"Dating the origin of the Orchidaceae from a fossil orchid with its pollinator"
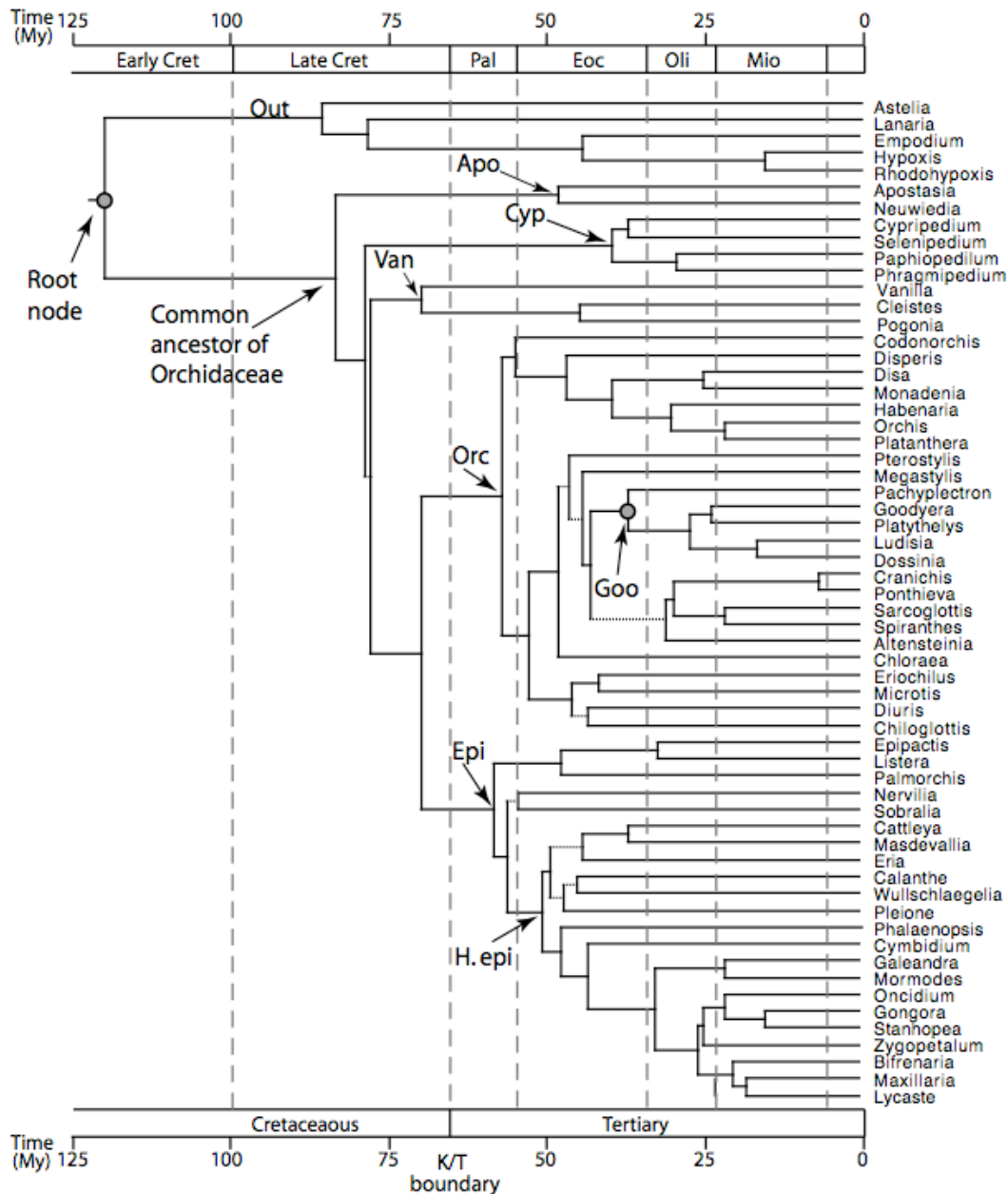
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1. SUPPLEMENTARY FIGURE AND LEGEND

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.   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 $\cong$ 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.   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.

Additional references*:

31. Burns-Balogh, P. Orchid pollen/pollinaria (IDC, New York 1991)

32. Pridgeon, A. M., Cribb, P. J., Chase, M. W. & Rasmussen, F. N.) *Genera Orchidacearum*, Vol. 3 (Oxford Univ. Press New York, 2003).

33. Schill, R. & Pfeiffer W. Untersuchungen an orchideenpollinien unter besonderer beruecksichtigung ihrer feinskulpturen. *Pollen et Spores* **19**, 5-118 (1977).

34. van der Cingel, N. A. *An atlas of orchid pollination: European orchids* (A.A. Balkema, Rotterdam, 1995).

35. van der Cingel, N. A. *An atlas of orchid pollination: America, Arica , Asia and Australia*. (A.A. Balkema, Rotterdam, 2001).

36. Williams, N. H. & Broome, C. R. Scanning electron microscope studies of orchid pollen. *Am. Orch. Soc. Bull.* **45**, 699-707 (1976).

37. Singer, R. B, Marsaioli A. J., Flach, A., Reis M. G. in *Floriculture, Ornamental and Plant Biotechnology* Vol IV (ed. Teixeira da Silva J.) 569-582 (Global Science Books, Middlesex 2006).

38. Chase, M. W. *et al* in *Monocots: Comparative Biology and Evolution excluding Poales* (eds. Columbus J. T., Friar E. A., Porter J. M., Prince L. M., Simpson M. G.) 63-75 (Allen Press, Rancho Santa Ana Botanic Garden, 2006).

39. Graham S. W. *et al* in *Monocots: Comparative Biology and Evolution excluding Poales* (eds. Columbus J. T., Friar E. A., Porter J. M., Prince L. M., Simpson M. G.) 3-21 (Allen Press, Rancho Santa Ana Botanic Garden, 2006).

40. Givnish T. J. *et al* in *Monocots: Comparative Biology and Evolution excluding Poales* (eds. Columbus J. T., Friar E. A., Porter J. M., Prince L. M., Simpson M. G.) 28-51 (Allen Press, Rancho Santa Ana Botanic Garden, 2006).

41. Walker, J. W., Walker, A. G. Ultrastructure of Lower Cretaceous angiosperm pollen and the origin and early evolution of flowering plants. *Ann. Mo. Bot. Gard.* **71,** 464–521 (1984).

42. Dettmann, M.E. Angiospermous pollen from Albian to Turonian sediments of Eastern Australia. *Spec. Publs. Geol. Soc. Aust.* **4,** 3-34 (1973).

43. Raine, J.I., Mildenhall, D.C. Kennedy, E.M. New Zealand fossil spores and pollen: an illustrated catalogue. 2nd edition. GNS Science miscellaneous series

no. 4.
http://www.gns.cri.nz/what/earthhist/fossils/spore_pollen/catalog/index.htm".
(2006).

44. Furness, C. A., Rudall, P. J. in *Monocots: Comparative Biology and Evolution excluding Poales* (eds. Columbus J. T., Friar E. A., Porter J. M., Prince L. M., Simpson M. G.) 191-196 (Allen Press, Rancho Santa Ana Botanic Garden, 2006).

45. Couper, R. A. Upper Mesozoic and Cainozoic spores and pollen grains from New Zealand. *New Zeal. Geol. Surv. Palaeontol. Bull.* **22**, 1-77 (1953).

46. Couper, R. A. New Zealand Mesozoic and Cainozoic plant microfossils. *New Zeal. Geol. Surv. Palaeontol. Bull.* **32**, 1-87 (1960).

47. Rudall, P. J., Furness, C. A., Chase, M. W., Fay, M. F. Microsporogenesis and pollen sulcus type in Asparagales (Lilianae). *Can. J. Bot.* **75,** 408–430 (1997).

48 Ruddall, P. J. Unique floral structures and iterative evolutionary themes in Asparagales: Insights from a morphological cladisitic analyses. *Bot. Rev.* **68**, 488-409 (2003).

49. Nadot, S. *et al* in *Monocots: Comparative Biology and Evolution excluding Poales* (eds. Columbus J. T., Friar E. A., Porter J. M., Prince L. M., Simpson M. G.) 197-203 (Allen Press, Rancho Santa Ana Botanic Garden, 2006).

50. Chmura, C. A. Upper Cretaceous (Campanian-Maastrichtian) angiosperm pollen from the western San Joaquin Valley, California, U.S.A. *Palaeontrographica*, *Abt. B.* **141,** 89-171 (1979).

51. Erdtman, G. *Pollen Morphology and Plant Taxonomy* (Hafner Publishing Co., New York, 1971).

52. Grimaldi, D. A., Shedrinsky, A., Ross, A., Baer, N. S. Forgeries of fossils in amber: history, identification and case studies. *Curator* **37,** 251-275 (1994).

53. Engel, M.S. The first fossil *Euglossa* and phylogeny of the orchid bees (Hymenoptera: Apidae: Euglossini). *Amer. Mus. Novitates* **3272**, 14 (1999).

54. Poinar, G. Jr. *Paleoeuglossa melissiflora* gen. n., sp. n. (Euglossine; Apidae), fossil orchid bees in Dominican amber. *J. Kan. Ent. Soc.* **71**, 29-34. (1999).

55. Pemberton R. W., Wheeler G. S. Orchid bees don't need orchids: evidence from the naturalization of an orchid bee in Florida. *Ecology* **87**,1995–2001 (2006).

56. Poinar, G. Jr. *The Amber Forest* (Princeton Univ. Press, New Jersey, 1999).