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LETTERS

Dating the origin of the Orchidaceae from a fossil orchid with its pollinator

Santiago R. Ramírez¹, Barbara Gravendeel², Rodrigo B. Singer³, Charles R. Marshall^{1,4} & Naomi E. Pierce¹

Since the time of Darwin¹, evolutionary biologists have been fascinated by the spectacular adaptations to insect pollination exhibited by orchids. However, despite being the most diverse plant family on Earth², the Orchidaceae lack a definitive fossil record and thus many aspects of their evolutionary history remain obscure. Here we report an exquisitely preserved orchid pollinarium (of *Meliorchis caribea* gen. et sp. nov.) attached to the mesoscutellum of an extinct stingless bee, *Proplebeia dominicana*, recovered from Miocene amber in the Dominican Republic, that is 15–20 million years (Myr) old³. This discovery constitutes both the first unambiguous fossil of Orchidaceae⁴ and an unprecedented direct fossil observation of a plant–pollinator interaction^{5,6}. By applying cladistic methods to a morphological character matrix, we resolve the phylogenetic position of *M. caribea* within the extant subtribe Goodyerinae (subfamily Orchidoideae). We use the ages of other fossil monocots and *M. caribea* to calibrate a molecular phylogenetic tree of the Orchidaceae. Our results indicate that the most recent common ancestor of extant orchids lived in the Late Cretaceous (76–84 Myr ago), and also suggest that the dramatic radiation of orchids began shortly after the mass extinctions at the K/T boundary. These results further support the hypothesis of an ancient origin for Orchidaceae.

Family Orchidaceae Juss., 1789
Subtribe Goodyerinae Klotzsch, 1846
Meliorchis caribea gen. et sp. nov.

Etymology. The generic name alludes to the plant's pollination mode by meliponine bees and incorporates the Greek name of an orchid (orchis: testicle). The specific epithet *caribea* refers to the Caribbean region.

Holotype. Museum of Comparative Zoology (Harvard University), catalogue number MCZ-31141.

Horizon and locality. Specimen was excavated in the year 2000 from a mine located east of Santiago, Cordillera Septentrional, Dominican Republic. Lignite and sandy clay beds, Early to Middle Miocene (15–20 Myr old; ref. 3).

Diagnosis. The species is separated from other members of Goodyerinae by the bent anther, large angular massulae (~100 per pollinarium), and tightly packed pollen units (20 × 20 μm). The amber piece (20 × 14 × 5 mm) contains a single inclusion of *Meliorchis caribea*. Two complete pollinia (each ~1,000 × 500 μm), belonging to a single pollinarium, are firmly attached to the mesoscutellum of a worker bee, *Proplebeia dominicana*⁷ (Fig. 1a). The tapering pollinia consist of >100 loosely packed angular massulae (~200 × 100 μm, Fig. 1b), each of which encapsulates several tetrads; obovoid pollen units are tightly packed.

These pollinarium features are found only in the Orchidoideae⁸. A survey of herbarium specimens of all Neotropical genera within this

subfamily showed that the size, shape and ornamentation of the fossil closely resemble those of modern members of the subtribe Goodyerinae, particularly the genera *Kreodanthus* and *Microchilus* (Supplementary Table 1). In addition, the position of the pollinarium on the fossilized bee enables us to make inferences about unique aspects of the flowers of *Meliorchis*, even in the absence of fossil flowers. Whereas in living Goodyerinae the pollinarium normally is attached to the mouthparts of pollinating bees⁹ (Fig. 2a), the

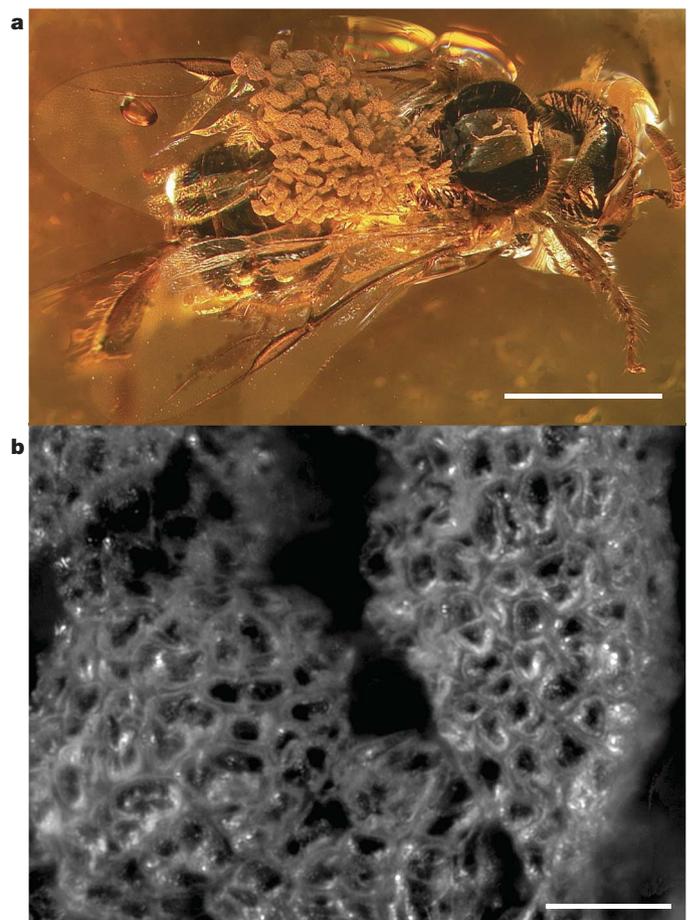


Figure 1 | Holotype of *Meliorchis caribea* gen. et sp. nov. This orchid pollinarium, carried by a worker stingless bee (*Proplebeia dominicana*), is preserved in amber from the Dominican Republic and represents the first definitive fossil record for the family Orchidaceae. **a**, General view of encapsulated specimen (scale bar, 1,000 μm). **b**, Detailed view of the pollinia surface showing pollen units (scale bar, 50 μm).

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pollinarium of *Meliorchis* is attached to the mesoscutellum (dorsal surface of the thorax) of worker bees of *P. dominicana*. This indicates that the flower of *M. caribea* was gullet-shaped, and, rather than the bee probing the lip of the flower with its tongue as in modern Goodyerinae (Fig. 2a), the anterior part of the bee would have had to enter the flower completely (Fig. 2b).

Because evidence of plant–pollinator interactions is exceedingly rare in the fossil record, our current knowledge of ancient pollination is indirectly inferred from specialized morphological features of fossilized insects^{10–12} and flowers^{13–15}. In addition, records of pollen grains on fossil insects and in coprolites provide circumstantial evidence for ancient insect–flower interactions^{5,6,10,12,14}, although these observations—with the exception of amber-preserved fig wasps carrying fig pollen⁶—do not exclude the possibility of flower visitation without pollination⁵. In contrast, because in most orchids the staminal filaments are fused to the style, the anatomical match required for a pollinator to remove the pollinarium is nearly identical to that necessary for its subsequent delivery (Fig. 2). Thus, *P. dominicana* bee workers were almost certainly pollinators of flowers of *M. caribea*. Because modern stingless bees pollinate numerous rainforest angiosperms¹⁶, including several tropical orchid species¹⁷, this fossil shows that adaptation by tropical orchids to specialized pollinators occurred at least as far back as the Miocene.

To explore the phylogenetic position of *Meliorchis* in relation to Modern orchid taxa, we constructed a morphological character matrix consisting of 25 characters and 15 taxa adapted from a previous study¹⁸ (see Supplementary Methods for details). Heuristic tree searches optimized by maximum parsimony yielded 129 equally short trees, all of which supported monophyly of both the subfamily Orchidoideae and the subtribe Goodyerinae (Fig. 3). The position of *Meliorchis* within Goodyerinae is supported by a bootstrap of 91%. Of the 129 recovered trees, none supported *Meliorchis* as a sister clade to the rest of the Goodyerinae genera. Together, these results indicate that *Meliorchis* represents a differentiated lineage within extant Goodyerinae. On the basis of estimated ages of Dominican amber³, a minimum age of 15–20 Myr can be assigned to the subtribe Goodyerinae.

Previously published putative orchid fossils have lacked diagnostic characters that would definitively assign them to Orchidaceae^{4,19}. In fact, in a thorough review of all known specimens, it was concluded that Orchidaceae have ‘no positive or useful fossil record’²⁴. This absence in the fossil record, most likely owing to their non-diagnostic leaves and lack of wind-dispersed pollen, has spurred considerable disagreement regarding orchids’ age of origin and timing of

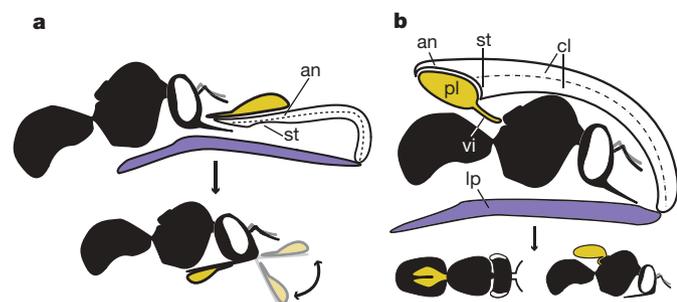


Figure 2 | Morphology and pollinarium placement of modern Goodyerinae and hypothetical reconstruction of floral morphology of *Meliorchis caribea*. **a**, The parallel lip (lp) and column (cl) and the erect anther (an) of extant Goodyerinae typically result in the pollinarium (pl) attachment on the pollinator’s mouthparts. **b**, The attachment of the pollinarium to the mesoscutellum (dorsal surface of thorax) of a worker bee is only possible when the lip and column of the flower are parallel but the anther is bent. Under this scenario, the distance between the lip and the column must be ~2.5 mm to enable a *P. dominicana* worker to crawl into the flower and remove the pollinarium with its mesoscutellum as it retreats; st, stigma; vi, viscidium.

diversification. Whereas orchids’ highly specialized pollination mechanisms, epiphytism and absence in fossil deposits were cited by early workers in support of a recent age^{4,20,21}, their worldwide distribution² and basal placement in the order Asparagales²² suggest an older age. Indeed, three recent molecular clock studies that broadly sampled angiosperm clades (including a few orchid representatives) obtained radically different age estimates for the Orchidaceae, ranging from ~26 Myr old²³ and ~40 Myr old²⁴ to ~110 Myr old²⁵. Such age discrepancies are most likely due to under-represented sampling and absence of internal calibration points. We here use both the age and phylogenetic position of *M. caribea* and other fossil monocots to estimate the timing of diversification for Orchidaceae.

We calibrated a molecular phylogenetic tree of Orchidaceae by implementing a relaxed-clock model through penalized likelihood and non-parametric rate smoothing (NPRS). We built a molecular phylogenetic tree of Orchidaceae that was based on plastid DNA sequences obtained from GenBank for 55 orchid genera representing all major lineages in the family, and five basal Asparagales genera as outgroup taxa. Our divergence time estimates using penalized likelihood suggest that extant Orchidaceae shared a most recent common ancestor in the Late Cretaceous, 76 ± 5 to 84 ± 6 Myr ago, depending on whether we use the oldest or youngest estimates of the ages of the fossils used to calibrate the relaxed molecular clock (Fig. 4). Similarly, age estimates obtained using NPRS suggest that crown Orchidaceae shared a common ancestor 76 ± 4 to 83 ± 4 Myr ago. Our results also suggest that stem lineages of all five orchid subfamilies were present early in the evolutionary history of Orchidaceae, before the end of the Cretaceous, ~65 Myr ago (Fig. 4). The extant lineages of the two largest orchid subfamily clades (Orchidoideae and Epidendroideae), which together encompass >95% of the living orchid species, began to diversify early in the Tertiary, although more thorough taxonomic sampling could result in older age estimates of their common ancestor.

The discovery of *Meliorchis caribea* and the internally calibrated molecular clock analyses presented here reject the hypothesis of a relatively recent (Eocene or younger) origin of Orchidaceae^{4,21}.

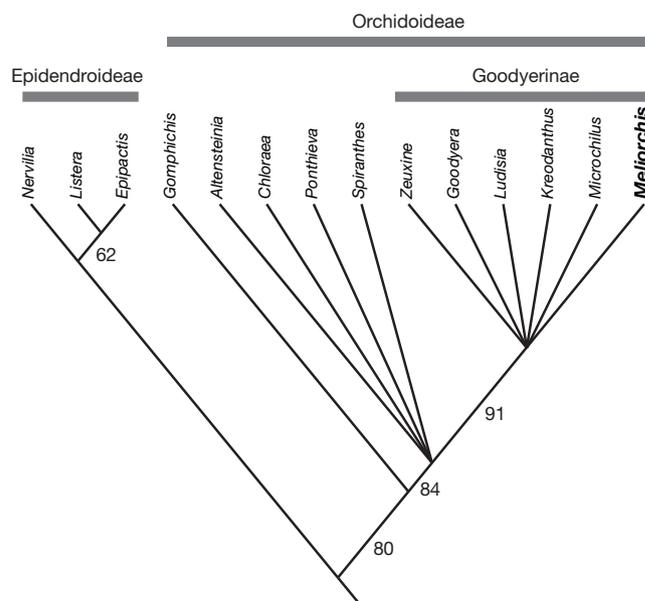


Figure 3 | Cladogram showing the estimated position of *Meliorchis* among modern clades in the orchid subfamily Orchidoideae. A strict consensus of the 129 shortest trees (tree length = 42, consistency index = 0.619, retention index = 0.660) obtained using 25 morphological characters for 15 taxa; values beside nodes correspond to bootstrap percentages (1,000 replicates). None of the shortest trees recovered *Meliorchis* as sister to all the other Goodyerinae included.

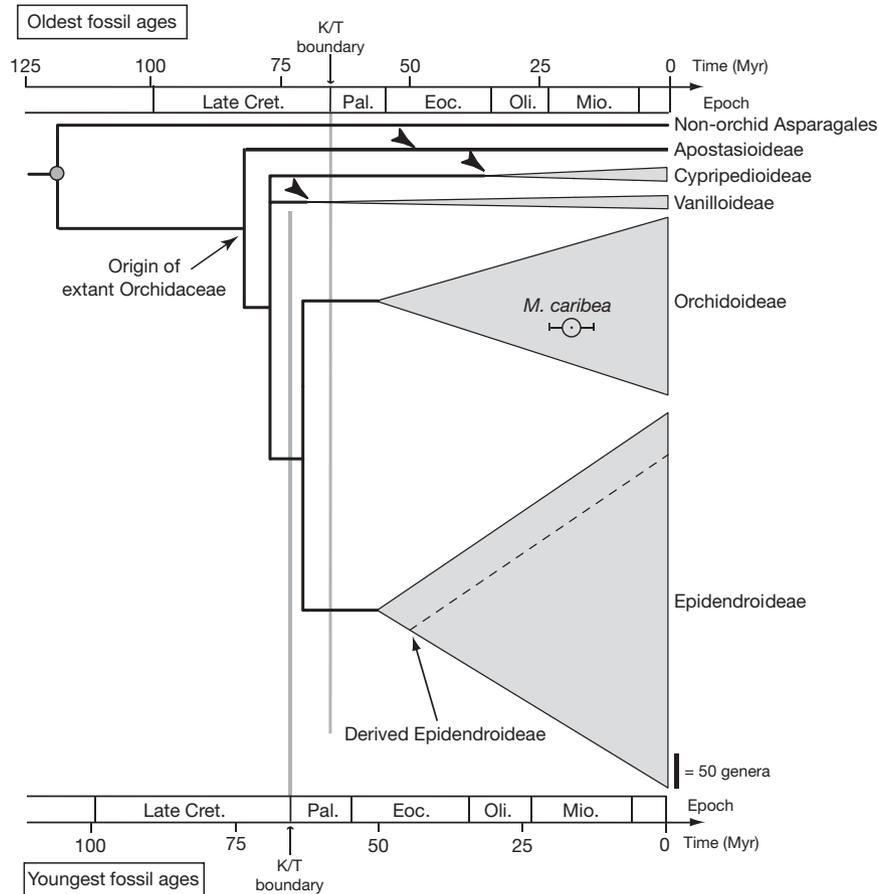


Figure 4 | Fossil-calibrated molecular clock chronogram of the family Orchidaceae, based on ~3 kilobases of plastid DNA (*matK* and *rbcL*). The relative size of each clade is proportional to the number of genera described in each orchid subfamily. Crown ages of small clades are indicated with arrow heads. Two sets of dates were used to calculate orchid divergence times: the oldest and youngest estimates of the ages of the fossils. The age boundaries of *M. caribea* (15–20 Myr old) relative to each timescale

Instead, our results favour the hypothesis of an ancient (Late Cretaceous)^{22,25} origin of extant Orchidaceae, but at the same time support a Tertiary radiation of the most diverse epiphytic clades. Our age estimates are younger than the oldest proposed for the family by previous studies²⁵, but we note that our age calculations should be regarded as minimum estimates, which could be pushed back with additional fossil discoveries. Our scenario corresponds to that previously proposed^{2,22}, is consistent with the observed disjunct pantropical distributions of the subfamily clades and the early-splitting genera (for example, *Vanilla*), and reinforces the possibility of a Late Cretaceous biotic exchange between tropical continents.

METHODS

Colour photomicrographs were taken with a JVC digital camera (KYF75U) mounted on a MZ16 Leica dissecting scope; black and white micrographs were taken with a Retiga EXi digital camera mounted on a Leica Leitz-dmrb compound microscope (objective $\times 40$). In both cases, 10 sequential shots at different focal depths were processed with the Auto-Montage software (Synchroscopy, 2002) to produce a single composite image.

The phylogenetic position of *Meliiorchis* was explored using morphological characters from flowers, pollinaria and pollen micro-morphology, all of which were directly observable or inferable from the type specimen of *M. caribea*. We treated all character states as unordered and weighted them equally. Because *Meliiorchis* unambiguously belongs to the subfamily Orchidoideae, we only included representative genera from this group. We selected outgroup taxa on the basis of previous studies that used both morphological¹⁸ and molecular²⁶ data. Heuristic tree searches were performed via maximum parsimony with

correspond to the distance between the circle's centre and the vertical bar. Additional monocot fossil records outside Orchidaceae were used to calibrate the root of the tree (node indicated by filled circle). Branch lengths were optimized under the maximum likelihood model of sequence evolution GTR+ Γ +I using a 95% majority-rule consensus tree (see Supplementary Information); node ages were estimated using a penalized likelihood method²⁹.

the TBR algorithm (100 random addition replicates). A total of 1,000 replicates were run to estimate bootstrap support; all analyses were performed in PAUP* v.4.0b.

Consensus phylogenetic trees of bayesian analyses were obtained with the software MrBayes v3.1.1 (for details, see Supplementary Materials). Our topologies agree with those obtained by previous studies^{27,28}. Divergence times were calculated by penalized likelihood and NPRS, using the truncated Newton algorithm in the software r8s v 1.71²⁹. Two sets of dates were used, corresponding to the youngest and oldest estimates of the ages of the fossils used as node age constraints. We applied (1) the age of *Meliiorchis* (15–20 Myr old; ref. 3) as a minimum age for the monophyletic Goodyerinae; (2) the age of the oldest known Asparagales (93–105 Myr old, see Supplementary Methods for details) as a minimum age constraint at the root of the tree; and (3) the age of the oldest known fossil monocot as the maximum age at the root of the tree (110–120 Myr old; ref. 30).

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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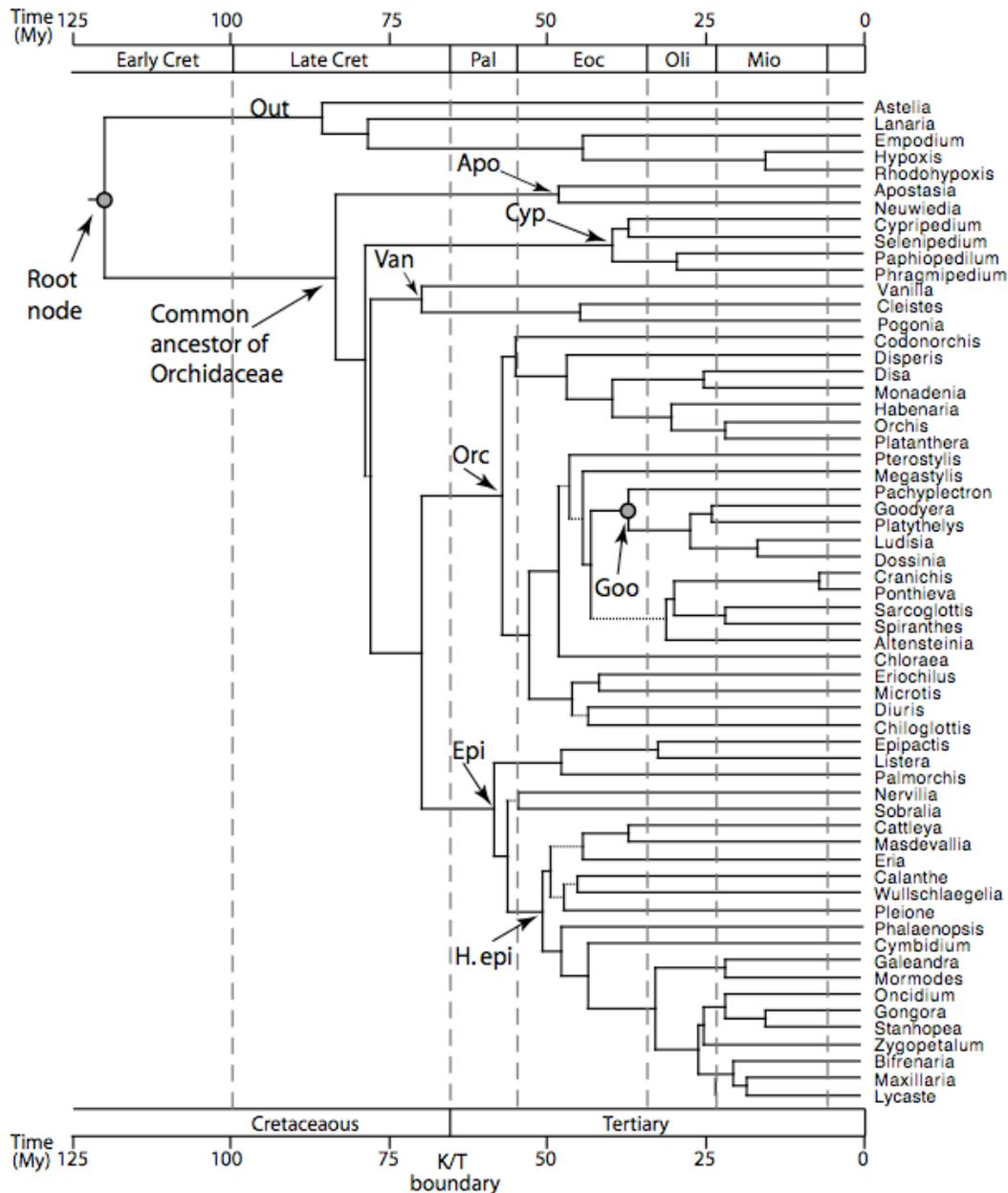
Author Contributions S.R.R., B.G. and N.E.P. procured and curated the specimen. S.R.R., B.G. and R.B.S. reviewed herbaria specimens, analysed ancestral floral morphology, and coded and analysed morphological characters. S.R.R. and C.R.M. designed dating approaches and considered their interpretation. S.R.R. wrote the paper. All authors discussed the results and commented on the manuscript.

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“Dating the origin of the Orchidaceae from a fossil orchid with its pollinator”

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1. SUPPLEMENTARY FIGURE AND LEGEND



Supplementary Figure 1. Molecular clock chronogram estimated via penalized likelihood using 50% Majority-Rule consensus topology (see Supplementary Methods) of the family Orchidaceae when using the oldest bound ages of fossil calibrations. Dashed branches subtend nodes with posterior probabilities below 0.95. Circles indicate age-constrained nodes. Out = Outgroups, Apo = Apostasioideae, Cyp = Cypripedioideae, Orc = Orchidoideae, Epi = Epidendroideae, H.epi = Higher Epidendroids, Goo = Goodyerineae.

2. SUPPLEMENTARY METHODS

1. Morphological character codes:

Floral features

- 01. Column and lip***
0 : parallel
1 : perpendicular
- 02. Stigma†****
0 : protruded
1 : flat or slightly convex
- 03. Anther orientation†***
0 : erect
1 : bent

Pollinaria

- 04. Caudicle†**
0 : absent
1 : present
- 05. Hamulus stipe†**
0 : absent
1 : present
- 06. Viscidium†**
0 : none
1 : diffuse
2 : detachable
- 07. Viscidium shape**
0 : pad-like
1 : elongated or U/V-shaped
- 08. Viscidium : pollinium ratio**
0 : viscidium << pollinium
1 : viscidium \cong pollinium
- 09. Tegula stipe†**
0 : absent
1 : present

10. Pollinium shape (dorsal profile)

- 0 : rhomboid
- 1 : clavate-obovoid

11. Pollinium orientation†

- 0 : juxtaposed
- 1 : superposed

12. Pollinium number†

- 0 : 2
- 1 : 4
- 2 : > 4

13. Pollinium texture†

- 0 : granular
- 1 : massulate

*Pollen micro-morphology***14. Massulae†**

- 0 : absent
- 1 : present

15. Massulae shape

- 0 : angular
- 1 : laminar
- 2 : rounded

16. Massulae across pollinia

- 0 : similar in size and shape
- 1 : variable in size and shape

17. Massulae : pollinia ratio

- 0 : $\leq 1:100$
- 1 : $\geq 1:50$

18. Massulae packaging

- 0 : loose
- 1 : tight

19. Tetrad packaging

- 0 : loose
- 1 : tight

20. Pollen unit†

- 0 : monad

1 : tetrad

- 21. Pollen sculpturing**
 0 : non reticulate
 1 : reticulate
- 22. Pollen grains**
 0 : baculate
 1 : tectate
- 23. Shape of pollen grains**
 0 : toroid
 1 : otherwise

Pollination

- 24. Pollinarium placement on pollinator**
 0 : mouthparts
 1 : mesothorax
 2 : legs
 3 : head
- 25. Pollination syndrome**
 0 : Coleoptera
 1 : Diptera
 2 : Hymenoptera
 3 : Lepidoptera
 4 : generalist

† Characters adapted from Freudenstein & Rasmussen (1999)¹⁸. The remaining characters were coded from the literature³¹⁻³⁶.

* The flower morphology constrains the placement of the pollinia onto different parts of the pollinator; attachment onto the mesoscutellum is achieved when the anther is bent and the lip and the column are parallel (Figure 2b). The lip and column of modern *Goodyerinae* are parallel, but the anther is erect (Figure 2a), which results in pollinia attachment to the pollinator's mouthparts. Hence, we can infer that *Meliorchis* displayed both a parallel lip and column, and bent anther (Fig. 2b).

**Orchids that display globose, indivisible pollinia have concave or sunken stigmatic surfaces, a feature that facilitates pollinia deposition into the concave stigmatic surface. Conversely, orchids with massulate pollinia display flat to slightly convex stigmatic surfaces, a feature that may promote cross-pollination

and multiple pollination events by individual pollinaria³⁷. Since *Meliorchis* had massulate pollinia, we infer that its stigmatic surface was flat to slightly convex.

Morphological data matrix

| | | | | | | | | | |
|--------------|-----|-----|-----|-----|-----|-----|-----|-----|---|
| Listera | 100 | 001 | 000 | 0?1 | 00- | --- | 010 | ?03 | 4 |
| Epipactis | ?00 | 001 | 010 | 001 | 00- | --- | 110 | ?0? | 4 |
| Nervilia | ?01 | 001 | 010 | 001 | 00- | --- | 110 | ?1? | 2 |
| Altensteinia | 000 | 002 | 100 | 101 | 002 | ?01 | 111 | 11? | ? |
| Chloraea | 000 | 000 | --0 | 101 | 002 | ?00 | 111 | 111 | 2 |
| Gomphichis | 100 | 002 | 000 | 001 | 001 | 001 | 011 | 11? | ? |
| Ponthieva | 000 | 002 | ?00 | 101 | 001 | 101 | 111 | 11? | 2 |
| Spiranthes | 000 | 002 | 100 | 001 | 002 | 101 | 111 | 010 | 2 |
| Zeuxine | 111 | 002 | 101 | 101 | 111 | ?10 | 111 | 11? | ? |
| Goodyera | 010 | 002 | 100 | 101 | 110 | 110 | 111 | 110 | 2 |
| Ludisia | 010 | 002 | 100 | 101 | 110 | 110 | 111 | 112 | 3 |
| Kreodanthus | 010 | 0?2 | 100 | 100 | 110 | 010 | ?1? | ??? | ? |
| Microchilus | 010 | 002 | 100 | 100 | 110 | 010 | 111 | 1?0 | 2 |
| Meliorchis | 011 | 002 | 100 | 100 | 110 | 010 | 111 | 101 | 2 |

The following are the current taxonomic positions of the genera used in the morphological matrix above³²:

Chloraeenae: *Chloraea*

Chranichidae: *Altensteinia*, *Gomphichis*, *Ponthieva*

Goodyerinae: *Zeuxine*, *Goodyera*, *Ludisia*, *Kreodanthus*, *Microchilus*

Spiranthinae: *Spiranthes*

Epidendroideae outgroups: *Listera*, *Epipactis*, *Nervilia*

2. Molecular phylogenetic methods:

We used ~3kb of plastid DNA sequences (1556 bp of *matK*, 1338 bp of *rbcL*) corresponding to 55 orchid genera belonging to all five orchid subfamilies as ingroup taxa. Several recent molecular studies³⁸⁻⁴⁰ have shown that Orchidaceae is sister to the rest of the Asparagales. Thus, theoretically, any non-orchid Asparagales could be used as the outgroup in our analyses. We chose five basal genera in the Asparagales as outgroups. All sequence data used in this study were obtained from *GenBank* (NCBI).

Likelihood analyses were implemented in a Bayesian framework with the software package *MrBayes* v3.1.1. We assumed a single model of sequence evolution for the entire dataset (GTR+ Γ +I) and ran the Monte Carlo Markov Chain (MCMC) for 1,000,000 generations, sampling every 100 generations for a total of 10,000 trees; model parameters were estimated during the run. Bayesian posterior probabilities were estimated as the proportion of trees sampled after discarding the trees corresponding to the first 1,000 generations (“burn-in”).

3. Molecular clock estimation:

We obtained a single, fully resolved topology by applying a 50% Majority-Rule (MR) consensus to all trees obtained in the Bayesian analyses. Few clades in the phylogeny had low support; we also obtained a 95% MR consensus tree in which poorly supported nodes (< 0.95 posterior probability) were collapsed into polytomies. We used both consensus trees (50% and 95%) in our estimation of divergence times. Our 50% MR consensus tree disagrees in the position of the subfamilies Vanilloideae and Cypripedioideae, but our 95% MR tree is entirely compatible with those obtained by previous studies^{27,28}.

We calculated branch lengths with maximum likelihood in the software package *PAUP**, optimized under the model of sequence evolution GTR+ Γ +I (molecular clock not enforced). Node divergence times were estimated with Penalized Likelihood (PL) and Non-Parametric Rate Smoothing (NPRS) with the TN algorithm in the software package *r8s* v1.71. Age standard deviations were calculated using non-parametric bootstrapping.

4. Fossil calibrations:

We used three different fossil calibrations in the molecular clock analyses presented here. Both maximum and minimum age constraints were enforced.

Meliorchis caribea gen. et sp. nov. was used as a **minimum** age calibration point for the monophyletic subtribe Goodyerinae (Supplementary Figure 1). Since the precise mine of origin of *Meliorchis caribea* is not known, we used both the oldest and youngest age bounds of Dominican amber (15-20 My)³ as minimum age constraints for the Goodyerinae.

We constrained the root of the tree with a **minimum** age corresponding to the oldest known fossil record for Asparagales. *Liliacidites* sp.1 and *Liliacidites* sp.2 from lower Upper Albian (~105 My) deposits of the Potomac Group⁴¹ and *Liliacidites* cf. *intermedius* and *L.* cf. *kaitangataensis* from Cenomanian-Turonian sediments of the Bathurst and Melville Islands of Eastern Australia (Late Cretaceous, 93-99 My)^{42,43} are the oldest records of the genus *Liliacidites*. Pollen grains of *Liliacidites* sp 2 (in Walker Walker⁴¹) are monosulcate-operculate, boat-shaped, thicotosulcate, reticulated irregularly into coarse and fine areas, and have psilate muri and dimorphic lumina. These characters unambiguously assign them to the monocotyledons⁴¹⁻⁴². The operculate pollen suggests an affinity with the monocot orders Asparagales, Liliales and Poales⁴⁴ and the trichotosulcus (*i.e.* single furrow divided into three branches in the distal pole^{45,46}) is almost invariably associated with simultaneous meiotic sporogenesis; both developmental and phylogenetic studies have shown that simultaneous sporogenesis is diagnostic of the Asparagales⁴⁷⁻⁴⁹. Additionally, the heterobrochate, mono-pluricolumellate reticula that diminish near colpi (*e.g.* *L. pollucibilis* from Late Cretaceous⁵⁰) suggest an affinity with the family Agavaceae (C. Jaramillo [STRI], pers. comm.), also in the Asparagales. Grains of *Liliacidites* cf. *intermedius* and *L.* cf. *kaitangataensis* from Bathurst and Melville Islands exhibit thickened exine in the equatorial zone, and also display a surface rupture opposite to the sulcus⁴², a feature that is present in pollen grains of the family Amarylidaceae (Asparagales)⁵¹. Although Walker and Walker⁴¹ reported an additional species of *Liliacidites* ("*L. minutus*") from Middle-Upper Albian Potomac Group deposits (~105 My), they conclude that this is "probably best treated as a distinct genus". Grains of "*L. minutus*" lack the diagnostic characters listed above and are therefore not used here. We use both the oldest and youngest age bounds of the sediments containing the earliest records of *Liliacidites* (93-105 My) with diagnostic characters of the Asparagales as minimum age constraints of the root of our tree.

We constrained the root of the tree (basal Asparagales) with a **maximum** age equal to the oldest known monocot fossil. Friis *et al* (2004)³⁰ recently described the earliest known monocot fossil pollen (*Mayoa portugallica*, Araceae) from sandy, lignitic horizons in the Almargem Formation of the Early Cretaceous of Portugal, a formation estimated to be 110-120 My old. We used both the oldest and youngest age bounds of *M. portugallica* (110 and 120 My) as a maximum age constraint for the root of the tree (see Supplementary Figure 1).

5. Identification of *Proplebeia dominicana* and authenticity of the amber inclusion.

The bee carrying the pollinarium of *Meliorchis* is unambiguously assigned to the well-known species of stingless bee *Proplebeia dominicana*. Three species of the extinct genus *Proplebeia* are known from Dominican amber, but *P. dominicana* is easily separated from the other two by the "short malar area (ca 1/2 diameter of scape); yellow stripe on paraocular area extending above the antennal alveolus;

[and the deep] emargination between [the] mandibular denticles”⁷. *P. dominicana* is known only from Dominican amber deposits and is now extinct, thereby strongly supporting the authenticity of the specimen. Careful examination of the amber piece revealed no cuts or evidence that the specimen had been re-embedded (see Grimaldi *et al.* [1994]⁵² for a discussion on amber authenticity).

6. Evidence for the presence of orchid bees and orchids in Hispaniola during the Miocene:

Two amber euglossine bees are known from Dominican amber^{53,54}. Extant euglossine bees (or orchid bees) are well known for their intricate associations with orchid flowers throughout the Neotropical Region. Male bees actively collect chemical fragrances from orchids flowers, store them in specialized hind leg pockets, and subsequently present them to females during courtship. In the process, male orchid bees pollinate a large number of orchid species that otherwise are not visited (nor pollinated) by any other group of pollinators. However, despite the intricate nature of this association, euglossine bees do not necessarily depend on their orchid hosts for reproduction. The strongest evidence for this comes from a recent study of a Mexican euglossine bee that was recently naturalized in the southern U.S. (Florida), an area where euglossine-pollinated orchids are absent. Because male bees gather fragrances from multiple non-orchid plant sources, this species of euglossine bee has been able to establish large, stable populations even in the absence of its customary orchid associates⁵⁵. Thus, the existence of euglossine bees in Dominican amber does not necessarily indicate that Hispaniola had a well developed orchid flora during the Miocene.

In *The Amber Forest*, Poinar (1999)⁵⁶ identifies an “infinitesimal seed [from Dominican amber] as possibly belonging to an orchid”; however, because of missing diagnostic characters, it cannot be unambiguously assigned to the family Orchidaceae⁵⁶.

3. SUPPLEMENTARY TABLES

Supplementary Table 1. Orchidoideae specimens examined for comparison (all specimens currently deposited in Harvard University Herbaria). Those closely resembling *Meliorchis caribea* are indicated with an asterisk (*).

| Orchid species | Tribe | Subtribe | Country | Voucher |
|---------------------------------------|--------------|---------------|--------------------|---------------------------------|
| <i>Cranichis muscosa</i> | Cranichideae | Cranichidinae | Ecuador | MacBryde 579 |
| <i>Fuertesiiella pterichoides</i> | Cranichideae | Cranichidinae | Cuba | Hioram 7615 |
| <i>Ponthieva racemosa</i> | Cranichideae | Cranichidinae | Venezuela | Steyermark 61230 |
| <i>Pterichis multiflora</i> | Cranichideae | Cranichidinae | Venezuela | Aristeguieta & Medine 3581 |
| <i>Aspidogyne multifoliata</i> | Cranichideae | Goodyerinae | Peru | Schunke Vigo 7369 |
| <i>Goodyera brachyceras</i> | Cranichideae | Goodyerinae | Mexico | Moore 5300 |
| <i>Goodyera striata</i> | Cranichideae | Goodyerinae | Mexico | Ostlund 2591 |
| <i>Goodyera striata</i> | Cranichideae | Goodyerinae | Mexico | Conzatti & Gonzalez 459 |
| <i>Kreodanthus casillasii*</i> | Cranichideae | Goodyerinae | El Salvador | Hamer 199 |
| <i>Kreodanthus crispifolius*</i> | Cranichideae | Goodyerinae | Ecuador | Drew E-355 |
| <i>Ligeophila jurvenensis</i> | Cranichideae | Goodyerinae | Colombia | Cuatrecasas 16280 |
| <i>Microchilus plantagineus*</i> | Cranichideae | Goodyerinae | Dominican Republic | Hodge 1940 |
| <i>Microchilus plantagineus*</i> | Cranichideae | Goodyerinae | Dominican Republic | Fennah 22 |
| <i>Platythelis querceticola</i> | Cranichideae | Goodyerinae | Guadeloupe | Proctor 20070 |
| <i>Platythelis querceticola</i> | Cranichideae | Goodyerinae | Cuba | Oakes Ames s.n. (Nov. 9th 1902) |
| <i>Stephanothelys xystophylloides</i> | Cranichideae | Goodyerinae | Ecuador | Steyermark 54818 |
| <i>Platanthera replicata</i> | Orchideae | Orchidinae | Cuba | Hodge et al. 4777 |
| <i>Beloglottis costaricensis</i> | Cranichideae | Spiranthinae | Peru | Klug 3718 |
| <i>Cyclopogon elatus</i> | Cranichideae | Spiranthinae | Argentina | Sosa et al 20 |
| <i>Eltroplectris calcarata</i> | Cranichideae | Spiranthinae | Jamaica | Howard & Proctor 13449 |
| <i>Eurystyles alticola</i> | Cranichideae | Spiranthinae | Dominican Republic | Gastony et al 597 |
| <i>Eurystyles ananassocomos</i> | Cranichideae | Spiranthinae | Peru | Schunke 533 |
| <i>Eurystyles domingensis</i> | Cranichideae | Spiranthinae | | |
| <i>Goodyera brachyceras</i> | Cranichideae | Spiranthinae | Mexico | Espejo 5586 |
| <i>Hapalorchis lineatus</i> | Cranichideae | Spiranthinae | Dominican Republic | NYBG (Liogier) 14549 |
| <i>Lankesterella longicollis</i> | Cranichideae | Spiranthinae | Brasil | Pabst 4319 |
| <i>Mesadenus polyanthus</i> | Cranichideae | Spiranthinae | Mexico | Dino 7251 |
| <i>Pelexia adnata</i> | Cranichideae | Spiranthinae | Mexico | Tamaulipas 671 |
| <i>Plexia adnata</i> | Cranichideae | Spiranthinae | Mexico | Roszinsky 1247 |
| <i>Pseudogoodyera wrightii</i> | Cranichideae | Spiranthinae | Cuba | Shafer 12212 |
| <i>Sarcoglottis acaulis</i> | Cranichideae | Spiranthinae | Surinam | Selby (Determann) 85-1142 |
| <i>Schiedeella amesiana</i> | Cranichideae | Spiranthinae | Dominican Republic | Krug & Urban 3005 |
| <i>Spiranthes vernalis</i> | Cranichideae | Spiranthinae | Mexico | Pringle 4192 |
| <i>Stenorrhynchos speciosum</i> | Cranichideae | Spiranthinae | Costa Rica | Standley 33907 |

Supplementary Table 2. Age estimates (in Millions of years, My) \pm standard deviations (SD) of major crown clades in the Orchidaceae calculated via two different methods: Penalized Likelihood (PL) and Non-Parametric Rate Smoothing (NPRS). Two different Majority-rule consensus trees (50% and 95%) resulting from the same Bayesian tree searches were used to calculate node ages. SD values were calculated via non-parametric bootstrapping.

| Taxon | 50% Majority-rule consensus tree (fully resolved) | | | | 95% Majority-rule consensus tree (with polytomies) | | | |
|----------------------------------|---|------------|---------------|------------|--|------------|---------------|------------|
| | Oldest ages | | Youngest ages | | Oldest ages | | Youngest ages | |
| | PL | NPRS | PL | NPRS | PL | NPRS | PL | NPRS |
| Family Orchidaceae | 84 \pm 6 | 83 \pm 5 | 77 \pm 5 | 76 \pm 4 | 84 \pm 6 | 83 \pm 5 | 76 \pm 5 | 76 \pm 4 |
| Subfamily Apostasioideae | 49 \pm 5 | 48 \pm 5 | 45 \pm 4 | 44 \pm 5 | 49 \pm 5 | 48 \pm 5 | 45 \pm 4 | 44 \pm 5 |
| Subfamily Vanilloideae | 71 \pm 5 | 67 \pm 4 | 65 \pm 5 | 62 \pm 4 | 71 \pm 5 | 67 \pm 4 | 65 \pm 4 | 62 \pm 4 |
| Subfamily Cypripedioideae | 40 \pm 5 | 56 \pm 6 | 35 \pm 5 | 52 \pm 6 | 37 \pm 4 | 52 \pm 6 | 34 \pm 4 | 47 \pm 5 |
| Subfamily Orchidoideae | 59 \pm 5 | 61 \pm 4 | 53 \pm 4 | 56 \pm 4 | 58 \pm 5 | 60 \pm 4 | 52 \pm 4 | 55 \pm 4 |
| Subfamily Epidendroideae | 61 \pm 8 | 68 \pm 4 | 53 \pm 7 | 63 \pm 4 | 59 \pm 8 | 68 \pm 4 | 51 \pm 7 | 62 \pm 4 |
| “Higher” Epidendroids | 53 \pm 8 | 59 \pm 5 | 45 \pm 7 | 54 \pm 4 | 50 \pm 7 | 56 \pm 4 | 42 \pm 6 | 51 \pm 4 |
| Subtribe Goodyerinae | 38 \pm 4 | 39 \pm 4 | 34 \pm 3 | 36 \pm 3 | 38 \pm 4 | 39 \pm 3 | 34 \pm 3 | 36 \pm 3 |

Supplementary Table 3. GenBank (NCBI) accession numbers of taxa used in this study.

| Subfamily | Genus | <i>matK</i> | <i>rbcl</i> |
|-----------------|----------------------|-------------|-------------|
| Outgroups | <i>Astelia</i> | AY368372.1 | Z77261 |
| | <i>Lanaria</i> | AY368376.1 | Z77313 |
| | <i>Empodium</i> | AY368376 | Y14987.1 |
| | <i>Hypoxis</i> | AY368375.1 | Z73702 |
| | <i>Rhodohypoxis</i> | AY368377.1 | Z77280 |
| Apostasioideae | <i>Apostasia</i> | AY557214.1 | Z73705 |
| | <i>Neuwiedia</i> | AY557211.1 | AF074200 |
| Vanilloideae | <i>Cleistis</i> | AJ310006 | AF074128 |
| | <i>Pogonia</i> | AJ310055 | AF074221 |
| | <i>Vanilla</i> | AF263687 | AF074242 |
| Cypripedioideae | <i>Cypripedium</i> | AF263649 | AF074142 |
| | <i>Paphiopedilum</i> | AY368379 | AF074208 |
| | <i>Phragmipedium</i> | AY368380 | AF074213 |
| | <i>Selenipedium</i> | AY368381.1 | AF074227 |
| Orchidoideae | <i>Altenstenia</i> | AJ309989 | AF074105 |
| | <i>Chiloglottis</i> | AJ310003 | AF074124 |
| | <i>Chloraea</i> | AJ310005 | AF074125 |
| | <i>Codonorchis</i> | AJ310007 | AY368338 |
| | <i>Cranichis</i> | AJ310013 | AF074137 |
| | <i>Disa</i> | AF263654 | AF274006 |
| | <i>Disperis</i> | AY370652.1 | AY370651 |
| | <i>Diuris</i> | AF263655 | AF074152 |
| | <i>Dossinia</i> | AJ543947.1 | AJ542405 |
| | <i>Eriochilus</i> | AJ310028 | AF074166 |
| | <i>Goodyera</i> | AF263663 | AF074174 |
| | <i>Habenaria</i> | AJ310036 | AF074177 |
| | <i>Ludisia</i> | AJ543911.1 | AJ542395 |
| | <i>Megastylis</i> | AJ310042 | AF074191 |
| | <i>Microtis</i> | AJ310045 | AF074194 |
| | <i>Monadenia</i> | AJ310047 | AY368344 |
| | <i>Orchis</i> | AY368385 | AF074203 |
| | <i>Pachyplectron</i> | AJ310051.1 | AF074205 |
| | <i>Platanthera</i> | AF263678 | AF074215 |
| | <i>Platythelys</i> | AY368386.1 | AF074216 |
| | <i>Ponthieva</i> | AJ310056 | AF074223 |
| | <i>Pterostylis</i> | AJ310062 | AF074224 |
| | <i>Sarcoglottis</i> | AJ310068 | AY368347 |
| | <i>Spiranthes</i> | AF263682 | AF074229 |

| | | | |
|--------------------------|------------------------|----------|----------|
| Lower Epidendroideae | <i>Epipactis</i> | AF263659 | Z73707 |
| | <i>Listera</i> | AF263668 | AF074184 |
| | <i>Nervilia</i> | AY368420 | AF074199 |
| | <i>Palmorchis</i> | AJ310052 | AF074206 |
| | <i>Sobralia</i> | AF263681 | AF074228 |
| Higher Epidendroideae | <i>Bifrenaria</i> | AY368394 | AF074112 |
| | <i>Calanthe</i> | AF263632 | AF264159 |
| | <i>Cattleya</i> | AF263638 | AF074122 |
| | <i>Cymbidium</i> | AF263648 | AF074141 |
| | <i>Eria</i> | AF263660 | AF074164 |
| | <i>Galeandra</i> | AY368408 | AF074171 |
| | <i>Gongora</i> | AY368409 | AY368358 |
| | <i>Lycaste</i> | AF263669 | AF074185 |
| | <i>Masdevallia</i> | AY368416 | AF074189 |
| | <i>Maxillaria</i> | AF239427 | AF074190 |
| | <i>Mormodes</i> | AY368417 | AF074196 |
| | <i>Oncidium</i> | AY368423 | AF074201 |
| | <i>Phalaenopsis</i> | AF263677 | AF074211 |
| | <i>Pleione</i> | AF263679 | AF264173 |
| | <i>Stanhopea</i> | AY368430 | AF074230 |
| | <i>Wulfschlaegelia</i> | AY368434 | AY368436 |
| <i>Zygopetalum</i> | AF263689 | AF074246 | |

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