Widespread evolution of poricidal flowers: A striking example of morphological convergence across flowering plants

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Total word count for main text: 3834; total tables: 3; total figures: 6 (all color)

Running title: Convergent evolution of poricidal flowers

Summary

- The evolution of tube-like floral morphologies that control pollen release via small openings (functionally poricidal flowers) represents a taxonomically and geographically widespread instance of repeated and independent evolution of a functionally similar morphology. Poricidal flowers are often closely associated with buzz pollination by bees, but we lack an updated angiosperm-wide survey of their phylogenetic distribution.
- We identify all presently known angiosperm genera containing poricidal flowers via a literature survey. We determined the phylogenetic distribution of poricidal flowers and minimum number of independent gains and losses via a genus-level and species level angiosperm-wide phylogeny. We estimated if evolution of poricidal flowers is associated with changes in speciation/extinction via diversification rate analyses.
- Poricidal flowers occur across at least 87 angiosperm families and 635 genera containing > 28,000 species. At the genus level, more than 200 independent gains and 145 independent losses of poricidal flowers occurred. Across angiosperms, genus-level analyses suggest poricidal flower evolution is associated with lower net diversification rates (origination-extinction). Species-level analyses argue no diversification linked to poricidal morphology but within the two focal families we found family-specific diversification effects.
- We present different hypotheses for the functional significance of poricidal flowers, including its association with buzz pollination, and suggest future directions for studies elucidating the extent to which morphological and functional convergence of poricidal flowers are correlated.

Keywords: Bees, buzz pollination, convergent evolution, diversification analysis, pollen, poricidal anthers, poricidal flowers.

Introduction

Patterns of convergent evolution are of fundamental interest in evolutionary biology. Broad convergence on functionally equivalent morphology across disparate groups is particularly common in plants, as in the case of succulent morphology or carnivorous pitcher plant traps, and has helped elucidate shared selective pressures, mechanisms, and developmental biases (Bennici, 2003; Ellison & Gotelli, 2009; Thorogood *et al.*, 2018). Likely the most geographically and taxonomically widespread pattern of convergent evolution is tubular floral morphology that restricts pollen dispersal via small openings, i.e., functionally poricidal floral morphology (hereafter 'poricidal flowers'; Fig. 1)(Vallejo-Marin & Russell, 2024). Previous studies estimate that approximately 22,000 plant species possess poricidal anthers ('poricidal species'), spread across 72 families and 544 genera (Buchmann, 1983). Additionally, flower traits that control pollen dispersal are key to angiosperm reproductive success (Hargreaves *et al.*, 2009) and many poricidal plant species are also agriculturally important, such as tomatoes, cranberries, blueberries, and kiwis (Cooley & Vallejo-Marín, 2021). Yet the phylogenetic distribution and evolutionary patterns of poricidal flowers has not been formally characterized and the latest published taxonomic survey of poricidal flowers is now nearly 45 years old (Buchman, 1978; Buchmann, 1983).

Poricidal flowers share a common functional morphology in which pollen is held within tubular floral structures with small openings (e.g., pores, valves, or slits; hereafter 'pores') through which the pollen is dispersed. In most cases, the tubular structure is composed of modified enlarged anthers, but may alternatively consist of modifications to the corolla or even the entire flower (Fig. 1) (Harris, 1905; Michener, 1962; Buchmann, 1983; De Luca & Vallejo-Marin, 2013; Russell *et al.*, 2017; Dellinger *et al.*, 2019b; Vallejo-Marin, 2019a). Poricidal flowers have been studied for over a century (Harris, 1905) and are frequently associated with a suite of floral characteristics (Buchmann, 1983; Renner, 1989; De Luca & Vallejo-Marin, 2013). Most poricidal plant species are animal pollinated, nectarless, and have dry, loose pollen (reduced pollenkitt lipids) and many are associated with enantiostyly (style handedness) and heteranthery (multiple kinds of stamens) (Buchmann, 1983; Vallejo-Marin *et al.*, 2010). Furthermore, poricidal flowers are often closely associated with the buzz pollination interaction (although not all poricidal flowers are buzz pollinated; see Vallejo-Marin and Russell (2024) for some exceptions), in which pollination is nearly exclusively performed by bee species capable of rapidly contracting their indirect flight muscles while on the flower, generating strong vibrations that expel pollen onto the bee's body (Macior, 1964; Buchmann, 1983; Proenca, 1992; Vallejo-Marin, 2019a; Vallejo-Marin & Russell, 2024). Indeed, poricidal flowers are thought to be a key pollen resource for these buzzing bees, particularly in tropical regions (Delgado *et al.*, 2023). Poricidal floral morphologies may facilitate speciation by filtering out less effective

59 pollinators (Cardinal *et al.*, 2018). Yet because of specialization on a subset of pollinators, poricidal
60 morphology may make these taxa more vulnerable to extinction (Renner, 1989; Pacheco Filho *et al.*,
61 2015; Dellinger *et al.*, 2019a; Vallejo-Marin, 2019b). Consequently, poricidal floral morphologies are
62 consistently suggested to be adaptations to pollinators that might be key traits affecting patterns of
63 speciation and extinction.

64 Previous attempts at comprehensively studying poricidal flowers were restricted to species
65 with pored anthers presumed to be buzz pollinated (Harris, 1905; Buchmann, 1983). However,
66 functionally poricidal floral morphologies are more diverse (Fig. 1). Additionally, clades of poricidal
67 species can comprise thousands of species (e.g., *Solanum*, Melastomataceae) and the rate of gain
68 and loss of poricidal floral morphologies has yet to be quantified. Although poricidal species are
69 extremely widespread, clades are often all poricidal or not and thus studies of small phylogenetic
70 clades suffer from the limitations of rarely seeing evolutionary transitions to and from poricidal
71 flowers. Consequently, large-scale analyses are needed to allow generalization and provide sufficient
72 statistical replication to test the effect of poricidal flower morphologies on diversification (Beaulieu
73 & O'Meara, 2018; Zenil-Ferguson *et al.*, 2019; Joly & Schoen, 2021). However, large-scale
74 phylogenetic analyses bring their own challenges (Helmstetter *et al.*, 2022). First, it is challenging to
75 obtain information on multiple traits for thousands of species (i.e., presence or absence of poricidal
76 flowers). Second, well resolved mega-phylogenies are required. Fortunately, recent angiosperm-
77 wide phylogenies have become available (Sauquet & Magallón, 2018; Jin & Qian, 2019; Janssens *et*
78 *al.*, 2020). Third, running complex diversification models in large phylogenies is computationally
79 demanding and proven methods continue to be developed to speed up large-scale and complex
80 analyses. Finally, the effects of a single trait in a large tree can be confounded with heterogeneity in
81 the process of diversification by other unmeasured factors (Beaulieu & O'Meara, 2018; Beaulieu &
82 O'Meara, 2019; Donoghue & Edwards, 2019).

83 Here, we investigate the evolutionary history of poricidal flowers across angiosperms to
84 address the following questions. **1)** What proportion of angiosperms possesses poricidal flowers? **2)**
85 How many times have poricidal flowers been gained and lost over the evolutionary history of
86 flowering plants at the genus level? **3)** Is the presence of poricidal flowers associated with changes in
87 origination of genera and diversification rates (origination-extinction)? And **4)** Is the presence of
88 poricidal flowers associated with diversification rates (speciation-extinction) across all angiosperms
89 and within some key families? We therefore conducted an extensive literature survey to identify all
90 presently known angiosperm genera containing species with poricidal flowers. We then used an
91 angiosperm-wide phylogeny at the genus level (as recently done in Meseguer *et al.*, 2022) to
92 determine the phylogenetic distribution of poricidal flowers, the geologic ages of poricidal lineages,

and the number of independent gains and losses of functionally poricidal floral morphology before the origination of genera. We also conducted diversification rate analyses at the species level to estimate if the evolution of poricidal flowers is associated with changes in speciation/extinction. Finally, we present an overview of both classic and novel hypotheses for why poricidal flowers evolve and suggest directions for future research about the extent to which convergence in poricidal flower morphology is accompanied by convergence in function.

Material and Methods

Literature survey

We defined "poricidal flowers" as flowers with tube-like floral structures (typically formed by individual anthers or groups of anthers, but sometimes by staminodes, sepals or corolla), restricting pollen release via an opening that can be described as a pore, small slit, or valve. We conducted our literature search between 2015 and November 2020 for English, German, and Portuguese language articles describing all presently known genera of functionally poricidal plant species. Sources included online books, online search engines (Web of Science, Google Scholar), dissertations, and primary research papers. Search terms included "anther", "stamen", or "flower" and "apical pore", "dehisce", "dehiscence", "dehiscent", "introrse", "longitudinal", "porandrous", "porate", "pore", "pored", "poricidal", "porose", "porous", "short slit", "thecal pores", "valve", or "valvular". The search was performed at the genus level because numerous well characterized genera are frequently monomorphic for the presence/absence of poricidal morphology (e.g., *Solanum*, *Senna*, *Miconia*) and information for all species in rarer and/or tropical genera is frequently sparse (although we also recognize that monomorphism within genera should not be taken for granted and there is evidence of potential exceptions, e.g., Renner, 1989; Gavrutenko *et al.*, 2020). For each genus, we documented the flower parts involved in forming the tubular structures that comprise poricidal flower morphology, as well as how pollen is released from poricidal anthers (Fig. 2, Table 1; Supporting Information Table S1). We also confirmed non-poricidal morphology for monomorphic genera when morphological information was available, but generally did not catalog the accompanying literature.

Angiosperm phylogeny and estimates of species number per genus

To estimate the incidence of poricidal taxa across angiosperms we cross-referenced our 638 poricidal genera against three angiosperm-wide databases. First, we used The Plant List (2013), which contained 271,405 species, 12,645 genera, and 396 families of angiosperms, after filtering out infraspecific classifications (i.e., subspecies and varieties). Next, we obtained two recent angiosperm time-calibrated phylogenies from the literature. The first phylogeny is the Nelsen et al. (2018) genus-

level phylogeny of angiosperms, which contains 10,785 genera in 409 families. Second, we used the Jin and Qian (2019) species-level, time-calibrated phylogeny of vascular plants, available through the *R* package *PhyloMaker* (Jin & Qian, 2019). This phylogeny contains 417 families, 10,363 genera and 74,531 species of angiosperms.

Phylogenetic comparative methods

State dependent diversification and origination analyses

We used two time-tree phylogenies to perform a hidden-state dependent speciation and extinction analysis at the genus level. First, we used the R package V.PhyloMaker by Jin and Qian (2019) that relies on the Smith and Brown (2018) angiosperm phylogenetic tree to obtain a phylogenetic tree at the genus level. The Smith and Brown (2018) tree was primarily created using mega tree methodology, that is, matching different large angiosperm phylogenies to generate a larger and more complete tree for genus-level data, but cannot be used for species-level data due to lack of resolution at that level. Therefore, for the angiosperm wide genus-level analysis, we pruned the tree to one species per genus (for a similar approach see Meseguer *et al.*, 2022). The second tree we used was the Meseguer et al. (2022) genus level-phylogeny, also created from the Smith and Brown (2018) tree. The published Meseguer et al. (2022) phylogenetic tree was presented as a bifurcating tree. However, we noted that when we pruned the Smith and Brown (2018) phylogeny we had to resolve polytomies in the genus-level tree, and we did this at random using the function multid2di() in R package ape (Paradis *et al.*, 2004). This is an important detail, because the algorithm used to resolve polytomies might change the estimation of diversification rates, and this is the main difference between the Meseguer *et al.* 2022 and our pruned tree. For both genus-level phylogenies we matched a total of 10,328 genera. Each tree was matched to binary states of poricidal status (0=not poricidal, 1=poricidal) using treeplyr (Uyeda & Harmon, 2020), using the genus-level character state obtained from the literature search. In addition, we chose four families from the Smith and Brown (2018) phylogenetic tree (Solanaceae, Melastomataceae, Ericaceae, and Fabaceae), and for each of the four families we obtained the species-level phylogeny and matched it with poricidal status.

The matching trees and states on the tips of the tree were entered as inputs for state-dependent diversification models. Using RevBayes (Höhna *et al.*, 2016), we wrote a binary hidden state speciation and extinction model HiSSE (Beaulieu & O'Meara, 2016) using a graphical model approach (Jordan, 2004) to estimate rates of net origination (origination of a genus minus extinction rate) for poricidal and not poricidal states associated with two hidden states A and B. We fitted this

158 model for the two genus level phylogenies, ours and the Meseguer *et al.* (2022) phylogeny for all
159 angiosperms, and for the family level phylogenies.

160 The HiSSE model is used to identify both differences in net diversification between poricidal
161 and not poricidal states, but also differences given by hidden states A and B. The difference between
162 hidden states is of particular interest, especially in large phylogenies, because it represents the
163 potential for diversification due to unmeasured factors (Beaulieu & O'Meara, 2016). Thus, estimating
164 this difference disentangles the effect of poricidal morphology on diversification from other
165 potential sources of diversification. We only fitted HiSSE and not the simplest version without the
166 hidden states (BiSSE, Maddison *et al.*, 2007), because in a large tree our null hypothesis should
167 always be one that has heterogeneity in the rates of diversification due to unobserved factors, and
168 not one where the trait of interest has all the signal. As discussed in the recent synthesis of SSE
169 (state-dependent speciation and extinction) models, phylogenies with large samples always find that
170 HiSSE is a preferred model over BiSSE (Helmstetter *et al.*, 2023).

171 We performed a second HiSSE analysis matching our information of poricidal states to the
172 species-level phylogeny of Janssens et al. 2020. The Janssens et al. (2020) time-calibrated phylogeny
173 was specifically designed to address species-level macroevolution analyses, which is the main
174 difference with the Brown and Smith (2018) tree. Although the latter tree has more species
175 sampling, the uncertainty of the placement of the species is much larger than the Janssens et al.
176 (2020) tree, hence our preference for Janssens et al. (2020) for our species-level analysis. Based on
177 matK (incl. trnK) and rbcL. plastids this phylogeny has 36,101 taxa representing 8,399 genera and is
178 dated using 56 angiosperm fossils. We matched this phylogeny to our species-level poricidal data
179 and managed to match 28,979 taxa. In addition, we found 267 small genera where all species had
180 the same tip value, but none of them were present in the tree. Hence, we added the trait value as
181 representative of the genus to maximize the information when using the R package phyndr (Pennell
182 *et al.*, 2016), resulting in a phylogeny of 29,246 tips with poricidal binary states. This represents the
183 largest plant phylogeny to date where a HiSSE analysis has been performed.

184 A Bayesian framework was used to obtain posterior probability densities via Markov chain
185 Monte Carlo (MCMC) for all HiSSE model parameters for genus-level and species-level phylogenies.
186 We checked for convergence of our MCMC algorithm by running our inference twice and observing
187 stationarity in the posterior distributions, as well as matching of convergence values in software
188 TRACER (Rambaut *et al.*, 2018). These two runs were not combined, and we retained only one run
189 for the results. Additionally, we verified that the effective sample size (ESS) for each parameter was
190 higher than 200. In the species-level analyses, convergence of the MCMC is very difficult due to

numerical precision at calculating likelihoods of the model. In this case we reached an ESS of more than 200 for the posterior distribution of the HiSSE after an 11-month long MCMC run, acknowledging that there are many local maxima for the posterior distribution that are very close, which makes estimation of diversification rates difficult. However, all these local maxima have the same consistent result in diversification, which is discussed in the Results section. The proportion of genera or species sampled was calculated according to the number of genera listed in The Plant List (2013), and the state of root was fixed to non-poricidal in the angiosperm-wide analysis and unknown for the family-level ones.

Ancestral state reconstruction and estimation of minimum number of transitions

We implemented ancestral state reconstruction in RevBayes using a HiSSE model, as proposed by Freyman and Höhna (Freyman & Höhna, 2018) for the genus level phylogenetic tree from Jin and Qian (2019) matched with our poricidal status data. To infer which of four states (non-poricidal A, non-poricidal B, poricidal A, poricidal B) is an internal node, we calculated the marginal posterior probability of each internal node and chose the state that maximized that probability as shown in Fig. 3 (Zenil-Ferguson *et al.*, 2019). Given these estimates for the internal states we calculated from node to node whether there has been a transition from not poricidal to poricidal or vice versa. This approach allows us to estimate the minimum number of expected transitions. An approximation to the exact number would require stochastic mapping that is numerically challenging when scaling a HiSSE model to large phylogenetic trees (Freyman & Höhna, 2019; Zenil-Ferguson *et al.*, 2023). All results and ancestral reconstructions are illustrated using the R package RevGadgets (Tribble *et al.*, 2021). Ancestral reconstructions were not computationally feasible for the species-level phylogenetic tree HiSSE analysis due to the large phylogeny size.

Results

Distribution of poricidal flowers across taxonomic levels

In a seminal paper, Buchmann (1983) estimated that 15,000-20,000 species of angiosperms in 72 families and 544 genera have anthers that dehisce through apical pores or slits (poricidal anthers). Here, we found that poricidal flowers, including species with poricidal anthers, are reported from 87 families and 638 genera (Fig. 2, Table 1; Supporting Information Table S1). These values correspond to approximately 21% of plant families and 5% of genera included in three different data sets of flowering plants analyzed here (Table 1). Using the 271,405 species of angiosperms in the largest database (The Plant List), which includes 635 of 638 genera identified in our study, yields an upper estimate of 28,218 poricidal species (5.02% of genera and 10.39% of species) (Table 1). We obtained a similar value from the other database that includes species-level information (Jin & Qian, 2019),

with an upper estimate of 10.93% (8,149 of 74,531) poricidal species and 5.06% (525 of 10363) poricidal genera. Our results therefore suggest that poricidal flower morphologies have evolved in a fifth of families and up to one in ten angiosperm species.

Different types of poricidal flowers across taxonomic levels

Flower parts forming the tubular structures that comprise poricidal flower morphology include poricidal anthers (an estimated 97% of poricidal species), corollas, or other functionally poricidal floral parts (i.e., staminodes or sepals) (Table 2). Additionally, how pollen is released from poricidal anthers is particularly diverse, although such classifications are morphological, rather than developmental, and may therefore be quite inaccurate. Morphological variation includes pores (60 families and 441 genera), short terminal slits (46 families and 125 genera), valves (15 families and 74 genera), and introrse dehiscence forming terminal pores or short slits (16 families and 53 genera) (Supporting Information Table S1). A given poricidal flower morphology is rarely restricted to a particular family or genus and even multiple kinds of poricidal anthers are known within a genus (Endress, 1996).

Diversification rates and transitions between poricidal and non-poricidal flowers

Ancestral character state reconstruction on the angiosperm wide HiSSE analysis at the genus level with our matched phylogeny from Jin and Qian (2019) indicated a minimum of 200 transitions from non-poricidal to poricidal flowers and 145 reverse transitions (poricidal to non-poricidal), when aggregated across hidden states (Fig. 3). This HiSSE analysis also suggests differences in rates of net origination (origination rate of genera is calculated as the birth rate minus the death rate per state in the HiSSE model) with and without poricidal flowers, depending on the state of the hidden character (Fig. 4). Specifically, the net diversification rate of poricidal genera is on average 26% lower than for non- poricidal taxa but only for the B state (credible intervals: 0.218 ± 0.0122 vs. 0.295 ± 0.0074, respectively), and similarly 31% lower when in combination with hidden state A (credible intervals: 0.0119 ± 0.0013 vs. 0.0172 ± 0.0011, for non-poricidal vs. poricidal, respectively) (Fig. 4). Interestingly, poricidal state B has a faster net diversification than non-poricidal state A, indicating that some, but not all poricidal lineages can have faster net diversification than some non-poricidal lineages (Fig. 3). Furthermore, poricidal genera appear early in angiosperm evolution and are substantially more common in older nodes versus more recent nodes (Fig. 5). However, both of these results should be interpreted with caution (for instance, because we do not test time-dependent diversification; Louca & Pennell, 2020).

256 Yet the relationship between poricidal flowers and origination rates varies when analyzed at
257 lower taxonomic levels (Supporting Information Fig. S1). In the species-level Solanaceae HiSSE
258 analysis, poricidal flowers diversified at a higher rate compared to non-poricidal flowers, but again,
259 this relationship varied with the state of the hidden character. In the HiSSE analysis of Ericaceae, we
260 found a higher diversification rate of poricidal flowers in combination with hidden character state A,
261 but lower when combined with character state B, indicating a strong context-dependence effect of
262 poricidal morphologies (Fig. S1). For the two other families analyzed at the species level
263 (Melastomataceae and Fabaceae), models did not converge after running the analyses for several
264 months due to high uncertainty on the branch lengths in the Melastomataceae and Fabaceae trees
265 and thus we lack results for these groups (additional discussion in Supporting Information). Using the
266 Meseguer et al. (2022) phylogeny, we found that poricidal states have faster net diversification for
267 poricidal in states A and B (Supporting Information Fig. S2). This difference might be due to
268 differences in how polytomies were resolved in the Meseguer et al. phylogeny. When using this
269 phylogeny, many local maxima were identified in the output of the MCMC algorithm, and when we
270 took the global maximum, we found that the ancestral state reconstruction at many nodes is highly
271 uncertain, indicating that the inference using this tree is highly uncertain as well.

272 For the species-level HiSSE analyses using the Jin and Qian (2019) phylogenetic tree with our
273 data, we found that there are no differences in net diversification (speciation minus extinction rates)
274 between non-poricidal and poricidal states (Fig. 6A). Instead, we only found differences between
275 hidden state net diversification rates with probability 0.99 (Fig. 6A), indicating that diversification
276 patterns come from unobserved factors and not our poricidal states. Traditionally, a model selection
277 procedure is used to compare HiSSE models against character independent models (CID) to test if
278 differences in diversification stem from hidden states. However, this is not necessary when using a
279 Bayesian framework, as the posterior distribution of HiSSE can be transformed to test the character
280 independent model hypothesis (see supporting information for a demonstration in Figs. S3 and S4).
281 This result is consistent with the synthesis on state-dependent diversification presented in
282 Helmstetter et al. (2023), where traits fitted to phylogenies with large sampling typically have no
283 correlation with diversification rates. Our diversification results in the species-level analysis along
284 with our genus-level HiSSE analysis indicate that the evolution of poricidal state might be key in the
285 generation of new angiosperm genera, but once those genera are established, poricidal state is not
286 as critical in diversification at the species level. Furthermore, we found that transitions from non-
287 poricidal to poricidal states are on average 33x and 6.6x slower than from poricidal back to non-
288 poricidal in A and B hidden states, respectively (95% credible intervals: non-poricidal to poricidal:
289 $q_{01}^{A} = (5.28x10^{-5}, 1.13x10^{-4})$, and $q_{01}^{B} = (2.65x10^{-4}, 5.88x10^{-4})$; poricidal back to non-

poricidal: $q_{10}^{A} = (1.75x10^{-3}, 3.81x10^{-3})$, and $q_{10}^{B} = (1.42x10^{-3}, 4.52x10^{-3})$), (Fig. 6B). This pattern of asymmetry in transition rates from a "specialized" poricidal state back to a "generalized" non-poricidal state was also found in recent state-dependent diversification analyses (Day *et al.*, 2016; Zenil-Ferguson *et al.*, 2023), where more evidence of evolving out of ecological specialization has been found.

Discussion

Taxa with functionally poricidal floral morphology comprise an estimated 10% of angiosperm species and this morphology has at least 200 independent origins at the generic level (Table 1, S1). Evidently, there is relatively strong selective pressure to evolve floral poricidal morphology. Given the diversity of angiosperm clades and ecosystems in which poricidal flowers have arisen, likely multiple non-mutually exclusive hypotheses explain their overall evolution (Vallejo-Marin & Russell, 2024). For instance, because poricidal flowers hold their pollen within tube-like morphology, their pollen is likely much better protected from environmental stressors such as ultraviolet radiation and rain, relative to the pollen of non-poricidal lineages (Table 3; c.f., Gottsberger & Silbauer-Gottsberger, 1988). Similarly, pollen is a nutrient-rich substrate for yeast and bacteria and poricidal morphology might limit microbial antagonists gaining access to the pollen (Table 3). Poricidal morphology is also frequently physically toughened, such as the endothecial thickenings and calcium oxalate crystal druses common to poricidal anthers (Gerenday & French, 1988; Carrizo García *et al.*, 2008; Burrieza *et al.*, 2010). Such modifications may conceivably reduce the theft of protein-rich pollen by chewing herbivores (Table 3; Coté & Gibernau, 2012; but see Paiva, 2021). Accordingly, we might expect the evolution and occurrence of poricidal flowers to be more common among angiosperm taxa living in regions experiencing greater pollen-related biotic and abiotic stressors.

Classical hypotheses for the evolution of poricidal morphology have highlighted the role of pollinators (Vallejo-Marin & Russell, 2024). Most, but not all species with poricidal flowers are pollinated by floral buzzing bees, which are prolific consumers of pollen (Michener, 1962; Buchmann, 1983; Hargreaves *et al.*, 2009; Danforth et al. 2019; Vallejo-Marin, 2022). Poricidal morphology might control the rate of pollen release, and thus reduce the tempo of pollen removal by pollen-consuming pollinators (Table 3; e.g., Larson & Barrett, 1999; De Luca *et al.*, 2014; Brito *et al.*, 2016). Likewise, poricidal morphology may result in more effective dispersal of pollen to conspecifics by pollinators, by enhancing the precision of pollen placement on the pollinator body and/or by filtering more generalized and less effective pollinators (Table 3; e.g., Pacheco Filho *et al.*, 2015; Mesquita-Neto *et al.*, 2018; Kemp & Vallejo-Marin, 2021; Vallejo-Marin & Russell, 2024). However, pollinator-mediated selection alone appears insufficient to explain the evolution of

323 poricidal morphology across diverse angiosperm clades. Poricidal species have diverse mating
324 systems, from selfing to outcrossing, and many poricidal flowers are wind pollinated, not animal
325 pollinated (e.g., in Poaceae and Halophytaceae;Pozner & Cocucci, 2006; Table S1). Although
326 vibration can be a key mechanism of wind-mediated pollen release (Timerman & Barrett, 2018), and
327 could function analogously to floral buzzing by bees for these poricidal species, the functional
328 benefits of poricidal morphology likely go well beyond pollen release, as discussed above and in
329 Vallejo-Marin and Russell (2024). While future work will be required to investigate the relative
330 contribution of each hypothesis to mediating the evolution of poricidal floral morphology across
331 diverse clades, we provide a list of key predictions and citations to facilitate the development of
332 these research directions (Table 3).

333 We also catalogue substantial diversity and bias in how poricidal morphology is achieved
334 (Fig. 1; Table 2). While floral parts such as anthers, corollas, sepals, and/or staminodes can be
335 involved, anther modifications are particularly common (~97% of poricidal genera Table 2, S1).
336 Within taxa, variation in which floral parts are used to achieve poricidal morphology is uncommon.
337 Additionally, there is substantial morphological diversity in poricidal anther among taxa, especially
338 with respect to anatomical differences in dehiscence (e.g., via slits, pores, or valves; Harris, 1905;
339 Buchmann & Hurley, 1978; Buchmann, 1983; Endress, 1996). These patterns strongly suggest
340 developmental biases, but the developmental pathways of poricidal morphology remain unexplored
341 to our knowledge. Finally, our study sets the stage for future work to investigate the evolutionary
342 history of other floral traits that have been repeatedly proposed to be potentially correlated with
343 poricidal floral morphology, such as loss of nectar, pore size, pollen size and exine ornamentation,
344 pollen quantity, or dry and loose pollen (Vogel, 1978; Dukas & Dafni, 1990; Cruden, 2000; Roulston
345 *et al.*, 2000; Freitas & Sazima, 2003; Brito *et al.*, 2016).

346 Despite the apparent adaptive benefits of poricidal morphology, we find significantly lower
347 origination rates for poricidal genera when considering angiosperms as a whole (31% - 26% lower,
348 for hidden states A and B respectively), suggesting that this trait is frequently costly. One obvious
349 potential cost is that poricidal morphology increases pollination specialization, reducing the capacity
350 for these plant taxa to persist or diversify (Renner, 1989; Dellinger *et al.*, 2019b). Specialization in a
351 variety of ecological systems (e.g., parasitism, herbivory, pollination) can be associated with reduced
352 diversification rates (Moran, 1988; Tripp & Manos, 2008; Day *et al.*, 2016; Raia *et al.*, 2016) although
353 this relationship may not be straightforward (Forister *et al.*, 2012; Armbruster, 2014; Hardy & Otto,
354 2014; Day *et al.*, 2016; Zenil-Ferguson *et al.*, 2023). As we described in the species-level analyses, we
355 found that poricidal specialization is not linked to diversification. However, it might be important in
356 the origination of some genera of angiosperms, as our genus-level state-dependent model shows

357 fast net diversification for poricidal state B. That this difference in diversification is present at both
358 the genus- and species-level indicates that evolving poricidal flowers is complex, but once the trait
359 has evolved and persists (either by more speciation or less extinction), a poricidal flower ancestor
360 can then establish a new genus (Fig. 4). At the same time, specialization is easy to reverse to a
361 generalist non-poricidal state (Figure 6B) and poricidal flowers might be costly to reacquire, which
362 could explain why poricidal states are not linked to recent diversifications for the majority of
363 angiosperms (Fig 6A). A main reason for the difficulty in reacquiring the poricidal state is that animal
364 pollination in poricidal plant species is frequently restricted to a subset of possible pollinators. In
365 fact, many poricidal plant taxa are pollinated by bees capable of floral buzzing or, rarely, by birds or
366 bees that activate a bellows-like mechanism in the stamen (Sazima *et al.*, 1993; Dellinger *et al.*,
367 2019b) and is likely often more specialized. Furthermore, the regional diversity of poricidal taxa and
368 buzzing bees can be strongly positively correlated (Pacheco Filho *et al.*, 2015), suggesting an even
369 greater degree of poricidal plant-bee specialization (Mesquita-Neto *et al.*, 2018).

370 The costs of poricidal morphology may also be related to pollen being the only reward
371 offered by the majority of poricidal species (Vogel, 1978; Buchmann, 1983; Endress, 1994; Vallejo-
372 Marin *et al.*, 2010). In addition to resulting in specialization on pollen-foraging pollinators, pollen
373 offered as a food reward might reduce its availability for export to conspecific flowers (Hargreaves *et*
374 *al.*, 2009). Furthermore, while separating male and female phases in flowers (dichogamy) reduces
375 selfing and/or interference with cross-pollinating mechanisms, dichogamy in pollen-only rewarding
376 species also potentially reduces visitation to the rewardless female phase flowers. If the costs of
377 dichogamy outweigh its benefits for pollen-only rewarding species, this may explain why the
378 simultaneous presentation of pollen and stigmas (homogamy) in pollen-only flowers is common
379 (Lloyd & Webb, 1986; Webb & Lloyd, 1986; Renner, 1989).

380 Notwithstanding the various potential costs of poricidal morphology and its associated
381 traits, some plant families have higher diversification rates associated with poricidal flowers,
382 although this is contingent on the state of hidden characters (e.g., Solanaceae, Ericaceae).
383 Accordingly, other factors that vary across angiosperm groups may be involved in the diversification
384 of poricidal taxa, such as flower modularity (Dellinger *et al.*, 2019a), abiotic and biotic selective
385 forces described in Table 3, the relative availability of potential pollinators (Pacheco Filho *et al.*,
386 2015; Mesquita-Neto *et al.*, 2018), or the presence of other pollen sources in the community (Kemp
387 *et al.*, 2022).

388 Poricidal morphology is thought to be an adaptive plateau and appears to constrain floral
389 evolution (Renner, 1989; Dellinger *et al.*, 2019b). Yet poricidal floral morphology is not necessarily a

390 dead end. We estimate 145 independent losses of this morphology on average, suggesting this trait
391 is evolutionarily labile. Multiple non-mutually exclusive hypotheses might explain a return to
392 loculicidal anther dehiscence. For example, the loss of key pollinators, a reduction in herbivory,
393 and/or reduced selection by microbial pathogens could all reduce the potential benefits of poricidal
394 versus non-poricidal morphology (Schwartz-Tzachor *et al.*, 2006; Brito *et al.*, 2017). A detailed
395 understanding of the biogeography of poricidal taxa and of pollinators and other selective agents
396 will likely be required to distinguish among these hypotheses. Given differences in how functional
397 poricidal morphology is achieved, certain poricidal types may also be more evolutionarily labile than
398 others. For example, the length of the distal slits in poricidal anthers often varies among taxa or even
399 over the lifetime of a given flower (e.g., in *Actinidia*; Goodwin, 1986), and thus evolutionary
400 transitions from partial to more and more complete longitudinal dehiscence for some floral forms
401 may be developmentally relatively simple to achieve.

402 In conclusion, given the hundreds of thousands of non-poricidal taxa and the tens of
403 thousands of poricidal taxa, transitions to poricidal morphology are relatively rare, but once evolved,
404 the probability of losing poricidal morphology is relatively high. Other possibilities for this pattern
405 include early bursts of speciation associated with poricidal morphology or greater chances for the
406 poricidal state to go extinct later, given that poricidal genera are proportionally more common early
407 in angiosperm evolution, but later become rarer relative to non-poricidal genera. Our results thus
408 suggest that selective forces driving the evolution and maintenance of poricidal morphology may
409 vary substantially over time and space. Given our coding of genera as poricidal or not, our estimates
410 for the independent loss and gain of poricidal morphology are also likely highly conservative and
411 should be interpreted cautiously, although we also recover this pattern of faster poricidal loss in the
412 species-level analysis with a much larger tree. Despite extensive documentation of poricidal
413 morphology (Supporting Information Table S1), detailed data across multiple species has only been
414 collected for some of the major lineages (Brito *et al.*, 2016; Dellinger *et al.*, 2019a; Dellinger *et al.*,
415 2019b). Furthermore, the pollinators or even pollination systems of poricidal species are frequently
416 incompletely documented. Additionally, poricidal flowers are associated with diverse pollination
417 systems, including many wind-pollinated Poaceae, fly and/or non-buzzing bee pollinated Araceae,
418 Rafflesiaceae (taxa with toothpaste-like pollen extrusion), and *Rhododendron* (taxa with clumped
419 pollen attached via sticky viscin threads), and bird pollinated *Agarista* and *Axinaea* (taxa with
420 bellows flowers). Considering such diversity, we believe that future studies investigating functions
421 beyond only the control of how and when pollen is released (see Table 3) will be invaluable in
422 understanding the selective pressures driving the gain and loss of poricidal morphology.

Acknowledgements

MVM was partially supported by grants RPG-2018-235 from The Leverhulme Trust and RGP0043/2022 from the Human Frontier Science Program. RZF supported by NSF-DEB 2323170. MVM and RZF greatly benefited from discussions supported by DiveRS-CESAB project AS/CESAB181003. SLB was partially supported by NSF-DEB 1929499. This paper is dedicated to the memories of James A. Harris, Charles D. Michener, and Stefan Vogel for their foundational efforts in elucidating the ecology of poricidal flowers and their bee pollinators.

Author Contributions

SLB, DDJ, RK, and ALR initially conceived and designed the study, with subsequent contributions from MVM on study design and framing. ALR & SLB conducted the literature review, and DDJ, MVM and RK collected taxonomic and phylogenetic data. MVM and RZF performed the diversification analyses and prepared figures. ALR and MVM wrote the first draft of the manuscript; other authors provided editorial advice.

Data Accessibility

The datasets and code supporting this article will be uploaded to Dryad and made publicly available upon publication.

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Tables and figures

Table 1. Incidence of poricidal flowers at the species, genus, and family levels, as estimated from three separate angiosperm-wide databases. Notice that the proportions of poricidal flowers at each taxonomic level is very similar, irrespective of the database analyzed. Poricidal = number of taxa in which at least one species or genus is reported as poricidal (genera treated as monomorphic). Non-poricidal = Number of taxa with no reported poricidal species or genera.

Database	Taxonomic level	Poricidal	Non-poricidal	Total	% Poricidal
The Plant List (2013)	**Family**	87	309	396	21.96%
	Genus	635	12010	12645	5.02%
	Species	28218	243187	271405	10.39%
Nelsen et al. (2018)	**Family**	86	323	409	21.02%
	Genus	521	10264	10785	4.83%
	Species	--	--	--	--
Jian and Qin (2019)	**Family**	87	330	417	20.86%
	Genus	525	9838	10363	5.06%
	Species	8149	66382	74531	10.93%

681 **Table 2.** Type of floral structure involved in forming a tube-like structure with a small opening
682 containing pollen grains (poricidal flower) exhibited by 639 genera. Two genera (*Tyleria* and
683 *Testulea*; Ochnaceae) contain species forming poricidal flowers via pores and staminodes and thus
684 are included more than once in the table. Data from Supporting Information Table S1.

Floral structure forming a tube	Number of genera (%)	Number of families	Example taxa
Anthers	**633 (99.6%)**	86	*Solanum* (Solanaceae) *Rhexia* (Melastomataceae)
Staminodes	**4 (0.62%)**	1	*Adrenarake, Sauvagesia, Testulea, Tyleria* (Ochnaceae)
Corolla	**3 (0.47%)**	2	*Pedicularis, Rhinanthus* (Orobanchaceae) *Ternstroemia* (Pentaphylacaceae)
Sepals	**1**	1	*Dalechampia* (Euphorbiaceae)

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686

687 **Table 3.** Hypotheses for the evolution of poricidal flowers discussed in the text (see also Vallejo-
688 Marin & Russell, 2024). The second column presents predictions arising from these hypotheses. The
689 different hypotheses are not mutually exclusive.

Hypotheses for the evolution of poricidal flowers	Predictions	References
Escalating response to pollen consumption by greedy pollinators	Poricidal species lose less pollen to pollinator consumption	No direct support, but see: (Dukas & Dafni, 1990; Harder & Barclay, 1994; Hargreaves *et al.*, 2009; Russell *et al.*, 2017; Russell *et al.*, 2021)
Filtering mechanism selecting subset of effective pollinators	Fewer, but more effective pollinators able to access pollen of poricidal species	(Pacheco Filho *et al.*, 2015; Mesquita-Neto *et al.*, 2018; Kemp & Vallejo-Marin, 2021)
Pollen dispensing mechanism	Pollen of poricidal species dispensed more gradually and/or more precisely to pollinators and on particular pollinator body parts	(Larson & Barrett, 1999; De Luca *et al.*, 2013; Dellinger *et al.*, 2019c; Brito *et al.*, 2021; Kemp & Vallejo-Marin, 2021; Pereira Nunes & Vallejo-Marín, 2022)

Protection of pollen from environmental stress (UV, rain, desiccation, etc.)	Pollen of poricidal species germinates more often; adaptations of pollen to environmental stress are reduced in poricidal species	No direct support, but see: (Buchmann, 1983; Gottsberger & Silbauer-Gottsberger, 1988; Zhang *et al.*, 2014; Koski & Ashman, 2015; Koski & Galloway, 2018)
Protection of pollen from herbivory	Poricidal morphology reduces pollen consumption	No direct support, but see: (Burrieza *et al.*, 2010; Coté & Gibernau, 2012; Paiva, 2021)
Protection from microbial antagonists	Adaptations of pollen to microbial antagonists are reduced in poricidal species	No direct support, but see: (Christensen *et al.*, 2021; Cullen *et al.*, 2021)

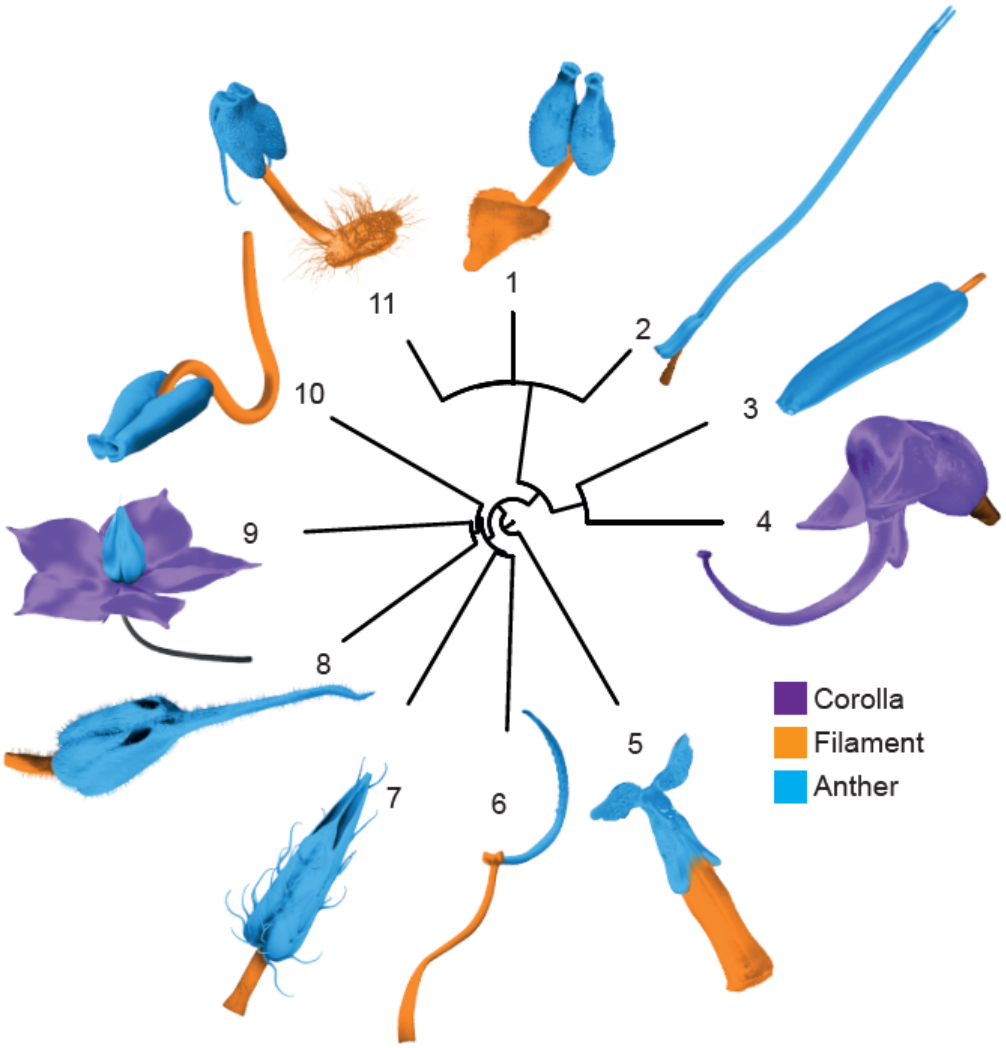


Figure 1. Diversity of poricidal flower structures illustrated across 11 taxa. The images depict stamens or whole flowers of taxa with poricidal flowers. For illustration of the diverse ways to build a poricidal flower, false colors indicate the structure or tissue involved: Corolla, Filament and accessory tissue (Filament), and Anther. The phylogeny at the center indicates relationships among taxa. Species as follows: 1 = *Chimaphila umbellata* (Ericaceae), 2 = *Demosthenesia cordifolia* (Ericaceae), 3 = *Solanum elaeagnifolium* (Solanaceae), 4 = *Pedicularis groenlandica* (Orobanchaceae), 5 = *Berberis sp.* (Berberidaceae), 6 = *Tibouchina urvilleana* (Melastomataceae), 7 = *Apeiba tibourbou* (Malvaceae), 8 = *Sloanea terniflora* (Elaeocarpaceae), 9 = *Sauvagesia capillaris* (Ochnaceae), 10 = *Senna* sp. (Fabaceae), 11 = *Arctostaphylos* sp. (Ericaceae). Redrawn from original 3D models by William Singleton and Daniel Hornung.

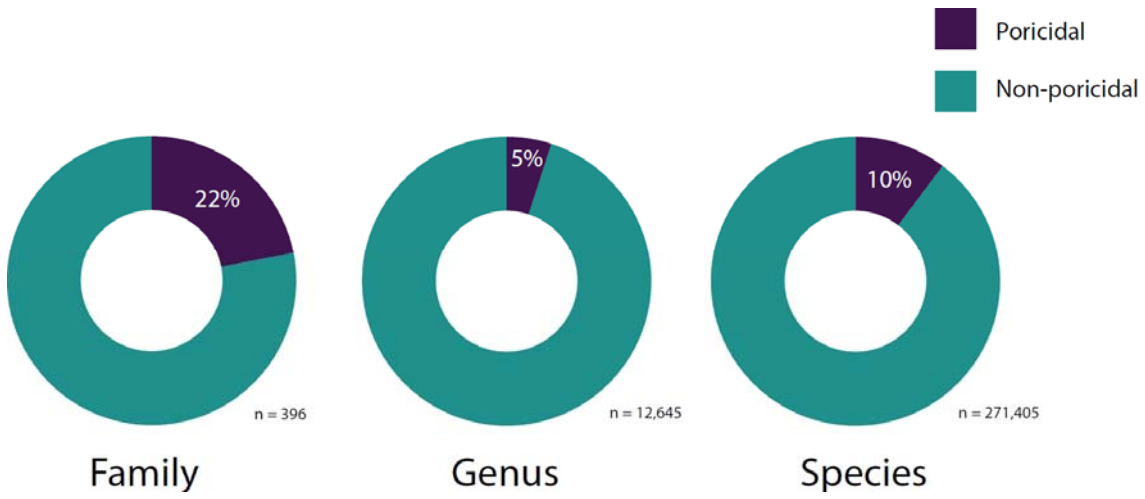


Figure 2. Proportion of taxa with poricidal flowers at the family, genus, and species level, estimated from The Plant List (2013) database.

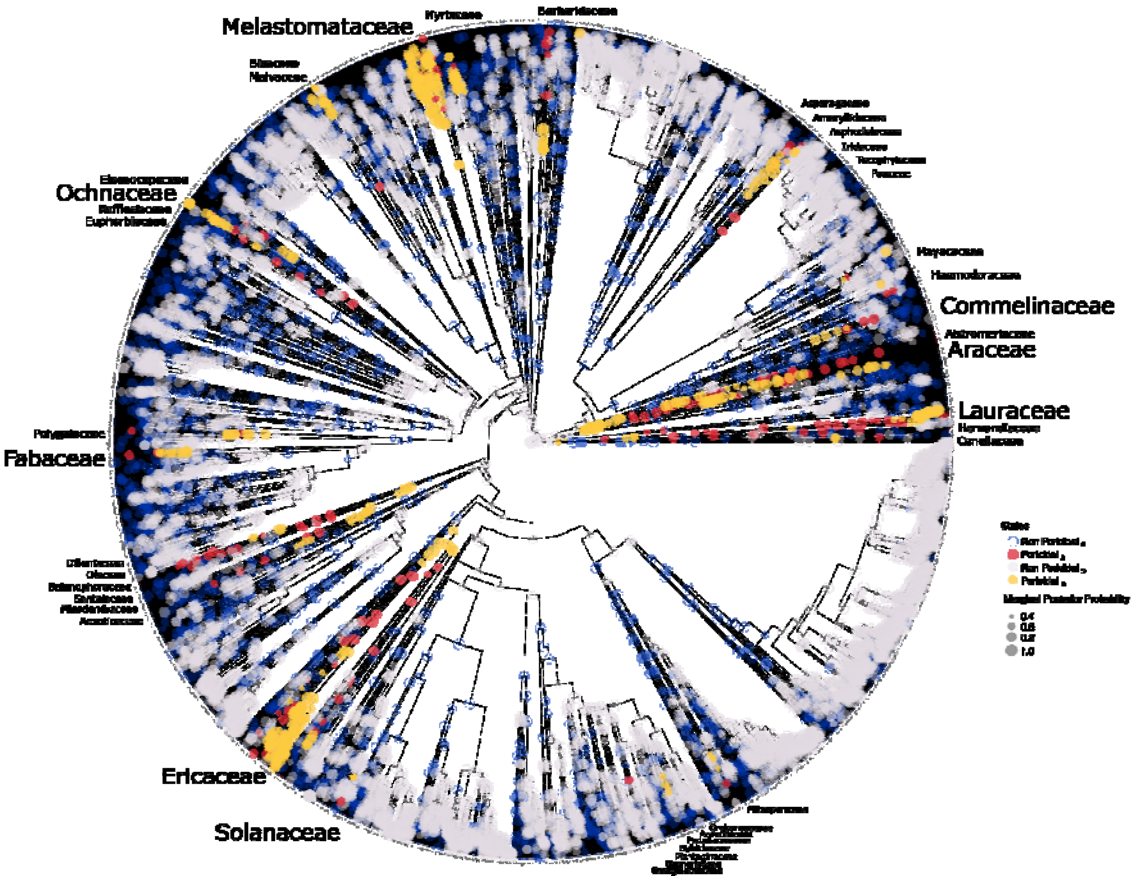


Figure 3. Ancestral character state reconstruction from the hidden state-dependent diversification model (HiSSE) on a genus-wide 10,328 tip phylogeny across angiosperms (see Methods). Non-poricidal states in hidden state B (light grey) diversify equally or more quickly than poricidal states in hidden state B (yellow). However, poricidal states in B (yellow) have diversified faster than other poricidal states and non-poricidal states in hidden state A (red and blue respectively). Even when non-poricidal states (light gray) represent the majority of the diversification in angiosperms, some yellow "bursts" of poricidal state originate genera in large families like Solanaceae, Ericaceae, Fabaceae, and Melastomataceae.

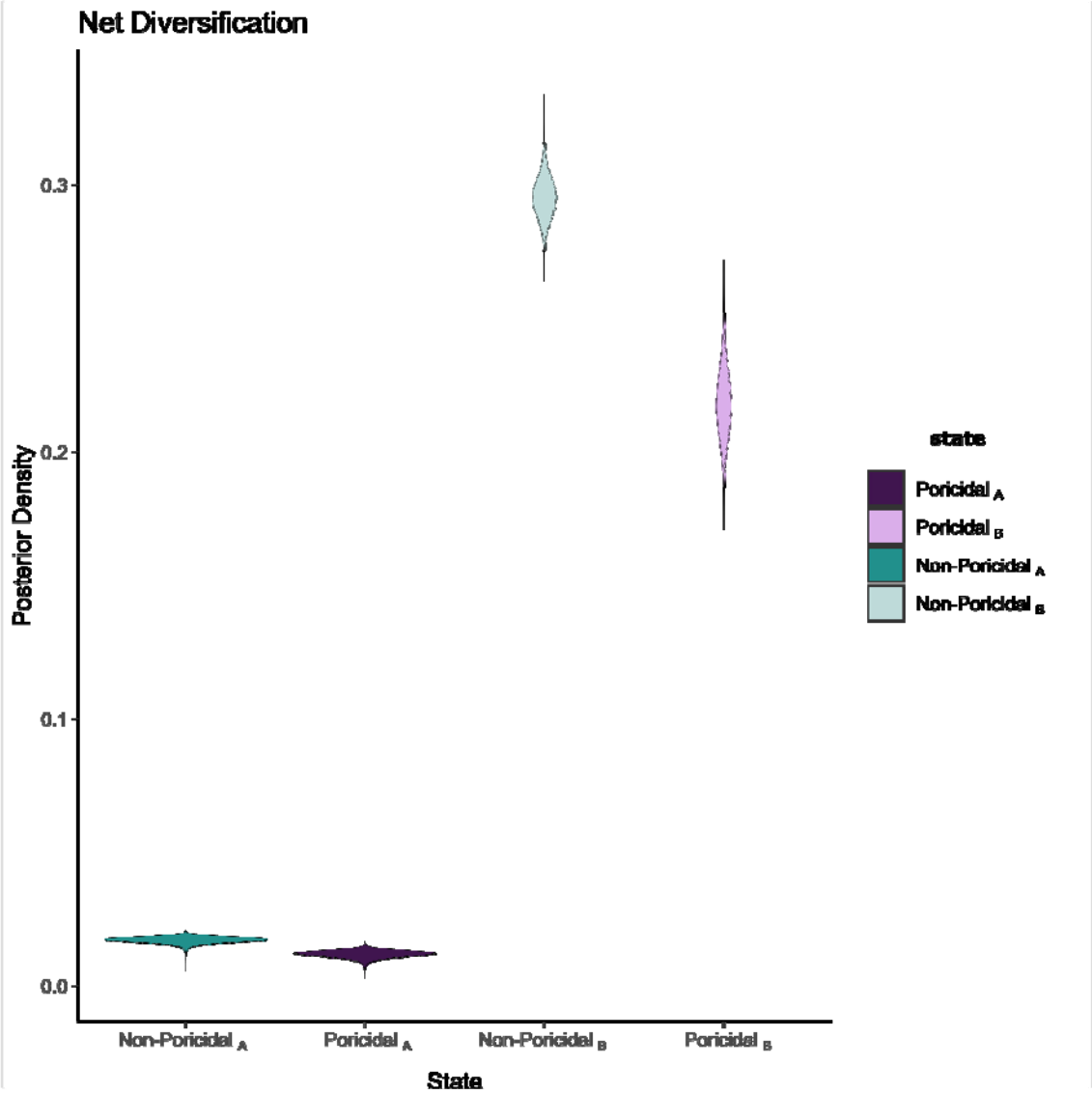


Figure 4. Posterior probability density of estimated diversification rates (origination – extinction) of poricidal vs. non-poricidal taxa (genus) in a hidden state dependent speciation and extinction model with two hidden states (A, B) in an angiosperm-wide genus-level phylogeny. States A and B represent other potential factors unmeasured by this study, but which affect diversification (Beaulieu and O'Meara, 2016, Zenil-Ferguson et al. 2019, Zenil-Ferguson et al. 2023). The states capture different paces of diversification, during which lineages can be poricidal or non-poricidal.

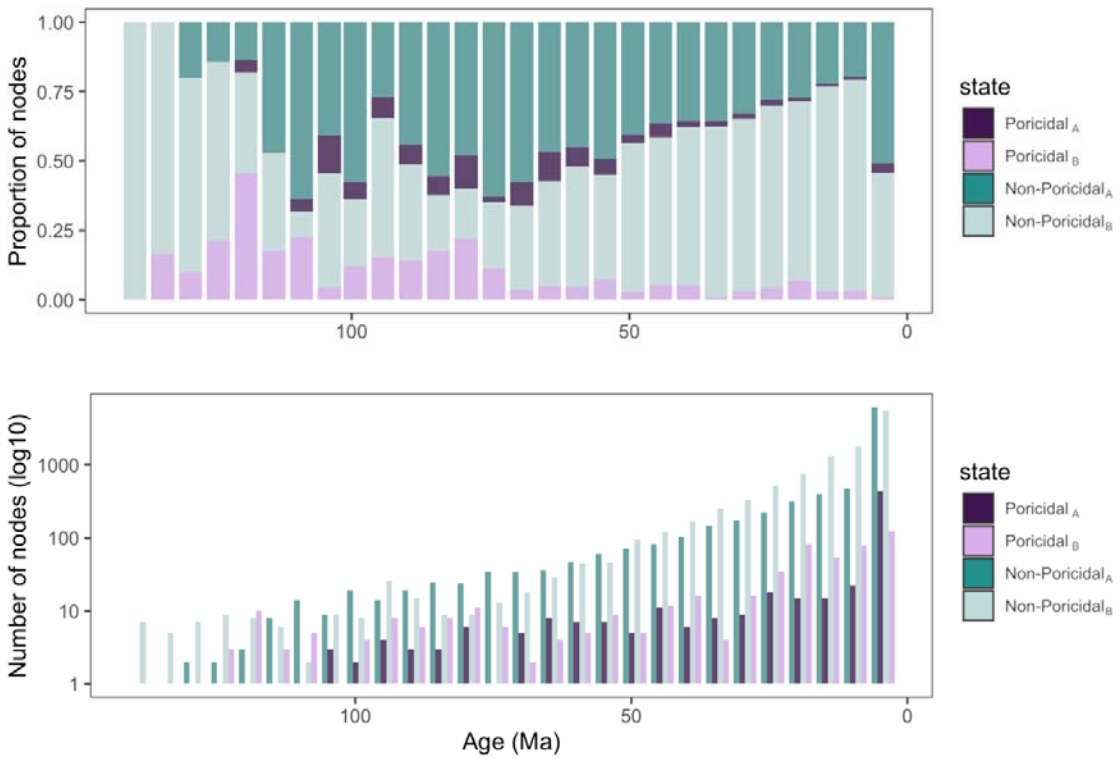


Figure 5. Proportion (top panel) and logged (bottom panel) estimated number of nodes over time (millions of years before present) of poricidal vs. non-poricidal taxa (genus) in a hidden state dependent speciation and extinction model with two hidden states (A, B) in an angiosperm-wide phylogeny.

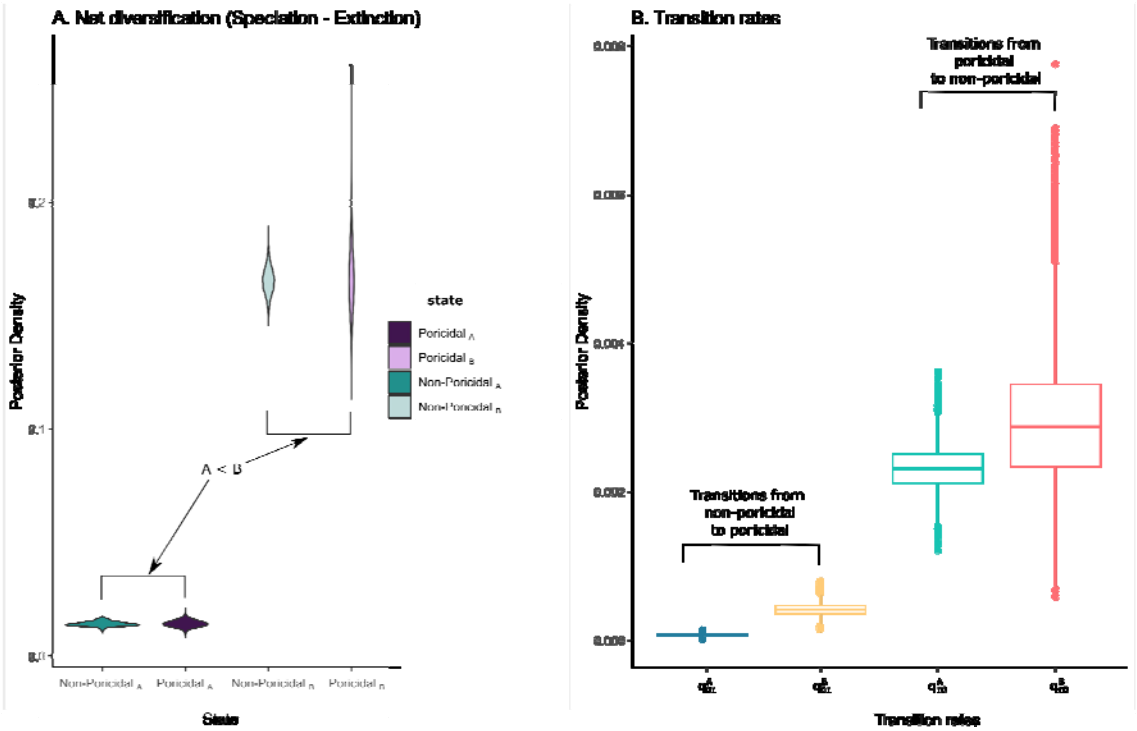
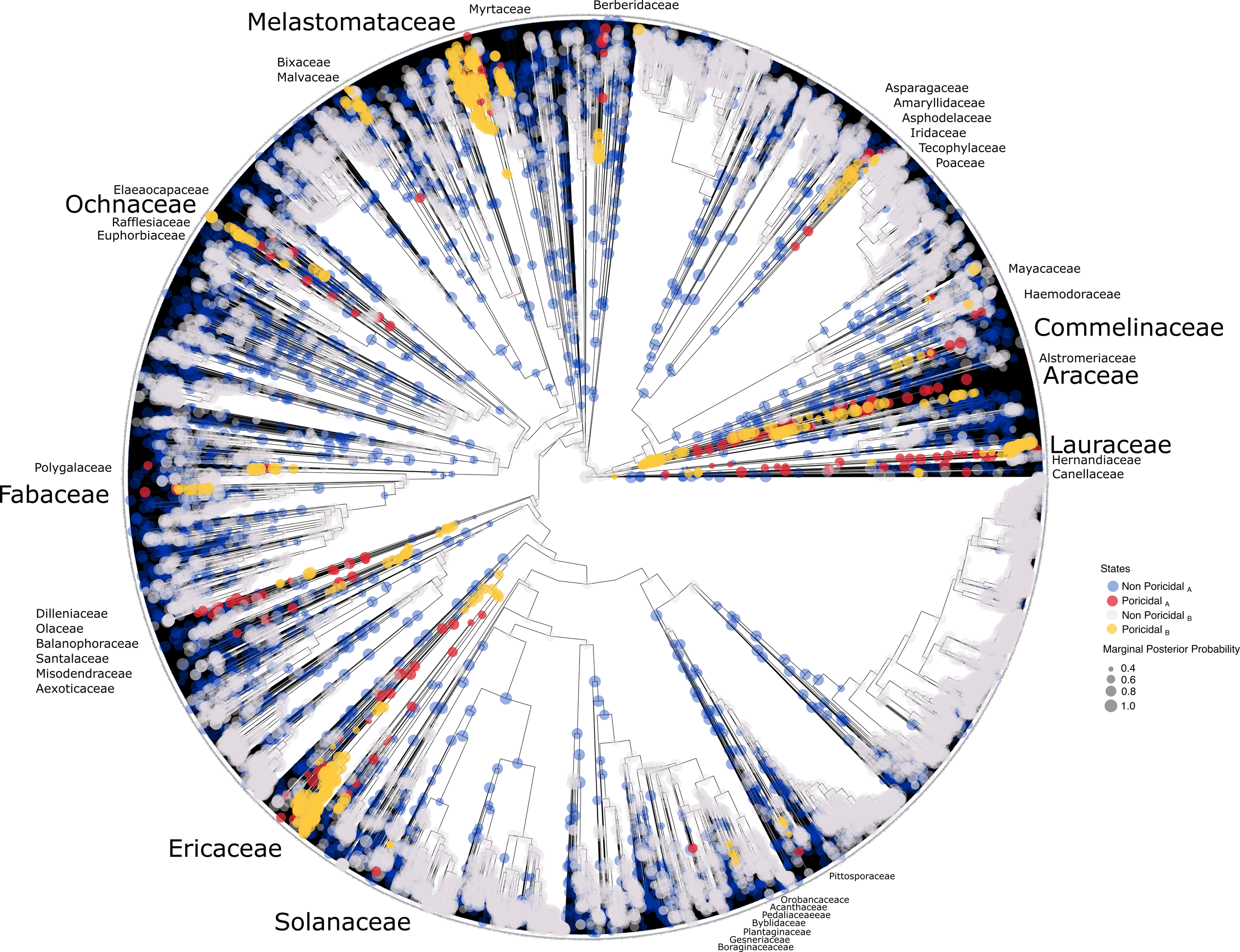


Figure 6. Hidden state dependent speciation and extinction model fitted to non-poricidal and poricidal states in a species-level phylogeny with 29,246 tips. (A) No differences in net diversification were found between poricidal and non-poricidal states in both hidden state A and B. (B) Transitions to poricidal states from non-poricidal are slower (NP P) than transitions from poricidal back to non-poricidal (P NP) for both hidden states A and B.


Melastomataceae
Myrtaceae
Berberidaceae
Bixaceae
Malvaceae
Asparagaceae
Amaryllidaceae
Asphodelaceae
Iridaceae
Tecophylaceae
Poaceae
Elaeaocapaceae
Ochnaceae
Rafflesiaceae
Euphorbiaceae
Mayacaceae
Haemodoraceae
Commelinaceae
Alstromeriaceae
Araceae
Lauraceae
Hernandiaceae
Canellaceae
Polygalaceae
Fabaceae
Dilleniaceae
Olaceae
Balanophoraceae
Santalaceae
Misodendraceae
Aexoticaceae
Ericaceae
Solanaceae
Pittosporaceae
Orobancaceace
Acanthaceae
Pedaliaceaeeae
Byblidaceae
Plantaginaceae
Gesneriaceae
Boraginaceaceae
States
Non Poricidal A
Poricidal A
Non Poricidal B
Poricidal B
Marginal Posterior Probability
0.4
0.6
0.8
1.0