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Precise age of *Bangiomorpha pubescens* dates the origin of eukaryotic photosynthesis

Gibson et al.

SUPPLEMENTAL METHODS

Stratigraphy and Sampling

Shale samples were collected from outcrops that lack evidence for secondary mineralization from hydrothermal activity and weathered regions that may have experienced alteration were also avoided. Sample sets comprise several 100–200 g samples excavated 10–30 cm from the outcrop surface to target fresh material. Samples were collected along strike from a narrow stratigraphic range (<10 cm), as well as from a vertical profile (up to 5 m). The most organic-rich and least visibly-weathered samples were then chosen for digestion and isotopic analysis.

Arctic Bay Formation samples are from Shale Valley (N 72° 45’ 04.8” W 83° 50’ 39.2”), ca. 180 m above the base of section T1413 (Arctic Bay-Adams Sound formations contact not exposed in this locale) and ca. 170 m below the base of the Ikpiarjuk Formation (Angmaat Formation equivalent; Fig. 1). Victor Bay Formation samples are from sections G1431 at Angmaat Mountain (N 72° 09’ 25.9” W 79° 02’ 05.5”) and MB1501 at Pingo Valley (N 72° 53’ 48.3” W 81° 24’ 45.02”). Both Victor Bay Formation sample sets are from within the same maximum flooding interval indicated by the finest-grained and most organic-rich horizon ca. 25 m above the contact with the Angmaat Formation.

Re-Os Geochronology Methods

Least-weathered samples from each sample set were selected and trimmed with a diamond-tipped lapidary saw blade to remove any weathered surfaces, then polished with a diamond pad to remove any metal contamination. After samples were dried at room temperature, 30–50 g aliquots were crushed to a fine powder (ca. 30 μm) using a SPEX #8506 zirconia ceramic puck and grinding container in a SPEX 8500 shatterbox to homogenize each sample (Kendall et al., 2009a). Analyses of Re and Os isotopic abundances and compositions were performed at the University of Alberta's Re-Os Crustal Geochronology Laboratory in the Department of Earth and Atmospheric Sciences following methodologies developed by Creaser et al. (2002), Selby and (2003), Kendall et al. (2004), and Cumming et al. (2013).

Between 0.2 and 0.5 g of each sample was digested and equilibrated with 8 ml of Cr^{VI}-H_2SO_4 along with a known quantity of mixed $^{185}Re + {}^{190}Os$ tracer solution (spike) in Carius tubes at 220 °C for 48 hrs. Digestion with Cr^{VI}-H_2SO_4 is known to preferentially liberate hydrogenous rather than detrital Re and Os in shale samples, resulting in more accurate and precise isochrons (Selby and Creaser, 2003; Kendall et al., 2004; Rooney et al., 2011). Osmium was isolated and purified by $CHCl_3$ solvent extraction and micro-distillation using HBr, and Re was purified using $(CH_3)_2CO$ solvent extraction and anion chromatography following protocols outlined by Selby and Creaser (2003) and Cumming et al. (2013). These Re and Os fractions were then loaded onto Ni and Pt filaments, respectively (Selby and Creaser, 2003; Selby et al., 2007), for analysis with a ThermoScientific TRITON instrument using negative thermal ionization mass spectrometry (NTIMS; Creaser et al., 1991). Re was analyzed via static Faraday collection and Os utilizing ion-counting with a secondary electron multiplier in peak-hopping mode.

Isochron ages were regressed using the Re and Os isotopic measurements, calculated 2σ

uncertainties for $^{187}Re/^{188}Os$ and $^{187}Os/^{188}Os$, and the associated error correlation function (rho) using Isoplot V. 4.15 (Ludwig, 1980; Ludwig, 2011) with a ^{187}Re decay constant (λ) of $1.666 \times 10^{-11}year^{-1}$ (Table DR1; Smoliar et al., 1996). A Re standard solution of normal isotopic composition was repeatedly analyzed to monitor long-term mass spectrometry reproducibility, using analysis amounts typical for shale samples (1–4 ng). For this solution, an average value for $^{185}Re/^{187}Re$ of 0.5973 ± 0.0007 (n = 52; 1σ) was obtained over the period of analysis, which overlaps the value of 0.5974 (Gramlich et al., 1973). A Johnson–Matthey Os solution is used as an in-house standard for Os, which yielded an average $^{187}Os/^{188}Os$ ratio of 0.10683 ± 0.00010 (n = 186; 1σ) by pulse-counting SEM measurement over the period of analysis, which is identical to values reported elsewhere (Li et al., 2010).

TABLE DR1. Re AND Os ABUNDANCES AND ISOTOPIC COMPOSITIONS

Sample	Formation	Re (ppb)	±2s	Os (ppt)	±2s	$^{187}Re/^{188}Os$	±2s	$^{187}Os/^{188}Os$	±2s	rho*	Os_i†
T1413-181.1§	Arctic Bay	67.31	0.25	1247.38	8.16	757.68	3.48	14.80	0.06	0.54	1.42
T1413-181.8§	Arctic Bay	21.62	0.08	488.94	3.55	490.77	2.97	10.12	0.05	0.73	1.45
T1413-182.0§	Arctic Bay	22.10	0.09	524.52	4.69	445.38	2.98	9.28	0.07	0.62	1.41
T1413-182.6§	Arctic Bay	48.49	0.18	1082.97	7.36	501.16	2.37	10.27	0.05	0.48	1.41
T1413-184.0§	Arctic Bay	16.76	0.07	404.74	3.11	432.12	2.94	9.07	0.06	0.76	1.43
T1413-185.0	Arctic Bay	50.76	0.19	1145.79	8.71	485.80	2.41	9.91	0.05	0.48	1.33
G1431-26.0b§	Victor Bay	0.76	0.01	33.38	0.49	166.36	4.32	4.15	0.10	0.66	1.22
G1431-26.0d§	Victor Bay	0.72	0.01	24.25	0.39	246.74	7.93	5.61	0.16	0.84	1.27
G1431-28.1§	Victor Bay	0.94	0.01	32.57	0.48	236.08	5.78	5.46	0.12	0.79	1.31
G1431-28.2	Victor Bay	0.94	0.01	32.79	0.43	229.48	5.36	5.21	0.10	0.83	1.18
MB1501-51.6a§	Victor Bay	16.73	0.04	406.59	3.41	416.96	2.52	8.58	0.06	0.75	1.24
MB1501-51.6b§	Victor Bay	15.52	0.04	384.67	4.13	403.94	3.25	8.39	0.09	0.69	1.28
MB1501-51.7§	Victor Bay	7.04	0.02	187.46	1.58	355.79	2.82	7.54	0.06	0.86	1.28
MB1501-51.9	Victor Bay	12.89	0.03	316.92	2.33	404.81	2.12	8.29	0.05	0.78	1.17

Note: Total procedural blanks analyzed during this study were 11 ± 3 pg Re and 0.25 ± 0.3 pg Os and $^{187}Os/^{188}Os$ of 1.3 ± 0.8 (1σ, n=5).

*Rho = associated error correlation (Ludwig, 1980).

†Os_i = Initial $^{187}Os/^{188}Os$ isotope composition calculated from $\lambda^{187}Re$ and isochron ages that utilize all samples (1051 Ma for Arctic Bay samples and 1047 Ma for Victor Bay formations samples; Figure DR4).

§Samples included in the isochrons that utilized a limited stratigraphic range (Fig. 2).

Cross-Calibrated Molecular Clock (BEAST2) Methods

In lieu of a complete fossil record, molecular clock analyses may be improved by increasing the amount of age data they incorporate. Cross-calibrated analyses leverage relative dating information using gene duplication events to increase the accuracy of divergence time estimates (Shih and Matzke, 2013). Molecular clock analyses were run on a concatenated dataset of proteins: AtpA, AtpB, AtpE, AtpF, AtpH, AtpI, Rpl2, Rpl16, Rps3, Rps12, and EfTu, as well as 16S rDNA. To generate the dataset, sequences were aligned using MAFFT (Katoh et al., 2005), then partitioned into the concatenated protein sequences and 16S nucleotide sequences. The base set of age calibrations implemented are primarily from on the fossil records of plants and algae and the molecular clock analyses of Smith et al. (2010). A summary of the various constraints used can be found in Table DR2. A uniform prior of 2.4–3.8 billion years ago (Ga) was used as a constraint for the last common ancestor. The only constraint that differed between the three analyses was the prior set on the green-red divergence, representing the oldest possible node for which *Bangiomorpha pubescens* can provide a direct constraint based on its position either derived within the Bangiales or perhaps as a stem-group red alga (e.g., Butterfield, 2000; Yang et al., 2016). In these analyses (Table 1), three constraints were tested to compare their effect on different interpretations of plastid endosymbiosis: 1) no prior (Run T07; Fig. DR1), 2) a prior based on the previously reported age for *Bangiomorpha pubescens* of 1.198 Ga (Run T08; Fig. DR2; Butterfield, 2000), and 3) a prior based on our geochronology data of 1.045 Ga (Run T09; Fig. DR3). As previously described (Shih et al., 2017), molecular clock analyses were estimated with the program BEAST2 (Drummond and Rambaut, 2007) using the CIPRES Science Gateway server (Miller et al., 2010). The CpREV model and the GTR + G model were used as the substitution model for the protein and nucleotide datasets, respectively. A lognormal

relaxed molecular clock model was implemented. For all analyses, three separate MCMC chains for 40–50 million generations were generated, sampling every 10,000 generations. The initial 20 million generations were discarded as burn-in, and maximum clade credibility trees were generated using TreeAnnotator v1.7.5. The analyses and dates of interest are summarized in the main text and Table 1.

TABLE DR2. SUMMARY OF CALIBRATION CONSTRAINTS USED IN THIS STUDY.

Divergence event	**Type of Distribution**	**Age Constraint (Ga)**
Angiospermae	Normal	0.217 ± 0.040 (1σ)
Land Plants	Normal	0.477 ± 0.070 (1σ)
Bangiomorpha pubescens	Uniform	1.174–1.222
"Rise of Oxygen"	Uniform	2.400–3.000
Last Common Ancestor	Uniform	2.400–3.800

Note: Angiospermae and land plant age constraints from Smith et al. (2010).

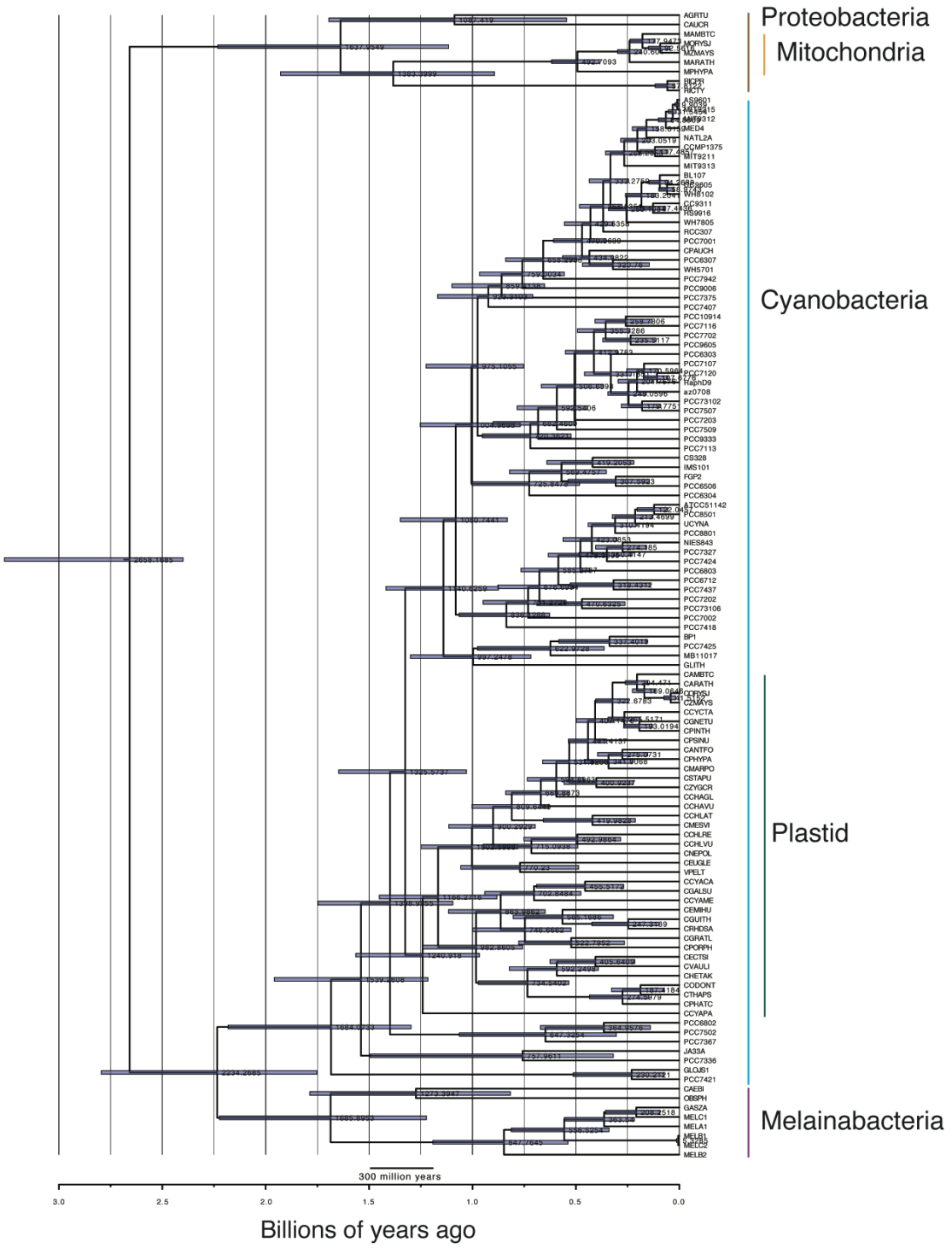

Figure DR1. **Divergence time estimates from T07 cross-calibrated BEAST2 run.** All land plant constraints were used; however, no *Bangiomorpha pubescens* constraint was utilized. Abbreviations are summarized in Table DR3.

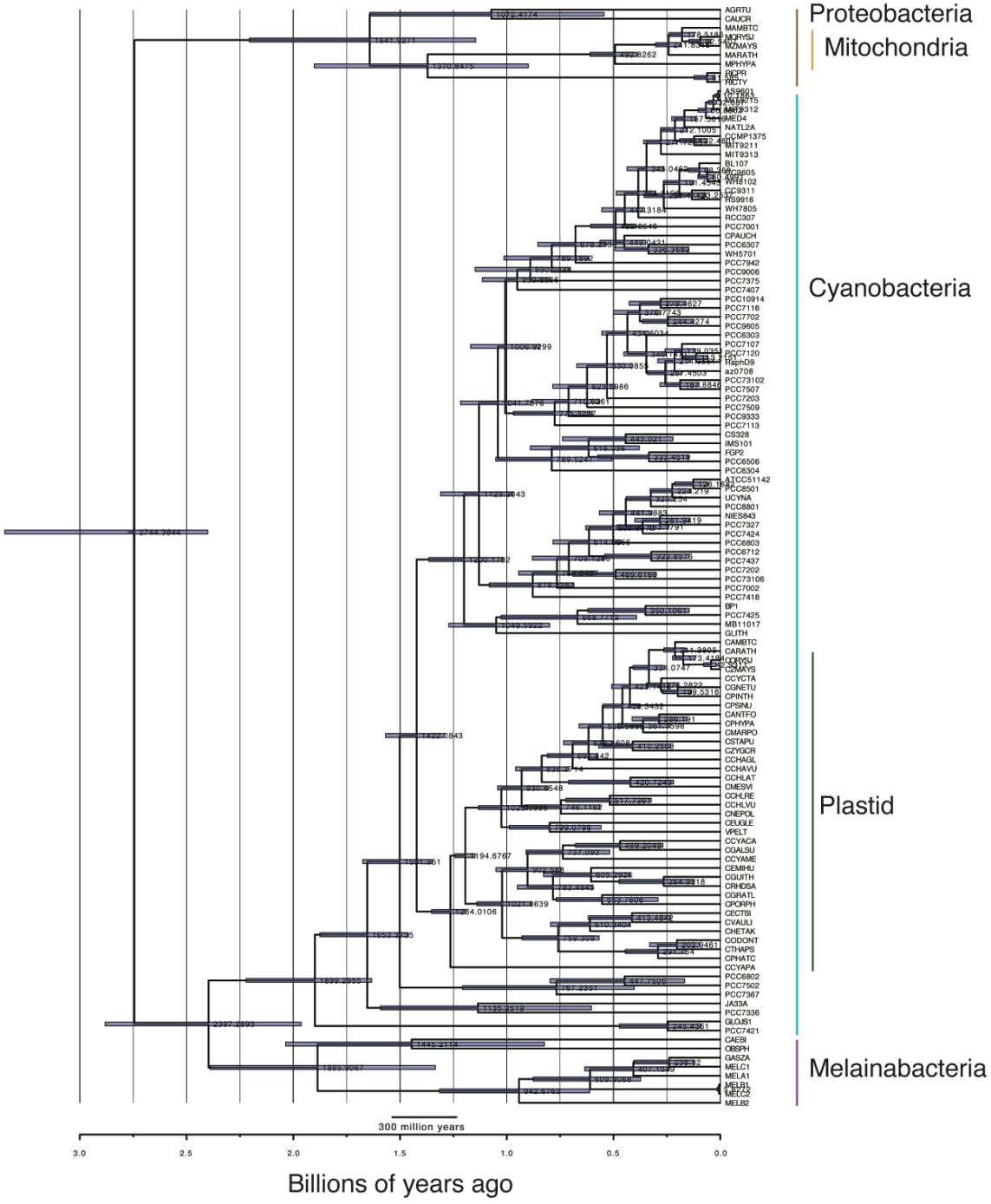

Figure DR2. **Divergence time estimates from T08 cross-calibrated BEAST2 run.** All land plant constraints were used. *Bangiomorpha pubescens* was constrained to the green-red divergence using the older and previously inferred age of 1.2 Ga. Abbreviations are summarized in Table DR3.

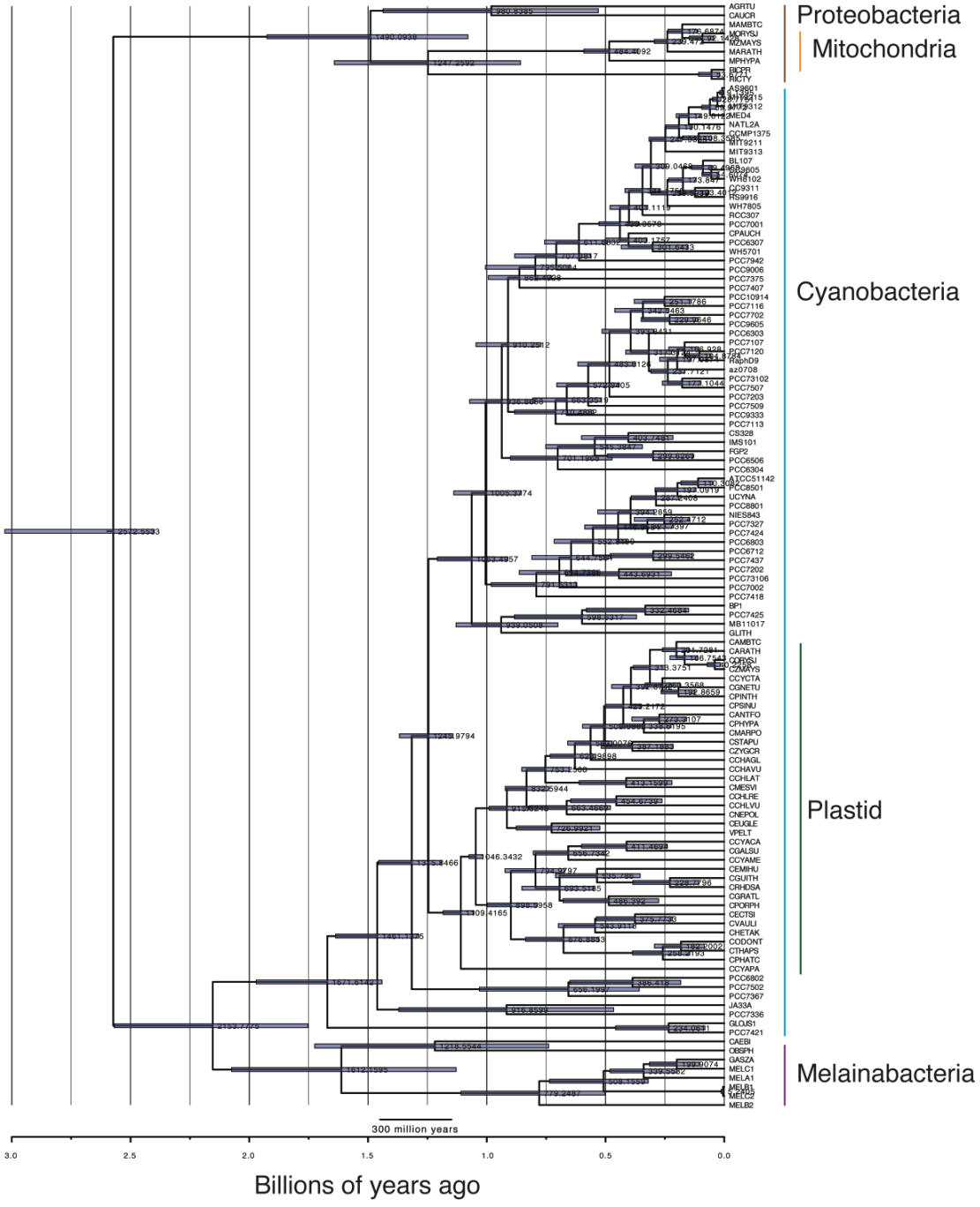

Figure DR3. Divergence time estimates from T09 cross-calibrated BEAST2 run. All land plant constraints were used. *Bangiomorpha pubescens* was constrained to the green-red divergence using the younger, revised age of 1.045 Ga. Abbreviations are summarized in Table DR3.

TABLE DR3. ABBREVIATIONS FOR SPECIES NAMES USED IN FIGURES DR1-3.

Species Name	Clade	Abbreviation
Gloeobacter violaceus PCC 7421	Cyanobacteria	PCC7421
Gloeobacter kilaueensis JS1	Cyanobacteria	GLOJS1
Synechococcus sp. PCC 7336	Cyanobacteria	PCC7336
Synechococcus sp. JA-3-3Ab	Cyanobacteria	JA33A
Pseudanabaena sp. PCC 7367	Cyanobacteria	PCC7367
Pseudanabaena sp. PCC 6802	Cyanobacteria	PCC6802
Synechococcus sp. PCC 7502	Cyanobacteria	PCC7502
Acaryochloris marina MBIC11017	Cyanobacteria	MB11017
Cyanothece sp. PCC 7425	Cyanobacteria	PCC7425
Thermosynechococcus elongatus BP-1	Cyanobacteria	BP1
Geitlerinema sp. PCC 7407	Cyanobacteria	PCC7407
Leptolyngbya sp. PCC 7375	Cyanobacteria	PCC7375
Prochlorothrix hollandica PCC 9006	Cyanobacteria	PCC9006
Synechococcus elongatus PCC 7942	Cyanobacteria	PCC7942
Cyanobium sp. PCC 7001	Cyanobacteria	PCC7001
Cyanobium gracile PCC 6307	Cyanobacteria	PCC6307
Synechococcus sp. WH 5701	Cyanobacteria	WH5701
Synechococcus sp. RS 9916	Cyanobacteria	RS9916
Synechococcus sp. CC 9311	Cyanobacteria	CC9311
Synechococcus sp. WH 7805	Cyanobacteria	WH7805
Synechococcus sp. BL 107	Cyanobacteria	BL107
Synechococcus sp. CC 9605	Cyanobacteria	CC9605
Synechococcus sp. WH 8102	Cyanobacteria	WH8102
Prochlorococcus marinus MIT 9313	Cyanobacteria	MIT9313
Prochlorococcus marinus, subsp. marinus CCMP 1375	Cyanobacteria	CCMP1375
Prochlorococcus marinus MIT 9211	Cyanobacteria	MIT9211
Prochlorococcus marinus MIT 9312	Cyanobacteria	MIT9312
Prochlorococcus marinus MIT 9215	Cyanobacteria	MIT9215
Prochlorococcus marinus AS 9601	Cyanobacteria	AS9601
Prochlorococcus marinus, subsp. pastoris CCMP 1986	Cyanobacteria	MED4
Prochlorococcus marinus NATL 2A	Cyanobacteria	NATL2A
Synechococcus sp. RCC307	Cyanobacteria	RCC307
Crinalium epipsammum PCC 9333	Cyanobacteria	PCC9333
Microcoleus sp. PCC 7113	Cyanobacteria	PCC7113
Chroococcidiopsis sp. PCC 6712	Cyanobacteria	PCC6712
Stanieria cyanosphaera PCC 7437	Cyanobacteria	PCC7437
Cyanobacterium stanieri PCC 7202	Cyanobacteria	PCC7202
Synechococcus sp. PCC 7002	Cyanobacteria	PCC7002
Gloeocapsa sp. PCC 73106	Cyanobacteria	PCC73106
Cyanothece sp. PCC 7424	Cyanobacteria	PCC7424
Microcystis aeruginosa NIES-843	Cyanobacteria	NIES843
Pleurocapsa sp. PCC 7327	Cyanobacteria	PCC7327
Synechocystis sp. PCC 6803	Cyanobacteria	PCC6803
Cyanothece sp. PCC 8801	Cyanobacteria	PCC8801
Crocosphaera watsonii WH 8501	Cyanobacteria	PCC8501
Cyanothece sp. ATCC 51142	Cyanobacteria	ATCC51142
Unidentified cyanobacterium UCYN-A	Cyanobacteria	UCYNA
Halothece sp. PCC 7418	Cyanobacteria	PCC7418
Chroococcidiopsis thermalis PCC 7203	Cyanobacteria	PCC7203
Synechocystis sp. PCC 7509	Cyanobacteria	PCC7509
Rivularia sp. PCC 7116	Cyanobacteria	PCC7116
Nostoc punctiforme PCC 73102	Cyanobacteria	PCC73102
Calothrix sp. PCC 7507	Cyanobacteria	PCC7507
Nostoc azollae 0708	Cyanobacteria	az0708
Raphidiopsis brookii D9	Cyanobacteria	RaphD9
Nostoc sp. PCC 7107	Cyanobacteria	PCC7107
Nostoc sp. PCC 7120	Cyanobacteria	PCC7120
Calothrix sp. PCC 6303	Cyanobacteria	PCC6303
Mastigocladopsis repens PCC 10914	Cyanobacteria	PCC10914
unidentified cyanobacterium PCC 7702	Cyanobacteria	PCC7702

TABLE DR3 *continued.* ABBREVIATIONS FOR SPECIES NAMES USED IN FIGURES DR1-3.

Species Name	Clade	Abbreviation
Fischerella sp. PCC 9605	Cyanobacteria	PCC9605
Oscillatoria acuminata PCC 6304	Cyanobacteria	PCC6304
Oscillatoria sp. PCC 6506	Cyanobacteria	PCC6506
Microcoleus vaginatus FGP-2	Cyanobacteria	FGP2
Arthrospira maxima CS-328	Cyanobacteria	CS328
Trichodesmium erythraeum IMS 101	Cyanobacteria	IMS101
Gloeomargarita lithophora	Cyanobacteria	GLITH
MEL.A1	Melainabacteria	MELA1
MEL.B1	Melainabacteria	MELB1
MEL.B2	Melainabacteria	MELB2
MEL.C1	Melainabacteria	MELC1
MEL.C2	Melainabacteria	MELC2
Rickettsia prowazekii strain, Madrid E	α-proteobacteria	RICPR
Rickettsia typhi strain ATCC VR-144	α-proteobacteria	ATPA_RICTY
Caulobacter crescentus strain ATCC 19089	α-proteobacteria	ATPA_CAUCR
Agrobacterium tumefaciens strain C58	α-proteobacteria	ATPA_AGRTU
Arabidopsis thaliana	Plastid	ARATH
Oryza sativa subsp. Japonica	Plastid	ORYSJ
Zea mays	Plastid	ZMAYS
Amborella trichopoda	Plastid	AMBTC
Pinus thunbergii	Plastid	PINTH
Cycas taitungensis	Plastid	CYCTA
Gnetum parvifolium	Plastid	GNETU
Psilotum nudum	Plastid	PSINU
Anthoceros formosae	Plastid	ANTFO
Marchantia polymorpha	Plastid	MARPO
Physcomitrella patens subsp. patens	Plastid	PHYPA
Zygnema circumcarinatum	Plastid	ZYGCR
Staurastrum punctulatum	Plastid	STAPU
Chaetosphaeridium globosum	Plastid	CHAGL
Chara vulgaris	Plastid	CHAVU
Chlamydomonas reinhardtii	Plastid	CHLRE
Chlorella vulgaris	Plastid	CHLVU
Nephroselmis olivacea	Plastid	NEPOL
Euglena gracilis	Plastid	EUGLE
Mesostigma viride	Plastid	MESVI
Chlorokybus atmophyticus	Plastid	CHLAT
Verdigellas peltata	Plastid	VPELT
Cyanophora paradoxa	Plastid	CYAPA
Cyanidioschyzon merolae	Plastid	CYAME
Cyanidium caldarium	Plastid	CYACA
Gracilaria tenuistipitata	Plastid	GRATL
Porphyridium purpureum	Plastid	PORPH
Galdieria sulphuraria	Plastid	GALSU
Thalassiosira pseudonana	Plastid	THAPS
Ectocarpus siliculosus	Plastid	ECTSI
Phaeodactylum tricornutum	Plastid	PHATC
Guillardia theta	Plastid	GUITH
Rhodomonas salina	Plastid	RHDSA
Vaucheria litorea	Plastid	VAULI
Heterosigma akashiwo NIES-293	Plastid	HETAK
Odontella sinensis	Plastid	ODONT
Emiliania huxleyi	Plastid	EMIHU
Paulinella chromatophora	Plastid	PAUCH
Arabidopsis thaliana	Mitochondria	ARATH
Zea mays	Mitochondria	ZMAYS
Oryza sativa	Mitochondria	ORYSJ
Amborella trichopoda	Mitochondria	AMBTC
Physcomitrella patens subsp. patens	Mitochondria	PHYPA

SUPPLEMENTAL TEXT

Rhenium-Osmium Results

Re and Os data from this study (see Table DR1) are within reported concentrations and isotopic ratios of other black shales and do not display evidence for post-depositional disturbance of the Re-Os system. Regression of Arctic Bay Formation samples excluding T1413-181.1 yields a nearly identical age of 1.054 ± 0.041 Ga and confirms that this sample does not disproportionally affect the isochron by "anchoring" its slope. Victor Bay Formation samples are from two correlative stratigraphic sections (G1431 and MB1501). Regression of G1431 Victor Bay Formation samples yield an imprecise Model 3 age of 1.077 ± 0.28 Ga due to an insufficient spread in initial $^{187}Re/^{188}Os$ and too much variation in initial $^{187}Os/^{188}Os$ values (see Table DR1) necessary to develop a precise isochron (Selby and Creaser, 2005; Kendall et al., 2009a). Therefore, samples from section MB1501 of the same maximum flooding interval in the lower Victor Bay Formation were also incorporated. Regression of MB1501 samples yielded an imprecise, but indistinguishable to G1431 (within uncertainty), Model 3 age of 0.995 ± 0.320 Ga. A sharp transgressive surface directly above the basin-wide Angmaat-Victor Bay unconformity marks a regional flooding event in the Milne Inlet Graben, and offers an unequivocally synchronous datum (Sherman et al., 2001). Sample set G1431 was collected from 26–28.1 m above this unconformity, and sample set MB1501 is from a slightly deeper-water, but time-correlative horizon 21.3–21.6 m above this unconformity. Robust stratigraphic evidence for depositional synchronicity and the similarity of their model ages enable regression of these samples as a combined data set to produce a significantly more precise age (Fig. DR4; Geboy et al., 2013). Combining these data sets is further supported by the relative precision and lower variance in the composite isochron, as well as its agreement with the Re-Os age for the Arctic

Bay Formation reported herein (Fig. 2).

Initial $^{187}Os/^{188}Os$ values for all Arctic Bay and Victor Bay samples range from 1.17–1.45 (average modern continental runoff $^{187}Os/^{188}Os$ = 1.5; Levasseur et al., 1999), consistent with a highly radiogenic Os flux dominated by evolved, continentally derived sediment and waters (Xu et al., 2009; Cumming et al., 2012; Cumming et al., 2013; Rooney et al., 2014). These data demonstrate that the Borden Basin had minimal communication with the global ocean during deposition of the sampled black shale units from the middle Arctic Bay (Turner and Kamber, 2012; Hahn et al., 2015) and lower Victor Bay formations and was strongly influenced chemically by runoff from the surrounding highly-evolved Archean to Paleoproterozoic orthogneiss and metasedimentary successions of the Rae Province (Crocker et al., 1993). However, abundant sulfate evaporite deposits, marine C, S, and Sr isotopic signatures, and evidence for tidal influence indicate that the Borden Basin was connected to a large ocean basin during deposition of the Angmaat Formation and other intervals of carbonate deposition (i.e. upper Victor Bay and Athole Point formations; Kah et al., 1999; Kah et al., 2001). Together these data demonstrate that the Borden Basin was periodically restricted from the open ocean and that the degree of restriction influenced sedimentation patterns, perhaps due to changes in the geochemical stratification of its basin waters. These interpretations may help characterize the environment in which *Bangiomorpha pubescens* evolved.

Precise Re-Os isochrons require samples of the same (or similar) age and with similar initial $^{187}Os/^{188}Os$ (Cohen et al., 1999; Creaser et al., 2002; Cohen, 2004). Sediment in restricted basins are known to exhibit highly variable $^{187}Os/^{188}Os$ as they are sensitive to short-term variability in weathering sources and runoff (McArthur et al., 2008; Cumming et al., 2012; Cumming et al., 2013; Tripathy et al., 2015). Therefore, on the condition that a sufficient spread

in $^{187}Re/^{188}Os$ is maintained, utilizing samples from a reduced stratigraphic interval, especially from restricted basins, can minimize age uncertainty by limiting the depositional timescale over which samples were deposited and thus stratigraphic variation in initial $^{187}Os/^{188}Os$ (Os_i; Xu et al., 2009; Cumming et al., 2012; Xu et al., 2014).

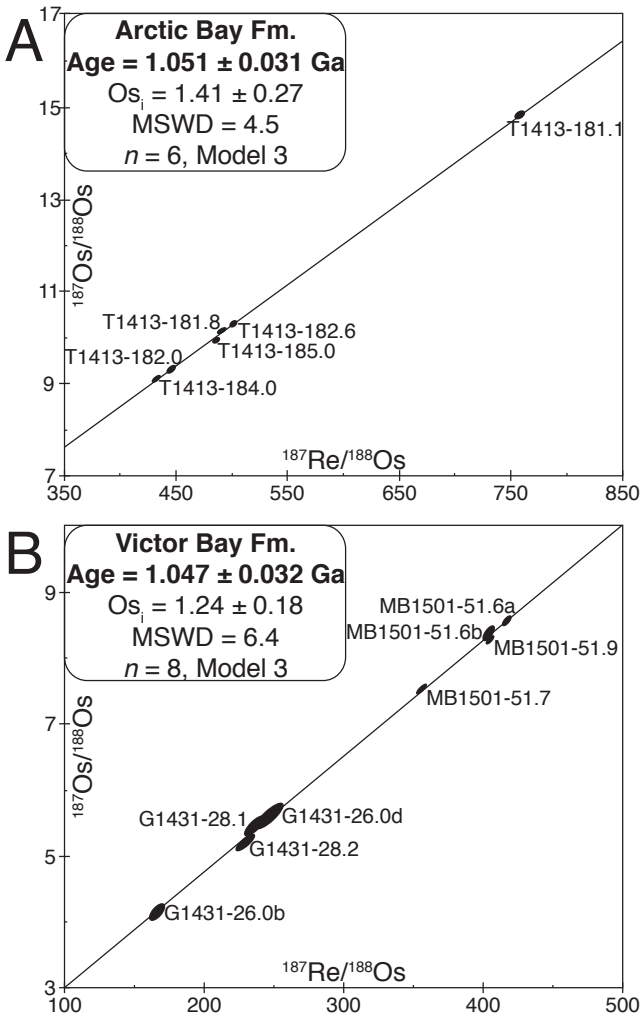

Figure DR4. **Re-Os geochronological data and isochron diagrams for all Arctic Bay (A) and Victor Bay (B) formations samples.** Mean square of weighted deviation (MSWD) values greater than unity (i.e., 1) indicate that geological factors rather than analytical error are responsible for scatter about the isochron (Mahon, 1996). Data-point error ellipses represent 2σ uncertainty. Elemental abundances and isotopic compositions are presented in Table DR1.

Previous Geochronology from the Bylot Supergroup

Pyrite Re-Os geochronology from the carbonate-hosted Nanisivik Pb-Zn deposit (Angmaat Formation equivalent; see Fig. 1) suggest approximately syn-depositional mineralization ca. 1.1 billion years ago (Ga), though the data span 1.151-1.013 Ga (Hnatyshin et al., 2016), which is broadly consistent with depositional ages presented from this study. Turner and Kamber (2012) conducted whole-rock U-Th-Pb analyses of Arctic Bay Formation black shales and calculated an age of 1.092 ± 0.059 Ga from the weighted mean of a ^{206}Pb-^{207}Pb isochron and ^{238}U-^{206}Pb and ^{232}Th-^{208}Pb errorchrons; however, a total of nine outlying samples were excluded in these calculations and stratigraphic heights are not reported.

Unpublished whole-rock, carbonate Pb-Pb geochronology of Angmaat Formation samples were reported to produce an age of 1.199 ± 0.024 Ga, and combined data from Angmaat, Victor Bay, and Athole Point formations samples an age of 1.204 ± 0.022 Ga (Kah et al., 2001). While these dates were often cited as the age of *Bangiomorpha* pubescens (ca. 1.2 Ga), they are older than and therefore incompatible with the calculated age of the underlying Arctic Bay Formation from Turner and Kamber (2012). Futhermore, Pb-Pb carbonate ages can overestimate depositional ages due to incorporation of basement-derived Pb during diagenesis (i.e., dolomitization), meteoric alteration, and metamorphism (e.g., Babinski et al., 2007). These incongruent ages highlight obstacles associated with the application of whole-rock U-Th-Pb and Pb-Pb geochronology to typical Precambrian samples. The utility of the black shale Re-Os geochronometer for yielding precise and accurate ages of Precambrian sedimentary successions, on the other hand, is corroborated by numerous recent studies (e.g., Selby and Creaser, 2003; Kendall et al., 2009b; Cumming et al., 2013; van Acken et al., 2013; Rooney et al., 2014; Rooney et al., 2015).

Age of the Chitrakoot Taxa

The Vindhyan Supergroup in central India has long been the center of debate regarding fossil discoveries and their ages (see Ray, 2006 for overview). This up-to 4-km-thick sedimentary succession primarily outcrops in the Son Valley and Rajasthan. The lower Vindhyan Semri Group has largely been studied in the Son Valley region where multiple interbedded volcanic tuffs offer robust U-Pb zircon depositional age constraints of ca. 1.6 Ga (Rasmussen et al., 2002; Ray et al., 2002; Bengtson et al., 2009). These ages are broadly consistent with the occurrence of microfossils such as *Grypania* (Kumar, 1995) which occur globally in strata of similar ages (Adams et al., 2017).

The Chitrakoot Formation occurs as a stratigraphic outlier in the Jankikund-Chitrakoot region to the north of the Son Valley, and is interpreted to record deposition within an isolated sub-basin that was disconnected from the main Vindhyan basin (Bose et al., 2015); however, discontinuous lateral exposure renders robust correlations, even within the Chitrakoot region, tenuous. The Chitrakoot Formation has been dated using whole-rock geochronological techniques, with whole-rock Rb-Sr ages from lower glauconitic facies spanning ca. 1.5–1.4 Ga (Kumar et al., 2001), and a whole-rock Pb-Pb age of 1.65 ± 0.089 Ga from the uppermost phosphatic Tirohan Dolomite (Bengtson et al., 2009). While these dates broadly support correlation between the Tirohan Dolomite of the Chitrakoot Formation and the Rohtas Limestone of the Semri Group (Bengtson et al., 2017), robust stratigraphic correlations between the Chitrakoot outlier and principal Vindhyan sections in the Son Valley are complicated by inconclusive chemostratigraphic signatures (Ray et al., 2003) and significant lithological and thickness differences between these successions (Chakraborty, 2006). Alternately, if the Tirohan Dolomite is equivalent to the next younger unit that directly overlies the Rohtas Limestone, it

would belong to the upper Vindhyan Kaimur Group which could be as young as ca. 1.07 Ga (Gregory et al., 2006)—similar to the age of the Angmaat Formation. Thus, the ca. 1.6 Ga age of the phosphatized fossils from the Tirohan Dolomite is primarily based on the internally inconsistent dates for the Chitrakoot Formation, and so further corroboration of the anomalously old age of these fossils requires the application of reliable, high precision geochronology to the Chitrakoot sections themselves.

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