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Dietary morphology of two island-endemic murid rodent clades is consistent with persistent, incumbent-imposed competitive interactions

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A lineage colonizing a geographic region with no competitors may exhibit rapid diversification due to greater ecological opportunity. The resultant species diversity of this primary-colonizing (incumbent) clade may limit subsequent lineages' ability to persist unless these non-incumbent lineages are ecologically distinct. We compare the diversity in diet-related mandibular morphology of two sympatric murid rodent clades endemic to Luzon Island, Philippines—incumbent Phloeomyini and secondary-colonizing Chrotomyini—to the mandibular morphological diversity of Sahul Hydromyini, the sister clade of Chrotomyini and the incumbent murid lineage on the supercontinent of Sahul. This three-clade comparison allows us to test the hypothesis that incumbent lineages can force persistent ecological distinction of subsequent colonists at the time of colonization and throughout the subsequent history of the two sympatric clades. We find that Chrotomyini forms a subset of the diversity of their clade plus Sahul Hydromyini that minimizes overlap with Phloeomyini. We also infer that this differentiation extends to the stem ancestor of Chrotomyini and Sahul Hydromyini, consistent with a biotic filter imposed by Phloeomyini. Our work illustrates that incumbency has the potential to have a profound influence on the ecomorphological diversity of colonizing lineages at the island scale even when the traits in question are evolving at similar rates among independently colonizing clades.

1. Introduction

In classic examples of adaptive radiation, a single lineage diversifies over time to occupy an array of novel niches, resulting in a group of ecologically and phenotypically distinct species [1–3]. In such cases, phenotypic diversification is facilitated by adaptive evolution into unoccupied niche space. However, the diversification dynamics that occur when two or more independent, ecologically similar lineages colonize the same system at different times require additional study across phylogenetic and temporal scales [4–8]. In this case, primary colonists (the incumbent clade) may prevent phenotypically similar species from invading the system, suggesting that only lineages that are sufficiently distinct from the primary colonists can invade and diversify [9,10]. This process of biotic filtering could enhance the overall ecological diversity of the system, while at the same time limiting the potential phenotypic diversity attained by secondary colonists. In other words, an incumbent clade may exclude very similar lineages from colonizing the system and prevent reasonably similar lineages from realizing otherwise attainable phenotypic diversity [11]. Alternatively, lineages that are ecologically similar may still be able to colonize the system, but exhibit rapid divergent ecological adaptation to specialize on the unoccupied areas of ecological and morphological space to minimize competition with the incumbent lineage [12,13].

In natural systems, testing whether incumbent lineages have such effects on the diversity of subsequent colonists is best accomplished in systems that meet certain rare requirements. First, the colonizing lineages should be ecologically similar enough that one could reasonably act as a biotic filter or competitor for the other. This requirement is most likely to be met by closely related lineages that colonize a system over a relatively short time span, because phylogenetic inertia tends to result in two lineages that resemble one another more than they do distantly related species [14–16]. Second, there should be some way to assess the potential phenotypic diversity that these lineages could attain were they to diversify apart from one another. Assessing the array of potential phenotypes achievable by any clade is a daunting challenge, and it is important to disentangle any intrinsic constraints on the 'evolvability' of a clade from external forces of interest, such as competition, that could limit the evolution of otherwise attainable phenotypes [17]. One way to meet this requirement is to examine diversification of a large clade, part of which is evolving in the presence of potentially competing lineages and part of which is not. Island archipelagoes, which bring together novel assemblages of organisms through different patterns of dispersal and colonization success [9,18], provide one such promising arena for investigation, and we present one example here.

We use three clades of murid rodents, specifically in subfamily Murinae (rats and mice), to assess whether incumbent lineages influence the success and ecomorphological diversity of subsequent colonists. Two of these clades are found on Luzon Island, the largest Philippine island, which has been colonized by murids at least five times [19,20]. The earliest two colonizations, Phloeomyini (*sensu* [21]) and Chrotomyini (*sensu* [20]), occurred approximately 12.8 and 8.4 Ma, respectively. The species diversity of just these two clades comprise the vast majority of native, non-flying mammal species on the island (approx. 85% [22]), providing a rich system to compare ecomorphological diversity in a phylogenetic framework. While direct, quantitative dietary information of these two clades is limited (e.g. [23–26]), their members exhibit extensive morphological disparity that corresponds to apparent differences in trophic ecology both between each clade and among genera within clades [22]. Decades of standardized field studies across the island of Luzon have established that members of these clades occur sympatrically, and in most cases, syntopically with one another, establishing the possibility that these lineages may compete for access to suitable habitat and food sources [22]. The extensive sampling of this island yields an understanding of community composition that is unmatched by other mammalian radiations.

The biogeography of murid rodents in Australasia also presents unique opportunities to test incumbency effects in Philippine murids. Notably, Chrotomyini is the sister clade to a large group of murid rodents that radiated throughout Australia, New Guinea and Melanesia [27,28]. This group, referred to here as the Sahul Hydromyini, is the first colonizing murid lineage on the islands they inhabit and includes both tiny and giant herbivorous rodents (resembling Phloeomyini) as well as shrew-like, primarily insectivorous rodents (resembling Chrotomyini) [29,30]. This system thus seems to meet both requirements for investigating whether incumbent lineages can limit phenotypic diversification: the two Luzon Old Endemic (LOE) clades, Phloeomyini and Chrotomyini, are sufficiently closely related to be likely competitors, and the Sahul hydromyine radiation's close relationship to Chrotomyini provides the best available opportunity to examine how Chrotomyini might have evolved had they colonized a similar system in the absence of murid competitors.

Here, we analyse mandibular morphology (as a proxy for dietary diversity) of these lineages in a phylogenetic comparative framework to test whether the incumbent lineage on Luzon (Phloeomyini) may have shaped phenotypic diversity of the secondary colonizing lineage (Chrotomyini) as compared to its sister taxon (Sahul Hydromyini). We focus on mandible (lower jaw) shape as an ecologically relevant trait that correlates with food-processing strategy and has proven to be useful for delineating dietary differences among species [31–34]. We then compare the variation in mandibular shape between the two sympatric clades to the variation between the two incumbent clades to determine whether the contemporary morphological diversity of Chrotomyini, when compared to that of Chrotomyini and Sahul Hydromyini together (hereafter referred to as Hydromyini, *sensu* [21]), is biased in a way that minimizes overlap with the areas occupied by incumbent Phloeomyini. Then, using a novel phylogeny of Hydromyini, we infer the ancestral morphology of Philippine chrotomyines to estimate its ecomorphological distinction from incumbent Phloeomyini at the time of chrotomyine colonization.

2. Material and methods

(a) Taxon sampling

Our molecular dataset consists of DNA sequence data obtained from several previous studies, with additional sequencing performed as needed (electronic supplementary material, Supplementary methods). We obtained 15 tissue samples from the American Museum of Natural History (AMNH), the Field Museum of Natural History (FMNH) and the Australian National Wildlife Collection (ANWC) to supplement existing molecular sampling. In total, our molecular phylogeny contained 132 samples representing 131 species of rodents endemic to the Philippines, rodents endemic to Sahul, six outgroup taxa from family Muridae, and *Chiropodomys gliroides*, which is the sister lineage to Chrotomyini and Sahul Hydromyini. Some lineages within Chrotomyini and Phloeomyini have dispersed to other islands in the Philippine archipelago. We included nine of these species in our phylogenetic analyses but did not include them in our morphometric analyses as they occur on islands other than Luzon, which have substantially different faunal, especially mammalian, community assemblages (compare e.g. [25,35]). Although these clade names technically refer to the old endemics distributed across the Philippine archipelago, throughout the manuscript, we use the names Chrotomyini and Phloeomyini to refer specifically to the Luzon members of these clades.

Our morphometric dataset comprised measurements of mandible shape from LOE and Sahul hydromyine rodents. For the LOE rodents, we used the mandibular morphometric dataset containing 337 specimens representing 41 rodent species (29 chrotomyine species and 12 phloeomyine species) obtained in a previous study [36]. This dataset included all described LOE rodent species and two undescribed chrotomyine species. The

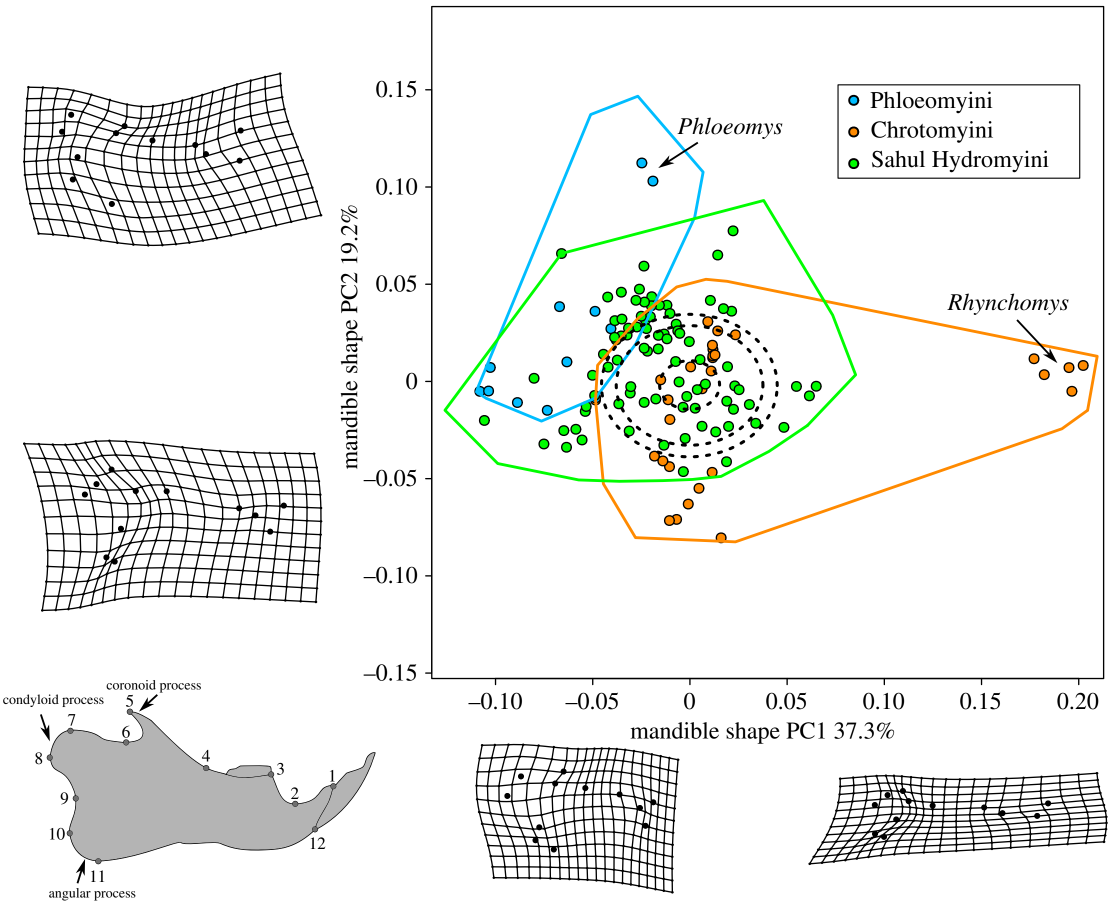

Figure 1. PC1 and PC2 of mandibular shape of LOE (Phloeomyini + Chrotomyini) and Sahul hydromyine rodents, with Sahul Hydromyini projected onto the LOE component space. Opaque enclosed circles represent species averages. Convex hulls indicate the extremes of each clade's individual specimens. Dashed ellipses indicate posterior density intervals for estimated ancestral state of Hydromyini (i.e. crown ancestor of Chrotomyini + Sahul Hydromyini) with increasing radii representing 50%, 90% and 95% highest posterior density, respectively. Percentages on axis labels indicate LOE (i.e. excluding Sahul Hydromyini) dataset variation explained by that axis. Thin-plate splines along axes show specimens with extreme scores and illustrate differences along these axes. Bottom left: lateral view of a mandible indicating landmarks taken. (Online version in colour.)

Sahul hydromyine dataset consisted of 270 specimens representing 85 species for approximately 58 and 86% coverage of all currently described species and genera, respectively. These Sahul specimens were obtained from the AMNH, FMNH and the United States National Museum of Natural History (USNM). We aged and photographed specimens as described by Rowsey *et al*. [36]. For the Sahul rodents, we attempted to sample four individuals per species with an even sex distribution but relaxed this constraint when suitable specimens were limited. The list of 607 sampled murid specimens and associated metadata can be found in the electronic supplementary material, table S1.

(b) Geometric morphometric analysis

We used the geometric morphometric analytical procedure previously described for LOE rodents [36] to collect and analyse morphometric data for the Sahul hydromyines. We collected 12 fixed landmarks along the outline of the mandible using the R package *geomorph* v. 3.0.5 [37–39] (figure 1). These landmarks were collected so as to be congruent to those collected by Rowsey *et al.* [36], where the pattern of morphometric dissimilarity between the two Luzon old endemic clades was first documented. In the cases where a specimen's mandible was damaged, we estimated the positions of missing landmarks using the thin-plate spline algorithm implemented in the estimate.missing function of *geomorph*, using other individuals of the same species as a reference when available or members of the same genus when conspecific specimens were unavailable [40]. The complete dataset of landmark configurations, including LOE landmark data, were subjected to a generalized Procrustes analysis (GPA [41,42]) from which we also retained log-transformed centroid size, or the average distance between each landmark and the centre of the landmark configuration, as a proxy for body size.

(c) Testing contemporary ecological distinction

We compared the overlap between Chrotomyini and Phloeomyini (sympatric clades) to Phloeomyini and Sahul Hydromyini (incumbent clades). To do this, we first averaged both the GPA-transformed landmark configurations and centroid size by species and performed a PCA on the correlation matrix using these average values. We then calculated the median pairwise distance between the 29 species in Chrotomyini and 12 species in Phloeomyini. This distance was compared to a distribution of distances between Phloeomyini and 10 000 permuted samples of 29 Sahul hydromyine species values to determine whether the distance between the two sympatric clades was significantly greater than that of the two incumbent clades.

(d) Phylogenetic inference

We used PartitionFinder v. 2.1.1 [43] to determine the best-fitting scheme of nucleotide partitions and substitution models in our seven-locus dataset (electronic supplementary material, Supplementary methods) using the Bayesian information criterion (BIC [44]). We selected locus-level candidate partitions, specified linked branch lengths among partitioning schemes and selected only substitution models that were supported by BEAST 2 [45], choosing the best scheme using the 'greedy' algorithm (electronic supplementary material, table S2). We used the resultant partitioning scheme along with secondary calibration points to infer the phylogeny of these rodents with divergence dates estimated in absolute time using BEAST v. 2.5.2 [45], (electronic supplementary material, Supplementary Methods).We placed a normal prior on the crown ages of Phloeomyini (mean: 11.1, standard deviation: 0.825), Chrotomyini (mean: 7.22, standard deviation: 0.506) and Sahul Hydromyini (mean: 9.00, standard deviation: 0.455) based on the distribution of ages inferred by Rowsey *et al.* [13], which were themselves dated based on thoroughly examined fossil data [14]. A recent study using simulated sequences illustrates that secondary calibrations may lead to node age estimates that are erroneously younger and exhibit lower variance, giving the false impression of greater certainty than primary calibrations [15]. We acknowledge this as a limitation of our sampling scheme, as no fossil data are available for the level of divergences necessary to calibrate divergences among our sampled clades. Nevertheless, we obtained nodal age estimates consistent with the study from which the secondary estimates were obtained [13].

(e) Testing ancestral ecological distinction

To test ancestral ecomorphological distinction between the two LOE clades, we needed to not only estimate a distribution of probable ancestral phenotypes of the common ancestor of Chrotomyini and Sahul Hydromyini, but also determine whether the contemporary distances between members of the two LOE (sympatric) clades were similar to the difference between Phloeomyini and the ancestor of the secondary-colonizing clade. This analysis was motivated by the observation that the LOE clades occupy almost entirely different regions of morphospace on PC 1–2, which together account for 56.5% of the variation in mandible shape in these two rodent clades [36]. We performed a PCA on LOE rodent mandibular shape (i.e. excluding Sahul Hydromyini) and used the rotation matrix from this PCA to project the Sahul hydromyine morphometric data into the LOE component space. We then took the average component score of each Sahul hydromyine species. We chose not to incorporate the Sahul Hydromyini in eigenvector computation because doing so would have caused variation in this allopatric clade to influence the major axes of variation of the two focal clades, and thus the extent of their overlap along this subset of component space. Instead, rotating the Sahul Hydromyini to the eigenvectors computed for the LOE clades displays how the allopatric, incumbent murid lineage varies along the major axes of mandibular shape of the sympatric focal clades.

We performed our ancestral state estimation procedure using MECCA, implemented in the *geiger* package, which employs a combination of MCMC and approximate Bayesian computation (ABC) algorithm to jointly estimate diversification rate parameters and trait evolution parameters [46]. See the electronic supplementary material, Supplementary methods for a detailed description of our MECCA workflow. Using the distribution of 950 estimated ancestral states for PC1 and PC2 from the 95% highest posterior density (HPD) of the ABC-MCMC model inference, we calculated the distance between extant Phloeomyini and these ancestral trait values using 10 000 combinations of ancestral state estimates along PC1 and PC2 sampled with replacement. We computed the distance between these ancestral states and contemporary Phloeomyini to allow for the greatest potential for overlap between this ancestral distribution and the incumbent Luzon clade and thus a more conservative test of biotic filtering. This distribution of distances was compared to the median pairwise distance in PC axes 1 and 2 of contemporary Phloeomyini and Chrotomyini.

(f) Testing adequacy of models of morphological evolution

We performed model adequacy tests in the *arbutus* package in R to determine how well the parameters of the single- and two-rate Brownian motion model inferred using MECCA describe the expected variation in these traits from the pruned time-scaled tree used to estimate ancestral chrotomyine morphological overlap [47]. We calculated six statistics describing aspects of trait evolution based on the mandibular morphometric data and compared these statistics to distributions generated by simulating trait evolution along the branches of the unit tree, which is a rescaled phylogenetic tree in which each branch is scaled equal to the amount of variance expected to accumulate along that branch (effectively standardizing the distribution of trait data to be equal to a Brownian motion model of trait evolution with a rate of 1 [47]). Of the six statistics modelled, we were particularly interested in those with potential influence on inferred ancestral states—C_{VAR}, S_{VAR} and S_{ASR}—that, respectively, correspond to unexplained variation in the model due to rate heterogeneity among branches, branch length error in the phylogenetic tree and evolutionary rate correlated with trait value. Recovering significant deviations in these statistics influences expected patterns of nodal states that could extend to the inference of the root node state in our analysis. For detailed information regarding this model adequacy procedure, as well as statistic definitions, see electronic supplementary material, Supplementary methods and table S3.

3. Results

(a) Testing incumbency-influenced morphospace exclusion

The two clades of Luzon rodents do not share mandibular shapes with each other, but Sahul hydromyines exhibit shapes that overlap broadly with both Luzon clades (figure 1; full PCA results in electronic supplementary material, table S4). Phloeomyine mandibles are typically stouter and more robust than chrotomyine mandibles, which are typically more gracile and exhibit greater concavity in the regions of the mandible between the coronoid, condyloid and angular processes. By contrast, Sahul hydromyines broadly overlap with both clades and include morphotypes similar to both Phloeomyini and Chrotomyini, although the range of Sahul hydromyine mandibular variation does not fully encompass that of both LOE clades combined. Placed in the context of previous work examining the correlation between diet and mandibular morphology in rodents specifically and mammals more broadly, stouter mandibles with broad, shield-like angular processes tend to be herbivorous whereas slender mandibles with long, narrow coronoid and angular processes tend to be insectivorous or carnivorous, suggesting that these differences in shape correspond to dietary differences [31,34].

We analysed the distribution of mandibular size independently as this trait may be evolving under a different evolutionary process compared to shape [36]. Nevertheless, distribution of mandibular size variation exhibits a similar pattern to that of shape: specimen-level data of Sahul hydromyine mandibular centroid size overlaps extensively with both Luzon clades, completely encompassing the variation of Chrotomyini and nearly encompassing Phloeomyini. Although Phloeomyini and Chrotomyini exhibit considerable overlap with one another, Phloeomyini occupies a 'large' size class to the exclusion of Chrotomyini (electronic supplementary material, figure S1).

We tested the significance of the contemporary distinction between the two LOE clades by comparing the average Euclidean distance among PC scores of mandibular form (i.e. shape and log centroid size combined). This multivariate distance-based permutation approach allows us to compare the shape along all component axes simultaneously without overparameterizing a regression model (as in covariance matrix-based MANOVA). The average distance of mandibular phenotype PC scores is significantly greater between the two sympatric clades than between Phloeomyini and the distribution of permuted samples of Sahul Hydromyini ($p = 0.0004$; figure 2*a*). This result was robust to the exclusion of *Rhynchomys*, which is highly distinct from the remaining rodents and may contribute to the inflation of average between-clade distances ($p = 0.0022$; figure 2*b*). These comparisons remain significant even when limited to shape variation along the first two component axes of mandibular shape (i.e. excluding size and component axes 3 to 24; electronic supplementary material, figure S2), and also when considering only those species found on New Guinea (electronic supplementary material, figure S3).

(b) Testing incumbency-influenced biotic filtering

To examine historical patterns of morphological variation, we estimated the evolutionary relationships among the three clades in a time-calibrated Bayesian phylogenetic framework (figure 3). We used this phylogenetic tree to estimate ancestral states for the stem ancestor of Chrotomyini (i.e. Chrotomyini + Sahul Hydromyini, forming the clade Hydromyini *sensu* [21]), which represents the earliest (and thus most conservative) estimate of ancestral phenotype from a lineage that could have colonized Luzon Island. The distribution of PC1 and PC2 scores of the ancestor of Chrotomyini and Sahul Hydromyini, like that of contemporary Chrotomyini, exhibits little overlap with the distribution of contemporary Phloeomyini on these axes (figure 1). The ancestral state of Hydromyini is intermediate to the crown ancestors of Sahul Hydromyini, which exhibits moderate overlap with Phloeomyini, and Chrotomyini, which exhibits no overlap (electronic supplementary material, figure S5). The contemporary distance along PC1-2 is not significantly greater than the distribution of 10 000 sampled distances between extant Phloeomyini and this ancestral state interval ($p = 0.44$; figure 4*a*). This result is robust even when we performed an additional model specification procedure where *Rhynchomys* was excluded ($p = 0.73$; figure 4*b*). The generating Brownian motion model adequately describes the distribution of PC1-2 scores except for underestimating the rate of morphological evolution for these clades and for estimating evolutionary rate correlated with node height along PC1, probably due to our overdispersed sampling scheme (which MECCA can account for, but *arbutus* cannot). Our lack of support for branch-specific rate heterogeneity, rates correlated with branch length and rates correlated with ancestral states suggest that our analyses need not invoke directional shifts of mandibular shape evolution in Hydromyini to describe the evolutionary patterns in these two clades (electronic supplementary material, table S3).

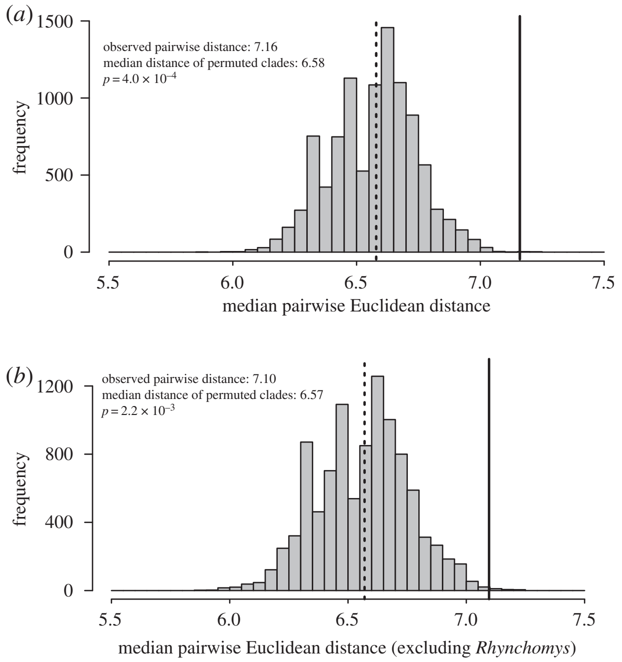

Figure 2. Histograms comparing average Euclidean distance in mandible form (i.e. shape and size) between Chrotomyini and Phloeomyini (solid black line) to the distribution of 10 000 average distances between Phloeomyini and permutations of Sahul Hydromyini (grey bars, median: dashed line). (*a*) *Rhynchomys* included, (*b*) *Rhynchomys* excluded.

4. Discussion

Our results provide evidence to support the hypothesis that the two LOE clades have exhibited persistent partitioning of morphological variation throughout their evolutionary history in sympatry. Chrotomyini only partially overlaps the morphological variation exhibited by its sister clade, the Sahul hydromyines, a clade that diversified in the absence of prior rodent competitors, such that chrotomyines do not exhibit the morphotypes typical of the Luzon-incumbent Phloeomyini. The distinction between the two Luzon 'Old Endemic' rodent clades is consistent with the hypothesis that Phloeomyini, as the incumbent murid clade in this system, may have been able to monopolize an area of morphospace that subsequent colonists (Chrotomyini) could not exploit, even though Chrotomyini's close relatives could in the absence of competition from an incumbent murid clade, and that ancestral ecological distinction subsequently facilitated successful colonization of Luzon by Chrotomyini. An alternative explanation for the patterns we observe is that the two Luzon lineages were ancestrally similar to one another but diverged through ecological character displacement over time to their contemporary distinction. Our results satisfy several criteria asserted by Schluter [48] in his evaluation of studies displaying evidence for ecological character displacement, namely greater divergence in sympatry than in allopatry, ruling out chance as the source of the pattern, and evidence that Chrotomyini may have exhibited a shift away from its hydromyine ancestors ([48] criteria 1–3) (electronic supplementary material, figure S5).

Regardless of resulting from ancestral or displacement-related distinction, contemporary chrotomyine morphological variation was likely influenced by phloeomyine incumbency. The emerging picture regarding incumbency effects in the evolution of Indo-Australian rodents is one of strong influence on the resulting ecomorphological diversity of the constituent clades, but weak influence on their rates of lineage diversification [5,20,49] and rates of trait evolution [36,50]. In other words, secondarily colonizing rodent clades appear to be

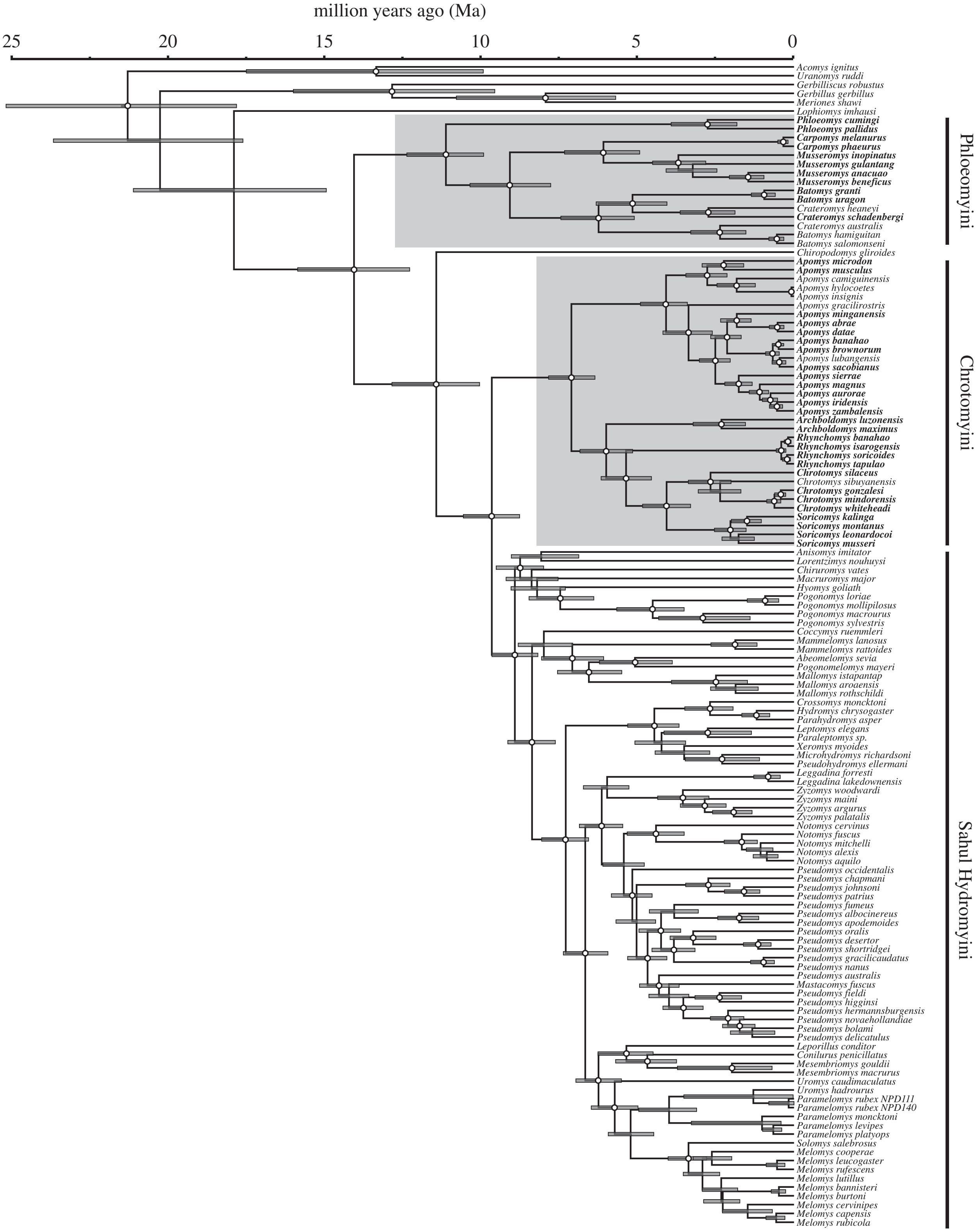

Figure 3. Maximum clade credibility tree from BEAST 2 analysis. Bars at nodes indicate 95% highest posterior density interval of node ages. Dots at nodes indicate posterior probability (PP) ≥ 0.95. Grey boxes surrounding clades indicate clades endemic to the Philippines, with bolded names indicating species occurring on Luzon Island.

able to diversify without a reduction in evolutionary rate provided they do not compete with existing lineages. However, the patterns observed among Indo-Australian rodents may not necessarily hold true in other clades. For example, in aquatic systems, secondary colonists can experience limited ecological opportunity and subsequent diversification compared to incumbent lineages, leading to depressed evolutionary rates in non-incumbent clades [7,51]. In North American canids, younger lineages appear to have driven incumbent lineages extinct, in a reversal of the expectations set by ecological incumbency conferring a competitive advantage [52]. These studies illustrate that clades vary in their evolutionary response to competition from ecologically similar lineages.

Our analysis also reveals the potential impact that geographic scale and isolation have on recovering patterns consistent with biotic filtering and subsequent clade-specific partitioning of morphospace. As one example, a previous

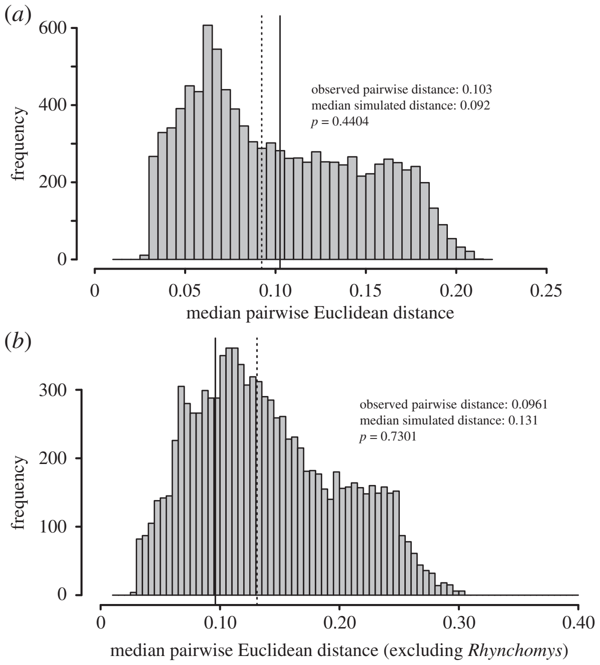

Figure 4. Histograms comparing average Euclidean distance in mandible shape along PC1–2 between extant Chrotomyini and Phloeomyini (solid black line) to 10 000 sampled ancestral states along PC1–2 from MECCA posterior distribution (grey bars, median: dashed line). (*a*) *Rhynchomys* included and (*b*) *Rhynchomys* excluded.

analysis on continent-wide assemblages of passerine birds revealed limited effects of colonization order and subsequent ecological evolution on the patterns of morphospace occupancy [11]. These results illustrate that incumbency effects can quickly become obscured by other factors, such as allopatric speciation and limited competition between focal clades, when examining ecological diversity and community assembly at increasingly broad geographic and taxonomic scales [53]. They also suggest that such effects may be heavily dependent on the relatedness and ecological similarity of the focal clades as well as the spatial scale at which these interactions occur.

Although our results strongly support the hypothesis of evolution influenced by a persistent biotic filter in the LOE rodents, we caution that our results should be interpreted in the light of two caveats, one methodological and one inherent to the comparative approach we used. These caveats present future avenues for study based on our conclusions as more data become available and methodological advancements are made for estimating ancestral states. The first caveat is that our approach is constrained by the lack of temporally relevant fossil information available for these three clades [54]. The availability of fossil information would enable inferences of directionality in mandibular evolution that would reduce ancestral state uncertainty and allow us to estimate ancestral states outside the range of observed values of Hydromyini [55]. The recovery of relevant fossils would help distinguish between Chrotomyini's contemporary morphological variation resulting from persistent ancestral distinction and ecological character displacement. Our model adequacy test illustrates that the extant variation in mandibular morphology is sufficiently described by a non-directional model of evolution (electronic supplementary material, table S3); however, the inability of *arbutus* to account for incomplete sampling of trait data means that these results may be sensitive to unsampled trait variation. The significant results of the inferred evolutionary rate and node height correlation along PC1 indicate that early hydromyine evolution occurred more rapidly than parameterized by the Brownian motion model, potentially including exploring areas of morphospace not currently occupied by the clade.

The second caveat of our study is that our results are consistent with the processes we sought to test but do not eliminate other potential explanations for the patterns we observe. For example, it is possible that these patterns of morphospace occupancy are the result of gradual takeover of phloeomyine morphospace by Chrotomyini. Fossil evidence of chrotomyine-like phloeomyine forms is necessary to test this hypothesis. If this is indeed the case, our recovery of significant overlap between Phloeomyini and the Sahul hydromyines would suggest that Chrotomyini may have been slowed in their ability to exploit the morphospace occupied by Phloeomyini, even as they drive similar forms extinct. Furthermore, the inference of constrained evolution in Chrotomyini compared to Sahul Hydromyini rests on the assumption that the three clades experience similar intrinsic (developmental) and extrinsic (ecological) macroevolutionary opportunity. In essence, we assume that Sahul hydromyines provide a realistic expectation for the evolution of Chrotomyini if this clade did not experience competition with Phloeomyini. One criticism of this assumption is that Sahul Hydromyini may have experienced greater ecological opportunity due to greater available land area and niche breadth, and thus cannot provide a comparison of realizable dietary diversity. However, the fact that this clade occupies an overlapping, but not eclipsing, area of LOE rodent morphospace, suggests that Sahul hydromyines did not have the ability to diversify into a substantially broader array of niches than the LOE.

An outstanding question resulting from this study is what processes contributed to generating the areas of morphospace filled by Luzon rodents unoccupied by Sahul hydromyines. Particularly considering Rowsey *et al.* [36] recovered strong support for a shift in evolutionary mode along the branch leading to the tweezer-snouted *Rhynchomys*, it seems likely that evolutionary rate heterogeneity has contributed to chrotomyine ecological radiation. We present two hypotheses that would make for additional and exciting areas of research. First, it seems plausible that although the initial colonizing chrotomyine lineage was dissimilar from Phloeomyini, competition between each clade in sympatry generated morphological innovation as a consequence of evolutionary pathways of least resistance [56]. If so, inter-clade competition may influence post-colonization morphological innovation in this system similar to patterns predicted by ecological character displacement. Second, the differing abiotic and biotic environments of Luzon Island and the Sahul supercontinent, the latter of which spans tropical, montane, arid subtropical and temperate biomes, may also contribute to Chrotomyini occupying areas not occupied by Sahul Hydromyini. For terrestrial lineages, island systems such as Luzon may have the potential to promote more rapid ecomorphological evolution than continental landmasses, even when these landmasses are more ecologically heterogeneous [57,58], due to evolution being influenced less by ecological opportunity and more rare dispersal events to ecologically similar habitats [59,60]. The murid rodents of the Indo-Australian archipelago may provide an excellent opportunity to explore this question given the exceptional ecological diversity and species richness of both continental- and island-endemic rodent lineages.

Our study examined incumbency effects from the standpoint of the secondary-colonizing Chrotomyini in part because of the easily-tractable sister-clade comparison between Chrotomyini and Sahul Hydromyini. However, our work raises the question as to what has influenced the morphological variation among phloeomyines. Phloeomyini diverged relatively early from the rest of 'crown' Murinae [20] and may exhibit intrinsic (plesiomorphic) constraints that limit their ability to evolve out of a herbivorous (including specialized folivory and granivory) morphotype. Testing this hypothesis would require comparing the evolutionary pattern and process between Phloeomyini and crown Murinae, which is incredibly species rich and geographically widespread. This sister relationship precludes a comparison of this nature due to conflation of geographic and ecological sources of evolutionary variability. However, if there are no intrinsic evolutionary constraints within Phloeomyini (i.e. Phloeomyini and Hydromyini exhibit the same potentially realizable trophic diversity), the absence of carnivorous phloeomyines may be related to limits placed on this clade by Chrotomyini (i.e. secondary colonists outcompeting incumbents). Testing this hypothesis is difficult unless a fossil or newly discovered extant species bearing a chrotomyine-like mandibular shape is discovered and definitively attributed to Phloeomyini.

Biotic filtering has been previously demonstrated to be an important factor in the community assembly of island-endemic and continentally distributed faunas alike [9,61]. The patterns of mandibular form variation in the three murid clades we studied illustrate that incumbency may establish a biotic filter for subsequently colonizing lineages in systems with repeated colonization as well. Furthermore, when added to the body of work examining incumbency effects among Luzon murids, our results indicate that such effects are more likely to influence the patterns of morphological diversity than the rates of lineage diversification or morphological evolution, with non-incumbent clades diversifying relatively freely after colonization [20,36]. We conclude that secondarily colonizing clades may be forced to exhibit persistent ecological distinction from the incumbent clade, but as long as this distinction is maintained, evolution may proceed relatively uninhibited.

Data accessibility. Luzon Old Endemic landmark data are available from the Dryad Digital Repository: https://dx.doi.org/10.5061/dryad.gb7k5d1 [62]. Tree distribution, concatenated sequence matrix, Sahul hydromyine landmark data, MECCA results and scripts necessary to conduct data analysis are available from the Dryad Digital Repository: https://dx.doi.org/10.5061/dryad.r7sqv9s7p [63]. This article contains supporting information online at the publisher's website.

Authors' contributions. D.M.R. and S.A.J. designed research; D.M.R. and R.M.K. conducted research and analysed data; D.M.R. and S.A.J. wrote the paper.

Competing interests. We declare we have no competing interests.

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