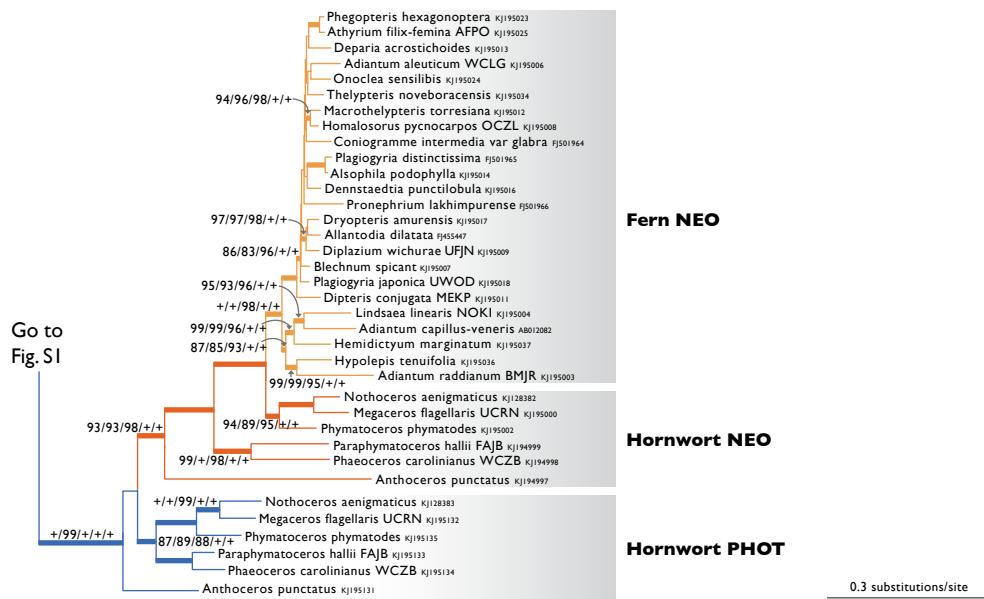
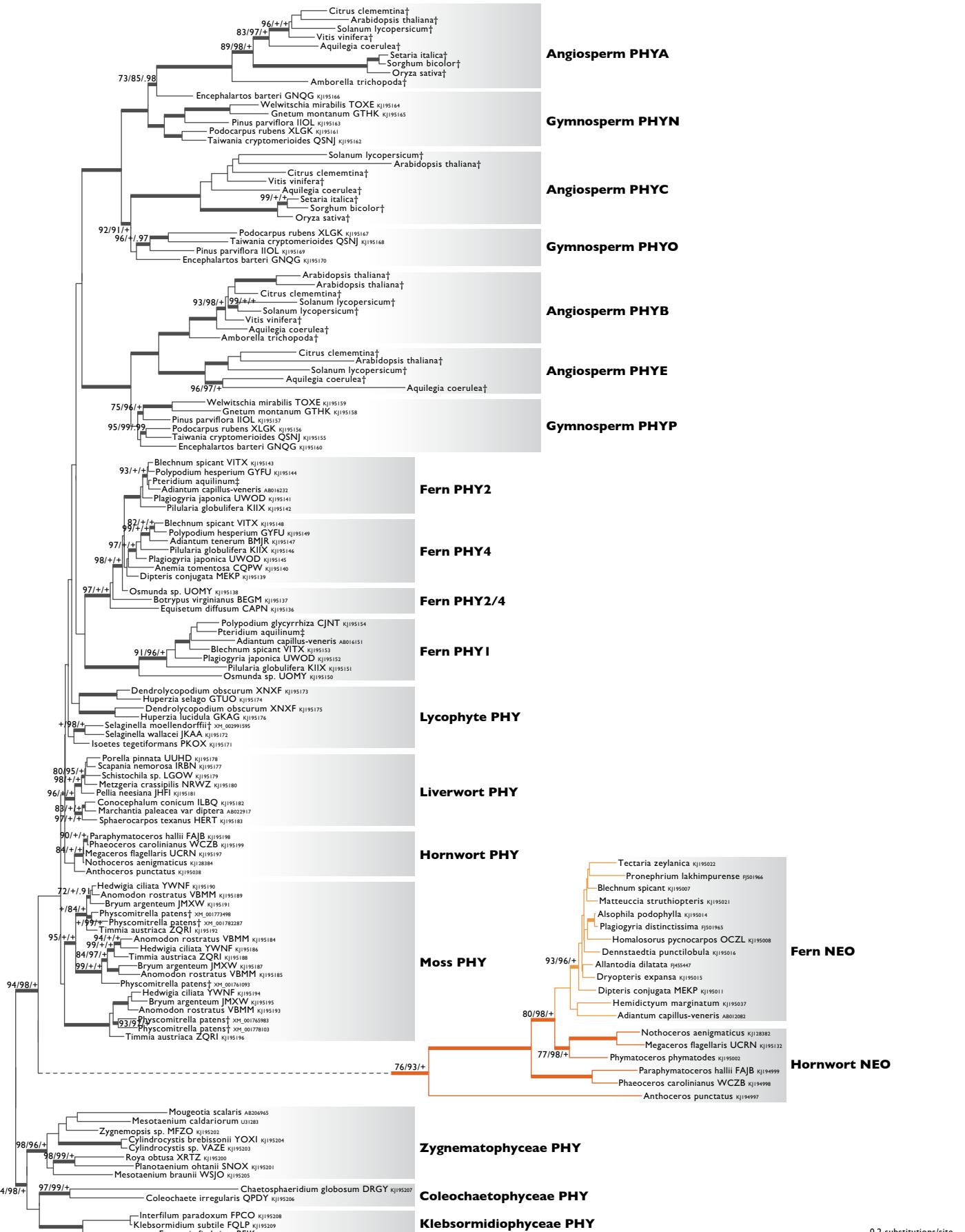


**Fig. S1.** Phylogenetic relationships of land plant and algal phototropin (PHOT) and the corresponding domains from hornwort, fern, and algal neochrome (NEO). Topology derived from the best maximum likelihood tree. The five support values associated with branches are maximum likelihood bootstrap values (BS) / BS from nhPhyML / aLRT supports under codon model (aLRT) / Bayesian posterior probabilities (PP) from MrBayes / PP from BEAST; these are only displayed (along with thickened branches) when BS > 70, aLRT > 70 and PP > 0.95. "+" denotes BS = 100, aLRT = 100 or PP = 1.00; thickened branches without numbers are "+/+/-/+". Alphanumeric codes following species names are the four-letter 1KP transcriptome identifiers, or Genbank accessions, or both; "†" indicates the sequence came from genome sequence data, and "‡" from *Pteridium aquilinum* transcriptome. The blue, orange and yellow branches represent hornwort phototropin, hornwort neochrome and fern neochrome, respectively.

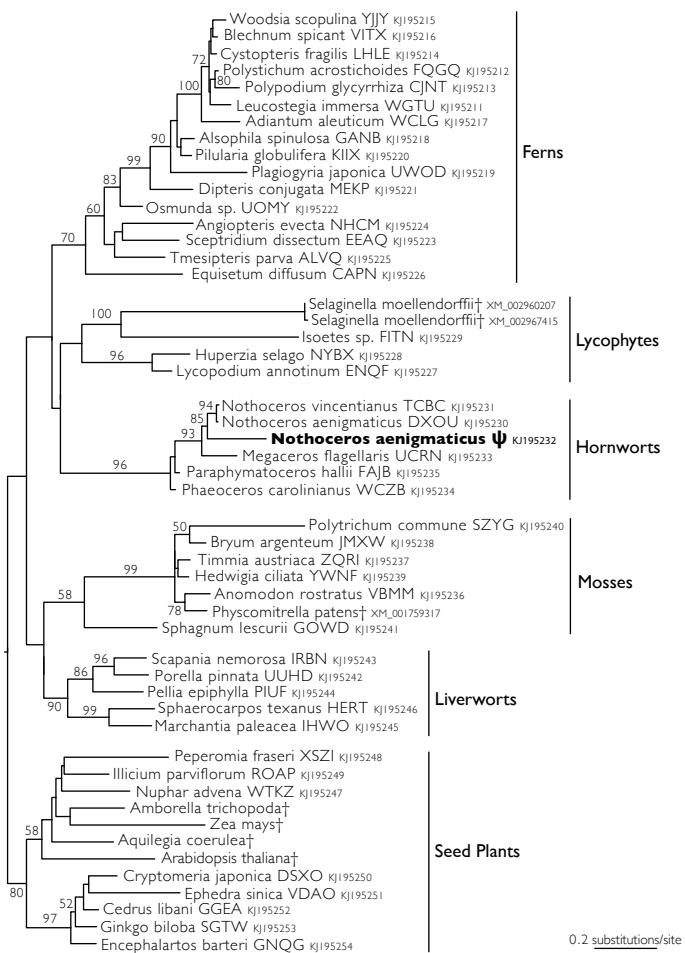
0.3 substitutions/site



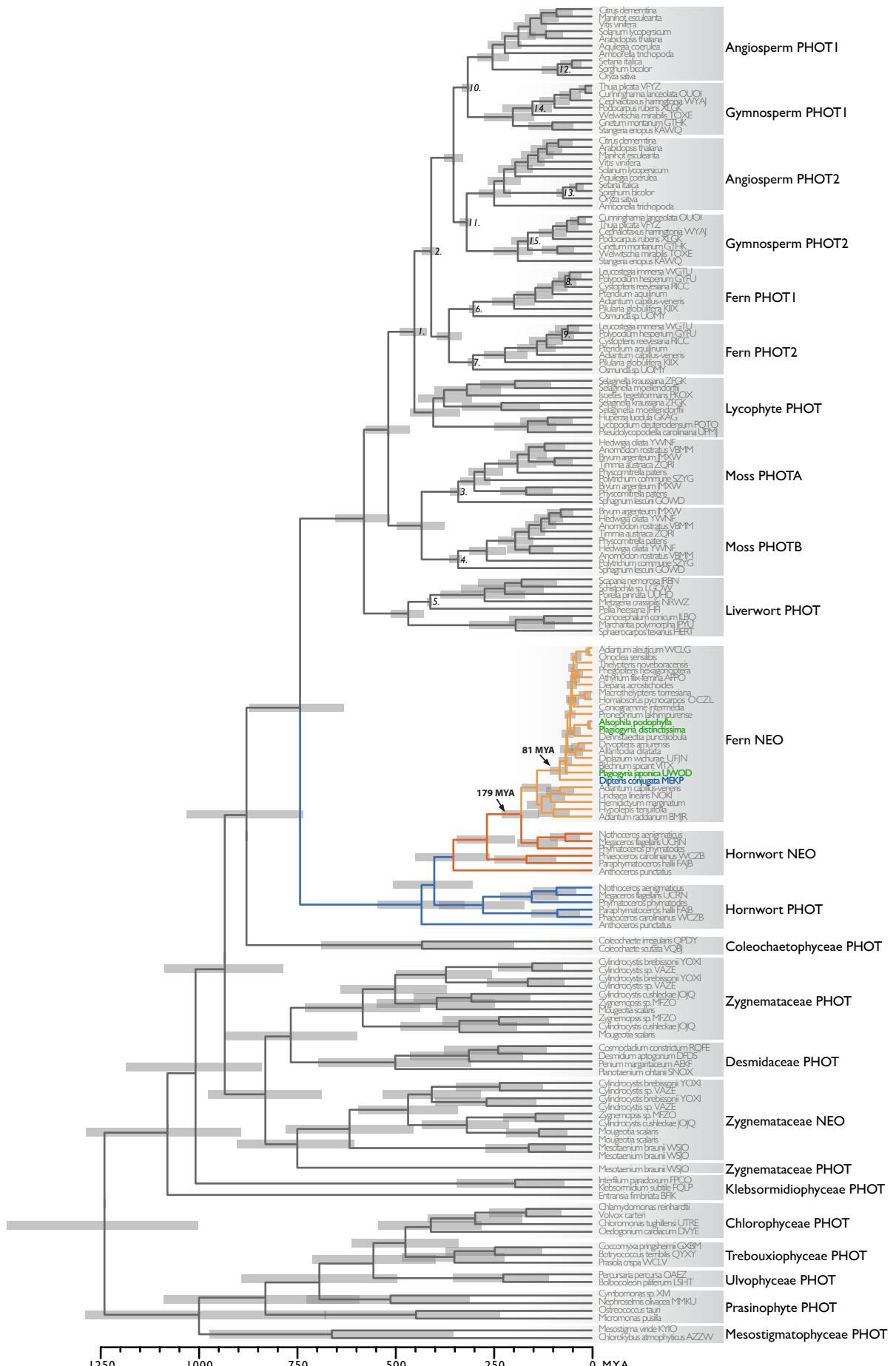
**Fig. S2.** Phylogenetic relationships of fern neochrome (NEO), hornwort neochrome and phototropin (PHOT). This figure is continued from Fig. S1 and follows the same conventions.



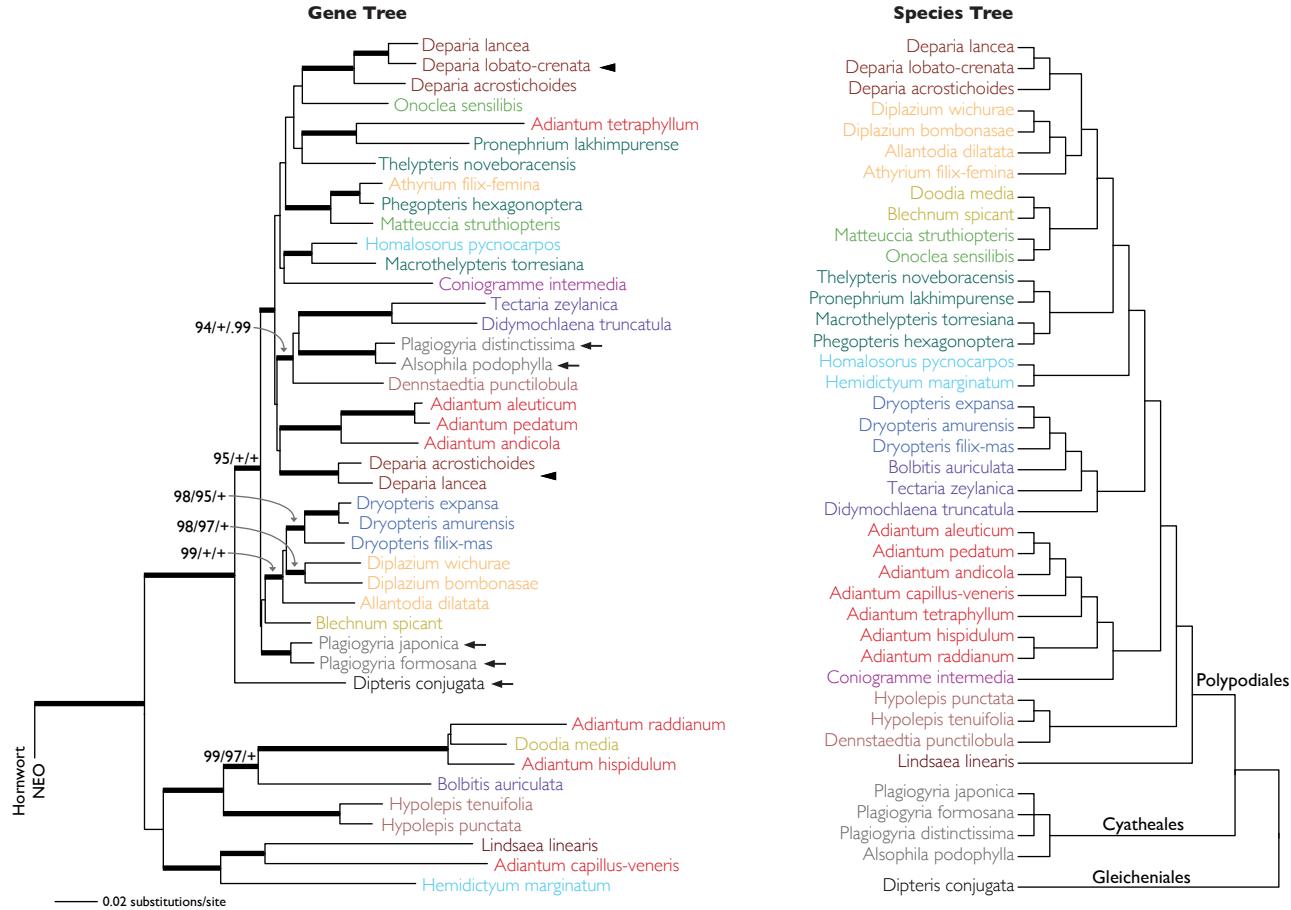
**Fig. S3. Phylogenetic relationships of land plant and algal phytochrome (PHY) and the corresponding domains from hornwort and fern neochrome (NEO).** Topology derived from the best maximum likelihood tree. The three support values associated with branches are maximum likelihood bootstrap values (BS) / aLRT supports under codon model (aLRT) / Bayesian posterior probabilities (PP); these are only displayed (along with thickened branches) if BS > 70, aLRT > 0.95 and PP > 0.95. "+" denotes BS = 100, aLRT = 100 or PP = 1.00; thickened branches without numbers are "+/+". Alphanumeric codes following species names are the four-letter 1KP transcriptome identifiers, or Genbank accessions, or both; "‡" indicates the sequence came from whole genome sequence data, and "‡" from *Pteridium aquilinum* transcriptome. For space considerations, the dashed line artificially extends the NEO clade and does not reflect true branch length. The orange and yellow branches represent hornwort neochrome and fern neochrome, respectively.



**Fig. S4.** Phylogenetic relationships of land plant imidazoleglycerol-phosphate dehydratase (IGPD). In the hornwort *Nothoceros aenigmaticus*, we conducted genome-walking downstream of neochrome and found a IGPD pseudogene (denoted by  $\Psi$ ). In a land plant phylogeny of IGPD our *N. aenigmaticus* pseudogene is most closely related to other hornwort IGPD. This relationship confirms that our hornwort neochrome sequence data were indeed derived from the hornwort genome, and not from symbiotic algae or fungi. Numbers associated with branches are maximum likelihood bootstrap support values. Alphanumeric codes following species names are the four-letter 1KP transcriptome identifiers, or Genbank accessions, or both; “†” indicates the sequence came from whole genome sequence data.

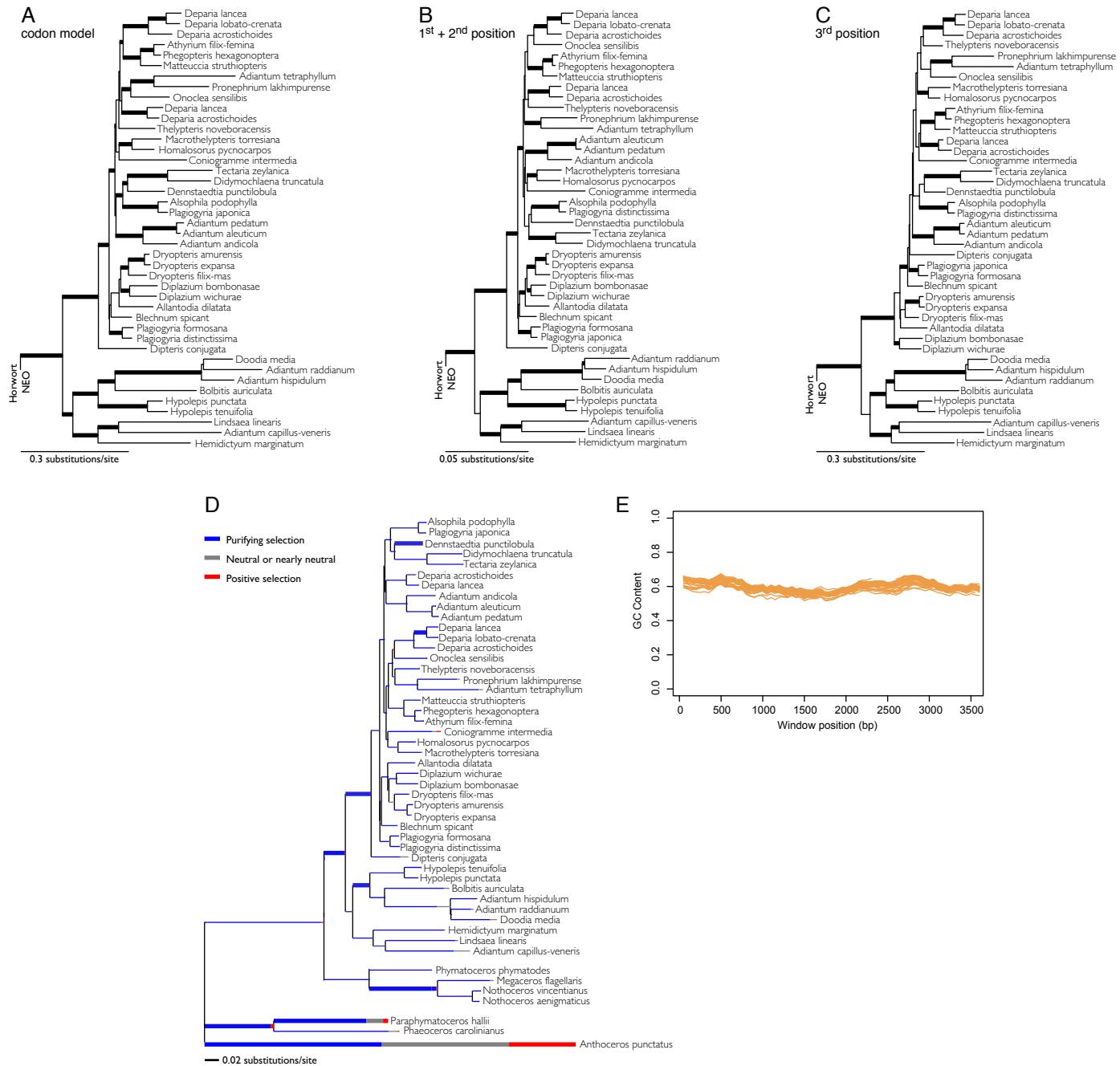


**Fig. S5.** Chronogram of land plant and algal phototropin (PHOT) and the corresponding domains from hornwort, fern, and algal neochrome (NEO). A simplified version of this figure is shown in Fig. 1B. Grey bars represent 95% highest posterior density intervals of the age estimates. Italicized numbers adjacent to nodes refer to the fossil or secondary time calibrations detailed in Table S2. Two divergence time estimates are highlighted: one marks the HGT event (179 MYA) and the other marks the split of Gleicheniales (blue taxon), Cyatheales (green taxa) and other neochromes (81 MYA).

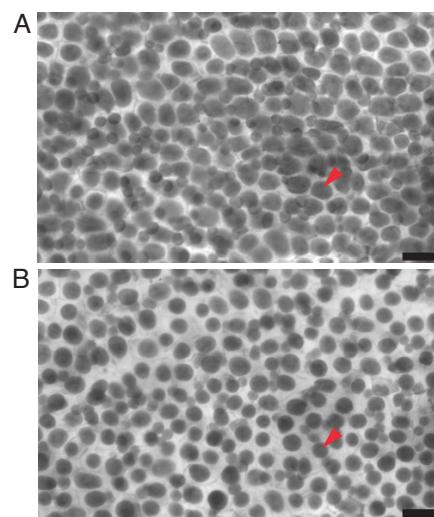


**Fig. S6.** Phylogenetic incongruence between fern neochrome gene tree and fern species tree. The gene tree topology is derived from the best maximum likelihood tree based on the nucleotide dataset, and the species tree summarized from Schuettpelz and Pryer (1), Kuo et al (2), Rothfels and Schuettpelz (3), and Rothfels et al (4). Tree inference based on codon models, 1st + 2nd and 3rd codon positions yielded similar topologies (Fig. S7). Closely related species/genera are coded with the same color. The neochrome gene tree is rooted with hornwort neochromes (not shown). Numbers above branches are maximum likelihood bootstrap values (BS) / aLRT supports under codon model (aLRT) / Bayesian posterior probabilities from MrBayes (PP), and are only displayed (along with thickened branches) if BS > 70, aLRT > 70 and PP > 0.95. "+" denotes BS = 100, aLRT = 100 or PP = 1.00; thickened branches without numbers are "+/+/" . Arrowheads point to the two divergent neochrome copies found in *Deparia* spp. Arrows point to neochromes from Gleicheniales and Cyatheales that appear nested among Polypodiales neochromes.

1. Schuettpelz E, Pryer KM (2007) Fern phylogeny inferred from 400 leptosporangiate species and three plastid genes. *Taxon* 56:1037–1050.
2. Kuo LY, Li FW, Chiou WL, Wang CN (2011) First insights into fern matK phylogeny. *Mol Phylogenet Evol* 59:556–566.
3. Rothfels CJ, Schuettpelz E (2013) Accelerated rate of molecular evolution for vittarioid ferns is strong but not driven by selection. *Syst Biol* 63:31–54.
4. Rothfels CJ et al. (2013) Transcriptome-mining for single-copy nuclear markers in ferns. *PLoS ONE* 8:e76957.



**Fig. S7.** Phylogeny, selection profile and GC content of fern neochromes. Maximum likelihood reconstructions of gene phylogeny based on (A) codon model, (B) first and second codon positions, and (C) third codon position. Thickened branches indicate aLRT supports (in A) or bootstrap supports (in B, C)  $> 70$ . (D) Selection profile displayed along phylogenetic branches for fern and hornwort neochromes. Tree topology derived from the best maximum likelihood tree (Fig. S6). The width of each color along a branch is proportional to the number of codon sites in the corresponding selection class. Thickened branches have experienced significant episodic positive selection ( $P < 0.05$ ). (E) Sliding window analysis of GC content for fern neochrome. Each line displays the GC content for each neochrome sequence. None of the ferns in our study were deviant in base composition for neochrome. Each window is 400bp in size and the window slides every 50bp.



**Fig. S8.** Hornwort chloroplasts (arrowhead) contract under strong light. (A) Before irradiation, chloroplasts of *Nothoceros aenigmaticus* occupy most of the cellular space. (B) After irradiation of blue light ( $57 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) for 2 hours, chloroplasts evidently reduced in size. Scale bar = 40  $\mu\text{m}$ .

**Table S1.** List of transcriptomes and genome sequences screened for neochrome, phototropin and phytochrome genes. All the transcriptomes were from 1KP ([www.onekp.com](http://www.onekp.com)), except for *Pteridium aquilum*. The four letter codes following species names are the 1KP transcriptome identifiers. Details of transcriptome tissue type and specimen voucher can be found at [www.onekp.com](http://www.onekp.com). \*\*\* denotes whole or draft genome sequences.

Angiosperms	Ferns	Lycophytes	Charophytes	Chlorophyceae	Prasinophytes
Amborella trichopoda*	Adiantum aleuticum WCLG	Dendrolycopodium obscurum XNFX	Bambusina borrii QWFV	Ankistrodesmus sp. OTQG	Bathyccoccus prasinos MCPK
Aquilegia coerulea*	Adiantum raddianum BMJR	Diphysastraum digitatum WAFT	Chaetophoridium globosum DRGY	Aphanocetea repens IJMT	Cymbomonas sp. XVI
Arabidopsis lyrata*	Anemia tormentosa COPW	Huperzia lucidula GKAG	Chara vulgaris MWXT	Asteromonas gracilis NTLE	Dolichomastix tenuelepis XOAL
Arabidopsis thaliana*	Angiopteris evecta NHCM	Huperzia myrsinifolia CBAE	Chlorokybus atropolyticus AZZW	Brachiomonas submarina GUBD	Mantoniella squamata QXSZ
Brachypodium distachyon*	Argyrochosma nivea XDDT	Huperzia selago GTUO	Closterium lunula DRFX	Carteria crucifera VIAU	Micromonas pusilla*
Capsella rubella*	Asplenium nidus PSKY	Huperzia selago NYBX	Coleochaete irregularis OPDY	Carteria obtusa RUIF	Monostromat opisthostigma BTFM
Carica papaya*	Asplenium platyneuron KIZG	Huperzia squarrosa GAON	Coleochaete scutata VQBJ	Chaetopeltis orbicularis BAZF	Nephroselmis olivacea MMKU
Citrus clementina*	Azolla caroliniana CVEG	Lycopodiella appressa ULKT	Cosmarium broomei HIDG	Chlamydomonas reinhardtii*	Nephroselmis pyriformis ISIM
Citrus sinensis*	Athyrium filix-femina URCP	Lycopodium annotinum ENQF	Cosmarium granatum MNMM	Chlamydomonas bilobata MULF	Ostreococcus tauri*
Cucumis sativus*	Athyrium filix-femina AFPO	Lycopodium deuterodensum PQTO	Cosmarium othoides HJVM	Chlamydomonas cribrum BCYF	Ostreococcus lucimarinus*
Eucalyptus grandis*	Blechnum spicant VITX	Phylloglossum drummondii ZZEI	Cosmarium othoides STKJ	Chlamydomonas moewisia JRGZ	Picocystis salinarum TGNL
Fragaria vesca*	Bolbitis repanda JBL	Pseudolycopodiella caroliniana UPMJ	Cosmarium subtumidum WDGV	Chlamydomonas noctigama VALZ	Prasinococcus capsulatus XMCL
Glycine max*	Botrypus virginianus BEGM	Selaginella moellendorffii*	Cosmarium tinctum BHBK	Chlamydomonas sp. TSQ	Prasinoderma coloniale HYHN
Gossypium raimondii*	Cibotium glaucum ORJE	Selaginella acanthonota ZYCD	Cosmoldium cf. constructum RQFE	Chlamydomonas sp. AOIJ	Pseudoscoleflex marina JMT
Linum usitatissimum*	Crepidomanes venosum TWZF	Selaginella apila LDQG	Cylindrocystis brebissonii YOXI	Chloromonas oogama IHOI	Pycnococcus provasoli MXEZ
Malus domestica*	Cryptogramma acrostichoides WQML	Selaginella kraussiana ZFGK	Cylindrocystis brebissonii RPGL	Chloromonas perforata QRTH	Pyramimonas parkeae TNAW
Manihot esculenta*	Culcitia macrocarpa PNZO	Selaginella lepidophylla ABJU	Cylindrocystis cushlaeckae JOIQ	Chloromonas reticulata LBRP	Scherrfelia dubia FMVB
Medicago truncatula*	Cyathea spinulosa GANB	Selaginella selaginoides KUXM	Cylindrocystis sp. VAZE	Chloromonas rosae AJUW	Tetraselmis striata HHXJ
Mimulus guttatus*	Cystopteris fragilis XHHP	Selaginella sphaerolepis ZZOL	Desmidium aptogonium FDFS	Chloromonas subdivisa GFUR	Tetraselmis coroides DUMA
Oryza sativa*	Cystopteris fragilis LHLE	Selaginella wallacei JKAA	Entrisia fimbriata BFK	Chloromonas tughilensis UTRE	Tetraselmis strata HHXJ
Panicum virgatum*	Cystopteris protrusa YOWV	Selaginella willdenowii KJYC	Euastrum affine GYRP	Chlorosarcinopsis halophilica KSFK	coccoid prasinophyte XJGM
Phaseolus vulgaris*	Cystopteris reevesiana RICC	Isoetes sp. PYHZ	Gonatozygon kinahanii KEYW	Dunaliella salina RHVC	
Populus trichocarpa*	Cystopteris utahensis HNDZ	Isoetes sp. FITN	Interfilum paradoxum FPCO	Eudorina elegans RNAT	
Prunus persica*	Davallia fejeensis OQWW	Isoetes tegetiformans POKX	Klebsormidium subtile FQLP	Fritschella tuberosa VFIV	
Ricinus communis*	Dennstaedtia davallioides MTGC		Mesostigma viride KYIO	Golenkinia longispicula BZSH	
Setaria italica*	Deparia lobato-crenata FCHS		Mesotaenium braunii WSJO	Gonium pectorale KUJJ	
Solanum lycopersicum*	Didymochlaena truncatula RFRB		Mesotaenium caldarium HKZW	Haematococcus pluvialis ODXI	
Solanum tuberosum*	Diplazium wickhurae UFJN		Mesotaenium endlicherianum WDCW	Haematococcus pluvialis AGIO	
Sorghum bicolor*	Dipteris conjugata MEKP		Mesotaenium kramstel NBYP	Hafniomonas reticulata FXHG	
Theobroma cacao*	Equisetum diffusum CAPN		Micrasterias fimbriata MCHJ	Helicodictyon planctonicum AJAU	
Vitis vinifera*	Equisetum hyemale JSVZ		Mougeotia sp. ZRMT	Heterochloramylonomas inaequalis IRYH	
Zea mays*	Gaga arizonica DCDT		Netrium digitus FGFR	Lobochlamys segnis OFUE	
Gymnosperms	Gymnopcarpium dryopteris HEGQ		Nucleoetanum efelense KMXN	Lobomonas rostrata JKKI	
Austrotaxus spicata BTTS	Hemitelia arifolia ZXJO		Onychonema laeve GGWH	Microcosra cf. tumidula FOYQ	
Callitris macleayana RMMV	Homalosorus pycnocarpos OCZL		Penium exiguum YSQT	Neochloris oleobundans EEOJ	
Cathaya agyrophylla NPLR	Hymenophyllum bivalve QIAD		Penium margaritaceum AEKF	Neochloris sp. GIY	
Cedrus libani GGEA	Hymenophyllum cupressiforme TRPJ		Phyamatodocis nordstadiana RPQV	Neochlorosarcina sp. USIX	
Cephalotaxus harringtonia WYAJ	Leucostegia immersa WGTU		Planotaenium ohtanii SNOX	Oedogonium cardiacum DVYE	
Cryptomeria japonica DSXO	Lindsaea linearis NOKI		Pleruolettaenium trabecula MOYY	Oedogonium foveolatum SDPC	
Cunninghamia lanceolata OUOI	Lindsaea microphylla YIXP		Roya obtusa XRTZ	Oogamochlamys gigantea XDL	
Cypressus dupreziana QNGJ	Lygodium japonicum PBUU		Spirogyna sp. HAOX	Pandoria morum RYJX	
Cycas micholitzii XZUY	Marattia sp. UKCS		Spirotaenia minuta NNHQ	Pediastrum duplex KXWQ	
Dioon edule WLIC	Myriopteris eatonii GSXD		Spirotaenia sp. TPHT	Pediastrum duplex XT0N	
Encephalartos barteri GNQG	Nephrolepis exaltata NWWI		Staurastrum baldwi ISHC	Phacotus lenticularis ZIVZ	
Ephedra sinica VDAO	Notholaena montanae YCKE		Stauromedes convergens WCQU	Gracilaria lemaniforme IKWM	
Ginkgo biloba SGTV	Notholaena montanae YCKE		Staurodesmus omeairi RPRU	Gracilaria filicina ZIOJ	
Glyptostrobus pensilis OGJ	Onclea sensibilis HTFH		Xanthidium antilopaeum GBGT	Grateloupia turuturu URSB	
Gnetum montanum GTHK	Ophioglossum petiolatum QHVS		Zygnemopsis sp. MFZO	Grateloupia chiangii PWKQ	
Juniperus scopulorum XMGP	Ophioglossum petiolatum WTJG			Gymnogongrus fibelliformis CKXF	
Keteleeria evelyniana JUWL	Osmunda javanica VIBO			Heterosiphonia pulchra YSD	
Larix species WVWN	Osmunda regalis YKSS			Kappaphycus alvarezii IHYJ	
Nothotsuga longibracteata AREG	Osmunda regalis UDYM			Mazzarella japonica WEJN	
Phyllocladus hypophyllus JRNA	Osmundastrum cinnamomeum BIVQ			Polyphysiphonia japonica XAXW	
Picea engelmannii AWQB	Pliliaria globulifera KIIK			Porphyridium yezoense ZULU	
Pinus jeffreyi MFTM	Pityrogramma trifolia UJT			Porphyridium cruentum OBUY	
Pinus parviflora IIOL	Plagiolepia japonica UWOD			Rhodella violacea RTLC	
Pinus ponderosa JBND	Pleopeltis polypodioides UWU			Rhodochaetea parvula JIZR	
Pinus radiata DZOM	Polypondium amorphum YLJA			Sinutobimorpha guangdongensis PYDB	
Podocarpus coriaceus SCEB	Polypondium glycyrrhiza CINT				
Podocarpus rubens XLKG	Polypondium hesperium GFYU				
Pseudolarix amabilis AQFM	Polypondium hesperium IXLH				
Pseudotaxus chienii YLPM	Phlebodium pseudoaureum ZQYU				
Pseudotsuga menziesii IOVS	Polystichum acrostichoides FQQG				
Sciadopitys verticillata YFZK	Psilotum nudum QVMR				
Stangeria eriopus KAWQ	Pteridium aquilinum <sup>1</sup>				
Taiwania cryptomerioides QSNJ	Pteris ensiformis FLTD				
Taxus baccata WWSS	Pteris vittata POPJ				
Taxus cuspidata ZYAX	Sceptrinium dissectum EEAQ				
Thujopsis dolabrata NKIN	Sticherus lobatus XDVM				
Torreya nucifera HQOM	Thelypteris acuminata MROH				
Torreya taxifolia EFMS	Thysanopteris elegans EWXK				
Tsuga heterophylla GAMH	Tmesipteris parva ALVQ				
Welwitschia mirabilis TOXE	Vittaria appalachiana NDUV				
Widdringtonia cedarbergensis AUDE	Vittaria lineata SKYV				
Wollemia nobilis RSCE	Woodsia ilvensis YQEC				
	Woodsia scopulina YJJY				
Mosses					
	Anomodon attenuatus QMWB				
	Anomodon rostratus BBMM				
	Atrichum angustum ZTHV				
	Aulacomnium heterostichum WNGH				
	Bryum argenteum JMKW				
	Buxbaumia aphyla HRWG				
	Ceratodon purpureus FFPD				
	Dicranum scoparium NGTD				
	Funaria sp. XWHE				
	Hedwigia ciliata YWNF				
	Hypnum subimplenis LNSF				
	Leucobryum albidum VMXJ				
	Leucobryum glaucum RGKI				
	Leucodon sciuroides ZACW				
	Neckera douglasii TMAJ				
	Orthotrichum lyellii CMEQ				
	Philonotis fontana ORKS				
	Physcomitrella patens*				
	Physcomitrium pyriforme YEPO				
	Pliagiomnium insigne BGXB				
	Polytrichum commune SZYG				
	Pseudotaxiphyllum elegans QKQO				
	Racomitrium varium RDOO				
	Rhynchostegium serrulatum JADL				
	Rhiztidiodelphus loreus WSPM				
	Scouleria aquatica BPSC				
	Schwetschkeopsis fabroniae IGUH				
	Sphagnum lescurii GOWD				
	Sphagnum palustre RCBT				
	Sphagnum recurvatum UHLI				
	Syntrichia princeps GRKU				
	Takakia lepidozoides SKQD				
	Thuidium delicatulum EEMJ				
	Timmia austriaca ZQRI				
Ulvophyceae					
	Acrosiphonia sp. JIWJ				
	Blastophysa cf. rhizophora VHJ				
	Bolbocileen piliferum LSHT				
	Bryopsis plumosa JTIG				
	Cephaloeris virescens YDCQ				
	Cladophora glomerata VBLH				
	Codium fragile GYBH				
	Cylindrocapsa geminella DZPJ				
	Entocladia endozooica OOQN				
	Halochlorococcus marinum ALZF				
	Ignatius tetrasporus KADG				
	Ochlochatea sp. COQP				
	Oltmannsiellopsis viridis PZBH				
	Oltmannsiellopsis viridis QJYX				
	Percursaria percusa OAEZ				
	Planophila laetevires CBNG				
	Planophila terrestris LETF				
	Trentepohlia annulata NATT				

<sup>1</sup>R.G. Wolf 923 (UTC); Norwich, UK

<sup>2</sup>D G Long s.n. (OXF); Edinburgh, UK.

**Table S2.** The calibrations used in dating the divergences within the phototropin gene family.

No.	Clade	Calibration	Date (MYA)	Prior	Reference	Justification
1	Tracheophyta	<i>Zosterophyllum sp.</i>	416	lognormal (mean: 3.5, STD: 1, offset: 416)	70, 74	Oldest unequivocal record of total group of lycopod; see Ref. 72 for detailed justifications
2	Euphyllophyta	<i>Ibyka sp.</i>	388.2	lognormal (mean: 3.5, STD: 1, offset: 388.2)	70, 75	Oldest unequivocal record of monilophyte based on protoxylem morphology; see Ref. 72 for detailed justifications
3	Bryophyta PHOT1	"type III" fragment	330.9-346.7	lognormal (mean: 1.5, STD: 1, offset: 330.9)	71	Oldest unequivocal record of crown Bryophyta; the fibrils and pores similar to those of <i>Sphagnum</i> water-storage cells
4	Bryophyta PHOTB	"type III" fragment	330.9-346.7	lognormal (mean: 1.5, STD: 1, offset: 330.9)	71	Oldest unequivocal record of crown Bryophyta; the fibrils and pores similar to those of <i>Sphagnum</i> water-storage cells
5	Jungermanniopsida	<i>Riccardiothallus devonicus</i>	407-411	lognormal (mean: 1.5, STD: 1, offset: 407)	72	Oldest unequivocal record of crown Jungermanniopsida; gross morphology similar to the extant <i>Riccardia</i> species
6	Polypodiopsida PHOT1	<i>Rastropteris pirtguanensis</i>	296	lognormal (mean: 1.5, STD: 1, offset: 296)	8, 76	Oldest unequivocal record of crown Polypodiopsida; see Ref. 8 for detailed justifications
7	Polypodiopsida PHOT2	<i>Rastropteris pirtguanensis</i>	296	lognormal (mean: 1.5, STD: 1, offset: 296)	8, 76	Oldest unequivocal record of Osmundaceae stem; see Ref. 8 for detailed justifications
8	Eupolypod PHOT1	imported secondary date	116.7	Normal (mean: 116.7, STD: 35.01)	8	A well-established time estimate for the divergence of Eupolypods
9	Eupolypod PHOT2	imported secondary date	116.7	Normal (mean: 116.7, STD: 35.01)	8	A well-established time estimate for the divergence of Eupolypods
10	Spermatophyta PHOT1	<i>Cordaiyylon iowensis</i>	306.2	lognormal (mean: 2.5, STD: 1, offset: 306.2)	70, 77	Oldest unequivocal record of Acrogymnospermae; see Ref. 72 for detailed justifications
11	Spermatophyta PHOT2	<i>Cordaiyylon iowensis</i>	306.2	lognormal (mean: 2.5, STD: 1, offset: 306.2)	70, 77	Oldest unequivocal record of Acrogymnospermae; see Ref. 72 for detailed justifications
12	Grass PHOT1	phytoliths in dinosaur coprolites	65-67	lognormal (mean: 1.5, STD: 1, offset: 65)	73	Oldest unequivocal record of PACMAD or BEP of grass; phytoliths morphology similar to subclades in PACMAD or in BEP
13	Grass PHOT2	phytoliths in dinosaur coprolites	65-67	lognormal (mean: 1.5, STD: 1, offset: 65)	73	Oldest unequivocal record of PACMAD or BEP of grass; phytoliths morphology similar to subclades in PACMAD or in BEP
14	Coniferae PHOT1	<i>Araucaria mirabilis</i>	147	lognormal (mean: 1.5, STD: 1, offset: 147)	70	Oldest unequivocal record of Cupressophyta crown; see Ref. 72 for detailed justifications
15	Coniferae PHOT2	<i>Araucaria mirabilis</i>	147	lognormal (mean: 1.5, STD: 1, offset: 147)	70	Oldest unequivocal record of Cupressophyta crown; see Ref. 72 for detailed justifications

**Table S3.** The primers and PCR protocols used in this study. See Table S4 for primer sequences.

Taxa	Gene	Primary PCR primers	Secondary PCR primers <sup>1</sup>	PCR program <sup>2</sup>	Specimen voucher
<b>Hornworts:</b>					
Phymatoceros phytatomoides	neochrome	neoF65 + neoR2818	neoF430 + neoR2776	a/a	J. Pittermann s.n. (DUKE)
Phymatoceros phytatomoides	neochrome	neoF65 + neoR4110	neoF2367 + neoR3456	a/a	J. Pittermann s.n. (DUKE)
Phymatoceros phytatomoides	neochrome	neoF65 + neoR4110	neoF3230 + neoR4110	a/a	J. Pittermann s.n. (DUKE)
Phymatoceros phytatomoides	phototropin	neoF65 + neoR4110	neoF2367 + neoR3456	a/a	J. Pittermann s.n. (DUKE)
Phymatoceros phytatomoides	phototropin	photF1856 + photR2508	photF1970 + photR2245	a/a	J. Pittermann s.n. (DUKE)
Phymatoceros phytatomoides	phototropin	photF2774 + photR4339	-	a	J. Pittermann s.n. (DUKE)
Megaceros flagellaris	neochrome	neoF65 + neoR902	-	b	B. Crandall-Stotler s.n. (ABSH)
Megaceros flagellaris	neochrome	neoF649 + neoR1950	-	b	B. Crandall-Stotler s.n. (ABSH)
Megaceros flagellaris	neochrome	neoF1844 + neoR2361	-	c	B. Crandall-Stotler s.n. (ABSH)
Megaceros flagellaris	neochrome	neoF2239 + neoR3300	-	b	B. Crandall-Stotler s.n. (ABSH)
Megaceros flagellaris	neochrome	neoF2361 + neoR4110	-	c	B. Crandall-Stotler s.n. (ABSH)
Megaceros flagellaris	phototropin	photF1856 + photR4339	photF1970 + photR4339	a/a	B. Crandall-Stotler s.n. (ABSH)
Nothoceros aenigmatus	neochrome	F5 + R1_T1	F565 + R1_T1	d/d	F.W. Li 1291 (DUKE)
Nothoceros aenigmatus	neochrome <sup>3</sup>	neoF4018 + AP1	neoF4110 + AP2	e/f	F.W. Li 1569 (DUKE)
Nothoceros aenigmatus	neochrome <sup>3</sup>	neoR429 + AP1	R3re_phYN + AP2	e/f	F.W. Li 1569 (DUKE)
Nothoceros aenigmatus	neochrome <sup>3</sup>	NaNEO_3-1_GM1 + AP1	NaNEO_3-1_GM2 + AP2	e/f	F.W. Li 1569 (DUKE)
Nothoceros aenigmatus	phototropin	SupF1 + R7	SupF2 + R7	c/g	F.W. Li 1291 (DUKE)
Nothoceros aenigmatus	phototropin <sup>4</sup>	F565 + I_R1	I_F2 + I_R2	d/d	F.W. Li 1291 (DUKE)
Nothoceros aenigmatus	phytchrome	F-200_Maphy + R4850_Maphy	F-3_Maphy + R4450_Maphy	h/h	F.W. Li 1291 (DUKE)
Phaeoceros carolinianus	neochrome	neoF65 + neoR877	-	c	B. Crandall-Stotler s.n. (ABSH)
Phaeoceros carolinianus	neochrome	neoF649 + neoR1950	-	b	B. Crandall-Stotler s.n. (ABSH)
Phaeoceros carolinianus	neochrome	neoF1576 + neoR4104	-	a	B. Crandall-Stotler s.n. (ABSH)
Anthoceros punctatus	neochrome	neoF67 + neoR832	-	c	D. Chamberlain s.n. (E)
Anthoceros punctatus	neochrome	neoF428 + neoR3049	neoF812 + neoR2938	a/g	D. Chamberlain s.n. (E)
Anthoceros punctatus	neochrome	neoF2938 + neoR4104-2	neoF3049 + neoR4104-2	a/a	D. Chamberlain s.n. (E)
<b>Ferns:</b>					
Adiantum andicola	neochrome	neoF20 + neoR4242	neoF20 + neoR2336	i/c	C.J. Rothfels 2641, DB5549 <sup>5</sup> (DUKE)
Adiantum hispidulum	neochrome	neoF58 + neoR4238	neoF651 + neoR3718	c/c	L. Huiet s.n., DB9529 (DUKE)
Adiantum hispidulum	neochrome	neoF20 + neoR4242	neoF651 + neoR3718	i/c	L. Huiet s.n., DB9529 (DUKE)
Adiantum pedatum	neochrome	neoF20 + neoR4242	neoF20 + neoR2336	i/c	C.J. Rothfels 3839, DB7517 (DUKE)
Adiantum pedatum	neochrome	neoF20 + neoR4242	neoF651 + neoR3718	i/c	C.J. Rothfels 3839, DB7517 (DUKE)
Adiantum tetraphyllum	neochrome	neoF20 + neoR4242	neoF651 + neoR3718	i/c	L. Huiet 105, DB2505 (UC)
Adiantum tetraphyllum	neochrome	neoF20 + neoR4242	neoF20 + neoR2236	i/c	L. Huiet 105, DB2505 (UC)
Adiantum tetraphyllum	neochrome	neoF1108 + neoR3065	-	k	L. Huiet 105, DB2505 (UC)
Alsophila podophylla	neochrome	neoF20 + neoR4242	neoF20 + neoR2336	i/l	E. Schuettpeilz 1201A, DB4948 (DUKE)
Alsophila podophylla	neochrome	neoF20 + neoR4242	neoF2115 + neoR4242	i/l	E. Schuettpeilz 1201A, DB4948 (DUKE)
Alsophila podophylla	neochrome	neoF20 + neoR4242	neoF538 + neoR4000	i/l	E. Schuettpeilz 1201A, DB4948 (DUKE)
Bolbitis auriculata	neochrome	neoF20 + neoR4242	neoF651 + neoR3718	i/c	F. Rakotondrainibe, DB3504 (P)
Dennstaedtia punctilobula	neochrome	neoF20 + neoR4242	neoF651 + neoR3718	i/c	C.J. Rothfels 4167, DB8975 (DUKE)
Dennstaedtia punctilobula	neochrome	neoF20 + neoR4242	neoF20 + neoR2336	i/l	C.J. Rothfels 4167, DB8975 (DUKE)
Dennstaedtia punctilobula	neochrome	neoF20 + neoR4242	neoF2115 + neoR4242	i/l	C.J. Rothfels 4167, DB8975 (DUKE)
Deparia acrostichoides	neochrome	neoF20 + neoR4242	neoF651 + neoR3718	i/c	C.J. Rothfels 3894, DB7797 (DUKE)
Deparia acrostichoides	neochrome	neoF20 + neoR4242	neoF20 + neoR2336	i/c	C.J. Rothfels 3894, DB7797 (DUKE)
Deparia acrostichoides	neochrome	neoF20 + neoR4242	neoF2115 + neoR4242	i/c	C.J. Rothfels 3894, DB7797 (DUKE)
Deparia lancea	neochrome	neoF20 + neoR4242	neoF651 + neoR3718	i/c	E. Schuettpeilz 298, DB2558 (DUKE)
Deparia lancea	neochrome	neoF20 + neoR4242	neoF2115 + neoR4242	i/c	E. Schuettpeilz 298, DB2558 (DUKE)
Didymochlaena truncatula	neochrome	neoF20 + neoR4242	neoF651 + neoR3718	i/c	E. Schuettpeilz 267, DB2435 (DULE)
Diplazium bombonasae	neochrome	neoF20 + neoR4242	neoF2115 + neoR4242	i/c	R.C. Moran 7493, DB3764 (DUKE)
Doodia media	neochrome	neoF20 + neoR4242	neoF651 + neoR3718	i/c	E. Schuettpeilz 295, DB2555 (DUKE)
Dryopteris amurensis	neochrome	neoF20 + neoR4242	neoF20 + neoR2336	i/c	A. Uchida 1392, DB7982 (TNS)
Dryopteris amurensis	neochrome	neoF20 + neoR4242	neoF2115 + neoR4242	i/c	A. Uchida 1392, DB7982 (TNS)
Dryopteris expansa	neochrome	neoF20 + neoR4242	neoF20 + neoR2336	i/c	A. Ebihara TH2007-507, DB7977 (TNS)
Dryopteris expansa	neochrome	neoF20 + neoR4242	neoF2115 + neoR4242	i/c	A. Ebihara TH2007-507, DB7977 (TNS)
Hemitictym marginatum	neochrome	neoF20 + neoR4242	neoF20 + neoR2336	i/j	M. Christenhusz 2476, DB3054 (CAY)
Hemitictym marginatum	neochrome	neoF20 + neoR4242	neoF2115 + neoR4242	i/j	M. Christenhusz 2476, DB3054 (CAY)
Hemitictym marginatum	neochrome	neoF20 + neoR4242	neoF651 + neoR3718	i/j	M. Christenhusz 2476, DB3054 (CAY)
Hemitictym marginatum	neochrome	neoF1108 + neoR3065	-	k	M. Christenhusz 2476, DB3054 (CAY)
Hypolepis tenuifolia	neochrome	neoF20 + neoR4242	neoF2115 + neoR4242	i/c	E. Schuettpeilz 286, DB2574 (DUKE)
Macrothelypteris torresiana	neochrome	neoF20 + neoR4242	neoF651 + neoR3718	i/c	Schuettpeilz 335, DB2980 (DUKE)
Macrothelypteris torresiana	neochrome	neoF20 + neoR4242	neoF20 + neoR2336	i/c	Schuettpeilz 335, DB2980 (DUKE)
Macrothelypteris torresiana	neochrome	neoF20 + neoR4242	neoF2115 + neoR4242	i/c	Schuettpeilz 335, DB2980 (DUKE)
Matteuccia struthiopteris	neochrome	neoF20 + neoR786	-	b	A. Larsson 258, DB7946 (DUKE)
Matteuccia struthiopteris	neochrome	neoF649 + neoR1950	-	b	A. Larsson 258, DB7946 (DUKE)
Matteuccia struthiopteris	neochrome	neoF1530 + neoR2300	-	m	A. Larsson 258, DB7946 (DUKE)
Matteuccia struthiopteris	neochrome	neoF2239 + neoR3300	-	m	A. Larsson 258, DB7946 (DUKE)
Matteuccia struthiopteris	neochrome	neoF2935 + neoR3720	-	m	A. Larsson 258, DB7946 (DUKE)
Matteuccia struthiopteris	neochrome	neoF58 + neoR4238	neoF651 + neoR3718	c/c	A. Larsson 258, DB7946 (DUKE)
Onoclea sensibilis	neochrome	neoF20 + neoR4242	neoF651 + neoR3718	i/c	E. Schuettpeilz 353, DB2998 (DUKE)
Onoclea sensibilis	neochrome	neoF20 + neoR4242	neoF2115 + neoR4242	i/c	E. Schuettpeilz 353, DB2998 (DUKE)
Phegopteris hexagonoptera	neochrome	neoF20 + neoR4242	neoF651 + neoR3718	i/c	M. Christenhusz 3844, DB2731 (TUR)
Phegopteris hexagonoptera	neochrome	neoF20 + neoR4242	neoF2115 + neoR4242	i/c	M. Christenhusz 3844, DB2731 (TUR)
Plagiogyria formosana	neochrome	neoF20 + neoR786	-	b	E. Schuettpeilz 1083A, DB4826 (DUKE)
Plagiogyria formosana	neochrome	neoF649 + neoR1950	-	b	E. Schuettpeilz 1083A, DB4826 (DUKE)
Plagiogyria formosana	neochrome	neoF1530 + neoR2300	-	m	E. Schuettpeilz 1083A, DB4826 (DUKE)
Plagiogyria formosana	neochrome	neoF2935 + neoR3720	-	m	E. Schuettpeilz 1083A, DB4826 (DUKE)
Tectaria zeylanica	neochrome	neoF20 + neoR4242	-	i	E. Schuettpeilz 514, DB3569 (GOET)
Thelypteris noveboracensis	neochrome	neoF20 + neoR4242	neoF2115 + neoR4242	i/l	C.J. Rothfels 4164, DB8972 (DUKE)

<sup>1</sup>The primer pair for secondary PCR in nested PCR reaction. "-" indicates no nested PCR was conducted.

a 98:30s, (98:10s, 70:30s, 72:90s)x35, 72:600s

<sup>2</sup>The PCR program used (primary PCR/secondary PCR, if nested PCR was used).

b 98:30s, (98:10s, 60:30s, 72:90s)x35, 72:600s

<sup>3</sup>Genome walking using Clontech Genome Walker kit.

c 98:30s, (98:10s, 72:120s)x35, 72:600s

<sup>4</sup>Genome walking using Inverse PCR.

d 98:30s, (98:10s, 72:180s)x7, (94:25s, 67:180s)x32, 67:420s

<sup>5</sup>Fern DNA Database number (<http://fernlab.biology.duke.edu>).

e (94:25s, 72:180s)x5, (94:25s, 67:180s)x20, 67:420s

f 98:30s, (98:10s, 67:30s, 72:90s)x35, 72:600s

g 98:30s, (98:10s, 67:30s, 72:90s)x35, 72:600s

h 98:30s, (98:10s, 67:30s, 72:150s)x35, 72:600s

i 98:30s, (98:10s, 68:30s, 72:120s)x35, 72:600s

j 94:300s, (94:60s, 60:60, 72:120s)x35, 72:600s

k 94:300s, (94:60s, 56:60, 72:240s)x35, 72:600s

l 98:30s, (98:10s, 70:30s, 72:120s)x35, 72:600s

m 98:30s, (98:10s, 55:30s, 72:90s)x35, 72:600s

**Table S4.** The primer sequences.

Primer	Sequence (5'-3')
SupF1	ATTACACAAATGTTGCCCGATGTGC
SupF2	CTGCACTCCTACTCGTTACCG
AP1	GTAATACGACTCACTATAAGGGC
AP2	ACTATAGGGCACCGGTGGT
F-200_Maphy	AGCGTGTAGCCTTGTCTGTAC
F-3_Maphy	GCGACAGCGGCAAAGTTGAAG
F5	GCGGCAGGCTGCTCAACTACAG
F565	TACACCGAAGGCTACAAGGCTAATG
I_F2	CAAGTGCAATCCAATGATGCCGC
I_R1	TTCTGTAGTTGAGCAGGCTGCCC
I_R2	GAGGAGTAGCCGGTCATGGTGAAG
NaNEO_3-1_GM1	TGTGGAACAAAGGCAACTGGGACGAA
NaNEO_3-1_GM2	ATGTGAAGGCTCAAGGCAATGTTACAAGT
neoF1108	GTGCAAGCTAACATKGAGCTGGA
neoF1530	TCBTRTTTGGTTYAGGTCRAYACTGC
neoF1576	CTGGACAGGGACGACGACTCTCG
neoF1844	CATTGAGGGACAAGGAGGAGTTACAGG
neoF20	CCAAGACGAAGCACAGCGTG
neoF2115	GGAGGTTGATTGGAGSCAAGTGC
neoF2239	AGGAAAGATGGYAGCWRYTTYGGAA
neoF2300	GCTRAGGTTDASCAAGTACACDGAGGG
neoF2361	CGGCACCCAGGACAAAGGTTCTG
neoF2367	CAGTCSCCTCATCAAGTACGAYGT
neoF2935	GTKCAGCTYATCCGAGATGCGT
neoF2938	CTGTCCTGGAGATCGTAACATACACC
neoF3049	CAACAGAAGGTGGCGGATTATGTTCC
neoF3230	CAGACCATCTATGGGTGCGGCATTG
neoF4018	ATCTTGCTCTACGGAGATGCTCTATGGC
neoF4110	TACATTCCAACCAGCATCCAGTGTAG
neoF428	GYACGGATSTGCGGATGCTCTTCAC
neoF430	ACGGATSTGCGGATGCTCTTCAC
neoF58	AGBGCGNGATGCMAGRCTYCATGC
neoF649	GATCDGDTGATGGCBTACAARTTYCA
neoF649	GATCDGDTGATGGCBTACAARTTYCA
neoF65	ATCGCAGGCTKAATGCCGCGTTGAG
neoF651	TCGGGTGATGCCCTACAAGTTCA
neoF67	GCGAGGCTGMATGCCGGYGTYYGAG
neoF812	ACAAGTTYCAYGAGGACGAGCACG
neoR1950	CCYCGAAYNGCYTCCATCCAYTCCTG
neoR2236	AGAAGYTGCTGCCRTCTTCTGT
neoR2361	CRGAAACCTTGTCTGGTGC
neoR2776	GCGAAGATGATGGGGTTGTCG
neoR2818	GCACCTCCCTCCCTGCTGTAACCTGTCAG
neoR2938	GGTAGTTCACGATCTCCAGGGACAG
neoR3049	GGAACATAATCCGCCACCTCTGTG
neoR3065	CTGHACTCCGATGAAGTACTGGA
neoR3300	GYARCTSGGATCTGWGATCAC
neoR3456	AGCATCATSGCCTTGTCCATG
neoR3718	TGACVCCCATGCACTGGAGGTACTC
neoR3720	GTTCTCBGGCTTSAGRTCBCGGTAGATG
neoR4104	ATGCTGGTGGGAATGTRAGCTCTTG
neoR4104-2	AYGCTGSTSGGAAKGTGAGCTCTTG
neoR4110	AGGCTCACTGGGATGCTGGTTGG
neoR4238	CGGATRAGAGGCCAGTYGATKYCTYGA
neoR4242	CGGATGAGAGGCCAGTCGATKYCT
neoR429	GAGTGAACAGCATCCGACATCCG
neoR786	GGTARTGCARGCCVAGRTAHGGCTCC
neoR832	GAGGCTGATGGCTGGTGGAG
neoR877	ATGTACTCGTGTGGCAACCGTGC
neoR902	GACGAGACGGAGCCATGTTGC
photF1856	CTGGTGSTCAAGGAGGAGCTGG
photF1970	GCTCTCCWCCTTCACGAGACG
photF2645	CTTCGCCCTCYGACCAYTTCTGG
photF2774	GGAGAGACGGGACATCACTGTGC
photR2508	AGCAGCGACAGAAATCCGAGGGAC
photR2901	GCTCGTACTCGTSCCRTCCAG
photR4102	ATGCTGSTSGRAATGAGCTCTTGTT
photR4339	TCYKCTCGTCCCACCTCAGRTC
R1_T1	ACCCAGGATCAAACACATCGCTG
R3re	GACGCATTCTCGCTATTGCCAGGAT
R4450_Maphy	CCATCCACCAAGGTTCTGAACAC
R4850_Maphy	AAAATGTCCAGGACCGTCAGGTT
R7	AGAGTGGTGGCCAAGTCAATTCC