ORIGINAL ARTICLE

DID TECTONIC ACTIVITY STIMULATE OLIGO–MIOCENE SPECIATION IN THE INDO-WEST PACIFIC?

Suzanne T. Williams[1,2] and Thomas F. Duda Jr.[3,4,5]

[1]*Department of Zoology, The Natural History Museum, London SW7 5BD, United Kingdom*

  [2]*E-mail: s.williams@nhm.ac.uk*

[3]*University of Michigan Museum of Zoology and Department of Ecology and Evolutionary Biology,*
Ann Arbor MI 48109

  [4]*E-mail: tfduda@umich.edu*

[5]*Smithsonian Tropical Research Institute, Apartado 0843-03092, Balboa, Ancón, Republic of Panama*

Analyses of molecular phylogenies of three unrelated tropical marine gastropod genera, *Turbo, Echinolittorina*, and *Conus*, reveal an increase in the rate of cladogenesis of some Indo-West Pacific (IWP) clades beginning in the Late Oligocene or Early Miocene between 23.7 and 21.0 million years ago. In all three genera, clades with an increased rate of diversification reach a maximum of diversity, in terms of species richness, in the central IWP. Congruence in both the geographical location and the narrow interval of timing suggests a common cause. The collision of the Australia and New Guinea plate with the southeast extremity of the Eurasian plate approximately 25 Mya resulted in geological changes to the central IWP, including an increase in shallow-water areas and length of coastline, and the creation of a mosaic of distinct habitats. This was followed by a period of rapid diversification of zooxanthellate corals between 20 and 25 Mya. The findings reported here provide the first molecular evidence from multiple groups that part of the present-day diversity of shallow-water gastropods in the IWP arose from a rapid pulse of speciation when new habitats became available in the Late Oligocene to Early Miocene. After the new habitats were filled, the rate of speciation likely decreased and this combined with high levels of extinction (in some groups), resulted in a slow down in the rate of diversification in the genera examined.

KEY WORDS: Biogeography, cladogenesis, diversification, gastropods, marine tropics.

The modern Indo-West Pacific (IWP) is the largest marine biogeographic province and is renowned for its biodiversity. A diversity maximum, in terms of species richness, is observed for many shallow-water organisms in the central IWP region bounded by the Philippines, Indonesia, and Papua New Guinea, with diversity declining with increasing distance from the center (Briggs 1999; Mora et al. 2003). However, although this area is now recognized as a "hot spot" of biodiversity, the location of this diversity maximum is a geologically recent phenomenon. In the Middle Eocene (49–41 Mya), the center of maximum known diversity for many

marine taxa was the western European Tethys (Vermeij 2001). At that time, central IWP carbonate platforms were not as extensive and were dominated by large, benthic foraminiferans and calcareous algae (Wilson and Rosen 1998) and the central IWP fauna was not as rich with respect to corals and shallow-water invertebrates as that of the Mediterranean and Caribbean (Budd et al. 1992; Wilson and Rosen 1998; Vermeij 2001).

The IWP was effectively isolated from the Atlantic and Mediterranean by the closure of the Tethys Seaway when a land barrier first formed in the Late Burdigalian between Eurasia and

the Arabian plate (about 18–19 Mya, Vrielynck et al. 1997; Rögl 1998; Harzhauser et al. 2002, with intermittent marine connections until the Late Miocene, Robba 1987), although the faunistic differentiation evident in some fossil assemblages predates the closure by at least 5 Myr (Harzhauser et al. 2007). After the closure of the Tethys Seaway, fossil evidence suggests that the diversity maximum shifted to the western Indian Ocean (with faunal elements from both western Tethyan and IWP regions), before moving to the central IWP (Harzhauser et al. 2007). As the IWP has been effectively isolated from other tropical marine realms since the closure of the Tethys Seaway, what is the origin of its present-day diversity of shallow-water tropical fauna? Continental Southeast Asia has remained in, or close to, tropical latitudes at all times, so changes through geological time in species richness are not due to climatic effects (Wilson and Rosen 1998). Fossil evidence suggests that diversification of zooxanthellate corals about 20–25 Mya (Wilson and Rosen 1998; Crame and Rosen 2002) was driven by tectonic events in the Late Oligocene and Early Miocene (Wilson and Rosen 1998).

One of the most important tectonic events in the IWP during the Oligo–Miocene was the collision of the Australia and New Guinea plate with the southeast extremity of the Eurasian plate and the Philippines-Halmahera-New Guinea arc system approximately 25 Mya (Hall 1998). This was followed soon afterwards by a collision between the Ontong Java plateau and the Melanesian arc. These two events caused significant geological changes in the central IWP that created a discontinuous land connection via the island arcs of Halmahera and the Philippines into Sulawesi, and also changed ocean currents (Hall 1998). The uplift of north-central Borneo caused sediments to be released into deltas of northern and southeastern Borneo (Hall 1998; Moss and Wilson 1998). A broad zone of shallow water occurred between Australia and Sulawesi, probably with numerous islands (Hall 1998). Long, discontinuous island arcs also linked Asia and Melanesia (Hall 1998). These tectonic events decreased the isolation of the central IWP from more peripheral regions and increased both the amount of shallow-water area and the length of coastline (Hall 1998; Wilson and Rosen 1998). Overall, they augmented the geographical complexity of the region, which in turn provided opportunities for the isolation and origination of new species (Wilson and Rosen 1998).

A concurrent increase in zooxanthellate corals in the central IWP contributed to the radiation of reef-associated organisms (Kohn 1990; Wilson and Rosen 1998). Similar patterns have also been found in other areas of the globe. Fossil evidence shows that bivalve diversity increased in the southwest Caribbean following an increase in the area of coral-reef carbonate platforms (Johnson et al. 2007). Similarly, an association between species-rich radiations and coral-reef habitat has been identified for tetraodontiform fish (Alfaro et al. 2007).

Recent studies based on molecular phylogenies imply that modern diversity in the IWP is due both to long-term persistence of ancient lineages with origins predating the closure of the Tethys Seaway and to speciation within clades, often of morphologically similar species, that radiated in the Late Oligocene or Miocene (e.g., Bellwood et al. 2004; Barber and Bellwood 2005; Williams 2007). If there is a causal link between species-level radiations in the central IWP and the tectonic events that began about 25 Mya, we can make the following predictions. First, we expect to see an increase in the rate of diversification within IWP clades beginning about 20–25 Mya. Increases in diversification rates over this time period should be limited to IWP clades of shallow-water or reef-associated species; there is no reason to expect deep-water taxa or East Pacific or Atlantic clades to show the same general pattern of increased diversification at this time. Second, we expect that the groups that underwent a rapid increase in the rate of cladogenesis should exhibit diversity maxima in the central IWP if species originated in this region during the Miocene. This pattern might still be identified in modern taxa if extinction has been low and geographically random during the last 25 Myr.

In a study of the circumtropical gastropod genus *Turbo*, Williams (2007) provided some of the first molecular evidence for a significant increase in the rate of diversification of one IWP subgenus in the Late Oligocene. However, it was not shown whether this clade has a diversity maximum in the central IWP. One study of squat lobsters showed a significant increase in the rate of diversification in the genus *Munida* approximately 7–14 Mya based on "average" rates of molecular divergence observed in other organisms (Machordom and Macpherson 2004). Likewise, several reef fish show evidence of radiations in the Miocene, but molecular phylogenetic data were not examined statistically to identify significant changes in rates of diversification (e.g., Bellwood et al. 2004; Barber and Bellwood 2005; Read et al. 2006). In the study of tetraodontiform fish by Alfaro and colleagues (2007), the authors postulated that radiations in five families were directly linked to coral reef associations.

In this article, we used published molecular datasets of three circumtropical, shallow-water gastropod genera to produce phylogenies to test the timing and tempo of lineage accumulation (speciation minus extinction) and to determine whether there have been any shifts in rates of diversification in these groups. We determine the timing of these diversification shifts and compare them with known tectonic events to understand how biodiversity has changed in response to geological changes. We also examine the number of species occurring in biogeographic regions to determine whether clades with increased rates of diversification show a maximum of diversity in the central IWP. Although chronograms were produced for all three datasets in their respective studies, different methods were used in each case to transform branch lengths, so we reanalyzed the data using Bayesian inference incorporating an

uncorrelated relaxed, log-normal clock. This method allows for coestimation of both phylogeny and divergence times in a consistent manner for all three datasets.

Materials and Methods
SAMPLING

Data were taken from three published studies on tropical gastropod genera: *Turbo* (285, 165 and COI, 34 ESUs, Williams 2007), *Echinolittorina* (285, 125 and COI, 60 ESUs, Williams and Reid 2004, D. G. Reid, unpubl. data), and *Conus* (calmodulin intron and 165, 100 ESUs, Duda and Kohn 2005). Sequences from the genus *Turbo* were obtained for mitochondrial genes for several specimens (usually 2–5) for each nominal species (Williams 2007), although only one representative of each species (or ESU) was used in this study. Five of the most widely distributed *Turbo* species (*T. argyrostomus*, *T. chrysostomus*, *T. crassus*, *T. petholatus*, and *T. setosus*) are currently the focus of large-scale population genetic studies and preliminary data indicate these are likely to include cryptic species (C. P. Meyer and G. Paulay, unpubl. ms.). The exact number of species in this genus is unknown, but we estimate that there are approximately 75 species based on the number of nominal species and allowing for an additional 20% cryptic species (based on Alf and Kreipl 2003; Williams 2007, and S. T. Williams, unpubl. data). Of nominal species, only four East Pacific and three Atlantic species are missing. All but one of the IWP subgenera were represented, and only eight nominal species were missing from the largest subgenus, *Marmarostoma,* and seven from all other IWP subgenera combined.

Almost all known species of the genus *Echinolittorina* were included in phylogenetic analyses by Williams and Reid (2004), with a total of 57 species represented in the three-gene tree. Two Atlantic species were represented only in the 12S tree and a new Atlantic species has only recently been identified (D. G. Reid, unpubl. data) (total $n = 60$). New COI sequences for these three species have been provided by D. G. Reid and these were included in this analysis for completeness (GenBank accession numbers AM941712-14), along with the 12S sequences from Williams and Reid (2004) for two species. The *Echinolittorina* study by Williams and Reid (2004) included at least two specimens of each species in individual gene analyses, and often more, although only one exemplar was used in the combined-gene dataset. Further studies of population variation in the most widely distributed species were undertaken by Reid et al. (2006, see also Reid 2007). These studies showed that one species, *E. vidua*, had several genetically distinct ESUs (separated by K2P distances 2.2–5.9%, Reid 2007; 1.9–4.0 Myr, this study) that were not able to be defined morphologically and so were retained in a single species. We analyzed two datasets in this study using *E. vidua* as

a single species ($n = 60$) and as six distinct entities ($n = 65$), but present results for the more conservative study based on 60 species.

Previous phylogenetic analyses of 135 *Conus* species, using one exemplar per species, revealed two divergent clades that separated early during the evolutionary history of this group (Duda and Kohn 2005). One clade predominantly contains species from the eastern Pacific and western Atlantic (the "small major clade"), whereas a second contains species from a variety of geographic regions but is presumably IWP in origin (the "large major clade"). The genus includes approximately 500 species in total and sampling in Duda and Kohn (2005) may be biased toward widespread and common IWP species.

NEW PHYLOGENETIC ANALYSES

We used BEAST (ver. 1.4.6, Drummond and Rambaut 2007) to reanalyze the combined datasets from each study (*Turbo*: 28S rRNA, 16S and COI; *Echinolittorina*: 28S, COI and 12S; *Conus*: calmodulin intron and 16S) using Bayesian inference incorporating an uncorrelated relaxed, log-normal clock. This method allows for coestimation of both phylogeny and divergence times in a consistent manner for all three datasets. It also calculates 95% highest posterior density (HPD) interval for node heights (ages). The 95% HPD is the shortest interval that contains 95% of the sampled values.

Sequence variation was partitioned among genes and gene-specific model parameters were used (GTR+G+I for all genes partitions except *Conus* calmodulin, which used HKY+G+I), with each gene evolving at different rates. We used the Yule tree prior, which assumes a constant speciation rate among lineages, with a log-normal prior for birth rate. We did a preliminary analysis for each dataset and on the basis of the results, changed Jeffries priors to log-normal priors for very low GTR base substitutions ($\leq 10^{-3}$) and used log priors for meanRate, ucld.mean, and individual gene siteModel.mu parameters.

The starting trees for the BEAST analyses were neighbor-joining trees with branches transformed using nonparametric rate smoothing (NPRS) and node heights scaled so that all calibration points fell within the limits defined by the priors (NPRS transformed and scaled using TreeEdit, ver. 1.0a10, A. Rambaut and M. Charleston, http://evolve.zoo.ox.ac.uk). Analyses were run twice (each run 20,000,000 generations for *Turbo*, 40,000 for *Echinolittorina*, and 50,000,000 for *Conus*) and with a sample frequency of 1000. Final trees were produced by determining a consensus among the combined sets of accepted trees (for *Turbo*, each burnin: 2001, total accepted: 36,000; for *Echinolittorina*, each burnin: 4001, total accepted: 76,000; for *Conus,* each burnin: 5001, total accepted: 90,000) with the "maximum clade creditability" option and mean node height using TreeAnnotator (ver. 1.4.6, Drummond and Rambaut 2007; http://beast.bio.

ed.ac.uk); trees were drawn using FigTree (ver. 1.1, A. Rambaut, http://tree.bio.ed.ac.uk/software/figtree).

MOLECULAR CLOCK CALIBRATION

The chronogram for the genus *Turbo* was calibrated as in Williams (2007) using the oldest known fossil belonging to the IWP subgenus *Marmarostoma* (*T. subsetosus*, 24–25 Myr, Chattian, Late Oligocene, Aquitaine basin, south-western France; Cluzaud and Cahuzac 2006) to date the age of the crown-group (mean: 24.0 Myr, SD: 0.15, 95% interval: 18.8–30.7 Myr). We also used three additional calibrations. A record of *T. radiatus* var. *naricus* from India (Lower Nari of Bhagothoro Hill in Sind; Vredenberg 1928, p. 4 and 404) is thought to date from the Early Oligocene (Rupelian, 28.5–33.9 Myr, Welcomme et al. 2001). This range of dates was used to date the split between *T. radiatus* and *T. jonathani* (mean: 28.5 Myr, SD: 0.05, zero offset: 3 Myr, 95% interval: 29.2–34.0 Myr).

The oldest fossil *Turbo* with a calcareous operculum fixed in place is from the Cenomanian or Albian of Santorens, France, 93.5–112 Mya (McLean and Kiel 2007). Other *Turbo*-like shells also occur in the Cretaceous. One specimen from the mid-Cretaceous in the Natural History Museum, London, was collected from Le Mans, France (Cenomanian, 93.5–99 Mya, reg. no. BMNH 32360). A second specimen (Late Albian, approx 100 Myr, Haldon, Devon, BMNH 988684) was associated with a fossil coral bed (N. J. Morris, pers. comm). A Cretaceous origin for the genus is also supported by additional specimens in the Muséum national d'Histoire naturelle, Paris, and by literature records (e.g., *T. schweinfurthi* and *T. innesi*, Campanian, 71.3–83.5 Mya, Ouady Am Rokam, Egypt; Fourtau 1903). For these reasons, the crown age of the genus was calibrated as Cretaceous in age (mean: 93.5 Myr, SD: 0.25, 95% interval: 62.0–141.1 Myr). The crown of the outgroup subgenus *Lunella* (*Lunella*) was constrained to be at least 34.2 Myr (mean: 34.2, SD: 0.1, zero offset 5 Myr, 95% interval: 34–45 Myr) corresponding to the Late Eocene. This calibration was based on the age of the oldest fossil of the subgenus *Lunella* from the southern Ryukyu Islands, Japan, *L. miyarensis*, from the Eocene Miyara group (MacNeil 1964). This group has been accurately aged using foraminiferans and can be placed in the Priabonian stage (37–34.2 Myr, Saito et al. 1984). Outgroup species from *Lunella* (see Williams 2007) were trimmed from the tree prior to further analyses.

The *Echinolittorina* tree was calibrated by Williams and Reid (2004) using three different methods and we use the same calibration points. First, the rise of the Isthmus of Panama at approximately 3.1 Mya (Coates and Obando 1996) was used to constrain the age of the two youngest geminate pairs (based on age estimates in Williams and Reid 2004) to be older than 3.1 Myr (mean: 3.1 Myr, SD: 1.0, zero offset: 3 Myr, 95% interval: 3.6–19.1 Myr). The second calibration point also relies on a biogeographic event.

The IWP was effectively isolated from the Atlantic and Mediterranean by the closure of the Tethys Seaway about 18–19 Mya (Vrielynck et al. 1997; Rögl 1998; Harzhauser et al. 2002), although the faunistic differentiation evident in fossil assemblages predates the closure by at least 5 Myr (Harzhauser et al. 2007). Therefore we dated the split between the IWP clade (corresponding to the subgenus *Granulilittorina*) and the East Pacific and Atlantic taxa to be in the range 18–27 Myr (mean: 18 Myr, SD: 0.15, zero offset 4 Myr, 95% interval: 18.1–27 Myr). The third calibration point relied on the oldest known fossil *Echinolittorina* (41 Myr), which was used to date the crown group of *Echinolittorina* (mean: 41 Myr, st dev: 0.1, zero offset 6 Myr, 95% interval: 40.8–54.3 Myr). Outgroups *Littorina* and *Afrolittorina* (see Williams and Reid 2004) were trimmed from the tree prior to further analyses.

Duda and Kohn (2005) used the closure of the Isthmus of Panama at approximately 3.1 Mya (Coates and Obando 1996) and the fossil record of closely related *Conus* species to calibrate the *Conus* phylogeny. Fossils of *C. lividius* occur in the Vigo Shale, Philippines (Dickerson 1921), estimated to be from about 5.2–12 Myr (Odin et al. 1997). *Conus quercinus* is known from the Tjilanang Beds of Java (Van der Vlerk 1931) from about 11 Myr (Shuto 1975; Odin et al. 1997). We follow Duda and Kohn (2005) and apply a calibration for the divergence of *C. lividus* and *C. quercinus* of at least 11 Myr (mean: 11 Myr, SD: 0.1, zero offset: 2 Myr, 95% interval: 11.3–15.0 Myr). Based on results in Duda and Kohn (2005) the split between the youngest geminate pair in previous analyses (*C. gladiator* and *C. mus*) was calibrated to be older than 3.1 Myr (mean: 3.1 Myr, SD: 1.0, zero offset: 3 Myr, 95% interval: 3.6–19.1 Myr). We also used one additional calibration point, based on the earliest *Conus* fossils (*C. concinnus* and *C. rouaulti*) from Lower Eocene deposits of England and France, respectively (approximately 55 Mya; Kohn 1990), to date the crown of the *Conus* tree (mean: 55 Myr, SD: 0.1, zero offset: 5 Myr, 95% interval: 51.7–69.8 Myr).

LOCATING CHANGES IN DIVERSIFICATION RATE

Two types of tests can locate changes in diversification rates within a phylogeny. The first relies exclusively on branching order. The second, more powerful class of tests incorporates estimates of branch length (corresponding to time in a phylogeny with a molecular clock enforced) to infer the timing of speciation events and compares the observed distribution of speciation events through time with that expected under a null model of random diversification (Sanderson and Donoghue 1996; Moore et al. 2004). As we are interested in the timing of speciation events, we used the latter class of tests, specifically, the relative cladogenesis (RC) statistic implemented in END-EPI (ver. 1.0, Rambaut et al. 1997), to identify any significant increases in diversification rates in the phylogenies. The RC statistic uses a constant-rates birth–death model

and calculates the probability that a particular lineage existing at time *t* will have *k* extant tips. This probability is compared to the total number of extant tips and the statistic identifies branches with higher than expected rates of cladogenesis. We also used two likelihood ratio-based statistics (Δ_1 and Δ_2) and the Slowinski and Guyer statistic (SG, Chan and Moore 2005) as implemented in SymmeTREE (ver. 1.1, Chan and Moore 2005) to identify shifts in diversification rates. These tests are based on topology only and do not include branch length information.

LINEAGES THROUGH TIME

Plots of the log of the number of lineages against node height ("lineages through time"; LTT) were used to illustrate the rate of diversification (using Genie, ver. 3.0, Pybus and Rambaut 2002). As the trees used in this study were chronograms, the node heights correspond to time (in Myr). Under a constant birth–death model we expect a straight line with slope *b–d* (where *b* is speciation rate and *d* extinction rate), and, if recent extinction is negligible, an upturn in the number of lineages toward the present (0 Myr) with slope *b* (Harvey et al. 1994; Pybus and Harvey 2000). New Bayesian trees based on data from Williams (2007), Williams and Reid (2004), and Duda and Kohn (2005) were used to plot the log of the number of lineages against node height to illustrate diversification through time for *Turbo, Echinolittorina*, and *Conus*.

We used the Constant Rate (CR) test, with γ-statistic, of Pybus and Harvey (2000) to determine whether the LTT plots were consistent with a constant net rate of diversification through time. Significant gamma values indicate that rates are not constant. Negative values for gamma indicate that nodes are too close to the root (a slowdown in the rate of diversification through time) whereas positive values indicate that nodes are too close to the tips (an increase in the rate of diversification through time), compared with expectations of a constant diversification model (Pybus and Harvey 2000). For a phylogeny, like that of *Echinolittorina*, with complete sampling of taxa and a constant rate of diversification, the γ–statistic has a standard normal distribution (Pybus and Harvey 2000). For phylogenies with incomplete taxon sampling, significance values have to be drawn from simulations using a Monte Carlo constant-rate (MCCR) Test (Pybus and Harvey 2000).

The phylogenies of *Turbo* and *Conus* were based on incomplete taxon sampling, so we simulated the gamma results for a phylogeny of all known extant taxa in these groups (approximately 75 for *Turbo*; 500 for *Conus*), of which only a fraction were sampled (34 for *Turbo*, 100 for *Conus*), with 10,000 replicates using the programme MCCRtest (Pybus and Harvey 2000). LTT plots were also produced for clades with higher than average rates of diversification (see below). In these clades we simulated the gamma results using estimates for the number of species in

Turbo subgenus *Marmarostoma* (30) and *Conus* "Clade X" (184; see below for definition of Clade X).

Three alternative models of lineage accumulation were also used to test the distribution of speciation events over time using models described by Paradis (1997). Using the programme Diversi in APE (ver. 2.0–1, Paradis 1997) running in R (ver. 2.6.0), we tested three different models. Model A assumes a constant rate of diversification over time. Model B assumes a gradual change in diversification over time. Application of this model permits calculation of the parameter β. Values of β < 1 indicate that diversification is increasing, either as a result of increased rates of speciation or decreased rates of extinction, whereas values of β > 1 suggest that diversification is slowing down. Model C assumes that there are two distinct rates of diversification, each with its own rate of speciation before (δ_1) and after (δ_2) a defined point in time (*Tc*). Models A and B can be compared using the hierarchical likelihood-ratio test or Akaike's Information Criterion (AIC), but Model C is not a nested model, and is compared using AIC. For example, if Model B is significantly better at explaining the data than Model A, then to compare Models B and C, the model with the lowest AIC is preferred.

PREDICTING SPECIES RICHNESS

We used the method of Magallón and Sanderson (2001) to determine whether estimates of present-day species richness of *Marmarostoma*, *Granulilittorina*, and *Conus* Clade X were significantly different from diversity levels predicted based on a rate of diversification across the whole genus (*Turbo*, *Echinolittorina*, and *Conus*, respectively). Diversification (r_ε) was modeled as a stochastic time-homogeneous birth–death process with diversification rate (*r*) given relative extinction rate (ε) (Magallón and Sanderson 2001). The relative extinction rate (ε) is given by the rate of extinction divided by the rate of speciation and can range from zero to infinity (Magallón and Sanderson 2001). We used the Geiger package (ver. 1.0–91, Harmon and Brock) in R to determine whether clades with increased rates of diversification are excessively species rich given their age and the estimated diversification rate for the genus as a whole. We did this by estimating a 95% confidence interval for the expected number of species in a hypothetical clade that diversifies with a rate equal to that obtained for the genus as a whole, for an interval of time equivalent to the crown age of the clade. Then, we compared the diversity of the real clades with the 95% confidence interval for expected species diversity for a clade of similar age. Confidence intervals were calculated for the crown age of the clade of interest assuming both extremely high (ε = 0.9) and low (ε = 0) rates of relative extinction ($r_0 - r_{0.9}$). Numbers of extant species that fall outside these confidence limits suggest higher or lower diversity than that predicted by the diversification rate determined for the entire genus.

SPECIES DISTRIBUTION

For each genus we counted the occurrence of species in each of 11 defined regions of the IWP: Red Sea, western Indian Ocean, eastern Indian Ocean, northwestern Australia, central IWP, mainland Japan, western Pacific (including the coast of Vietnam and Japanese offshore islands), Marquesas, Easter and Pitcairn Islands and Hawaii (based on regions used in Williams and Reid (2004), with the addition of a new temperate zone around southern Australia). Exact boundaries of biogeographic regions in the IWP vary among publications by different authors, but our aim here is only to test for a general pattern.

Distribution data were obtained from the literature and museum records (*Turbo*, museum records and field guides including Alf and Kreipl 2003; *Echinolittorina*, Reid 2007; *Conus*, Röckel et al. 1995; Preece 1995 and A.J. Kohn's *Conus* Biodiversity Website, http://biology.burke.washington.edu/conus/). We plotted and compared distributions of species in clades that exhibit significant increases in rates of diversification and those that do not. We also counted the number of species endemic to each region, counting only those species whose entire distribution fell within one biogeographic region.

For *Turbo* we counted 45 IWP species including 40 nominal species plus five cryptic species that have been identified genetically but not yet named. Distributions of these cryptic species remain to be determined, and these species were counted only for those regions where they are currently known to occur. One species, *T. petholatus* is likely to include several cryptic species (C. P. Meyer and G. Paulay, unpubl. data; Williams 2007) but was counted as a single species in this study because of difficulties in defining distributions. Additional cryptic species within the IWP are likely and have been allowed for in the CR test, but were not considered when analyzing distributions. The distributions of all IWP *Echinolittorina* species (subgenus *Granulilittorina*) have been illustrated in detail by Reid (2007). All species were included in our distribution plots. For *Conus* we counted the occurrence of 335 IWP species and subspecies discussed by Röckel et al. (1995) and on the *Conus* Biodiversity Website (http://biology.burke.washington.edu/conus/). Approximately 146 species are known or predicted (on the basis of morphology or unpublished mitochondrial gene trees) to occur in *Conus* Clade X.

SOURCES OF ERROR

There are three main sources of error in this study: the use of an incorrect tree (both topology and branch length), inappropriate calibration, and incomplete taxon sampling. We have attempted to overcome the first problem by the use of Bayesian inference incorporating a relaxed normal clock. This method allows for co-estimation of both phylogeny and divergence times and is thought to result in better trees (Drummond et al. 2006). This is illustrated

by the fact that trees in this study differ only slightly from previously published trees, but nodes generally receive higher support. The use of a single phylogenetic method also provides a more standardized approach to compare results for the three genera. The second source of error, resulting from the use of an inappropriate calibration, was minimized by the use of multiple calibration points, each with a logarithmic prior, which allows for a range rather than a point calibration. As an indication of uncertainty, 95% highest posterior density intervals were also determined for node ages. The final source of error, incomplete taxon sampling, is inevitable in nearly all studies. It is rare that every species in a genus can be sampled for a phylogeny, and even when all nominal species are included, cryptic species may still be missed. Tests to identify shifts in diversification are sensitive to this source of error; however, we have complete sampling of all (known) species for *Echinolittorina* and almost half the species of *Turbo*.

Results

LOCATING CHANGES IN DIVERSIFICATION RATE

Three significant increases in diversification rate were identified within the phylogeny for *Turbo* using the RC statistic. All of these shifts occurred within an entirely IWP clade corresponding to the subgenus *Marmarostoma*. Only the first and the last of these are well-supported nodes and the first coincides with the appearance of *Marmarostoma* (23.7 Myr, posterior probability, $PP = 100\%$, RC test, $P_{RC} = 0.002$; 18.9 Myr, $PP = 55\%$, RC test, $P_{RC} = 0.016$; 14.7 Myr, $PP = 98\%$, RC test, $P_{RC} = 0.025$; Fig. 1). These same branches were shown to have a significant increase in diversification rate in Williams (2007), but an additional three shifts in diversification rate reported by Williams (2007) were not found in this study.

One branch at the base of the *Echinolittorina* phylogeny exhibits a significant increase in diversification rate (28.4 Myr, $PP = 88\%$, RC test, $P_{RC} = 0.011$, $\Delta_1, P = 0.006$, $\Delta_2, P = 0.010$, SG, $P = 0.013$). The branch leading to a single clade containing all IWP species corresponding to the subgenus *Granulilittorina*, shows at approximately 21.0 Myr a substantial, but nonsignificant, increase in the rate of diversity ($PP = 100\%$, RC test, $P_{RC} = 0.074$; Fig. 1). The same branch leading to *Granulilittorina* shows a significant increase in diversification rate when the analysis is performed on a phylogeny including the six *E. vidua* ESUs ($PP = 100\%$, RC test, $P_{RC} = 0.037$; data not shown). Using the tree in Williams and Reid (2004), two significant shifts in diversification rate were identified; the branch leading to *Granulilittorina* and the next internal branch (data not shown).

Eight branches that exhibited an increased rate of diversification were identified in the *Conus* phylogeny. All have HPD intervals that overlap with the 20–25 Myr time period (Fig. 1). These include the branch leading to the clade known as the large
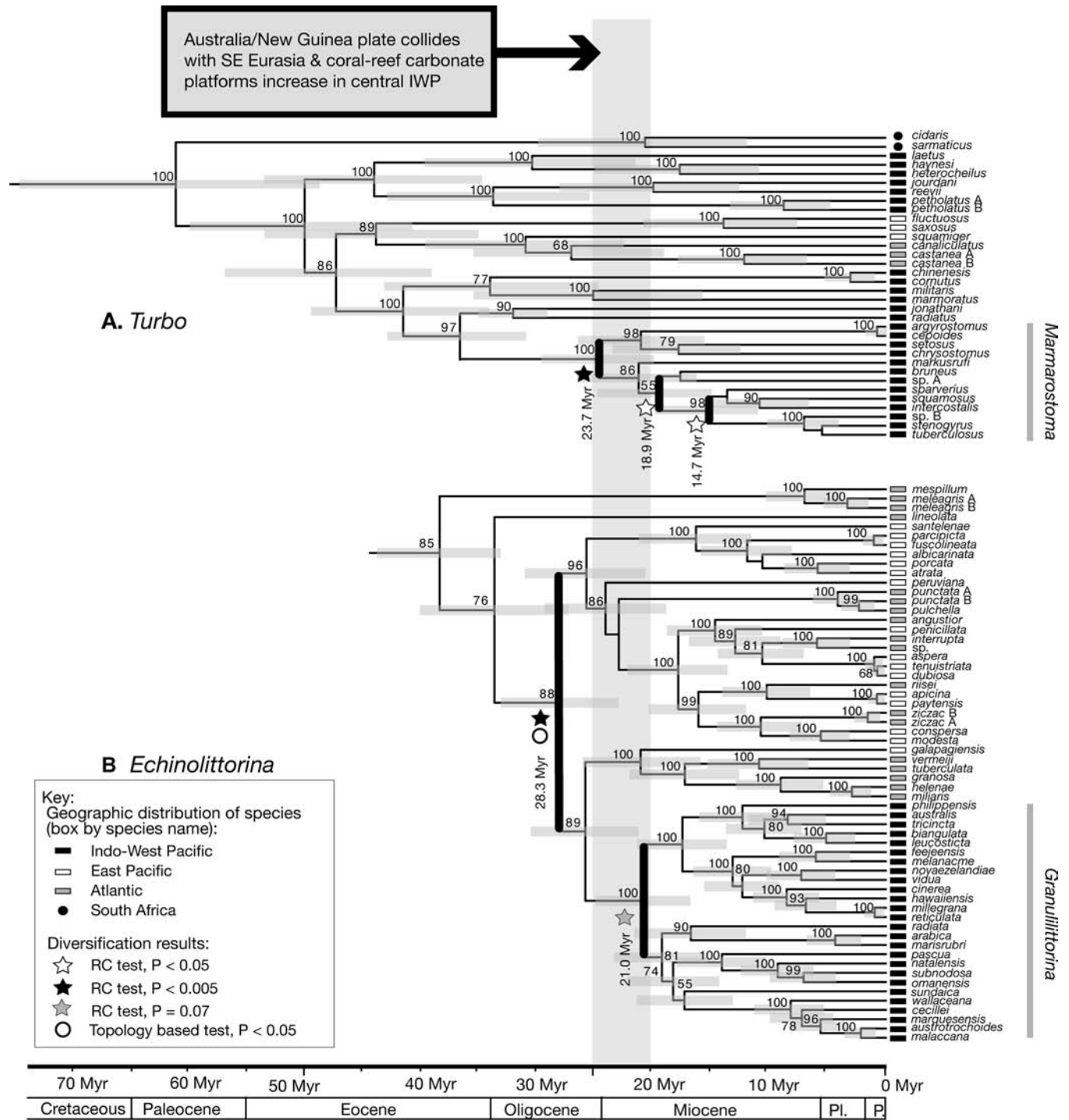
Figure 1. Chronograms, with branch lengths proportional to time (scale below in millions of years), for three gastropod genera. All trees are drawn to the same scale. Clades with significantly increased rates of diversification are indicated with thickened, black lines. Stars indicate levels of significance using the relative cladogenesis statistic (see Key in figure). Circles indicate significant increases in diversification using tests based on topology only (Δ_1, Δ_2, SG—see Results for details). Support values are posterior probabilities (*PP*, above branches); only values $\geq$ 50% are shown. Thick, horizontal, light-gray bars correspond to 95% highest posterior density (HPD) interval for node heights (ages). The 95% HPD is the shortest interval that contains 95% of the sampled values. Note that 95% HPD bars are only given where node has PP $\geq$ 50%. Geographic distribution of species is indicated by a box next to the species name (see Key for details). A. *Turbo.* B. *Echinolittorina.* C. *Conus.*
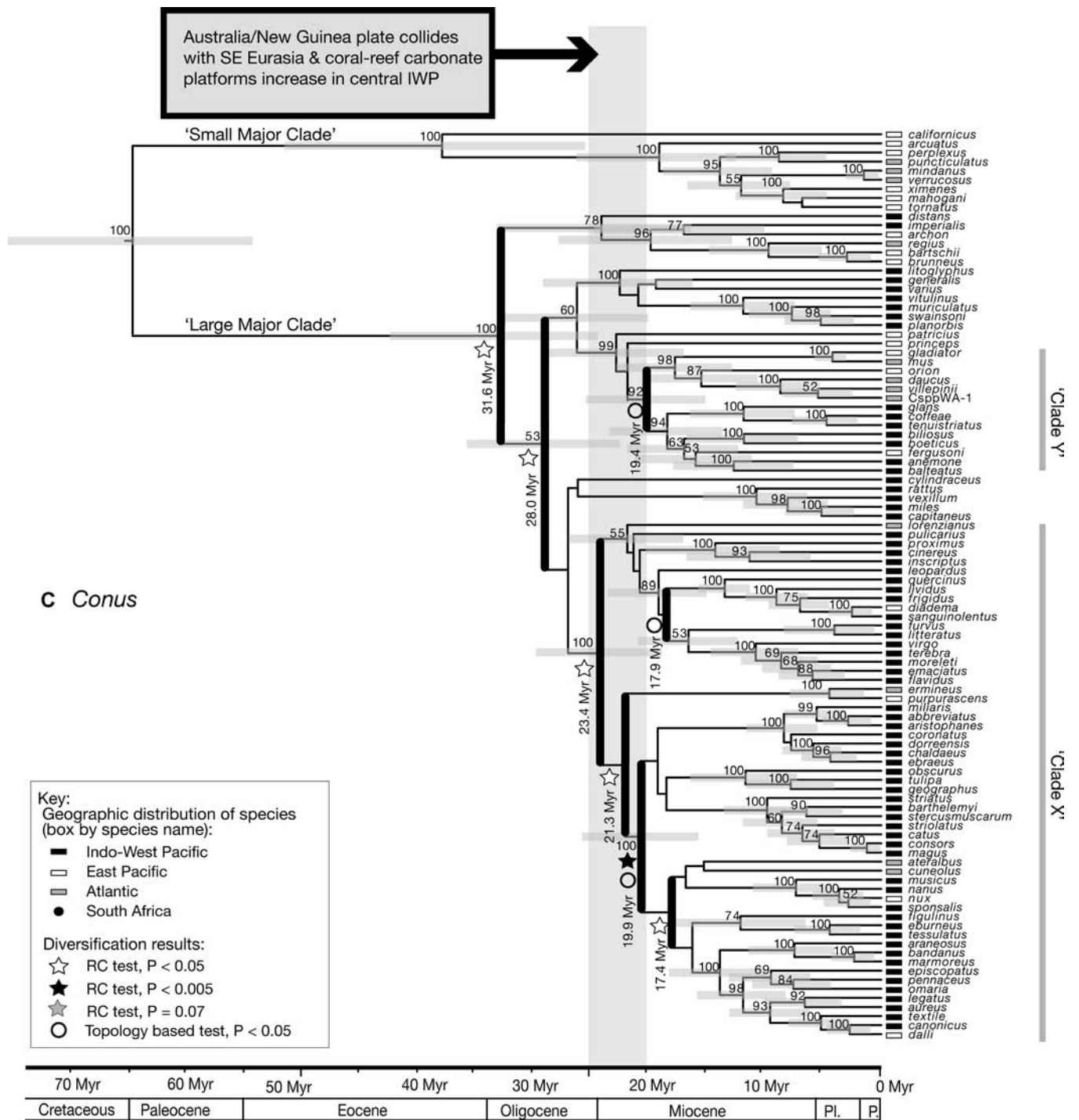
Figure 1. Continued.

major clade in Duda and Kohn (2005), which is thought to be IWP in origin (31.6 Myr, $PP = 100\%$, RC test, $P_{RC} = 0.02$), and the next (poorly supported) internal branch (28.0 Myr, $PP = 53\%$, RC test, $P_{RC} = 0.01$). Four of the six remaining diversification shifts occur 19–25 Mya. Five shifts occur with a large clade comprised almost entirely of IWP species, except for eight species, that diversified 23.4 Mya (Clade X: 23.4 Myr, $PP = 100\%$, RC test, $P_{RC} = $ 0.02; 21.3 Myr, $PP < 50\%$, RC test, $P_{RC} = 0.03$; 19.9 Myr, $PP = 100\%$, RC test, $P_{RC} = 0.003$, Δ_1, $P = 0.050$, Δ_2, $P = 0.056$, SG, $P = 0.014$; 17.9 Myr, $PP = 92\%$, Δ_1, $P = 0.045$, Δ_2, $P = 0.045$, SG, $P = 0.045$; 17.4 Myr, $PP < 50\%$, RC test, $P_{RC} = 0.03$). The last diversification shift was identified only using topology-based methods and occurred at the root of the branch leading to a clade with both IWP and East Pacific and Atlantic species ("Clade Y":

19.5 Myr, $PP = 92\%$, Δ_1, $P = 0.033$, Δ_2, $P = 0.033$, SG, $P = 0.033$). Trees presented in Duda and Kohn (2005) were based on single gene datasets and differ in the taxa included, so we were unable to make direct comparisons with trees in this study.

AGES OF CLADES

In this study we estimate the age of *Marmarostoma* to be 23.7 Myr (95% highest posterior density [HPD] interval for node heights/ages: 19.5–28.3 Myr), *Granulilittorina* is estimated to be 21.0 Myr (95% HPD: 17.2–25.0 Myr) and *Conus* Clade X is estimated to be 21.3 Myr (95% HPD: 18.4–28.5 Myr). An additional analysis for *Turbo*, using all calibration points except the fossil calibration for *Marmarostoma* also results in a similar age (23.8 Myr, 95% HPD: 18.2–29.4 Myr; tree not shown). Estimates from previous studies are similar to those found in this study, and fall within 95% HPD intervals for *Marmarostoma* and *Conus* (*Marmarostoma*, 25 Myr, Williams 2007; *Granulilittorina*, 25.6 Myr, Reid and Williams 2004; *Conus* Clade X, 28.5 Myr, Duda and Kohn 2005).

LINEAGES THROUGH TIME

The LTT plot for the phylogeny of *Turbo* did not differ significantly from a constant net rate of diversification (speciation–extinction) over time (CR test: species sampled, $x = 34$; estimated total number of species, $y \approx 75$; experimental $\gamma = -2.74$; critical value of γ at $P = 0.05$, one-tailed test, $\gamma_{0.05} = -2.75$; Fig. 2). The survivorship analysis, however, suggested that Model B, with a gradual slowdown in the rate of lineage accumulation ($\beta = 3.25$) fitted the data better than a constant rate model (Model A vs. Model B, χ^2 test, $P < 0.001$) or Model C, which assumes two distinct rates of diversification. Like *Turbo*, an analysis of the LTT plot for *Marmarostoma* also showed no significant deviation from a constant rate of diversification (CR test: $x = 13$, $y \approx 30$, $\gamma = -2.17$; $\gamma_{0.05} = -2.22$; Fig. 2). The survivorship analysis, however, suggested that Model B, with a gradual slowdown in the rate of lineage accumulation ($\beta = 1.74$) fitted the data better than a constant rate model (χ^2 test, $P = 0.03$) and had lower AIC values than Model C.

The LTT plots for both *Echinolittorina* and *Granulilittorina* in Williams and Reid (2004) were convex and had significant negative values of the γ-statistic of the CR test suggesting that there has been a slowdown in the rate of diversification. The LTT plot for *Echinolittorina* based on the Bayesian tree used in this study is almost straight but lacks an upturn toward the present. It also had a significant negative value of the γ-statistic ($\gamma = -2.26$, $P < 0.05$, one-tailed test, standard normal distribution; Fig. 2). The survivorship analysis also suggested that the data best-fit Model B, with $\beta > 1$ ($\beta = 4.24$, $P < 0.001$); this is consistent with a
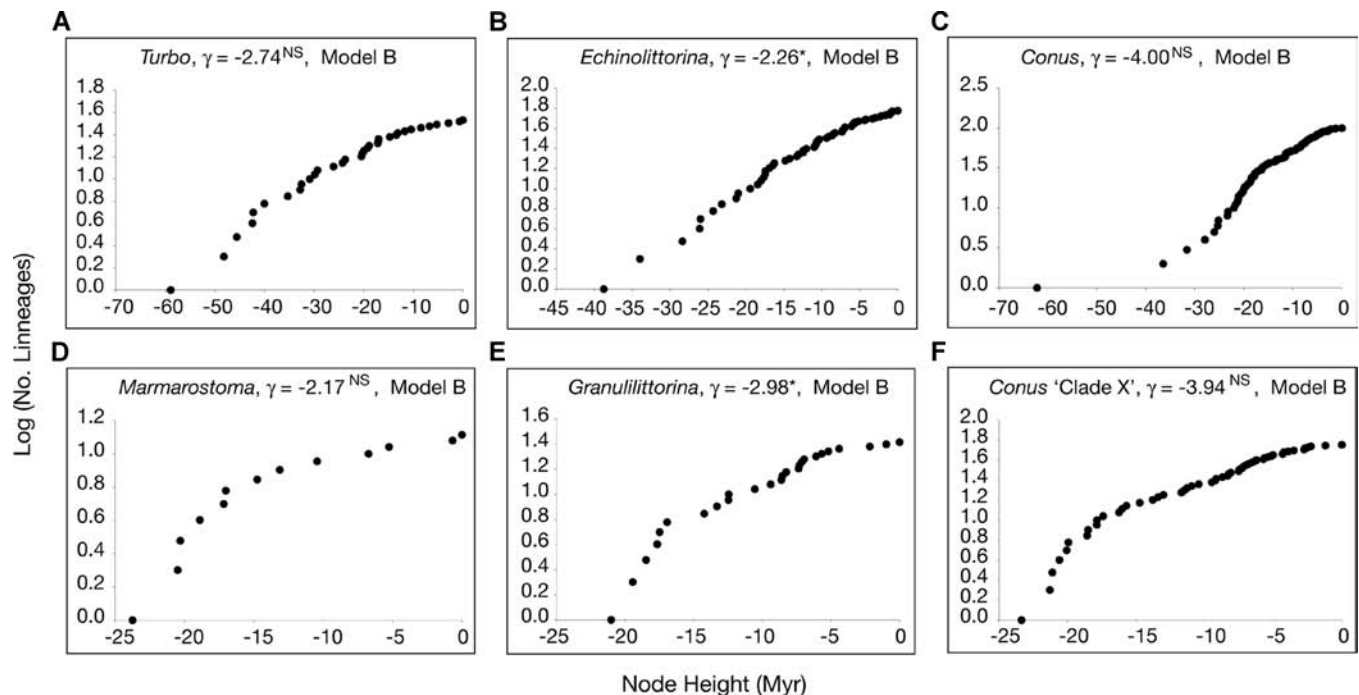

Figure 2. LTT plots for three gastropod genera (A–C), and for three predominantly IWP clades (D–F) that show an increased rate of diversification at their origin in the Late Oligocene or Early Miocene. As the trees used in this study were chronograms, the node heights on the x-axis correspond to time (in Myr). A. *Turbo*. B. *Echinolittorina*. C. *Conus*. D. *Turbo*, subgenus *Marmarostoma* E. *Echinolittorina* subgenus *Granulilittorina*. F. *Conus* 'Clade X'. In these examples Model B assumes a gradual decrease in diversification over time. * indicates significant value for γ of the CR test at $P < 0.05$ and NS indicates a nonsignificant value.

gradually decreasing rate of lineage accumulation. An LTT plot for the *Granulilittorina* alone showed, as for the whole genus, a significant decrease in diversification over time ($\gamma = -2.98$, $P < 0.05$; Fig. 2). Similarly, the survivorship analysis suggested that the IWP data best-fit Model B, with $\beta > 1$ ($\beta = 2.20$, $P < 0.001$), consistent with a gradually decreasing rate of lineage accumulation.

The initial part of the LTT plot for *Conus* was concave, suggesting that diversification rate initially increased over time, but then approached a constant rate. The CR test suggested that the diversification rate did not differ significantly from a constant rate (CR test: $x = 100$; $y \approx 500$; $\gamma = -4.00$; $\gamma_{0.05} = -5.76$, one-tailed test; Fig. 2). The survivorship analysis suggested that the data best fit Model B, with $\beta > 1$ ($\beta = 8.56$, $P < 0.001$). This model was also preferred to one with two different rates of diversification. Similar results were also obtained for the large major clade (CR test: $x = 91$; $y \approx 450$; $\gamma = -5.07$; $\gamma_{0.05} = -5.79$; Model B; plot not shown). Although the LTT plot for *Conus* Clade X (the predominantly IWP clade with five significant shifts in rates of diversification) was convex, the CR test showed no significant deviation from a constant rate of diversification (CR test: $x = 57$; $y \approx 273$; $\gamma = -3.94$; $\gamma_{0.05} = -4.56$; Fig. 2). However, the survivorship analysis suggested that Model B is the best fit to the data with $\beta > 1$ ($\beta = 2.55$, $P < 0.001$); this is consistent with a gradually decreasing rate of lineage accumulation.

PREDICTING SPECIES RICHNESS

The rate of diversification determined for the entire genus *Turbo* ranged from a maximum 0.062 net speciation events per million years in the absence of extinction ($r_0 = 0.062$) to a minimum of 0.035 net speciation events per million years under a high relative extinction rate ($r_{0.9} = 0.035$). Using these rates to determine the 95% confidence intervals for the expected number of species in the *Marmarostoma* clade ($t = 23.7$ Myr), we calculated an expected number of species that ranged from 3–23 species using r_0, 1–31 species for $r_{0.5}$, and 1–55 species for $r_{0.9}$. We estimate that there are 30 species of *Marmarostoma* (of which 25 are either nominal or already identified cryptic species) making it a species-rich clade under low-to-moderate levels of relative extinction.

The rate of diversification determined for the genus *Echinolittorina* was slightly higher than that observed in *Turbo*, and ranged from $r_0 = 0.088$ to $r_{0.9} = 0.048$. Using these rates to determine the 95% confidence intervals for the expected number of species in the *Granulilittorina* clade ($t = 21.0$ Myr), we calculated values that ranged from 3–34 species for r_0, to 2–73 for $r_{0.9}$. The upper limit of both of these intervals exceeds the number of extant species in *Granulilittorina* whether *E. vidua* is counted as one species or as six ($n = 26$ or $n = 31$) and the upper limit of the last interval is greater than the number of extant species in the entire genus of *Echinolittorina* ($n = 60$). Because other tests show that

Granulilittorina has undergone a substantial shift in the rate of diversification, it is possible that the lower than expected number of species is because extinction has been high in this group over the last 25 Myr.

Conus had similar rates of diversification to those observed for *Echinolittorina*. Rates ranged from $r_0 = 0.089$ to $r_{0.9} = 0.062$. Using these rates to determine the 95% confidence intervals for the expected number of species in the *Conus* Clade X clade ($t = 23.4$ Myr), we calculated values that ranged from 3–43 species for r_0, to 2–132 for $r_{0.9}$. We estimate that there are 273 species in *Conus* Clade X making it a species-rich clade under all levels of relative extinction and genus level rates of diversification. However, given that the rate of evolution differs between the small major clade and the large major clade (Duda and Kohn 2005) and evidence in this article that the entire large major clade experienced a significant increase in diversification rate, we also determined the rate of evolution for the large major clade only, and used this to predict the number of species likely to occur in *Conus* Clade X.

The rate of diversification for the *Conus* large major clade was higher than that determined across the whole genus, ranging from $r_0 = 0.171$ to $r_{0.9} = 0.119$. Using these rates to determine the 95% confidence intervals for the expected number of species in *Conus* Clade X ($t = 23.4$ Myr), we calculated values that ranged from 15–304 species for r_0, to 5–598 for $r_{0.9}$. The upper limit of both of these intervals exceeds the estimated number of extant species in *Conus* Clade X ($n \sim 273$) and the upper limit of the last interval is greater than the number of extant species in the entire genus of *Conus* ($n \sim 500$). As we suggest for *Granulilittorina*, because other tests show that *Conus* Clade X underwent an increase in its rate of diversification, the lower than expected number of species may reflect a higher extinction rate in this group over the last 25 Myr.

A partial explanation for less than the predicted numbers of species in *Granulilittorina* and *Conus* Clade X, may be that the number of species in each group has been underestimated. Numbers of species are typically underestimated in marine groups (Knowlton 1993), and this is particularly likely for *Conus*, because species in the phylogeny were represented by only a single sample and population level studies of several IWP *Conus* have detected cryptic species (T. F. Duda, unpubl. data). Another partial explanation has to do with the age of the clades relative to the age of the genus. Magallón and Sanderson (2001) report that if a clade's rate of diversification decreases through time (which is true of all three genera in this study), estimates of average rates based on the assumption of a constant rate, will underestimate the rate of diversification during the early evolution of the clade, but will overestimate the rate during its old age. Thus, in both *Conus* and *Echinolittorina*, which are younger than *Turbo*, diversification rates may have been slightly overestimated.

Table 1. Numbers of species occurring within broad biogeographic regions. Species distributions are determined from literature. Biogeographic regions defined by Williams and Reid (2004), with the addition of a temperate 'Southern Australia' region. Widespread species occurring in more than one region count once for each region in which they are found. Endemic species are defined as species whose entire distribution falls within one biogeographic region. Areas of maximum diversity within each clade are indicated with a gray box and bold type. Biogeographic regions and species counts for *Turbo* subgenus *Marmarostoma*, *Echinolittorina* subgenus *Granulilittorina*, and *Conus* 'Clade X' are illustrated in Figure 3.

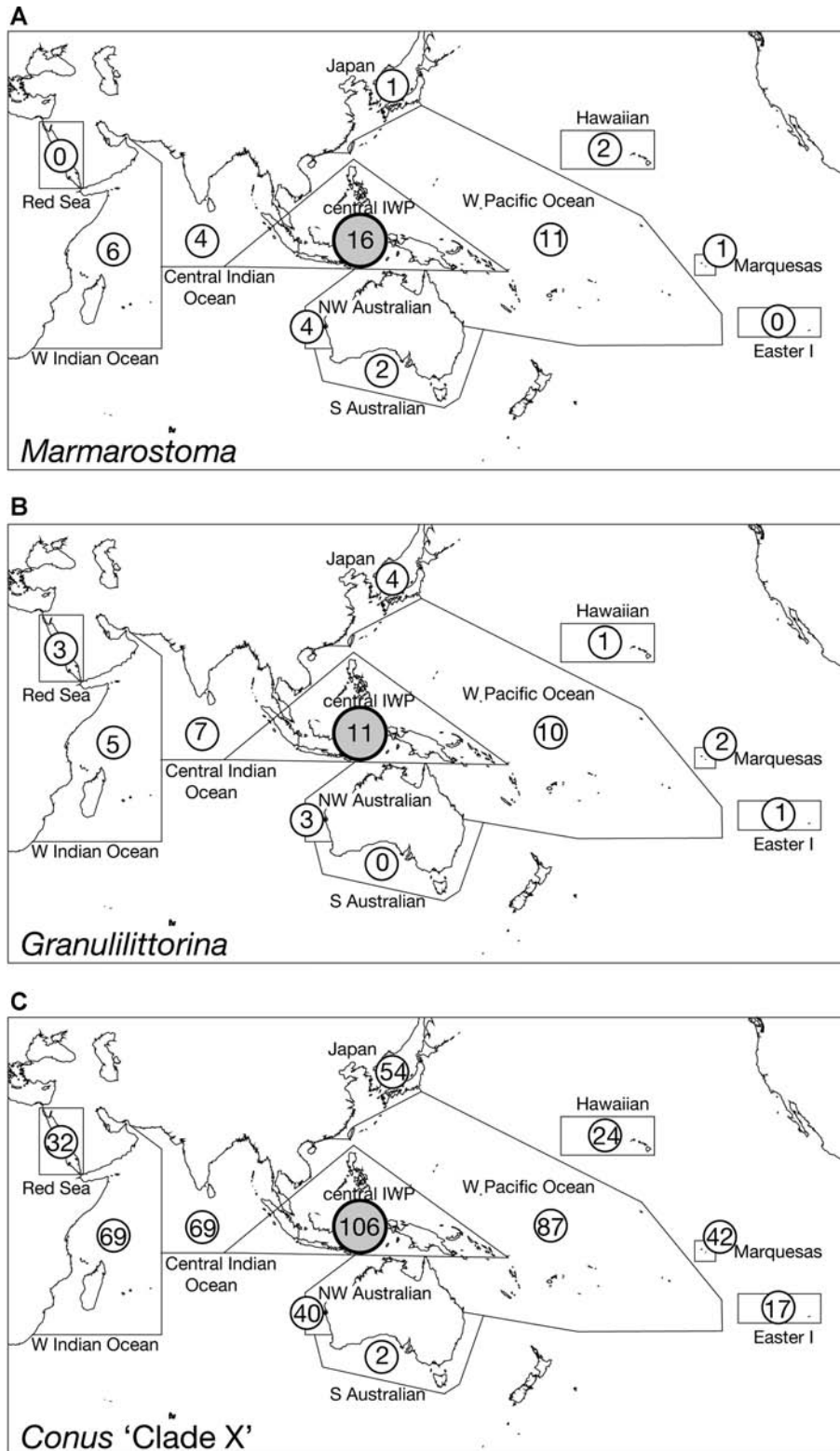| Clade	Red Sea	Western Indian Ocean	Central Indian Ocean	North-western Australia	Southern Australia	Central IWP	Japan	West Pacific	Hawaii	Marquesas	Easter I. and Pitcairn I.
Turbo n=45	2	13	7	9	6	**21**	6	17	2	1	1
Turbo (endemics only) n=25	1	6	0	1	3	**8**	2	1	2	0	0
Turbo subgenus *Marmarostoma* n=25	0	6	4	4	2	**16**	1	11	2	1	0
Turbo subgenus *Marmarostoma* (endemics only) n=15	0	2	0	0	1	**8**	0	1	2	0	0
Turbo, excluding *Marmarostoma* n=20	2	**7**	3	5	4	5	5	6	0	0	1
Turbo, excluding *Marmarostoma* (endemics only) n=10	1	**4**	0	1	2	0	2	0	0	0	0
Echinolittorina subgenus *Granulilittorina* n=26	3	5	7	3	0	**11**	4	10	1	2	1
Echinolittorina subgenus *Granulilittorina* (endemics only) n=15	2	2	2	1	0	**4**	0	1	1	1	1
Conus n=335	53	135	135	77	16	**218**	116	197	38	60	22
Conus (endemics only) n=101	4	21	13	4	4	**28**	5	17	2	3	0
Conus 'Clade X' n=146	32	69	69	40	2	**106**	54	87	24	42	17
Conus 'Clade X' (endemics only) n=40	2	7	6	2	0	**14**	1	5	1	2	0
Conus, excluding 'Clade X' n=189	21	66	66	37	13	**112**	62	110	14	18	5
Conus, excluding 'Clade X' (endemics only) n=61	2	**14**	7	2	4	**14**	4	12	1	1	0

Figure 3. Numbers of species in three gastropod clades determined from literature and molecular studies that occur within 11 broad biogeographic regions and provinces. Regions are based on those in Williams and Reid (2004) with the addition of a temperate Southern Australia region. Widespread species occurring in more than one region count once for each region in which they are found. Distribution maps figured are for those clades with a significant increase in diversification rate in the Late Oligocene or Early Miocene. A. *Turbo* subgenus *Marmarostoma*. B. *Echinolittorina* subgenus *Granulilittorina*. C. *Conus* Clade X. Figure for *Granulilittorina* modified from Williams and Reid (2004), with species counts based on new data in Reid (2007).

SPECIES DISTRIBUTION PLOTS

All three genera exhibit a diversity maximum within the central IWP (Table 1), as do clades with increased rates of diversification (*Marmarostoma*, *Granulilittorina*, and *Conus* Clade X; Fig. 3). Analysis of *Turbo*, excluding the subgenus *Marmarostoma*, shows that species numbers of the other IWP subgenera are highest in the western Pacific and western Indian Ocean (Table 1). On the other hand, although *Conus* Clade X shows a diversity maximum in the central IWP, so also do the remainder of the *Conus* IWP taxa (Table 1). These comparisons were not made for *Echinolittorina* because there was only one clade consisting of all IWP *Echinolittorina* species (subgenus *Granulilittorina*) and this entire clade exhibited an increased rate of diversification.

These diversity patterns are not unduly influenced by the presence of widespread species. Mapping the number of species endemic to each region also shows a maximum of diversity in the central IWP for *Marmarostoma*, *Echinolittorina*, and *Conus* Clade X (Table 1).

Discussion

Analyses of three unrelated gastropod genera show that there has been a substantial increase in the rate of diversification in some, but not all, IWP clades, despite generic-level LTT plots that are consistent with either a slowdown or a constant rate of diversification. This pattern is likely the result of heterogeneity among diversification rates within the phylogeny (Moore et al. 2004). An irregular distribution of diversification rates over time is consistent with a punctuated evolutionary model of cladogenesis (Moore et al. 2004). Determination of the cause of this "punctuation" is then of particular interest.

Multiple instances of increased rates of diversification were found in all three genera. Most of the branches that show significant increases in rates of diversification are well supported in their respective molecular phylogenies and some correspond to subgeneric groupings. The timing of the first shift in the rate of diversification in three well-supported ($PP = 100\%$), predominantly IWP clades (the *Turbo* subgenus *Marmarostoma*, the *Echinolittorina* subgenus *Granulilittorina* and *Conus* Clade X) is Late Oligocene to Early Miocene (23.7–21.0 Mya). In the case of *Conus*, localized diversity increases in the Asian region of Eurasia in the Late Oligocene and major Miocene radiations in the Indo-Australian region are also supported by fossil data (Kohn 1990; Lozouet 1997).

The congruence of both location (IWP) and the narrow interval of timing (beginning 23.7–21.0 Mya) in three independently calibrated chronograms implies a common cause for the rapid increase in diversification in some IWP clades in *Turbo*, *Echinolittorina*, and *Conus* (clades corresponding to *Marmarostoma*, *Granulilittorina*, and *Conus* Clade X). We propose that the rate of speciation increased in these clades as a result of increased availability of habitat generated by the collision of the Australia and New Guinea plate with SE Eurasia about 20–25 Mya and the concurrent increase in coral-carbonate platforms. If this is so, then the clades that show an increase in diversification rates should include mainly IWP taxa and the species radiations should be focused in the area of the tectonic collision and increase in coral carbonates. In fact, members of all three clades are predominantly, or entirely, IWP in distribution and two are reef-associated. Moreover, *Marmarostoma*, *Granulilittorina*, and *Conus* Clade X all have a diversity maximum in the central IWP (Table 1; Fig. 3). These patterns are not entirely due to widespread species. Mapping the number of species that are endemic to each region also shows a maximum of diversity in the central IWP for *Marmarostoma*, *Granulilittorina*, and *Conus* Clade X (Table 1) consistent with species origination in the central IWP.

An alternative explanation that the diversification rate increased as a result of lowered rates of extinction relative to speciation rates in the central IWP (the survival-of-survival hypothesis) is not preferred, even though there is some fossil evidence to suggest that extinction has been lower in the IWP than in other tropical regions (Vermeij 1987). Lowered levels of extinction cannot explain the pattern seen in the subgenus *Granulilittorina*, because this clade, despite showing a substantial increase in the rate of cladogenesis, is species-poor based on diversification rates calculated for the genus. This pattern fits the time heterogeneous model of Strathmann and Slatkin (1983), whereby a short initial period in which the speciation rate is much higher than the extinction rate is followed by a longer period in which speciation and extinction rates are more equal. A similar result is also obtained for *Conus* Clade X when species richness is predicted using rates of diversification calculated for the large major clade. Moreover, fossil evidence for *Conus* also suggests that a large proportion of species went extinct in the Late Miocene-Pliocene (only 11% of Miocene and 33% of Pliocene species survive; Kohn 1990).

The diversity maximum of *Turbo* in the central IWP is largely due to the distribution of *Marmarostoma* species (Fig. 3). Exclusion of the subgenus *Marmarostoma* from the analysis shows that species numbers are highest in the western Pacific and western Indian Ocean for the remaining IWP *Turbo* subgenera (Fig. 3; Table 1). *Marmarostoma* are the most common in the intertidal (often in crevices or under coral-rocks exposed at high tide) to shallow subtidal zone on coral-reef carbonate platforms, habitats that increased in area after tectonic activity. Of the non-*Marmarostoma* IWP subgenera, three are also found in association with coral reef-platforms (*Turbo*, *Lunatica*, and an unnamed subgenus occurring in the west Indian Ocean, Williams 2007), but species in the remaining IWP subgenera predominantly occur in temperate or deeper water, or are not reef-associated (*Batillus*, *Carswellana*, and *Euninella*).

It was not possible to determine with certainty all species that belong to *Conus* Clade X, so our distribution maps for this clade are tentative, pending further studies. However, the distribution of species belonging to both *Conus* Clade X and the subset including the remainder of the *Conus* IWP taxa (non-CladeX) reach a diversity maximum in the central IWP, although non-Clade X endemics are equally diverse in the West Indian Ocean (Fig. 3; Table 1). As *Conus* is very under-sampled in all molecular phylogenetic analyses to date, it is possible that increased taxon sampling will reveal other clades with rapid diversification rates (Clade Y is discussed below). In fact, the entire large major clade (which is thought to be IWP in origin, Duda and Kohn 2005) shows a significant increase in diversification at its origin and most *Conus* species are common in shallow-water habitats and are associated with coral reefs.

One clade in the *Conus* phylogeny, *Conus* Clade Y (crown age 19.4 Myr) shows a significant increase in diversification in topology-based tests. The clade includes two subclades: one mostly IWP and one of East Pacific and Atlantic species. The clade originates 21.1 Mya and diversifies approximately 19.4 Mya, corresponding to the closure of the Tethys Seaway and the isolation of the IWP from the Mediterranean and Atlantic. An older clade in *Echinolittorina* (28.3 Myr) also shows a significant increase in diversification. It too includes one East Pacific and Atlantic clade and one mostly IWP clade. Separation of faunal assemblages began prior to the complete closure of the Tethys (Harzhauser et al. 2007), so it is possible that in both cases the increase in diversification was in response to events leading to the closure of the Tethys Seaway.

Other factors affecting species richness in the central IWP include the overlapping of biogeographic provinces centered in the Indian Ocean and in the Pacific Ocean (the center-of-overlap hypothesis; Ekman 1953; Woodland 1983; McMillan and Palumbi 1995), the dispersal of species from the periphery into the center (the "center-of-accumulation" hypothesis; Ladd 1960; Jokiel and Martinelli 1992), isolation of populations during periods of lowered sea levels (e.g., McCoy and Heck 1976; McManus 1985; Randall 1998; Williams and Benzie 1998; Bellwood and Hughes 2001; Santini and Winterbottom 2002), and a mid-domain effect (Bellwood et al. 2005). However none of these alone can explain the rapid increase in diversification that we observed in three unrelated gastropod genera around 20–25 Mya. Our results are most consistent with the "center-of-origin" hypothesis (Ekman 1953; Briggs 1999), which suggests that speciation events are concentrated in the central IWP. However, more specifically, this study suggests that the central IWP was a "historical center-of-origin" with many species-level radiations originating in the Late Oligocene and Early Miocene.

If tectonic events are responsible for the rapid diversification observed in the three gastropod genera in this study, other taxa should also exhibit this pattern. Species richness in five families of reef-associated tetraodontiform fish exceeds that predicted based on rates of diversification determined for the entire order of fish (Alfaro et al. 2007). Consistent with radiations following tectonic activity, three of these five families and two clades in a fourth radiated approximately 20–25 Mya (Ostraciidae, 20.8 Myr; Balistidae, 22.9 Myr; Monocanthidae, 24.6 Myr; clade "K" in Tetraodontidae, 19.4 Myr, unnamed clade in Tetraodontidae, ∼21 Myr fig. 5 in Alfaro et al. 2007). All four families are highly diverse in the IWP, with 30–57% of all nominal species in each family found within the central IWP (calculated from data in Carpenter and Niem 2001). Many other likely examples exist, some of which, like the gastropod family Cypraeidae, have been the focus of recent molecular studies (Meyer 2003), but have not yet had a molecular clock applied to published trees.

In this study, we found significant increases in the rate of diversity in two-reef associated clades (*Marmarostoma* and *Conus* Clade X) and evidence of a substantial increase in diversification in one rocky-shore clade (*Granulilittorina*). An explanation for the increase in diversification rate in *Echinolittorina* includes the fact that the central IWP is a geologically complex area that exhibits not only a long coastline, but also a mosaic of many distinct, nonoverlapping habitat types. Much of this complexity probably arose when the Australia and New Guinea plate collided with SE Eurasia forming new mountains with their associated rivers and terrestrial run-off as well as new bays and headlands. Williams and Reid (2004) suggested that the rocky shore habitat is partitioned among *Echinolittorina* species according to a nutrient or productivity regime. Species differ in habitat preferences, with defined ranges along a continuum from continental to oceanic conditions. Similar ecological limitations on species boundaries are well known in other gastropods and probably affect all three genera examined. In the case of *Echinolittorina*, sister species are always allopatric and allopatry persists for millions of years (Williams and Reid 2004). The fidelity of species to a particular habitat type over time, as evidenced by the persistence of allopatry, may also have contributed to the increase of lineage accumulation in a spatially variable environment (Kozak et al. 2006).

Rates of diversification averaged over lineages within *Granulilittorina*, *Marmarostoma*, and *Conus* Clade X have not continued to increase but have remained constant or have gradually decreased over time. Incomplete taxon sampling at the species level (affecting both *Turbo* and *Conus*) can bias the placement of nodes away from the present, which is interpreted as an apparent decline in diversification rates over time (Pybus and Harvey 2000). However, this cannot explain the effect in *Echinolittorina* because all known members of this group were examined in LTT analyses. Moreover, while *Marmarostoma* and *Conus* Clade X are both more species rich than would be expected using diversification rates averaged across genera; *Granulilittorina* is not and neither is *Conus* Clade X when diversification rates are calculated for the

large major clade only. This suggests that a period of rapid diversification was followed by decreased rates of speciation and/or high levels of extinction, further reducing the number of extant species in *Granulilittorina* and *Conus* Clade X. LTT plots and fossil evidence of Late Miocene-Pliocene extinctions in *Conus* (Kohn 1990) are consistent with high rates of extinction.

It has been shown in other groups (e.g., salamanders, Kozak et al. 2006) that ecological and genetic interactions limit the distributional overlap of species and can account for a shift to a lower diversification rate following a period of rapid diversification (Schluter 2000; Rüber et al. 2003; Rüber and Zardoya 2005; Kozak et al. 2006). Our results suggest that once major lineages became established in new habitats in the IWP during the Early Miocene, opportunities for speciation became more limited as ecological and geographical niches were filled and this led to an overall slowdown in the tempo of evolution.

SUMMARY

The Oligo–Miocene was a time of rapid diversification in the tropical IWP. Our results provide the first molecular evidence to suggest that at least some of the species radiations in the tropical IWP were due to tectonic events that changed the geology and ecology of the region. The closure of the Tethys Seaway may have resulted in increased diversification rates in some groups, but of particular importance was the increase in coastline and shallow-water area associated with the collision of the Australia New Guinea plate with SE Eurasia. These events resulted in increased habitat complexity, which along with the concurrent increase in coral-carbonate platforms contributed to the origination of new species. However, speciation did not continue at an increased pace in the genera studied, but slowed down once the new habitats were filled. We identified three independent examples that demonstrate this effect among tropical gastropod genera, although the increase in diversification was stronger in two reef-associated clades than one rocky-shore clade. There are likely to be many other examples, and reef-associated organisms are particularly good candidates for further studies.

ACKNOWLEDGMENTS

LITERATURE CITED

Alf A., and K. Kreipl. 2003. The family Turbinidae, subfamily Turbininae, genus *Turbo*. Conchbooks, Hackenheim, Germany; photos by Guido Poppe.

Alfaro, M. E., F. Santini, and C. D. Brock. 2007. Do reefs drive diversification in marine teleosts? Evidence from the pufferfish and their allies (Order Tetraodontiformes). Evolution 61:2104–2126.

Barber, P. H., and D. R. Bellwood. 2005. Biodiversity hotspots: evolutionary origins of biodiversity in wrasses (*Haliocheres*: Labridae) in the Indo-Pacific and new world tropics. Mol. Phylogenet. Evol. 35:235–253.

Bellwood, D. R., and T. P. Hughes. 2001. Regional-scale assembly rules and biodiversity of coral reefs. Science 292:1532–1534.

Bellwood, D. R., L. van Herwerden, and N. Konow. 2004. Evolution and biogeography of marine angelfishes (Pisces: Pomacanthidae). Mol. Phylogenet. Evol. 33:140–155.

Bellwood, D. R., T. P. Hughes, S. R. Connolly, and J. Tanner. 2005. Environmental and geometric constraints on Indo-Pacific coral reed biodiversity. Ecol. Lett. 8:643–651.

Briggs, J. C. 1999. Coincident biogeographic patterns: Indo-West Pacific Ocean. Evolution 53:326–335.

Budd, A. F., T. A. Stemann, and R.H. Stewart. 1992. Eocene Caribbean reef corals: a unique fauna from the Gatuncillo Formation of Panama. J. Paleontol. 66:570–594.

Carpenter, K. E., and V. H. Niem. 2001. FAO species identification guide for fishery purposes. The living marine resources of the Western Central Pacific. Volume 6. Bony fishes part 4 (Labridae to Latimeriidae), estuarine crocodiles, sea turtles, sea snakes and marine mammals. FAO, Rome. Pp. 3381–4218.

Chan, K. M. A., and B. R. Moore. 2005. SymmeTREE: whole tree analysis of differential diversification rates. Syst. Biol. 51:855–865.

Cluzaud A., and B. Cahuzac. 2006. Révision de deux espèces peu connues de *Turbo* (Gastropoda) du Tertiaire d'Aquitaine (suite). Deuxième partie— *Turbo subsetosus* d'Orbigny, 1852. Bulletin de la Société Linnéenne de Bordeaux 141:103–134.

Coates, A. G., and J. A. Obando. 1996. The geologic evolution of the Central American Isthmus. Pp. 21–56 *in* J. B. C. Jackson, A. G. Coates, and A. Budd, eds. Evolution and environment in tropical America. Univ. of Chicago Press, Chicago, Illinois.

Crame J. A., and B. R. Rosen. 2002. Cenozoic palaeogeography and the rise of modern biodiversity patterns. Pp. 153–168 *in* J. A. Crame and A. W. Owen, eds. Palaeobiogeography and biodiversity change: the Ordovician and Mesozoic-Cenozoic radiations, Volume 194. Geological Society, Special Publications, London.

Dickerson, R. E. 1921. Notes on a fauna of the Vigo Group and its bearing on the evolution of marine molluscan faunas. Proc. Calif. Acad. Sci. 11:1–26.

Drummond, A. J., and A. Rambaut. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7:214.

Drummond, A. J., S. Y. W. Ho, M. J. Phillips, and A. Rambaut. 2006. Relaxed phylogenetics and dating with confidence. PLoS Biol. 4:e88.

Duda, T. F. Jr, and A. J. Kohn. 2005. Species-level phylogeography and evolutionary history of the hyperdiverse marine gastropod genus *Conus*. Mol. Phylogenet. Evol. 34:257–272.

Ekman, S. 1953. Zoogeography of the sea. Sidgwick and Jackson, London.

Fourtau, R. 1903. Contribution à l'étude de la faune crétacique d'Égypte: Institut d'Egypte. Bulletin 4:231–349 and 5 plates.

Hall, R. 1998. The plate tectonics of Cenozoic SE Asia and the distribution of land and sea. Pp. 99–131 *in* R. Hall and J. D. Holloway, eds. Biogeography and geological evolution of SE Asia. Backhuys Publishers, Leiden.

Harvey, P. H., R. M. May, and S. Nee. 1994. Phylogenies without fossils. Evolution 48:523–529.

Harzhauser M., A. Kroh, O. Mandic, W. E. Piller, U. Gohlich, M. Reuter, and B. Berning. 2007. Biogeographic responses o geodynamics: a key study all around the Oligo-Miocene Tethyan Seaway. Zoologischer Anzeiger 246:241–256.

Harzhauser M., W. E. Piller, and F. F. Steininger. 2002. Circum-Mediterranean Oligo-Miocene biogeographic evolution—the gastropods' point of view. Palaeogeog. Palaeoclimatol. Palaeoecol. 183:103–133.

Johnson, K. G., J. A. Todd, and J. B. C. Jackson. 2007. Coral reef development drives molluscan diversity increase at local and regional scales in the late Neogene and Quaternary of the southwestern Caribbean. Paleobiology 33:24–52

Jokiel, P., and F. J. Martinelli. 1992. The vortex model of coral reef biogeography. J. Biogeogr. 19:449–458.

Knowlton, N. 1993. Sibling species in the sea. Annu. Rev. Ecol. Syst. 24:189–216.

Kohn, A. J. 1990. Tempo and mode of evolution in Conidae. Malacologia 32:55–67.

Kozak, K. H., D. W. Weisrock, and A. Larson. 2006. Rapid lineage accumulation in a non-adaptive radiation: phylogenetic analysis of diversification rates in eastern North American woodland salamanders (Plethodontidae: *Plethodon*). Proc. R. Soc. B 273:539–546.

Ladd, H. S. 1960. Origin of the Pacific island molluscan fauna. Am. J. Sci. 258A:137–150.

Lozouet, P. 1997. Le domaine atlantique européen au Cénozoique moyen: diversité et évolution des gastéropodes. Ph.D. Thesis, Muséum National d'Histoire Naturelle, Paris, France.

Machordom, A., and E. Macpherson. 2004. Recent radiation and cryptic speciation in squat lobsters of the genus *Munida* (Crustacea, Decapoda) and related genera in the South West Pacific: molecular and morphological evidence. Mol. Phylogenet. Evol. 33:259–279.

MacNeil, S. 1964. Eocene megafossils from Ishigaki-shima Ryuku-retto. Geological Survey Professional Paper 399-B.

Magallón, S., and M. J. Sanderson. 2001. Absolute diversification rates in Angiosperm clades. Evolution 55:1762–1780.

McCoy, E. D., and K. L. Heck. 1976. Biogeography of corals, seagrasses and mangroves: an alternative to the centre of origin concept. Syst. Zool. 25:201–210.

McLean, J. H., and S. Kiel. 2007. Cretaceous and living Colloniidae of the redefined subfamily Petropomatinae, with two new genera and one new species, with notes on opercular evolution in turbinoideans, and the fossil record of Liotiidae (Vetigastropoda: Turbinoidea). Palaontologische Zeitschrift 81/3:254–266.

McManus, J. W. 1985. Marine speciation, tectonics and sea-level changes in southeast Asia. Pp. 133–138. Proceedings Fifth International Coral Reef Congress. Vol. 4., Moorea, French Polynesia.

McMillan, W. O., and S. R. Palumbi. 1995. Concordant evolutionary patterns among Indo-West Pacific butterflyfishes. Proc. Roy. Soc. Lond. B 260:229–236.

Meyer, C. P. 2003. Molecular systematics of cowries (gastropoda: Cypraeidae) and diversification patterns in the tropics. Biol. J. Linn. Soc. 79:401–459.

Moore, B. R., K. M. A. Chan, and M. J. Donoghue. 2004. Detecting diversification rate variation in supertrees. Pp. 487–533 *in* O. R. P. Bininda-Emonds, ed. Phylogenetic supertrees: combining information to reveal the tree of life. Kluwer Academic, Dordrecht, The Netherlands.

Mora, C., P. M. Chittaro, P. F. Sale, J. P. Kritzer, and S. A. Ludsin. 2003. Patterns and processes in reef fish diversity. Nature 421:933–936.

Moss, S. J., and M. E. J. Wilson. 1998. Biogeographic implications of the Tertiary paleogeographic evolution of Sulawesi and Borneo. Pp. 133–163 *in* R. Hall and J. D. Holloway, eds. Biogeography and geological evolution of SE Asia. Backhuys Publishers, Leiden.

Odin, G. S, A. Montanari, and R. Coccioni. 1997. Chronostratigraphy of Miocene stages: a proposal for the definition of precise boundaries. Pp. 597–629 *in* A. Montanari, G. S. Odin, R. Coccioni, eds. Miocene stratigraphy: An integrated approach. Elsevier, Amsterdam.

Paradis, E. 1997. Assessing temporal variations in diversification rates from phylogenies: estimation and hypothesis testing. Proc. R. Soc. Lond. B. 264:1141–1147.

Preece, R. C. 1995. The composition and relationships of the marine molluscan fauna of the Pitcairn Islands. Biol. J. Linn. Soc. 56:339–358.

Pybus, O. G., and P. H. Harvey. 2000. Testing macro-evolutionary models using incomplete molecular phylogenies. Proc. R. Soc. Lond. B 267:2267–2272.

Pybus, O. G., and A. Rambaut. 2002. GENIE: estimating demographic history from molecular phylogenies. Bioinformatics 18:1404–1405.

Randall, J. E. 1998. Zoogeography of shore fishes of the Indo-Pacific region. Zool. Stud. 37:227–268.

Rambaut A., P. H. Harvey, and S. Nee. 1997. End-Epi: an application for reconstructing phylogenetic and population processes from molecular sequences. Comp. Appl. Biosci. 13:303–306.

Read, C. I., D. R. Bellwood, and L. van Herwerden. 2006. Ancient origins of Indo-Pacific biodiversity: a case study of the leopard wrasses (Labridae: Macropharyngodon). Mol. Phylogenet. Evol. 38:808–819. (DOI:10.1016/j.ympev.2005.08.001)

Reid, D. G. 2007. The genus *Echinolittorina* Habe, 1956 (Gasteropoda: Littorinidae) in the Indo-West Pacific Ocean. Zootaxa 1420:1–161.

Reid, D. G., K. Lal, J. MacKenzie-Dodds, F. Kaligis, D. T. Littlewood, and S. T. Williams. 2006. Comparative phylogeography and species boundaries in *Echinolittorina* snails in the central Indo-West Pacific. J. Biogeogr. 33:990–1006.

Röckel D., W. Korn, and A. J. Kohn. 1995. Manual of the living Conidae. Vol. I, Indo-Pacific. Christa Hemmen Verlag, Wiesbaden.

Rögl, F. 1998. Palaeogeographic considerations for Mediterranean and Paratethys seaways (Oligocene to Miocene). Annalen des Naturhistorischen Museums in Wien 99A:279–310.

Rüber, L., and R. Zardoya. 2005. Rapid cladogenesis in fish revisited. Evolution 59:1119–1127.

Rüber, L., J. L. Van Tassell, and R. Zardoya. 2003. Rapid speciation and ecological divergence in he American seven-spined gobies (Gobiidae, Gobiosomatini) inferred from a molecular phylogeny. Evolution 57:1584–1598.

Saito, T., H. Okado, and K. Kaiho. 1984. Biostratigraphy and international correlation of the Paleogene system in Japan. Publication Department Earth Science, Faculty of Science, Yamagata University, Japan.

Sanderson, M. J., and M. J. Donoghue. 1996. Reconstructing shifts in diversification rates on phylogenetic trees. Trends Ecol. Evol. 11:15–20.

Santini, F., and R. Winterbottom. 2002. Historical biogeography of Indowestern Pacific coral reef biota: is the Indonesian region a centre of origin? J. Biogeogr. 29:189–205.

Schluter, D. 2000. The ecology of adaptive radiation. Oxford Univ. Press, Oxford, UK.

Shuto, T. 1975. Preliminary correlation of the Neogene molluscan faunas in Southeast Asia. Geology and Paleontology of Southeast Asia 15:289–301.

Strathmann, R. R., and M. Slatkin. 1983. The improbability of animal phyla with few species. Paleobiology 9:97–106.

Van Der Vlerk, I. M. 1931. Caenozoic Amphineura, Gastropoda, Lamelli-branchiata, Scaphopoda. Leidsche Geologische Mededeelinger 5:206–296.

Vermeij, G. J. 1987. The dispersal barrier in the tropical Pacific: implications for molluscan speciation and extinction. Evolution 41:1046–1058.

Vermeij, G. J. 2001. Community assembly in the sea: geological history of the living shore biota. Pp. 39–60 *in* M. D. Bertness, S. D. Gaines and M. E. Hay, eds. Marine community ecology. Sinauer Associates, Inc, Sunderland, MA.

Vredenburg E. 1928. Descriptions of Mollusca from the post-Eocene Tertiary formation of north-western India: Gastropoda (in part) and Lamel-libranchiata. Memoirs Geolog. Surv. India 50:351–507.

Vrielynck, B., G. S. Odin, and J. Dercourt. 1997. Miocene palaeogeography of the Tethys Ocean; potential global correlations in the Mediterranean. *in* A. Montanari, G. S. Odin and R. Coccioni, eds. Miocene stratigraphy: an integrated approach. Elsevier Science.

Welcomme, J.-L., M. Benammi, J.-Y. Crochet, L. Marivaux, G. Métais, P.-O. Antoine, and I. Baloch. 2001. Himalayan Forelands: palaeontological evidence for Oligocene detrital deposits in the Bugti Hills (Balochistan, Pakistan). Geol. Mag. 138:397–405.

Williams, S. T. 2007. Origins and diversification of Indo-West Pacific marine fauna: evolutionary history and biogeography of turban shells (Gastropoda, Turbinidae). Biol. J. Linn. Soc. 92:573–592.

Williams, S. T., and J. A. H. Benzie. 1998. Evidence of a biogeographic break between populations of a high dispersal starfish, congruent regions within the Indo-West Pacific defined by colour morphs, mtDNA and allozyme data. Evolution 52:87–99.

Williams, S. T., and D. G. Reid. 2004. Speciation and diversity on tropical rocky shores: a global phylogeny of snails of the genus *Echinolittorina*. Evolution 58:2227–2251. (DOI: 10.1554/03–565).

Wilson, M. E. J., and B. R. Rosen. 1998. Implications of paucity of corals in the Paleogene of SE Asia: plate tectonics or centre of origin? Pp. 165–195 *in* R. Hall and J. D. Holloway, eds. Biogeography and geological evolution of SE Asia. Backhuys Publishers, Leiden.

Woodland, D. J. 1983. Zoogeography of the Siganidae (Pisces): an interpretation of distribution and richness patterns. Bull. Mar. Sci. 33:713–717.

Associate Editor: W. O. MacMillan