic diversity (Harper and Hawksworth, 1994; Purvis and Hector, 2000), but the relationships between various measures of biodiversity remain poorly known. Morphological diversity is one of the more intuitive measures of biodiversity (Williams et al., 1994), but richness of species or higher taxa can be a poor predictor of morphological diversity, both for living biota as well as for extinct taxa (Foote, 1995, 1997; Roy and Foote, 1997; Roy et al., 2001; McClain et al., 2004). To date, studies of morphological diversity patterns in a phylogenetic framework have focused on vertebrates (Hulsey and Wainwright, 2002; Harmon et al., 2003; Ricklefs, 2004, 2005), freshwater mollusks (Wilson et al., 2004) or fossil taxa (e.g., Wagner, 1995, 1996); such analyses are lacking for living marine invertebrates.

The morphological variation seen within the marine gastropod family Strombidae (conchs and their kin) make them fine candidates for examining how morphological diversity scales with species richness. All strombids exhibit determinate shell growth (Abbott, 1960; Vermeij and Signor, 1992), providing an unambiguous gauge of adult size and shape. The two most species-rich of traditionally defined strombid genera are *Lambis* Röding 1798 and *Strombus* Linné 1758 (Abbott, 1960, 1961). Species in both of these genera are herbivores associated with shallow-water reefs and grass beds. Both possess similar soft tissue

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doi:10.1016/j.ympev.2006.05.027

anatomies, egg masses, and radulae (Abbott, 1961), which led Kronenberg (1998) to suggest that *Lambis* and *Strombus* belong together in a group within the Strombidae, even though their shells show striking morphological differences (Fig. 1). In a previous study, Roy et al. (2001) examined the relationship between spatial patterns of morphological diversity and species richness in *Strombus* and *Lambis* but, because of the lack of a well-supported phylogeny, could not examine how species richness of individual clades of strombid gastropods related to their morphological diversity.

The last major taxonomic revisions of *Strombus* and *Lambis* were undertaken almost half a century ago (Abbott, 1960, 1961). Since then, some subspecies have been elevated to species status (e.g., Mienis, 1971; Kronenberg and Vermeij, 2002), subgenera have been raised to genera (e.g., Kronenberg, 1998), putative species have been revealed as hybrids (Kronenberg, 1993, 1999), and species have been carved from existing genera to create new monotypic genera (e.g., *Mirabilistrombus*, Kronenberg, 1998; *Tridentarius*, Kronenberg and Vermeij, 2002). Few studies, however, have addressed relationships among subgenera (sensu Abbott). Stone (2001) explored relationships among nine species of *Lambis* and three *Strombus* outgroups using morphological characters. One *Lambis* species (*L. crocata*) fell among the outgroups, suggesting that *Lambis* was paraphyletic and *Strombus* was polyphyletic, although bootstrap support values for all relationships were too low (<40%) for any robust conclusions. At a higher taxonomic level, Kronenberg and Vermeij (2002) recognized a shell character (glazing of the outer lip) shared by all Neotropical species as well as some Indo-Pacific (sub)genera, including *Euprotomus* and *Tridentatus*. Kronenberg and Vermeij (2002) also agreed with Stone (2001) that *Lambis* and *Strombus sensu lato* (*s.l.*) were polyphyletic or paraphyletic.

Here, we 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 molecular phylogenetic relationships among species and superspecific taxa within *Lambis* and *Strombus*. We then use this molecular phylogeny in conjunction with quantitative measures of shell shape to examine the relationship between taxonomic and morphological diversity within this group. We also assess whether the strombids of the New World are a monophyletic radiation, or a polyphyletic assemblage of species collected from a more ancient and species-rich Indo-West Pacific fauna.

2. Materials and methods

2.1. Selection of taxa

This study includes 31 species of *Strombus*, representing 10 of the 11 extant subgenera of Abbott (1960), and 3 of the 9 species of *Lambis* representing 2 of the 3 extant subgenera (Supplementary material Table 1). Our taxon sampling is nearly complete for the Eastern Pacific and Western Atlantic regions (lacking only the Brazilian *S. goliath* of 12 species in total), but less so for the Indo-Pacific. Like most other invertebrate groups, the total number of Indo-Pacific species belonging to Strombidae is currently unknown but our sample of 23 species represents about a third of the 72 named species and subspecies of Abbott (1960, 1961). However, because our study includes representatives from nearly all traditionally defined subgenera (sensu Abbott, 1960, 1961) of *Strombus* and *Lambis*, the resulting phylogeny should reflect the relationships among the main lineages within Strombidae.

2.2. DNA extraction, amplification, and sequencing

Genomic DNA was extracted using protocols that varied with the quality and age of samples (see Supplementary material Table 1 for collection information). For well preserved museum samples and for fresh tissue, we 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

Fig. 1. Shell morphologies for three strombid species. *Strombus raninus* is a Caribbean species placed by Abbott (1960) in the subgenus *Tricornis*. *Strombus taurus*, also in *Tricornis*, is found in the Marshall and Marianas Islands of the central Pacific. *Lambis lambis* ranges from eastern Africa to the central Pacific. Illustrations by Ben Anders.

extraction kit (QIAGEN, Chatsworth, CA, USA) was used. Most of our samples were collected fresh or came from recent (<5 years post-collection) museum specimens, but we had success with one sample (*S. canarium*, ANSP 104315) preserved in ethanol for over 90 years (collected in 1910).

We used 1–2 μL of DNA from each genomic extraction as template for amplification via the polymerase chain reaction (PCR). All reactions were carried out at 50 μL volumes in a PTC-100 or PTC-200 thermal cycler (MJ Research, Inc, Watertown, MA) under the following conditions: a hot start of 94 °C, a first cycle of 94 °C for 3 min, 50 °C for 2 min, and 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 min 15 s.

A 350 bp region of H3 was amplified and sequenced using primers from Colgan et al. (1998) (H3A = 5′-ATG GCTCGTACCAAGCAGACVGC-3′, H3B = 5′-ATATC CTTRGGCATRATRGTGAC-3′). A 710 bp region of COI was amplified using the primers of Folmer et al. (1994) (LCO1 = 5′-CGTCAACAAATCATAAAGATATTGG-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′-AGCTCCTGATATRGCY TTYCC-3′, HCO2004 = 5′-CTCAAACTGTATDCCYCG YCAYC-3′).

PCR reactions were visualized on 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 Strategene's PCR Polishing Kit, and then cloned using Invitrogen's (Carlsbad, CA) Zero Blunt-II TOPO Cloning Kit. Plasmids containing inserts of the desired size were directly sequenced using M13f and M13r primers provided with the TOPO Cloning Kit. All products were sequenced in both directions using fluorescently labeled dye-terminators (ABI, Foster City, CA) on an ABI 377 DNA Sequencer at LSU's Museum of Natural Science. All sequences are available from GenBank (H3: DQ525242–DQ525277, COI: DQ525207–DQ525241).

2.3. Phylogenetic analyses

Six-hundred and forty basepairs of COI and 325 bp of H3 were aligned by eye. No insertions or deletions were present in either marker, and both fragments remained in open reading frames over their total respective lengths. *Aporrhais pespelecani*, a member of the family Aporrhaidae, was used as the outgroup in our analysis because strombids and aporrhaids are generally thought to be closely related (Roy, 1996). In preliminary work we also examined sequences from other potential outgroups that have been placed in the Strombidae (*Terebellum terebellum*, *Tibia fusus*), but *Aporrhais* proved to be closer to the ingroup taxa than these other species.

To determine the DNA substitution models that most closely fit our data, each of the character sets (COI, H3, and combined) were analyzed in Modeltest (Posada and Crandall, 1998) and MrModeltest (Nylander, 2002) using hierarchical likelihood ratio tests (HLRTs, Huelsenbeck and Crandall, 1997) for several different variations of the data matrices. Both full data sets and truncated 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 4.0b10 (Swofford, 2001) for H3 (TrN + I + Γ) and COI (TvM + I + Γ) 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 as a partition homogeneity test) was performed on the resulting H3 and COI trees, which were found to be congruent ($p = 0.99$), justifying analysis of a combined data set (Cunningham, 1997). A phylogeny was derived from the combined data set under the HLRT model (TvM + I + Γ) 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 (using GTR + I + Γ) was performed on the H3, COI, and combined data set using MrBayes 3.04 (Huelsenbeck and Ronquist, 2001). Each data set was run independently five times for 1.2 million generations per run, with parameter values and trees saved once every 100 generations. The estimated parameters for each run were graphed to assess parameter stationarity (Leaché and Reeder, 2002) and to determine the appropriate number of generations to exclude for burn-in. The first 600,000 generations (COI) or 800,000 generation (H3 and combined) were excluded, as this many iterations were required for all parameters to reach stationarity (data not shown); transition rates required over 20 times as many generations to do so as did likelihood scores. The remaining trees from all five runs were imported into PAUP where majority rule consensus trees were created (spanning 3 million generations for COI, 2 million for H3 and combined).

2.4. Morphospace analyses

We used shell shape, a complex trait with considerable functional significance (Vermeij, 1978, 1987; Savazzi, 1991), to compute morphological diversity metrics. We used the same method and specimens as Roy et al. (2001) to quantify morphological diversity, except the present study uses only 32 species (*S. latus* and *S. wilsoni*, which were not included in the initial analysis, were excluded here) represented in the molecular phylogeny to define the strombid morphospace. Briefly, we used Elliptical Fourier (EFA) analyses and computed 10 harmonics for each specimen using digitized shell outline and available

software (Rohlf and Ferson, 1992; Isaev, 1995; Isaev and Denisova, 1995; see Roy et al., 2001 for details of methods). A principal components analysis (PCA) using the 10 harmonics was used to define the axes of a shape morphospace. As in Roy et al. (2001), we used two different measures of morphological diversity: (i) geometric mean of the ranges of scores on each of six PCA axes was used to measure the volume of morphospace occupation and (ii) the geometric mean of the variance of scores on each axis was used to measure the dispersion among taxa (Foote, 1997).

We tested whether the relationship between taxonomic and morphological diversity is constrained by the phylogenetic relationships by comparing the observed patterns against a null model. First we identified all subclades (defined as monophyletic groups supported in our combined phylogenetic analysis by Bayesian posterior probabilities of 0.85 or greater) and computed the morphological diversity of the subclade measured using the two metrics described above. We then compared the observed relationships between taxonomic richness and morphological diversity of subclades with the null expectations derived by computing the morphological diversities of randomly generated subclades (based on 1000 iterations) of the same richness levels.

3. Results

3.1. H3

Of the 325 base pairs of H3 sequenced and aligned for all 36 species, 64 sites were variable and 45 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 et al., 2002); we detected no signs of heterozygosity in any of the chromatographs for H3 sequences. The deepest well-supported node in the H3 tree (Supplementary material Fig. 1), with a maximum likelihood bootstrap (MLB) value of 79 and Bayesian posterior probability (BPP) of 99, unites a clade that includes all Eastern Pacific and Atlantic (EPA) species and some Indo-West Pacific (IWP) species (including all *Lambis*). *Canarium*, *Labiostrombus*, *Laevistrombus*, and *Doxander* fall into a poorly resolved paraphyletic IWP grade. The three *Lambis* species sampled occur in a monophyletic clade (MLB 52, BPP 97) as sister to two IWP *Tricornis* species (themselves monophyletic: MLB 80, BPP 100). The EPA strombids, which includes *S. latus*, the only species from the Eastern Atlantic, are monophyletic (MLB 71, BPP 99), with all EPA *Tricornis* falling into a single clade (MLB 61, BPP 97). Because the Indo-Pacific *Tricornis* are more closely related to *Lambis* than to EPA *Tricornis*, the subgenus *Tricornis* as defined by Abbott (1960) appears to be polyphyletic. The three *Lentigo* species do not appear monophyletic in the H3 tree either, although their polyphyly is not supported statistically. The Bayesian H3 tree (not shown) places *S. lentiginosis* as sister to a clade of EPA + (*Lambis* and IWP *Tricornis*), again without support. Otherwise, topologies of the ML and Bayesian H3 trees are identical except for a few basal taxa for which resolution in the Bayesian analysis was 0.50 posterior probability.

3.2. COI

Strombus (*Lentigo*) *lentiginosus* consistently failed to amplify for COI. For the 35 other species, 260 sites of the total 640 aligned base pairs of COI were variable and 229 bp were parsimony informative. The topology of the Bayesian COI tree was identical to that of the ML COI tree for all clades with >0.50 BPP support. Similar tree topologies were recovered by both ML (Supplementary material Fig. 2) and Bayesian methods (identical to the ML tree for all clades with >0.50 BPP support, not shown). The relatively rapid evolution of COI provides resolution at several terminal nodes. *Strombus* (*sensu stricto*) (MLB 98, BPP 100) and *Euprotomus* (MLB 99, BPP 100) are well supported as monophyletic subgenera. The ML tree again places EPA and IWP *Tricornis* in different clades, with the Neotropical *Tricornis* again supported as monophyletic (MLB 64, BPP 93). The genus *Lambis* is well supported as a monophyletic clade (MLB 91, BPP 100). The COI data statistically supported only one node that was in potential conflict with the H3 data: four *Canarium* species (*S. maculatus*, *S. mutabilis*, *S. microurceus*, and *S. labiatus*) supported as the sister clade to most other species sampled in the H3 tree (BPP 81) instead fall into a larger *Canarium* clade (MLB 72, BPP 100) in the COI tree.

3.3. Combined H3 and COI

The congruency of the H3 and COI trees ($p = 0.99$) allowed these markers to be combined into a single analysis (Cunningham, 1997). The combined tree provides better resolution of overall topology than either H3 or COI separately (Fig. 2). As in the H3 tree and, to a lesser extent the COI tree, a deep node (MLB 78, BPP 96) splits the tree into a large clade containing all EPA and some IWP subgenera, and an unresolved paraphyletic group with subgenera found only in the Indo-West Pacific. The monophyletic IWP subgenus *Euprotomus* (MLB and BPP 100) is supported (MLB 70, BPP 98) as the sister to a monophyletic (MLB 98, BPP 100) EPA radiation. *Lambis* nests as a monophyletic group (MLB 99, BPP 100) inside *Strombus* (*s.l.*), with IWP *Tricornis* supported as a sister clade by a BPP of 93. As in the H3 and COI trees, Abbott's (1960) subgenus *Tricornis* is polyphyletic in the combined tree. Neotropical *Tricornis* are more closely related to Neotropical species from other subgenera, than to IWP *Tricornis*, which are closely allied to *Lambis*. The representatives of *Lentigo* also fall into different places on both the combined and H3 trees, although the

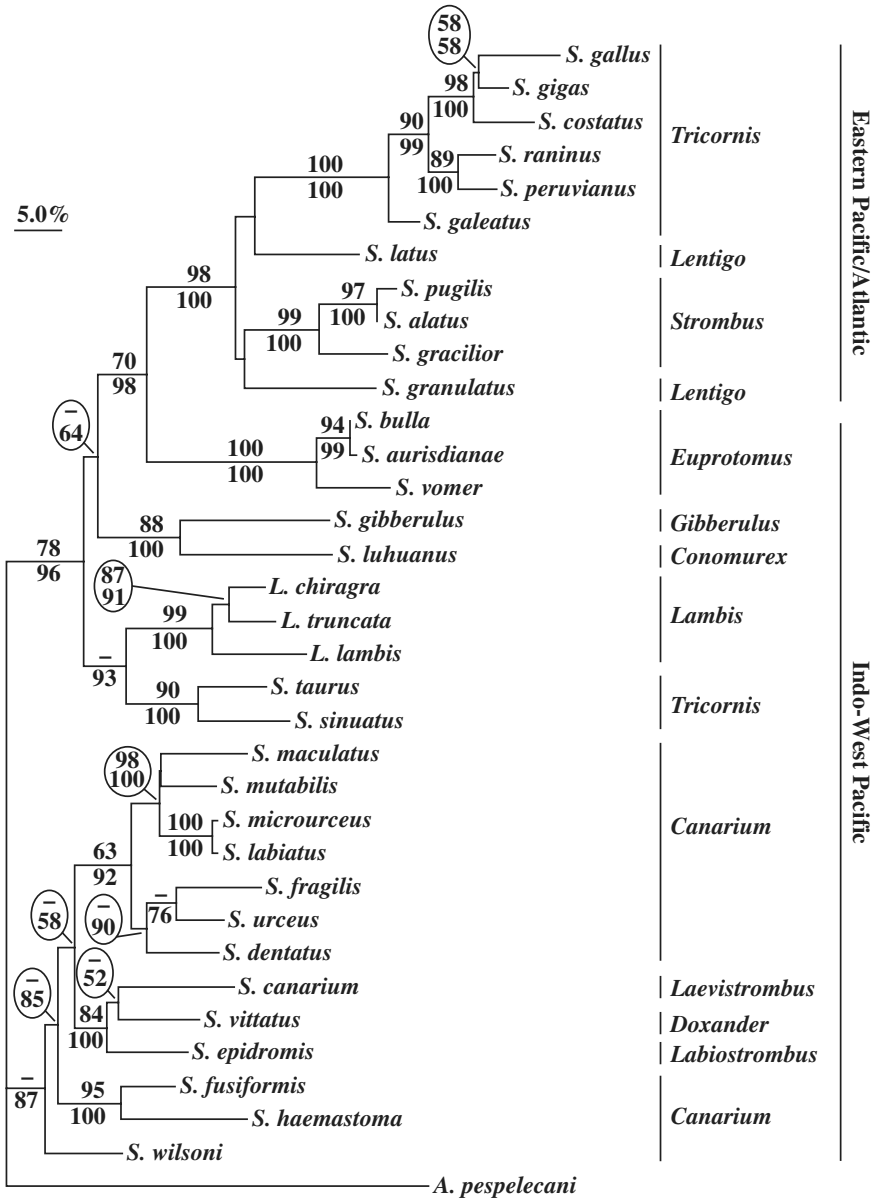

Fig. 2. Maximum likelihood (ML) tree constructed from a combined data set consisting of 325 bp H3 and 640 bp COI. ML bootstrap support (100 replicates) is given above corresponding branches, with Bayesian posterior probabilities (×100) shown beneath.

COI tree places them together in an unsupported clade. Thus *Lentigo*, as proposed by Abbott (1960), may also be polyphyletic although additional data are required to resolve the relationship. The Bayesian combined tree had a topology nearly identical to that of the ML combined tree, differing only in the placement of *S. latus* and *S. maculatus*, neither of which has support in either analysis.

3.4. Scaling of taxonomic and morphological diversity

The first six principal components explained 89.6% of the variance in shell shape and we used these axes to calculate the morphological diversity metrics. Species traditionally assigned to the *Strombus s.l.* have very different shell shapes compared to those assigned to *Lambis* (Fig. 1) and these differences are reflected in their distributions in the morphospace defined here (Fig. 3). Both metrics of morphological diversity show a general increase with taxonomic richness (Fig. 4). The relationship between geometric mean of the range of PCA scores and species richness of subclades is stronger and more linear (Spearman Rho 0.720, $p = 0.0007$) than that between the geometric mean of variance of PCA scores and richness (Spearman Rho 0.456, $p = 0.04$). Comparisons of the observed relationships between the taxonomic and morphological diversity with the null expectations show little evidence of phylogenetic constraints; neither measure of morphological diversity exhibits an overall trend significantly different from that expected from sample size alone, based on a random draw across the entire phylogeny (Fig. 4).

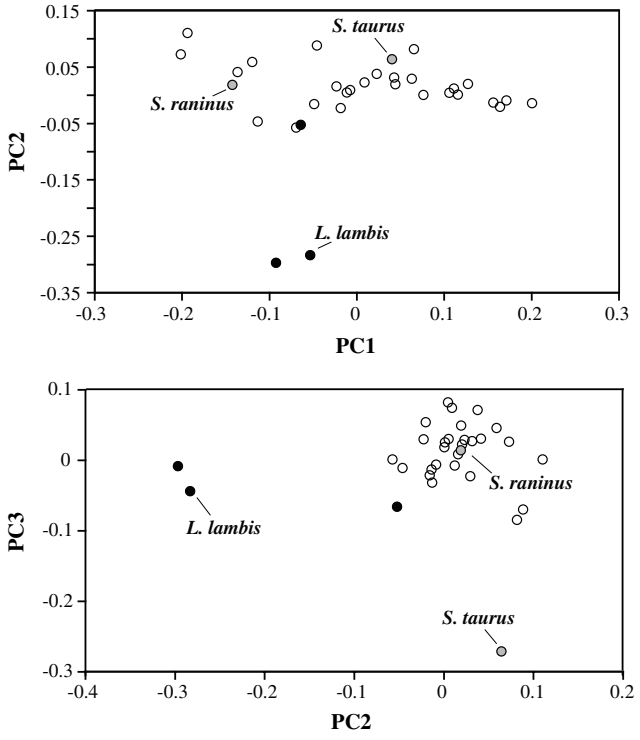

Fig. 3. Distribution of *Strombus* species (open circles) and *Lambis* species (closed circles) along the first three principal component axes. The three species illustrated in Fig. 1 are indicated; the circle for *S. raninus* in the lower panel is shaded. Note the large separation between *Strombus* and *Lambis* in morphospace.

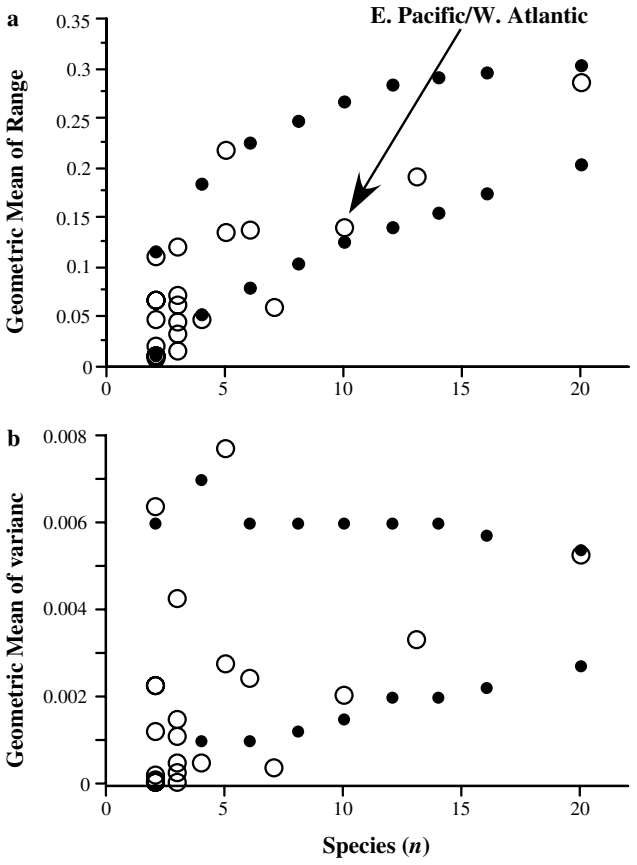

Fig. 4. The relationship between species richness and two different metrics of morphological diversity of subclades on the combined phylogenetic tree. (a) Geometric mean of the ranges of scores on the first six principal component axes and (b) geometric mean of the variance of scores on the first six PC axes. The arrow marks the subclade consisting of the Eastern Pacific and Western Atlantic species. The lines on each plot mark the 95% confidence interval for the expected relationship between the two variables when subclades are defined randomly (see text for details).

4. Discussion

4.1. Phylogenetic relationships within the genus Strombus

Our molecular data provide the first estimate of relationships within the diverse group including *Strombus s.l.* and *Lambis* that is independent of morphological data. The slowly evolving nuclear gene H3 resolved some deep nodes, while the more rapidly evolving mitochondrial COI showed complementary strengths, recovering a tree with better resolution toward the branch tips. Slowly evolving nuclear genes typically provide less information than more rapidly evolving mitochondrial genes, but also tend to show less homoplasy (Clabaut et al., 2005). Poor resolution of deeper nodes by COI was not unexpected; synonymous substitutions appear to be near saturation for the most divergent taxa in our COI data (not shown), and 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 and combine to provide a reasonable account of the phylogeny of these two genera, although the lack of resolution at some deeper nodes (e.g., the base of the IWP radiation) suggests a need for additional data from slowly evolving nuclear genes.

The phylogenetic analyses strongly supports the inclusion of *Lambis* within *Strombus* (*s.l.*); thus *Strombus* (*s.l.*) as defined by Abbott (1961) is paraphyletic. Stone (2001) had suggested previously that *Strombus* (*s.l.*) was polyphyletic, with *L.* (*Lambis*) *crocata* falling outside an otherwise monophyletic *Lambis* (*s.l.*) we were unable to obtain tissue for *L. crocata*. Otherwise, our *Lambis* relationships are consistent with the systematics of Stone (2001), with *L.* (*Lambis*) *truncata* falling closer to *L.* (*Harpago*) *chiragra* than to *L.* (*Lambis*) *lambis* (Fig. 4), contrary to the subgeneric classification proposed by Abbott (1961). The suggestion that the IWP *Tricornis* may be the sister to *Lambis* (supported by BPP of 0.93, Fig. 2) is consistent with the digitated flared lip seen in some IWP *Tricornis* (e.g., *S. taurus*, Fig. 1) and their position in morphospace (Fig. 3b).

Our analyses also provide mixed support for the uniting of many strombid subgenera, including all EPA species, based on the possession of a glazed edge of the shell's outer lip (Kronenberg and Vermeij, 2002). Indo-West Pacific (sub)genera possessing this character include *Euprotomus*, *Gibberulus*, *Tricornis*, and *Lambis* (*s.l.*), all of which fall in a clade that includes a monophyletic EPA radiation (Fig. 2). However, one taxon lacking outer lip glazing (*Conomurex*)

also falls into this clade with strong support, while others possessing a glazed outer lip (*Laevistrombus*, *Labiostrombus*) fall within the basal group of Indo-West Pacific taxa (Fig. 2). Although additional taxa and genetic markers would likely increase resolution within the basal IWP clade, our data suggest that lip glazing may be homoplasious among strombids.

Our taxon sampling is not sufficient to test for the monophyly of all the subgenera defined by Abbott (1960, 1961), but our results show that some of these are likely not monophyletic. For example, *Tricornis* includes Neotropical species that are apparently more closely related to Neotropical species in different subgenera than to their Indo-West Pacific consubgeners (Fig. 2). Kronenberg and Vermeij (2002) suggested that *Lentigo* as defined by Abbott (1960) was also polyphyletic based on morphological and paleontological criteria. We were unable to obtain COI sequence for Abbott's *Lentigo* type species (*S. lentiginosus*), but H3 sequences from that species suggest that it does not fall into the same clade as the EPA *Lentigo* species *S. latus* and *S. granulatus* (Appendix Fig. 1).

Relationships revealed by sequence data can be especially helpful when morphological characters are few and likely homoplasious, as in *Canarium*, which includes several species with small fusiform shells. Our data suggest that *Canarium* as defined by Abbott (1960) is not monophyletic, nor is *Fusicanarium* (*S. terebellatus*, *S. fragilis*, *S. dentatus*, and *S. fusiformis*) of Romanga Manoja (1980a,b), a taxon of debatable status (Kronenberg and Vermeij, 2002). Only one of the *Canarium* species (*S. fragilis*) moved to a new genus (*Terestrombus*) by Kronenberg and Vermeij (2002) was sampled here; it falls within the broader *Canarium*, and may not be closely related to either *Gibberulus* or *Conomurex* despite several similarities in shell shape noted by Kronenberg and Vermeij (2002) (Fig. 2). Few clades receive strong support however, despite sampling a reasonable 12 of Abbott's 17 nominal full extant species in *Laevistrombus*, *Doxander*, *Labiostrombus*, and *Canarium*. Future efforts to resolve species relationships among these species in these groups should explore additional sources of sequence data as well as more complete taxon sampling.

4.2. Biogeography

From a biogeographic perspective, our results strongly suggest a monophyletic Eastern Pacific/Atlantic clade derived from the IWP radiation, as suggested by Kronenberg and Vermeij (2002). *Euprotomus*, a subgenus restricted to the IWP, appears to be more closely related to the EPA clade than to other IWP subgenera (Fig. 2). This geographic pattern is different from those seen in some clades of other tropical marine gastropods. In *Conus*, IWP and EPA species often occur within the same clade, suggesting multiple radiations in both regions (Duda and Kohn, 2005). In *Echinolittorina*, IWP species belong to a single clade, with Eastern Pacific and Western Atlantic species being more basal (Williams and Reid, 2004). However, a pattern of Eastern Pacific or EPA monophyly nested within a larger IWP or circum-tropical species radiation has been noted previously in several reef fishes, including wrasses (Barber and Bellwood, 2005; Westneat and Alfaro, 2005), needlefish (Banford et al., 2004), and angelfish (Bellwood et al., 2004).

4.3. Scaling of morphological and taxonomic diversity

The relationship between taxonomic and morphological diversity seen here is qualitatively similar to that for all species of Indo-Pacific strombids analyzed by Roy et al. (2001), even though the previous study defined taxonomic diversity using species richness of geographic assemblages rather than richness of subclades used here. Geometric mean of the range of PC scores increases with species richness, suggesting that the volume of occupied morphospace increases as new species are added. This pattern is consistent with the idea that as richness increases, species tend to be added to the margins of morphospace (Ricklefs and Miles, 1994; Roy and Foote, 1997; Roy et al., 2001). In addition, the positive relationship between the geometric mean of variance of PC scores and species richness suggests that the internal packing of species in morphospace also changes as taxa are added. However, a positive relationship between species richness and the morphological diversity measures used here is also expected from sample size effects alone with subclades representing random groupings of species and we cannot reject such a sampling expectation (Fig. 4). Thus, although we think that the failure to reject the null hypothesis is largely a function of the small sample size of this study rather than a biological signal, these alternatives cannot be tested until a more complete phylogeny of strombid species is available. The results of the current study do suggest that overall phylogenetic constraint on the evolution of shell shape is at best fairly weak within the strombids, except perhaps within *Lambis*. This is further supported by the fact that the scaling of taxonomic and morphological diversity seen here is similar to that of Roy et al. (2001) who used geographic rather than monophyletic groupings.

4.4. Caveats

Inferences about diversification and patterns of morphological evolution using molecular phylogenies cannot incorporate information about past extinctions. Hence analyses such as those presented here assume that extinction is not biased in terms of morphology or clade membership. If, on the other hand, past extinctions are morphologically, phylogenetically or ecologically selective, then conclusions about rates and patterns of evolution (morphological or otherwise) based solely on molecular phylogenies may not be accurate. For strombid gastropods, there is some evidence that past extinctions were morphologically selective (Freiheit and Geary, 2001) but the effect of such selectivity on the results presented here is unknown at present.

Secondly, at this point we cannot rule out the possibility that the weak phylogenetic constraint on the evolution of shell shape seen here is, at least partly, a reflection of incomplete taxon sampling; a more complete sampling of species within individual lineages of strombids could potentially reinforce inter-clade differences and hence reveal stronger phylogenetic constraints on morphological evolution of this group. Quantitative tests of these possibilities would require integrating sequence information from more species of living strombids with the excellent fossil record of this group.

Acknowledgments

We thank the many people who helped us obtain tissue samples, including J. Slapcinsky at the Florida Museum of Natural History, M. Harasewych at the Smithsonian, G. Rosenberg at the Academy of Natural Sciences of Philadelphia, the Delaware Museum of Natural History, S. Merino at the Instituto Nacional de Desenvolvimento das Pescas in Cabo Verde, P. Marko, J. Schulz, and especially E. Garcia and K. Hutsell. P. Arbour-Reiley helped with laboratory work. M. Hafner and L. Anderson provided constructive comments on early drafts of the manuscript. This work was funded in part by an NSF grant (DEB-0075382) to MEH.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2006.05.027.

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