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LETTERS

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

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Since the time of Darwin[1], evolutionary biologists have been fascinated by the spectacular adaptations to insect pollination exhibited by orchids. However, despite being the most diverse plant family on Earth[2], 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) old[3]. This discovery constitutes both the first unambiguous fossil of Orchidaceae[4] 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 Orchidoideae[8]. 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 bees[9] (Fig. 2a), the

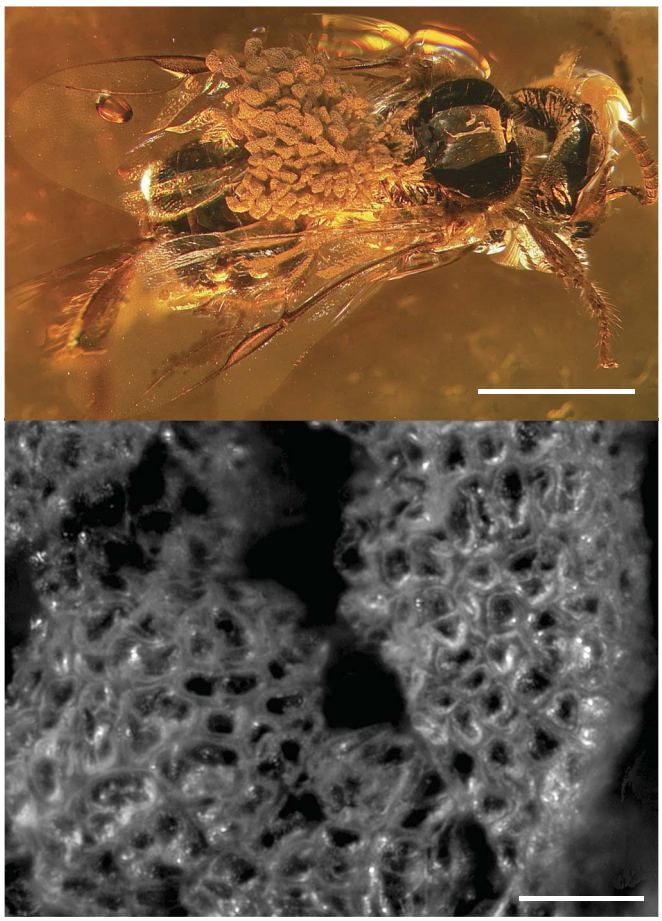

Figure 1 | Holotype of *Meliorchis caribea* gen. et sp. nov. This orchid pollinarium, carried by a worker stingless bee (*Proplebeia dominicana*), is preserved in amber from the Dominican Republic and represents the first definitive fossil record for the family Orchidaceae. **a**, General view of encapsulated 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 pollen[6]—do not exclude the possibility of flower visitation without pollination[5]. 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 angiosperms[16], including several tropical orchid species[17], 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 study[18] (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 amber[3], 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'[4]. 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 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 distribution[2] and basal placement in the order Asparagales[22] 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 old[23] and ~40 Myr old[24] to ~110 Myr old[25]. 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].

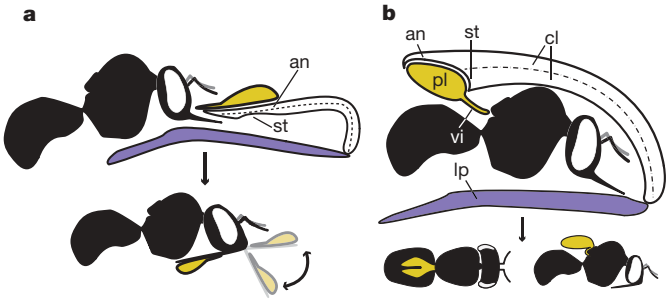

Figure 2 | Morphology and pollinarium placement of modern Goodyerinae and hypothetical reconstruction of floral morphology of *Meliorchis caribea*. **a**, The parallel lip (lp) and column (cl) and the erect anther (an) of extant Goodyerinae typically result in the pollinarium (pl) attachment on the pollinator's 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.

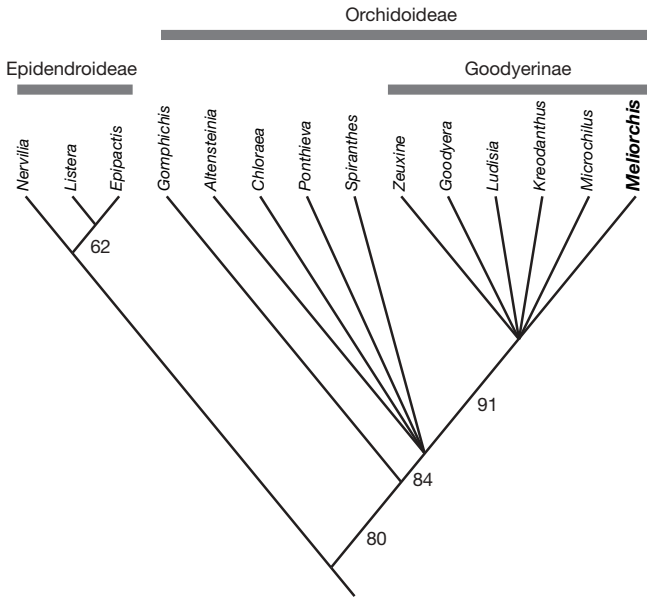

Figure 3 | Cladogram showing the estimated position of *Meliorchis* among modern clades in the orchid subfamily Orchidoideae. A strict consensus of the 129 shortest trees (tree length = 42, consistency index = 0.619, retention index = 0.660) obtained using 25 morphological characters for 15 taxa; values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

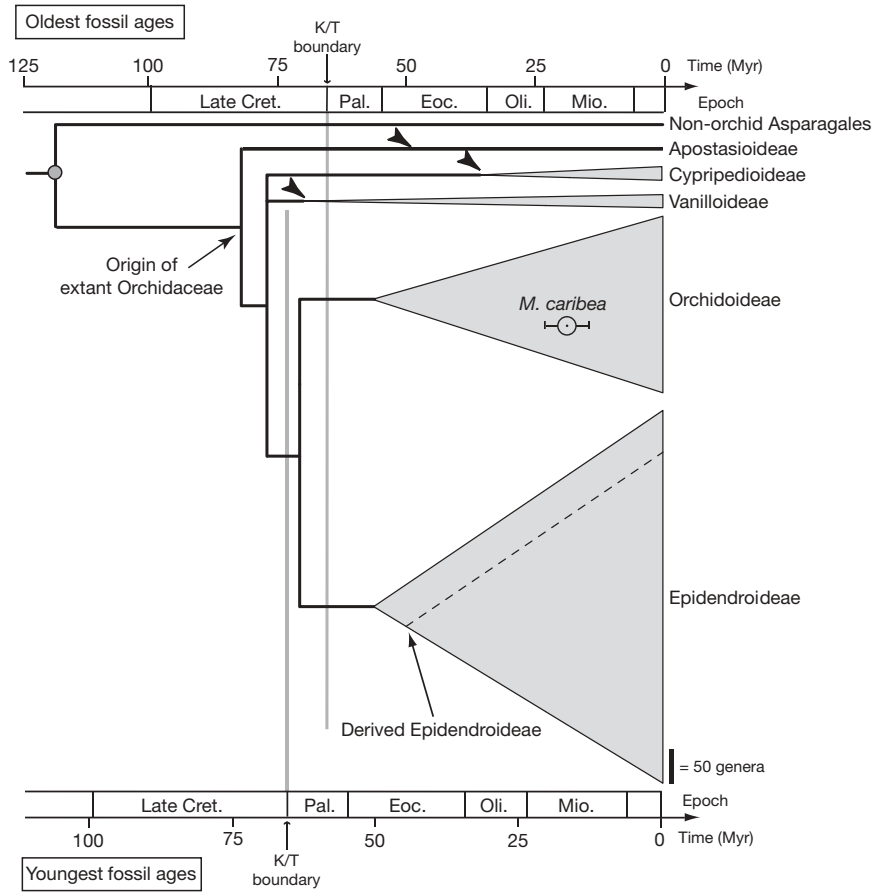

Figure 4 | Fossil-calibrated molecular clock chronogram of the family Orchidaceae, based on ~3 kilobases of plastid DNA (*matK* and *rbcL*). The relative size of each clade is proportional to the number of genera described in each orchid subfamily. Crown ages of small clades are indicated with arrow 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 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 method[29].

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 studies[25], 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 ×40). In both cases, 10 sequential shots at different focal depths were processed with the Auto-Montage software (Syncroscopy, 2002) to produce a single composite image.

The phylogenetic position of *Meliorchis* 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 *Meliorchis* 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 morphological[18] and molecular[26] data. Heuristic tree searches were performed via maximum parsimony with 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 1.71[29]. 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 *Meliorchis* (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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SUPPLEMENTARY INFORMATION

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

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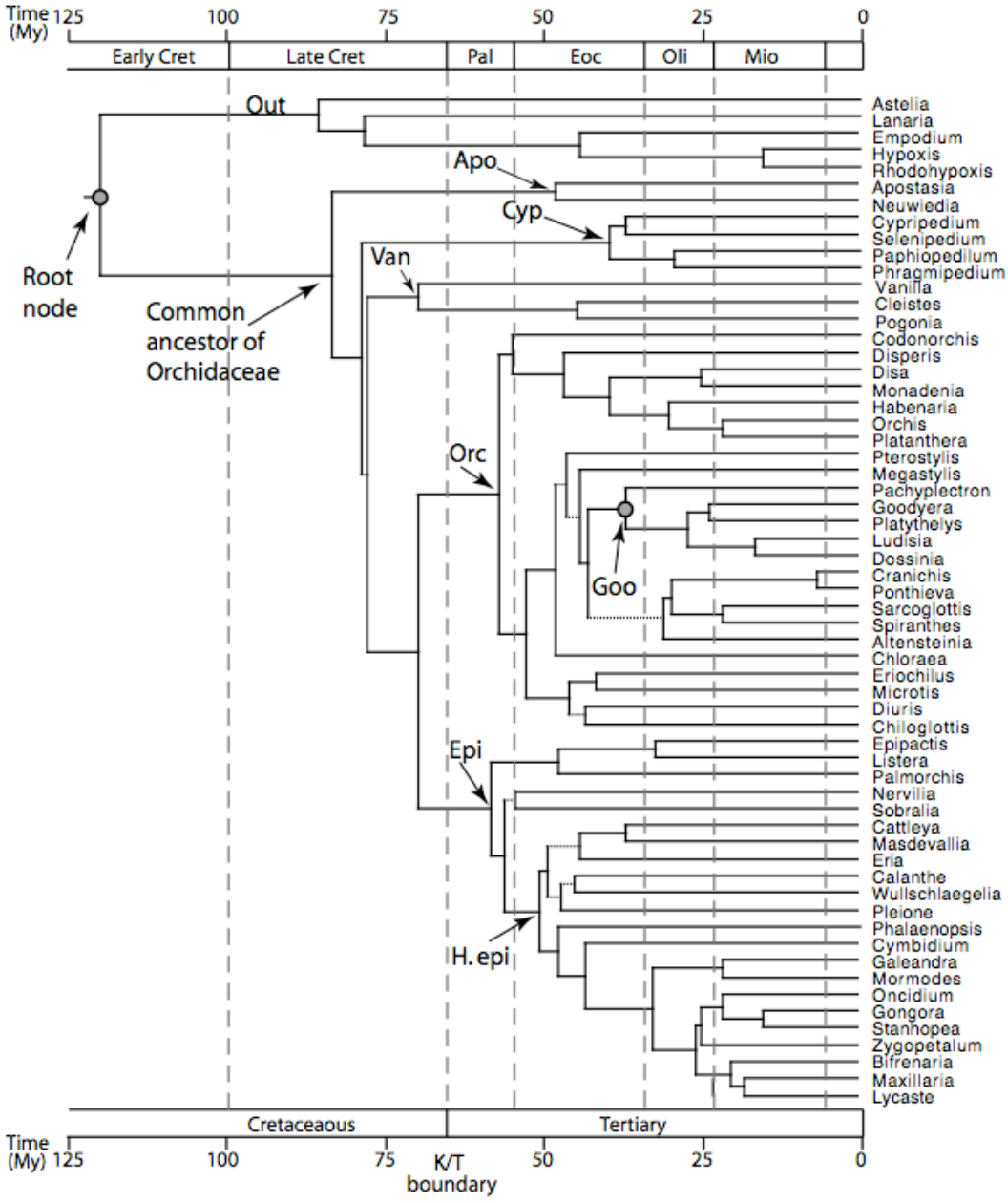
1. SUPPLEMENTARY FIGURE AND LEGEND



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 Epidendroinds, 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. Caudicles†
0 : absent
1 : present

05. Hamulus stipe†
0 : absent
1 : present

06. Viscidium†
0 : none
1 : diffuse
2 : detachable

07. Viscidum shape
0 : pad-like
1 : elongated or U/V-shaped

08. Viscidium : pollinium ratio
0 : viscidium << pollinium
1 : viscidium ≅ 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. Pollnium 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 : ≤ 1:100
1 : ≥ 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 literature[31-36].

* 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 pollinaria[37]. 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 above[32]:

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 *mat*K, 1338 bp of *rbc*L) corresponding to 55 orchid genera belonging to all five orchid subfamilies as ingroup taxa. Several recent molecular studies[38-40] 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 Group[41] 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 Walker[41]) are monosulcate-operculate, boat-shaped, thicotomosulcate, reticulated irregularly into coarse and fine areas, and have psilate muri and dimorphic lumina. These characters unambiguously assign them to the monocotyledons[41-42]. The operculate pollen suggests an affinity with the monocot orders Asparagales, Liliales and Poales[44] and the trichotomosulcus (*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 Asparagales[47-49]. Additionally, the heterobrochate, mono-pluricolumellate reticula that diminish near colpi (*e.g. L. pollucibilis* from Late Cretaceous[50]) 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 sulcus[42], a feature that is present in pollen grains of the family Amarylidaceae (Asparagales)[51]. Although Walker and Walker[41] 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 ½ 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 associates[55]**.** 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 Orchidaceae[56].

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

Supplementary Table 2. Age estimates (in Millions of years, My) ± 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 ± 6	83 ± 5	77 ± 5	76 ± 4	84 ± 6	83 ± 5	76 ± 5	76 ± 4
Subfamily Apostasioidae	49 ± 5	48 ± 5	45 ± 4	44 ± 5	49 ± 5	48 ± 5	45 ± 4	44 ± 5
Subfamily Vanilloidae	71 ± 5	67 ± 4	65 ± 5	62 ± 4	71 ± 5	67 ± 4	65 ± 4	62 ± 4
Subfamily Cypripedioideae	40 ± 5	56 ± 6	35 ± 5	52 ± 6	37 ± 4	52 ± 6	34 ± 4	47 ± 5
Subfamily Orchidoidae	59 ± 5	61 ± 4	53 ± 4	56 ± 4	58 ± 5	60 ± 4	52 ± 4	55 ± 4
Subfamily Epidendroideae	61 ± 8	68 ± 4	53 ± 7	63 ± 4	59 ± 8	68 ± 4	51 ± 7	62 ± 4
"Higher" Epidendroids	53 ± 8	59 ± 5	45 ± 7	54 ± 4	50 ± 7	56 ± 4	42 ± 6	51 ± 4
Subtribe Goodyerinae	38 ± 4	39 ± 4	34 ± 3	36 ± 3	38 ± 4	39 ± 3	34 ± 3	36 ± 3

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

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

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

4. SUPPLEMENTARY NOTES.

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