Maternal investment evolves with larger body size and higher diversification rate in sharks and rays

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Running head: Reproductive Mode Evolution of Sharks

Abstract

Across vertebrates, live-bearing has evolved at least 150 times from the ancestral state of egg-laying into a diverse array of forms and degrees of prepartum maternal investment. A key question is how this diversity of reproductive modes arose and whether reproductive diversification underlies species diversification? To test these questions, we evaluate the most basal jawed vertebrates, Chondrichthyans, which have one of the greatest ranges of reproductive and ecological diversity among vertebrates. We reconstructed the sequence of reproductive mode evolution across a time-calibrated molecular phylogeny of 610 chondrichthyans. We find that egg-laying is ancestral, and that live-bearing evolved at least seven times. Matrotrophy (i.e. additional maternal contributions) evolved at least 15 times, with evidence of one

reversal. In sharks, transitions to live-bearing and matrotrophy are more prevalent in larger-bodied species in the tropics. Further, the evolution of live-bearing is associated with a near-doubling of the diversification rate, but, there is only a small increase in diversification associated with the appearance of matrotrophy. The chondrichthyan diversification and radiation, particularly throughout the shallow tropical shelf seas and oceanic pelagic habitats, appears to be associated with the evolution of live-bearing and the proliferation of a wide range of maternal investment in their developing offspring.

Keywords: Live-bearing; Matrotrophy; Chondrichthyan; Shark; Trait Evolution; Diversification

Introduction

A key transition in the evolution of vertebrate life is the appearance of live-bearing and the evolution of maternal investment (Dulvy and Reynolds 1997; Goodwin et al. 2002; Blackburn 2015b). Live-bearing is thought to have evolved to increase offspring survival in the face of environmental and biological challenges (Clutton-Brock and Godfrey 1991). The retention of eggs inside the maternal body cavity provides a “safe harbor” for developing embryos (Shine 1978). For example, in montane reptiles, the transition to live-bearing potentially protects eggs from freezing (Shine 1995) or predation risk (Guillette 1993). However, mothers may suffer costs from increased energetic investment, reduced fecundity, or greater predation risk and reduced foraging ability due to pregnancy (Goodwin et al. 2002; Shine 2004). Body

size is important as internal body cavity space can limit female reproductive output. Hence, live-bearing should be correlated with increasing maternal body size and the space required to carry developing offspring to term in larger species (Qualls and Shine 1995; Goodwin et al. 2002). In addition to body size, temperature may strongly influence the evolution of reproductive modes in ecotherms (Shine 1995). In reptiles, viviparity has evolved in cold climates because of the risk of freezing and lower survival of eggs (Shine 2004).

Sister to all jawed vertebrates, the Class Chondrichthyes (hereafter 'shark and rays'), exhibit live-bearing and varying modes of maternal investment (Wourms 1981; Dulvy and Reynolds 1997; Reynolds et al. 2002). Similar to reptiles, reproductive transitions in chondrichthyans may be temperature-dependent but potentially for different reasons (Compagno 1990). Sharks and rays that nourish embryos solely via the yolk-sac (e.g. egg-laying or lecithotrophic live-bearing) are predominantly found in colder habitats either in deep water or high latitude seas. Whereas matrotrophic live-bearing, with maternal contribution beyond the yolk-sac, is more prevalent in shallow tropical habitats, potentially reflecting temperature effects on the rate and efficiency of development (Yampolsky and Scheiner 1996; Dulvy 1998; Gillooly et al. 2002). Under this hypothesis, low maternal investment strategies, such as lecithotrophic live-bearing, are more likely in colder environments where metabolic rate (and hence maintenance costs) are lower, leaving a greater fraction of energy available for embryonic growth. By contrast, the greater metabolic maintenance costs in warmer environments may require additional maternal investment through increased ovum size or post-fertilization trophic input to produce

a similar-sized offspring (Dulvy 1998). This temperature dependent – reproductive investment hypothesis is consistent with thermal physiology (Gillooly et al. 2002), but remains untested, simply because – until now – suitable molecular phylogenies necessary for ancestral state reconstruction have been unavailable (Dulvy and Reynolds 1997; Musick and Ellis 2005).

Reproductive mode evolution has the potential to influence speciation and diversification dynamics through colonization of novel habitats (Yoder et al. 2010) and / or parent-offspring conflicts (Zeh and Zeh 2000; Crespi and Semeniuk 2004). Live-bearing sharks and rays tend to have larger geographic range sizes, potentially spanning a greater range of habitats compared to smaller species (Goodwin et al. 2005). Speciation and diversification can also be driven by conflict between maternal and offspring genomes in live-bearing species (Zeh and Zeh 2000), whereby conflict over resource allocation during gestation drives antagonistic coevolution between maternal and paternal genomes increasing the rate of species divergence. It is in the father's best interest to have large, well-provisioned offspring, whereas it is in the mother's best interest to weigh current offspring investment against her own survival and future reproductive success (Zeh and Zeh 2000). Live-bearing provides an arena in which this conflict can arise, and morphological adaptations for matrotrophic nutrient transfer also provide an opportunity for embryos to influence maternal input during gestation (Crespi and Semeniuk 2004). This resulting conflict over resources could increase the rate of genetic divergence within populations, ultimately resulting in speciation (Zeh and Zeh 2000). Hence, the potential for parent-offspring conflict in live-bearing species may drive higher rates of diversification that may vary over the

range of maternal investment mechanisms. Increased rates of diversification associated with live-bearing have been noted in squamate lizards (Pyron and Burbrink 2014) and teleost fishes (Helmstetter et al. 2016), although within live-bearers, the degree to which matrotrophy versus lecithotrophy is related to species diversification has yet to be tested.

Sharks and rays are an ideal group for studying the evolution of reproductive mode and maternal investment. Sharks and rays exhibit egg-laying, live-bearing, and multiple forms of matrotrophy along a continuum of maternal post-fertilization input with yolk-sac live-bearing (lecithotrophy) at one end of the spectrum, with lipid histotrophy, oophagy, and placentotrophy representing the extreme forms of matrotrophy at the other. There remains considerable debate on whether the first chondrichthyan laid eggs (Dulvy and Reynolds 1997) or gave birth to live young (Musick and Ellis 2005).

The availability of a new molecular phylogeny of 610 chondrichthyan species (Stein et al. 2018) with complete information on reproductive mode and habitat provides a new opportunity to reconstruct the evolutionary history of reproductive mode in chondrichthyans. We test the following predictions: (1) that the most recent common ancestor of sharks and rays laid eggs based on the basal position of Chimaeriformes; the evolution of live-bearing and varying forms of matrotrophy is related to (2) larger body size, and (3) a radiation into shallow-water tropical habitats (Compagno 1990). Finally, (4) that the evolution of live-bearing and matrotrophy is associated with increasing rates of species diversification.

Methods

Trait Data and Phylogeny

Data on the reproductive mode and habitat type were collected for the 610 chondrichthyan species in our phylogeny, from primary literature and species catalogues (Ebert et al. 2013; Dulvy et al. 2014; IUCN 2014). Chondrichthyans exhibit eight distinct reproductive modes (Dulvy and Reynolds 1997), though we focus the evolution of live bearing and maternal investment, therefore species were categorized into three distinct modes: egg-laying, lecithotrophic live-bearing, and matrotrophic live-bearing where embryos are nourished via the initial yolk-sac investment and additional maternal contributions during gestation (oophagy, histotrophy, and placentotrophy). We note that lecithotrophic live-bearing has also been called yolk-sac viviparity, aplacental viviparity, or ovoviparity (Wourms 1981). We collected data on maximum body size and depth ranges (minimum, mean, median, and maximum) from species field guides and catalogues and primary literature. Minimum and maximum latitudinal range was collected from species Extent Of Occurrence geographic range maps from the International Union for the Conservation of Nature (IUCN) Red List of Threatened Species database (IUCN 2014). Median latitude was calculated as the midpoint between minimum and maximum latitude, and was expressed as an absolute value to represent distance north or south from the equator. All continuous trait values were standardized, centered, and divided by two standard deviations, using the `rescale` function in the `arm` (version 1.9-3) (Gelman and Su 2016) package prior to analyses to facilitate comparison of coefficients.

144 We conducted all analyses using a distribution of trees from a new 610 species
145 chondrichthyan molecular phylogeny (Stein et al. 2018). This phylogeny covers 51%
146 of all known species from every order, 98% of families, 88% of genera, and all
147 described character states are represented. Because the distribution of trees represents
148 a gradient of variation in root and node dating for the maximum likelihood tree, we
149 sequentially selected 21 trees, every 25th tree from one to 500 to account for the full
150 range of temporal calibrations. Results were pooled across all trees.
151
152 *Ancestral State Reconstruction and Diversification*
153 We reconstructed the evolutionary origins and sequence of reproductive mode and
154 habitat while estimating state dependent diversification rates using the multistate
155 speciation and extinction (MuSSE) method with maximum likelihood implemented
156 with `musse` (Pagel 1994) in the `diversitree` (version 0.9-9) package in R
157 (Fitzjohn 2012). Rabosky and Goldberg (2015) pointed out that state dependent
158 diversification models (including MuSSE) are susceptible to inflated false positives
159 when there is unmodeled heterogeneity in diversification rates – thereby associations
160 can be falsely detected between rates and states even when the diversification
161 dynamics are unrelated to the traits being considered. To minimize false positives, we
162 adopted the analytical approach of Uyeda et al. (2017) and integrated a hypothesis-
163 testing approach (i.e., MuSSE) with a more data-driven approach (what Uyeda et al.
164 refer to as "phylogenetic natural history"); in this way, we are able to tease out the
165 signal for our hypotheses that our focal traits are influencing diversification rates

while conditioning on the fact that there is likely substantial background variation in these rates across the tree.

More specifically, we first used the medusa algorithm (as implemented in Geiger v2.0.6; Pennell et al. 2014) to detect background variation in diversification rates unrelated to our traits of interest. First, we infilled those species missing from the molecular phylogeny (n = 582) using taxonomic constraints so that tips represented unresolved clades. Importantly for earlier divergences, our sampling did not miss any variability in reproductive mode (e.g. there are no live-bearing chimaeras) so missing samples will not affect our conclusions. Under some circumstances, medusa may not reliably correctly identify the placement of shifts (May and Moore 2016). However, this issue does not pertain to our analysis because we are not making any inferences about specific events or specific clades and are only interested in detecting broad-scale differences in diversification dynamics. Our Medusa analysis only revealed three clades with consistent increases in diversification rates across all trees: skates (Rajiformes), South American freshwater stingrays (Potamotrygonidae), and ground sharks (Carcharhiniformes). For the MuSSE analyses, we then partitioned the tree into four diversification regimes (these three known radiations plus the background) and fit a MuSSE model in which the diversification rate parameters were allowed to vary among these four partitions. We also assigned each partition its own sampling fraction based on the most up-to-date taxonomic treatment (Weigmann 2016; Stein et al. 2018). Ideally, we would have fit an integrated model that included heterogeneity in rates due to both the trait and to unmodeled “background” variation (Beaulieu and

188 O'Meara 2016; Uyeda et al. 2017) but unfortunately no such approach is available for
189 multi-state traits.

190 As we described above, speciation (λ), extinction (μ), and transition rates (*q*) were
191 estimated for each of the four partitions separately. Because the estimations of
192 extinction rates from molecular phylogenies can be difficult (Rabosky 2010), we ran
193 two models: with state dependent extinction rates (1) unconstrained ($\mu_{egg\text{-}laying} \neq \mu_{live\text{-}}$
194 $_{bearing} \neq \mu_{matrotrophic}$) and (2) constrained to be equal ($\mu_{egg\text{-}laying} = \mu_{live\text{-}bearing} = \mu_{matrotrophic}$).
195 We report findings from the unconstrained model as there was no significant
196 difference between models, and we are interested in how variation in this rate may
197 affect overall diversification. Additionally, the main difficulty with estimating
198 extinction rates arises from unaccounted for diversification rate heterogeneity, which
199 we have minimized by *a priori* identification with medusa and subsequent
200 partitioning. We report speciation (λ), extinction (μ), net diversification ($r = \lambda - \mu$),
201 and transition rates (*q*). We treated reproductive mode as an ordinal multistate
202 character and accordingly did not allow unlikely transitions such as directly between
203 egg-laying and matrotrophic live-bearing. Models were run for 10,000 generations
204 with the first 1,000 generations discarded as burn-in, using an exponential prior with
205 a rate of $1/(2r)$ where *r* is the character state independent diversification rate (Fitzjohn
206 2012). We checked that all parameter estimates had effective sample sizes (ESS: the
207 number of independent draws from an MCMC chain) greater than 200. To account
208 for autocorrelation in the estimates of state dependent diversification, we also
209 examined the posterior distribution of differences in state dependent rates across all
210 chains.

211

212 *Evolutionary Covariation with Ecological Traits*

213 We used a threshold model to test for the evolutionary covariation between
214 reproductive mode and continuous ecological traits. The threshold model assumes
215 that state changes in an ordinal variable (e.g. egg-laying to live-bearing to
216 matrotrophy) occur when a threshold value of an underlying continuous latent
217 variable, such as body size, is reached. Thus, it can be used to model the evolutionary
218 covariation between ordinal and continuous traits (Felsenstein 2012). Accurate
219 estimation of evolutionary covariation requires a suitable number of transitions and
220 distribution of traits across the phylogeny (Maddison and Fitzjohn 2015). We focus
221 on sharks (superorders Galeomorphii and Squalomorphii; n = 292) to evaluate
222 evolutionary covariance between reproductive transitions and three ecological traits
223 (body size, depth, and latitude), because there is only one transition in parity and few
224 appearances of matrotrophy within Chimaeriformes and rays (Batoideii). We
225 estimated the evolutionary covariance using Bayesian methods, sampling from the
226 posterior distribution using a special Reduced Animal Model implemented in a mixed
227 effects modeling framework while accounting for phylogeny, using the package
228 `MCMCglmmRAM` (version 2.24) in R (Hadfield 2015). This approach is equivalent to
229 estimating evolutionary covariance using the threshold model (Felsenstein 2012;
230 Revell 2014). These models are a special case of generalized linear mixed effects
231 models where heritability, akin to Pagel's λ, is set to a value of one corresponding to
232 Brownian motion with respect to the phylogenetic tree (Freckleton et al. 2002;
233 Housworth et al. 2004). Twenty chains were run for 2 million generations with the

234 first 200,000 iterations discarded as burn-in, using priors with an inverse-Wishart
235 distribution and the residual covariance matrix set to zero (Hadfield 2015). Samples
236 were drawn every 500 iterations to avoid temporal autocorrelation in parameter
237 estimates. Chains were visually inspected to ensure convergence using `coda` (version
238 0.19-4) (Plummer et al. 2006), and posterior samples were summarized to generate
239 mean and 95% highest posterior densities (HPD) with effective samples sizes greater
240 than 1000. Models were run using three different treatments of reproductive mode
241 with the threshold family: binary parity mode (egg-laying versus live-bearing), binary
242 embryo trophic mode (lecithotrophic versus matrotrophic), and ordinal multi-state
243 reproductive mode (egg-laying, lecithotrophic live-bearing, and matrotrophic live-
244 bearing).
245
246 **Results**
247 *(1) Ancestral chondrichthyans laid eggs*
248 The first chondrichthyans almost certainly laid eggs, as there is a high level of
249 support for egg-laying as the ancestral state of reproductive mode in chondrichthyans
250 (>99% probability; Figure 1). There are multiple independent origins of live-bearing
251 (seven) and matrotrophy (15), from the superordinal to subgeneric level, with few
252 instances of reversals (one reversal from matrotrophy to lecithotrophic live-bearing;
253 Figure 2). Specifically, live-bearing appears to have evolved from egg-laying at: (a)
254 base of Rhinopristiformes and Myliobatiformes, (b) base of Squalomorphii, (c) base
255 of clade encompassing Brachaeluridae, Orectolobidae, and Rhincodontidae, (d) base
256 of Ginglymostomatidae, (e) within the genus *Bythaelurus*, (f) in *Galeus polli*, and (g)

257 basal to clade encompassing Pseudotriakidae, Triakidae, Hemigaleidae, and
258 Carcharhinidae (Figure 1). We found no evidence of reversals from live-bearing to
259 egg-laying (Figure 2A).
260 Matrotrophy appears to have evolved independently from lecithotrophic live-
261 bearing at least 15 times with one reversal: (a-c) one to three origins within guitarfish
262 and wedgefish (Rhinopristiformes), (d) basal to stingrays (Myliobatiformes), (e-f) one
263 to three origins within sleeper sharks (Somniosidae), (g) great lanternsharks
264 (*Etmopterus princeps*), (h) tawny nurse shark (*Nebrius ferrugineus*), (i) mackerel
265 sharks (Lamniformes), (j) Pseudotriakidae, (k-m) one to three origins within
266 houndsharks (Triakidae), and (n) base of requiem sharks (Carcharhinidae). There was
267 evidence of a single instance of reversal from matrotrophy to lecithotrophic live-
268 bearing in the sharptooth houndshark (*Triakis megalopterus*). Overall transitions from
269 egg-laying to live-bearing and to matrotrophy occurred at higher rates then reversals
270 across all partitions that contained multiple reproductive modes (Figure 2B,C).
271
272 *(2) Live-bearing and matrotrophy evolve with increasing maternal size*
273 Reproductive mode was related to body size, such that larger bodied species had a
274 higher probability of live-bearing and matrotrophic investment (Figure 3A). We
275 found positive covariation with body size, using the threshold model to test for
276 evolutionary covariance between discrete values of reproductive mode and
277 continuous ecological traits, (median = 0.16, 95% CI = 0.09 to 0.22; Effective Sample
278 Size = 3351) indicating transitions in reproductive mode are more prevalent in
279 lineages with larger body size (Figure 3A). The relationship with larger body size was

280 slightly stronger for the transition from egg-laying to live-bearing (0.37 [0.17 to
281 0.55]; ESS = 3332) than for lecithotrophy to matrotrophy (0.3 [0.14 to 0.48]; ESS =
282 3600).
283
284 *(3) Live-bearing and matrotrophy evolve in wide-ranging species at low latitudes*
285 Live bearing species are more prevalent in the tropics, specifically transitions
286 from egg-laying to live-bearing are more prevalent in lineages at lower latitudes
287 (minimum latitude: -0.68 [-1.25 to -0.01], ESS = 2915, Figure 3C; median latitude: -
288 0.46 [-0.9 to -0.03]; ESS = 3189; Figure 3D). However, there was little evidence that
289 transitions in reproductive mode are related to either median latitude (-0.06 [-0.16 to
290 0.04], ESS = 3600) or median depth (0.06 [-0.04 to +0.15]; ESS = 3600; Figure 3
291 B,D). Transitions to live-bearing and in reproductive mode were more prevalent in
292 lineages with larger latitudinal ranges (0.058 [0.08 to 1.0], ESS = 2580; 0.11 [0.04 to
293 0.18], ESS = 3956; Figure 3E).
294
295 *(4) Diversification rate is highest in matrotrophic lineages*
296 Overall, the evolution of live-bearing and matrotrophy is associated with greater
297 diversification (Figure 4A-C), mainly due to a high relative extinction rate in egg-
298 laying species (Figure 4A,B). The evolution of matrotrophy is associated with greater
299 diversification than egg-laying (2.4 times), and there is weak evidence for higher
300 diversification than lecithotrophic live-bearing lineages (1.27 times; Figure 5A). Egg-
301 laying lineages have high turnover driven by high extinction rates because speciation
302 rate is highest in egg-laying lineages (mean 0.046 lineages/MY) compared with

303 lecithotrophic live-bearing (mean 0.03 lineages/MY) and matrotrophic lineages
304 (0.026 lineages/MY; Figure 4A-C).
305 Within the three radiations, the connection between reproductive mode and
306 diversification is more nuanced. Within the ground sharks (Carcharhiniformes), egg-
307 laying lineages have a higher diversification rate than lecithotrophic live-bearing
308 lineages (Figure 4D-F, Figure 5B), driven by high speciation in egg-laying cat sharks
309 that are found mainly in deepwater. Speciation is particularly high in both the skate
310 radiation (0.078 lineages/MY; Figure 4G) and the South American freshwater
311 stingray radiation (0.070 lineages/MY; Figure (4J), potentially reflecting their
312 colonization of novel deep-water and freshwater habitats, respectively.
313
314 **Discussion**
315 Here, we reveal the first chondrichthyan was an egg-layer and there have been
316 numerous transitions toward live-bearing and matrotrophy. The evolution of live-
317 bearing and matrotrophy covaries with increasing body size and is more prevalent in
318 shallow waters of tropical latitudes. Further, the evolution of live-bearing, and to a
319 lesser extent matrotrophy, appears to have resulted in greater species diversification.
320 Next, we consider three questions: What is the sequence of reproductive mode
321 evolution? What ecological factors have driven the evolution of live-bearing and
322 matrotrophy? Is chondrichthyan speciation and diversification explained by
323 viviparity-driven conflict or by novel ecological opportunity?
324
325 *The sequence of reproductive mode evolution*

326 We find support for egg-laying as the ancestral reproductive state, with numerous
327 independent origins of live-bearing and matrotrophy with few instances of reversals.
328 Previous analyses of reproductive evolution in chondrichthyans have been limited by
329 the available morphological phylogenetic hypotheses, particularly on the
330 phylogenetic position of rays (Shirai 1992; Naylor et al. 2012). Some morphological
331 analyses placed the rays as a highly derived group within the Squalimorph sharks, but
332 the emerging consensus from molecular data is that rays and sharks are sister taxa
333 (Naylor et al. 2012; Stein et al. 2018). With this new molecular tree, we find
334 transitions in reproductive mode are generally toward live-bearing or matrotrophy
335 though this not a strictly linear progression as reversals are infrequent though
336 plausible. We expect that improved biological sampling and phylogenetic
337 reconstructions may yield further examples, particularly in groups displaying
338 subgeneric transitions that are currently poorly phylogenetically resolved (e.g. within
339 catsharks, Scyliorhinidae). We find no support for live-bearing being ancestral nor
340 evidence of a high rate of reversals to egg-laying from live-bearing that this would
341 necessitate, as suggested previously (Musick and Ellis 2005). Interestingly, a similar
342 controversy has occurred in squamates, a group with highly labile reproductive
343 modes. The most likely model suggested an early origin of viviparity with a high rate
344 of reversals (Pyron and Burbrink 2014), though this result has been questioned based
345 on the choice of phylogenetic hypotheses and morphological features of live-bearing
346 lineages (Blackburn 2015a,b; Wright et al. 2015). Consequently, support remains for
347 the conventional hypothesis that the ancestral state was oviparity (Blackburn 2015b).
348 Live-bearing chondrichthyans, particularly lecithotrophic live-bearers, develop within

349 an egg envelope or candle which is similar to, but thinner than, that found in egg-
350 laying species (Castro 2009; Conrath and Musick 2012). This retention of the
351 morphological machinery for egg production could make reversals to egg-laying
352 feasible, and may allow for some of the subgeneric reproductive diversity seen within
353 catsharks (Scyliorhinidae). But these data, along with the emerging consensus in
354 reptiles, suggests that egg-laying is the ancestral vertebrate condition.

355 Transitions between matrotrophic modes can also occur, for example in hound
356 sharks (Family Triakidae) muccoid histotrophy can be used in lieu of, or in concert
357 with, placentotrophy (Hamlett et al. 2005) – resulting in intergeneric reproductive
358 diversity (López et al. 2006). Similarly, lecithotrophic live-bearing and some forms
359 of matrotrophy, particularly muccoid histotrophy found in Rhinopristiformes and
360 some sharks, may not represent discrete character states but rather a continuum that
361 will be revealed only with more detailed histological work in this lineage. Muccoid
362 histotrophy can be difficult to distinguish due to a paucity of uterine morphological
363 specializations and accurate measurements of ash-free dry weight of embryos and
364 ova. In several species of squaliform sharks the change in organic mass from ovum to
365 embryo has been used to identify muccoid histotrophy (Paiva et al. 2011; Cotton et al.
366 2014), though there is uncertainty given the exact threshold value of change in mass
367 that should be used to distinguish between modes (Frazer et al. 2012). Thus, large
368 groups predominantly composed of lecithotrophic live-bearing species (i.e.
369 Rhinopristiformes, Squalimorphii) may actually contain a greater diversity of
370 maternal investment than currently measurable or dealt with in this analysis. Despite
371 this uncertainty, chondrichthyans still exhibit remarkably labile reproductive modes

372 compared with other vertebrate groups, more similar to the transition rates seen in the
373 much larger clade Squamata (~10,000 species with >150 origins of live-bearing and 6
374 origins of matrotrophy) (Blackburn 2015a).
375
376 *Evolutionary correlates of reproductive mode*
377 Here we show that in sharks live-bearing and maternal investment has evolved in
378 association with larger body size, tropical latitudes, and increasing latitudinal range,
379 while there is little evidence of association with depth. We speculate that body size
380 may, in effect, be capturing allometric differences in predation pressure and access to
381 food resources, which have been hypothesized as drivers of reproductive evolution in
382 smaller more tractable freshwater livebearers (Trexler and Deangelis 2003; Marsh-
383 Matthews and Deaton 2006; Van Dyke et al. 2014). It seems that, in chondrichthyans
384 at least, the origin of live-bearing necessitates an increase in body size to
385 accommodate retained embryos throughout gestation in limited internal body space, if
386 larger offspring size is optimal (Blackburn 2015a). As a result, lecithotrophic live-
387 bearing sharks and rays typically have fewer, but larger, pups that are presumably
388 subject to less predation pressure and juvenile mortality as a result (Conrath and
389 Musick 2012; Kindsvater et al. 2016).
390 Despite detecting a relationship between reproductive mode and body size and
391 latitude in extant chondrichthyans (Rigby and Simpfendorfer 2013), our ability to test
392 for evolutionary covariation is hindered by the phylogenetic clustering of traits and
393 the use of depth and latitude as proxies for environmental temperature and resource
394 availability. As a result, it is currently difficult to disentangle evolutionary

395 hypotheses, as large lineages with similar character states may reflect a single origin
396 and subsequent coinheritance rather than a functional evolutionary covariation
397 (Maddison and Fitzjohn 2015). Rays exhibit an interesting transition – consistent with
398 the relationship we have revealed in sharks – from deep egg-laying skates to shallow
399 shelf and coastal live-bearing lineages, including: electric rays (Torpediniformes),
400 guitarfishes, wedgefishes, and sawfishes (Rhinopristiformes), and matrotrophic
401 stingrays (Myliobatiformes). However, our power to test for plausible correlations
402 between reproductive evolution and depth or latitude due to thermal physiology
403 (Dulvy 1998), predation (Harper and Peck 2016), and productivity are limited
404 because the batoids lineage contains a single origin of live-bearing and only one
405 certain origin of matrotrophy.
406
407 *Viviparity-driven conflict versus novel ecological opportunity*
408 At the large, coarse scale there appears to be evidence that radiations into novel
409 deepwater (Sorenson et al. 2014) or novel and highly dynamic fragmented freshwater
410 habitats (Lovejoy 1996; Lovejoy et al. 1998, 2006), rather than of reproductive mode
411 generally affecting diversification rates across all chondrichthyans. While
412 diversification rates are higher in live-bearing and matrotrophic lineages across the
413 main partition of the tree, resulting genomic conflicts may be a weak driver of
414 diversification compared to ecological forces in chondrichthyans.
415 The elevated diversification rates seen in skates (Rajiformes), South American
416 Freshwater stingrays (Potamotrygonidae), and ground sharks (Carcharhiniformes)
417 appear to be related to colonization of new ecological space. However, in each case,

418 the ecological space is different – skates radiated into deepwater habitats with the
419 opening up of the Atlantic Ocean (McEachran and Miyake 1990), subsequent
420 isolation of ocean basins (Long 1994), limited egg and adult dispersal ability
421 (McEachran and Miyake 1990), and a high degree of spatial niche differentiation
422 (Bizzarro et al. 2014; Humphries et al. 2016). By contrast the Neotropical
423 potamotrygonid stingrays (and other fish lineages) colonized freshwater upon the
424 closure of the isthmus of Panama (Lovejoy 1996, 1997; Lovejoy et al. 1998; de
425 Carvalho and Lovejoy 2011), radiating into novel and highly fragmented freshwater
426 habitat potentially facilitated by the emergence of unique morphological adaptations
427 (Kolmann et al. 2016). The increased diversification in ground sharks is driven by
428 both reinvasion of deepwater by catsharks (Scyliorhinidae) and the colonization of
429 shallow water coastal habitats (coral reefs and associated inshore and estuarine
430 habitats) by requiem sharks (Carcharhinidae) (Sorenson et al. 2014). At present, the
431 weight of evidence suggests that clade-specific increases in diversification rate are
432 associated with new ecological space, rather than being systematically driven by
433 reproductive evolution *per se* – nevertheless, we await stronger tests of this
434 hypothesis when more extensive phylogenies become available. Future investigations
435 of genomic conflict-driven speciation may require refined measurements of
436 ecological variation (Burin et al. 2016) or life history (Marki et al. 2015) as drivers of
437 diversification with tests restricted to smaller groups.
438
439 *Conclusions*

The evolution of chondrichthyan reproductive modes, ranging from egg-laying to live-bearing and matrotrophy, appears strongly related to body size and temperature-related biogeography. While patterns of species diversification in three major radiations appear to be more strongly driven by colonization of novel habitats, the evolution of the diversity of reproductive modes remains a fruitful area of research. Parent-offspring conflict over resources during development and subsequent antagonistic coevolution is an intriguing potential driver of reproductive mode evolution worthy of further investigation. Chondrichthyans are an ideal group to test for this given the diversity of reproductive modes and the frequency of polyandry, though this requires a better understanding of maternal-fetal interactions across a wider range of species. Future research could focus on improved measures of maternal investment, particularly for identifying the continuum on which lecithotrophic live-bearing and histotrophic matrotrophy may be expressed. Combined with further refinement of phylogenetic hypotheses with more extensive taxon sampling will help to clarify patterns of energetic investment, the degree of income breeding in live-bearing species, and inter and intra specific plasticity. More generally, we anticipate that this taxon will yield profound insights into the interplay between reproductive life history evolution, ecology, and the biogeographic patterning of species diversity across the Earth's oceans.

Ethics Statement

No live animals or human subjects were used in this study.

Data Availability

All phylogenetic trees are available at www.vertlife.org/sharktree.

Acknowledgements

We thank A. Mooers and B. Crespi for helpful discussion regarding this study. We thank J. Hadfield and L. Revell for their guidance on the analysis of evolutionary covariation. This study was funded by the Natural Science and Environment Research Council Discovery grants to NKD and MWP, and a Canada Research Chair to NKD. KEY acknowledges funding from UNCW and a CMS Pilot Project Grant during the writing of this manuscript.

Author Contributions

CGM, KEY and NKD conceived the study; CGM and MWP designed the analytical approach; CGM performed the analyses; CGM, MWP, KEY and NKD drafted the manuscript. All authors gave final approval for publication.

Competing Interests

We have no competing interests.

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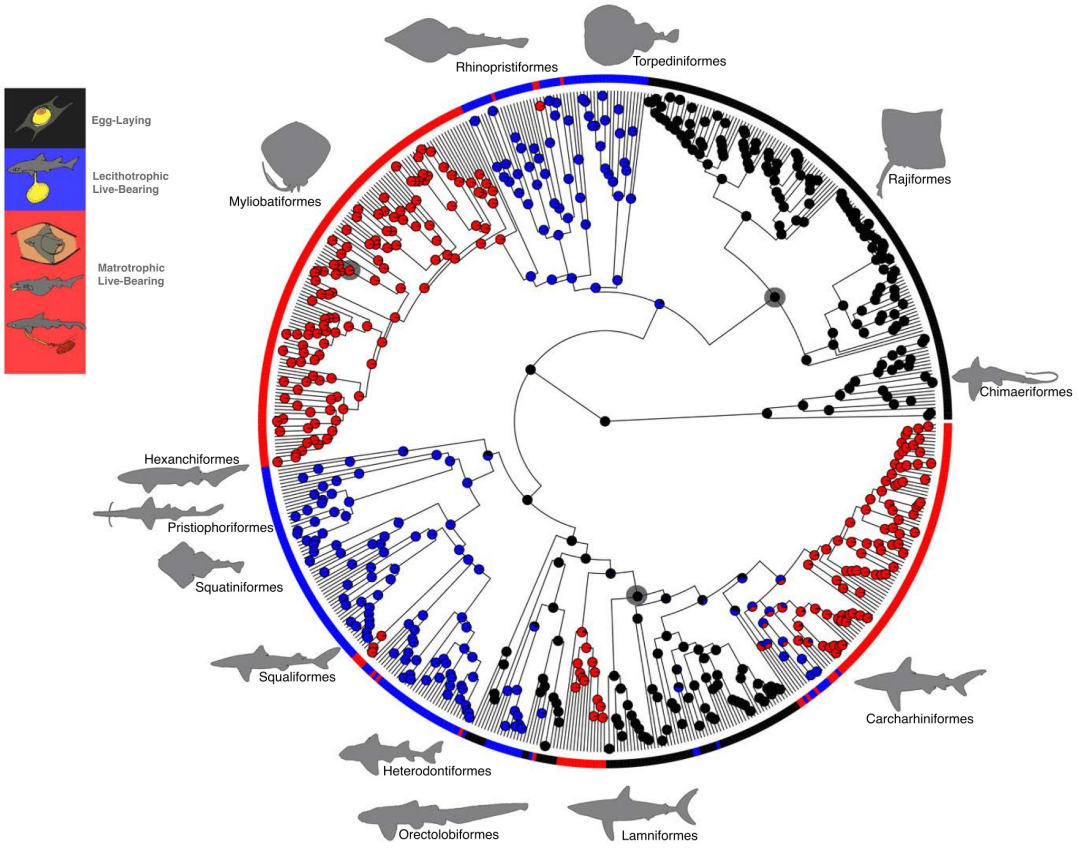
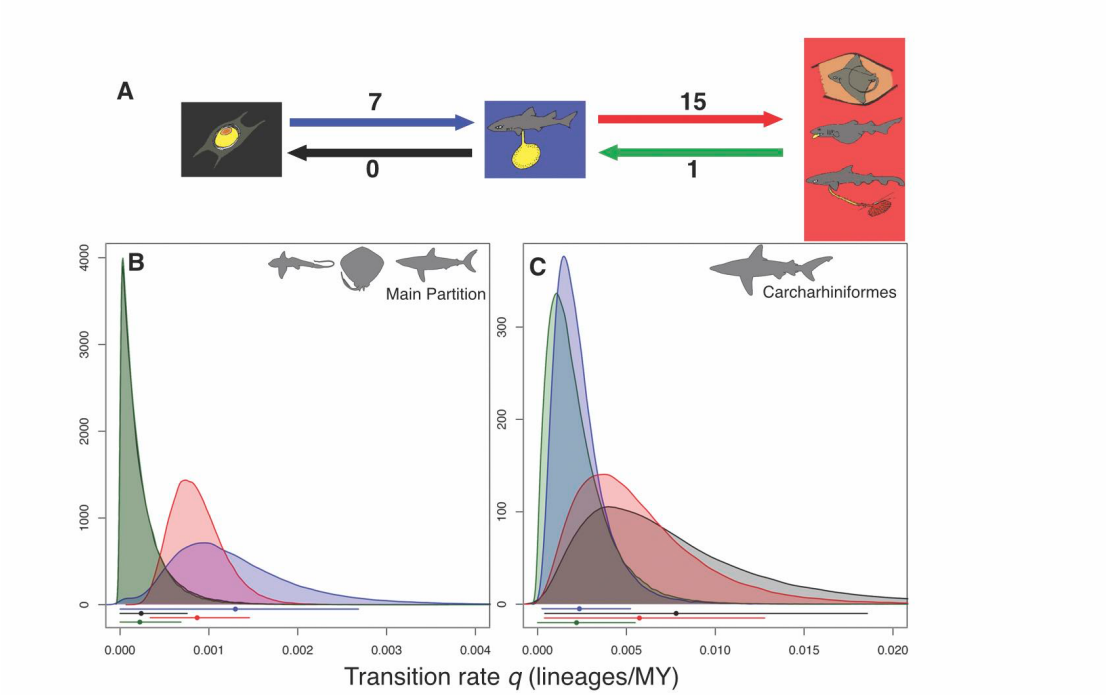
688 **Figure Legends**
689 **Figure 1**. Ancestral state reconstruction reproductive mode on a representative tree of
690 610 species of chondrichthyans. Pie symbols represent the likelihood of the character
691 state for each node being egg-laying (black), live-bearing (blue), or matrotrophic
692 (red). Dark grey symbols denote the partitions encompassing diversification rate
693 shifts. Silhouettes depict representative species from the major orders.
694
695 **Figure 2.** The number (A) evolutionary transitions in reproductive mode across
696 chondrichthyans and transition rates between modes in (B) main partition of the tree
697 and (C) within Carcharhiniformes. Origins of live-bearing from egg-laying are
698 depicted in blue with reversals in black, and origins of matrotrophy from
699 lecithotrophic live-bearing are depicted in red with reversals in green. Bars and
700 shaded regions in represent the 95% posterior density of transition rate estimates.
701
702 **Figure 3.** Coefficient plots of evolutionary covariation between (A) body size, (b)
703 median depth, (c) minimum latitude, (d) median latitude, and (e) latitudinal range
704 from MCMCglmm models. Black circles denotes egg-laying vs. live-bearing, grey
705 circles denote live-bearing vs matrotrophy, and open circles denote reproductive
706 mode as an ordinal variable with all three character states. Horizontal bars represent
707 the 95% confidence intervals of the mean posterior estimate.
708
709 **Figure 4.** Posterior densities of parameter estimates from MuSSE model showing
710 state dependent speciation (λ), extinction (μ), and net diversification rate (*r*) from the
711 main partition of the tree (A-C), within the order Carcharhiniformes (D-F), within the
712 order Rajiformes (G-I), and within the family Potamotrygonidae (J-L). Egg-laying is
713 depicted in black, live-bearing in blue, and matrotrophy in red. Bars and circles
714 represent the mean and 95% confidence interval of the posterior mean estimate.
715
716 **Figure 5.** Differences between posterior densities of parameter estimates from
717 MuSSE model showing the differences in reproductive mode dependent net
718 diversification rates (*r*) from the main partition of the tree (A), and within the order
719 Carcharhiniformes (B). Live-bearing relative to egg-laying is depicted in black,
720 matrotrophy relative to live-bearing in blue, and matrotrophy relative to egg-laying in
721 red. Bars and circles represent the mean and 95% confidence interval of the posterior
722 mean estimate. The vertical lines denote no difference between the diversification
723 rates for the reproductive modes being compared.
724

Figure 1.


Egg-Laying
Lecithotrophic Live-Bearing
Matrotrophic Live-Bearing
Rhinopristiformes
Torpediniformes
Rajiformes
Myliobatiformes
Chimaeriformes
Hexanchiformes
Pristiophoriformes
Squatiniformes
Squaliformes
Carcharhiniformes
Heterodontiformes
Orectolobiformes
Lamniformes

728 **Figure 2.**



729
730

Figure 3.

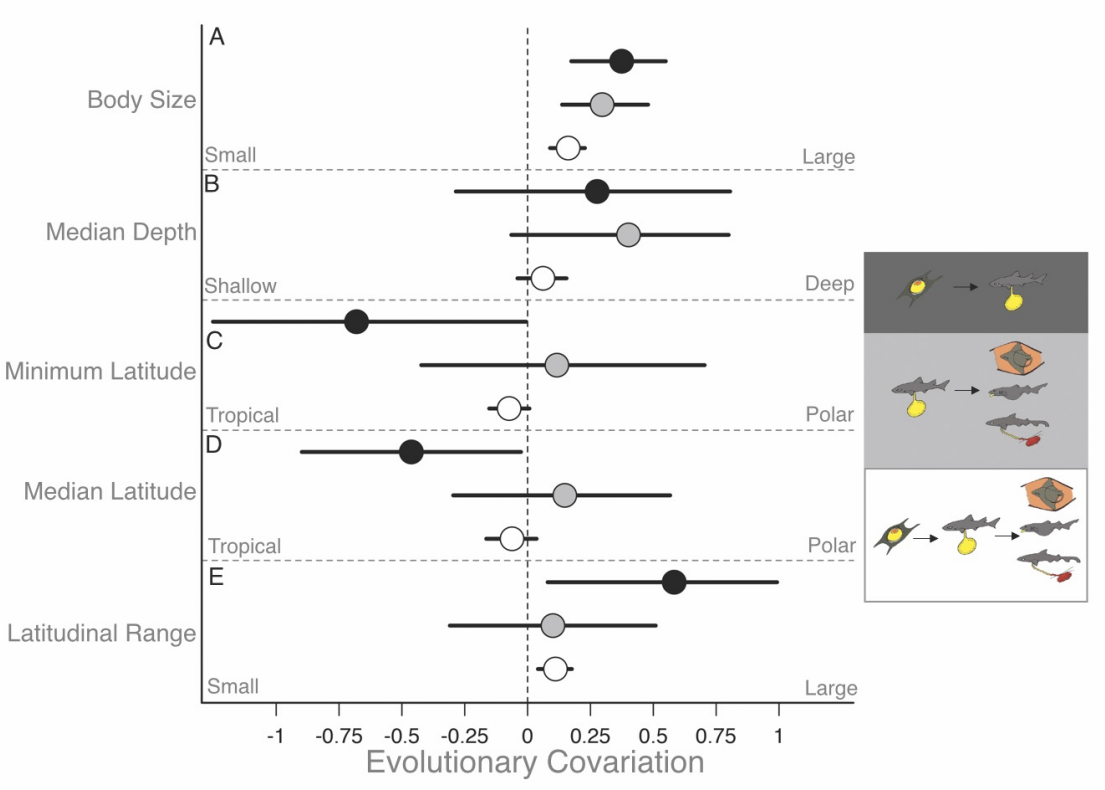
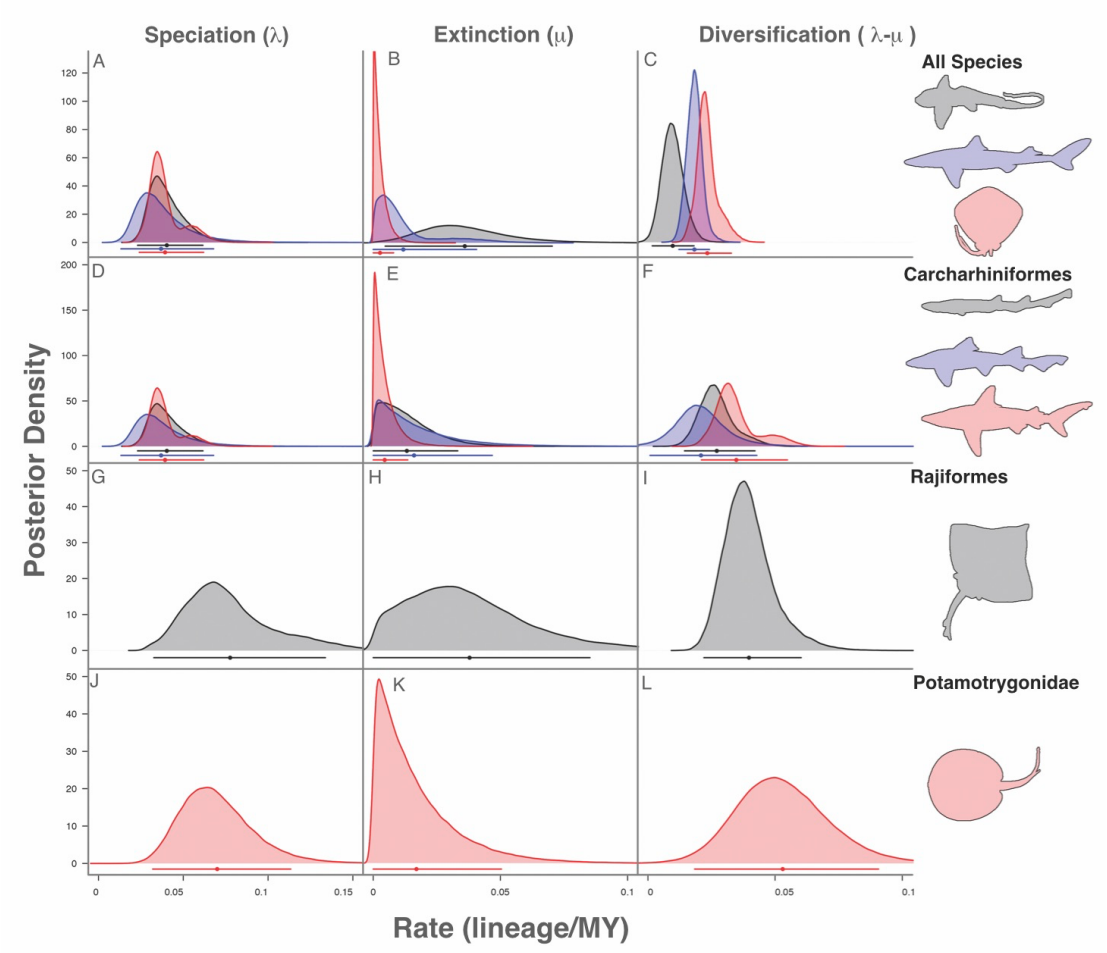
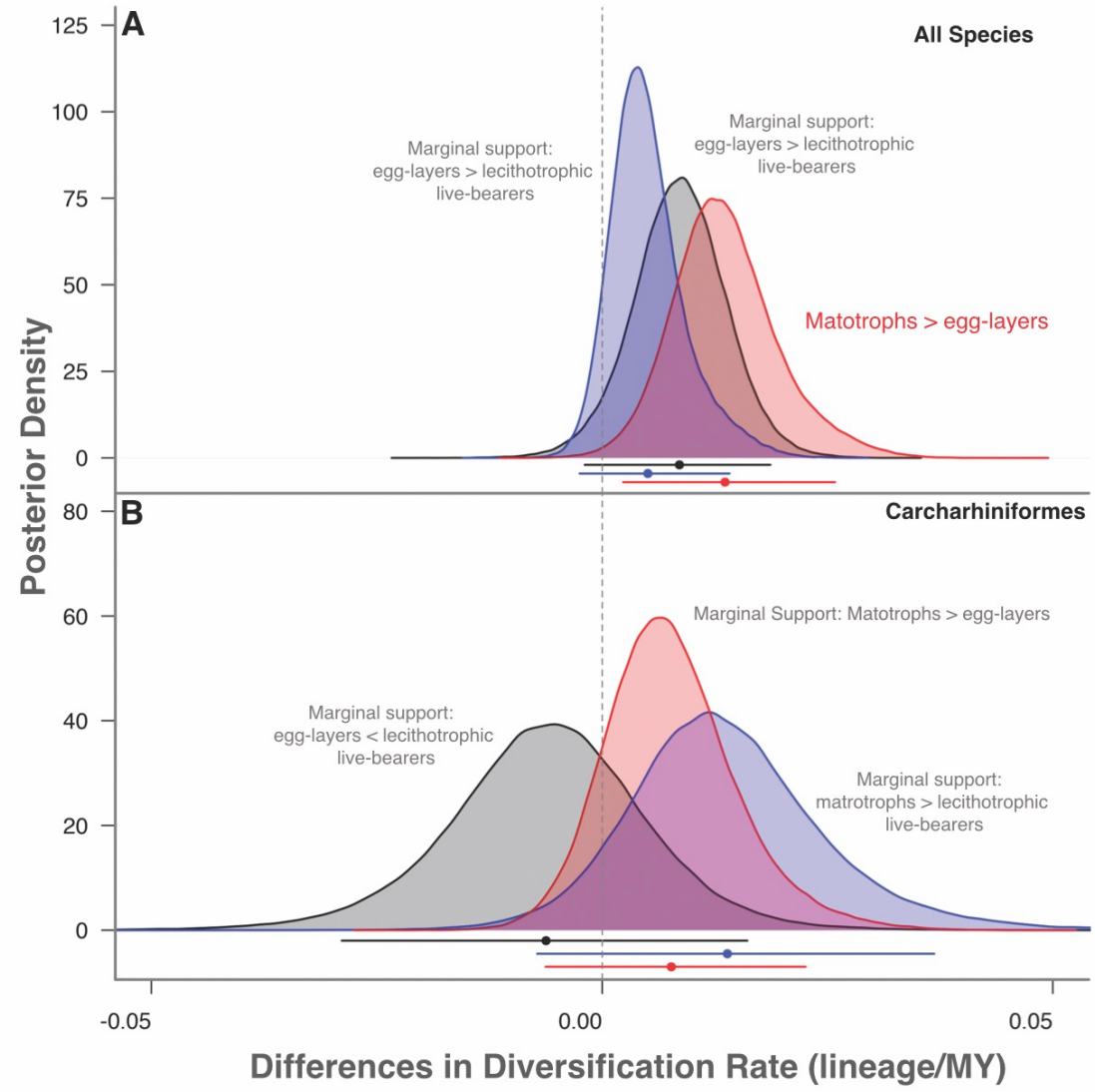

A
Body Size
Small
Large
B
Median Depth
Shallow
Deep
C
Minimum Latitude
Tropical
Polar
D
Median Latitude
Tropical
Polar
E
Latitudinal Range
Small
Large
-1
-0.75
-0.5
-0.25
0
0.25
0.5
0.75
1
Evolutionary Covariation

Figure 4.


Speciation (λ)
Extinction (μ)
Diversification (λ-μ)
All Species
Carcharhiniformes
Rajiformes
Potamotrygonidae
Posterior Density
Rate (lineage/MY)
A
B
C
D
E
F
G
H
I
J
K
L

737 **Figure 5.**



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