

Appendix from C. J. Rothfels et al., “Natural Hybridization between Genera That Diverged from Each Other Approximately 60 Million Years Ago”

(Am. Nat., vol. 185, no. 3, p. 433)

The appendix contains supplementary data, including voucher table, calibration dates, and best-fitting parametric distributions for posterior node age estimates from the first dating analysis, applied as priors to their respective nodes in the second dating analysis.



Figure A1: Left, *Gymnocarpium dryopteris* (Rothfels 4048.3 [DUKE]. Maine, USA); right, *Cystopteris fragilis* (Smith 1 [DUKE]. Colorado, USA); middle, *Cystocarpium roskamianum* (Roskam s.n. [DUKE]. Cultivation).

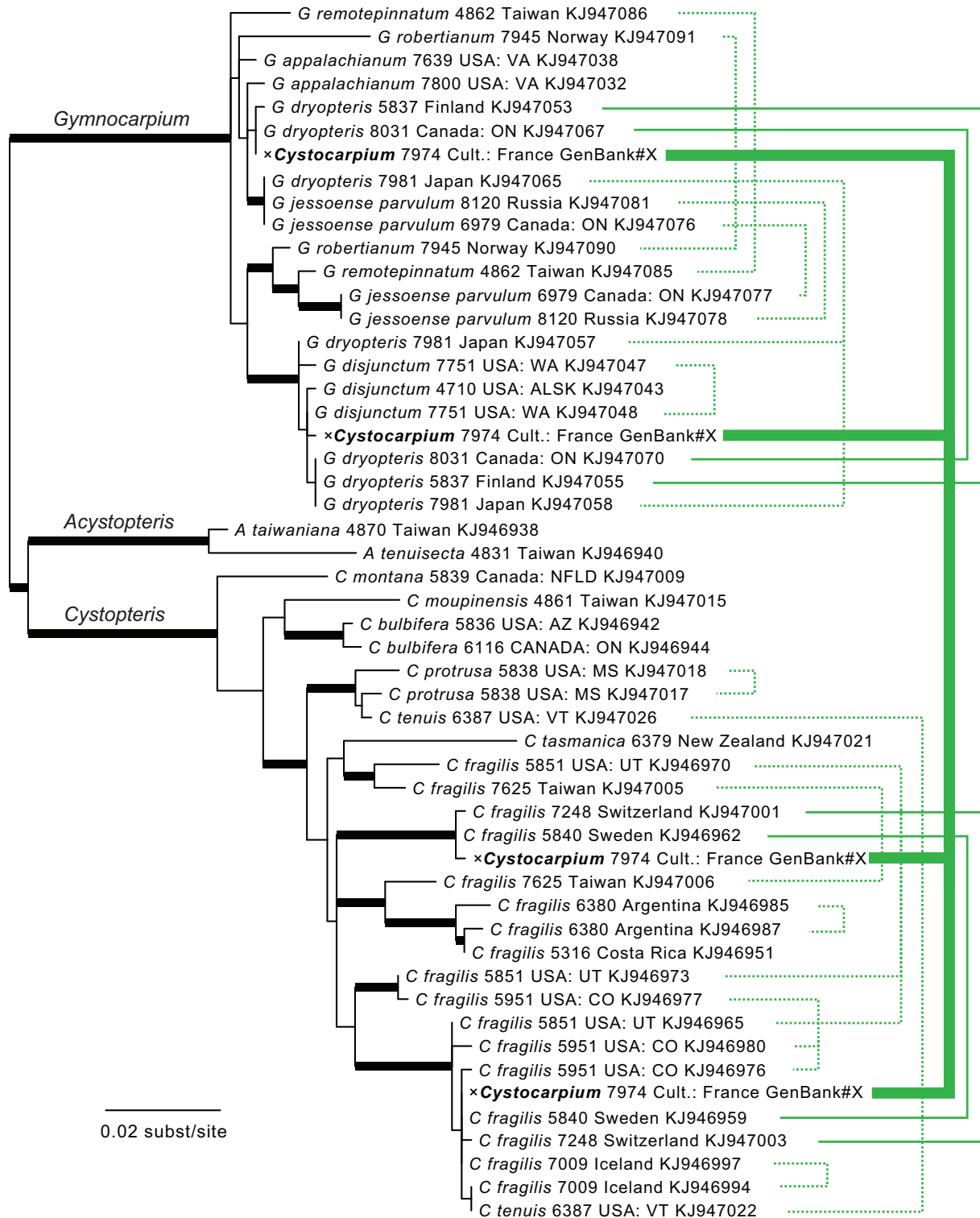


Figure A2: Maximum likelihood (ML) phylogram of *gapCp* alleles (reduced from the original total of 271 sequences; see “Methods” and Rothfels et al. 2014). Sequences from *xCystocarpium* are in boldface. Thickened branches have $\geq 70\%$ ML bootstrap support. Vertical lines on the right-hand side connect sequences from individual accessions: those of *xCystocarpium* are joined by a thick line, thin lines join those of allopolyloid accessions most consistent with their being the progenitors of *xCystocarpium*, and dashed lines connect sequences from individuals not involved in the formation of *xCystocarpium*. Numbers following the specific epithets are accession numbers from the Fern Lab Database (<http://fernlab.biology.duke.edu>).



Figure A3: A single most parsimonious phylogram of all unique *gapCp* sequences attained. Numbers following the specific epithets (*Gymnocarpium*, *Acystopteris*, and *Cystopteris* spp.) are accession numbers from the Fern Lab Database (<http://fernlab.biology.duke.edu>), followed by country or region of origin and then a list of the clones yielding each sequence (for example, “c3_6_7” indicates that clones 3, 6, and 7 had that sequence; a total of 271 clones are represented here). The final numbers, where present, indicate the length of that sequence, in base pairs.

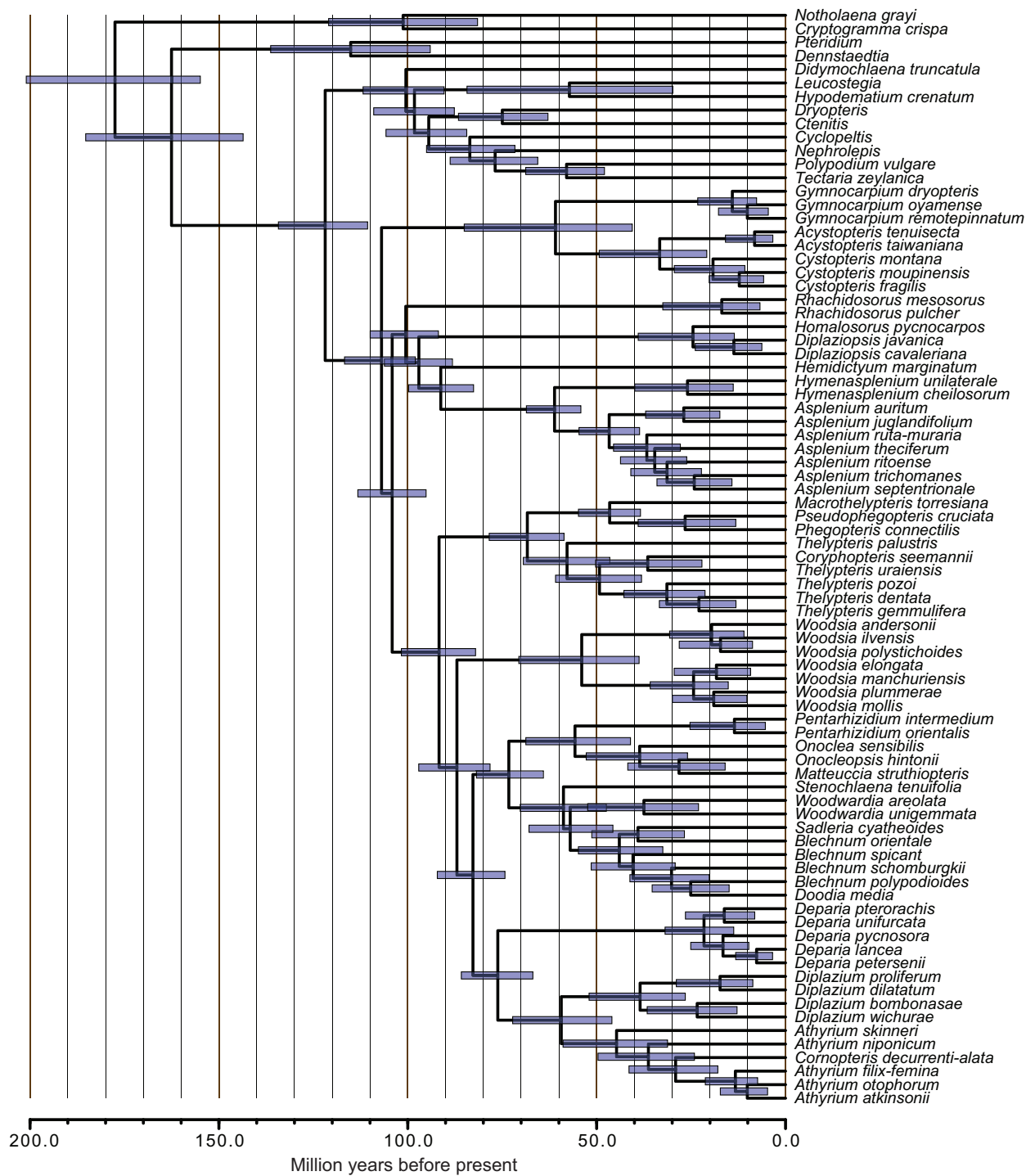


Figure A4: Chronogram of the eupolypod II sample from Rothfels et al. (2012a), with tips labeled, corresponding to figure 1B. Node bars indicate 95% credibility intervals of divergence time.

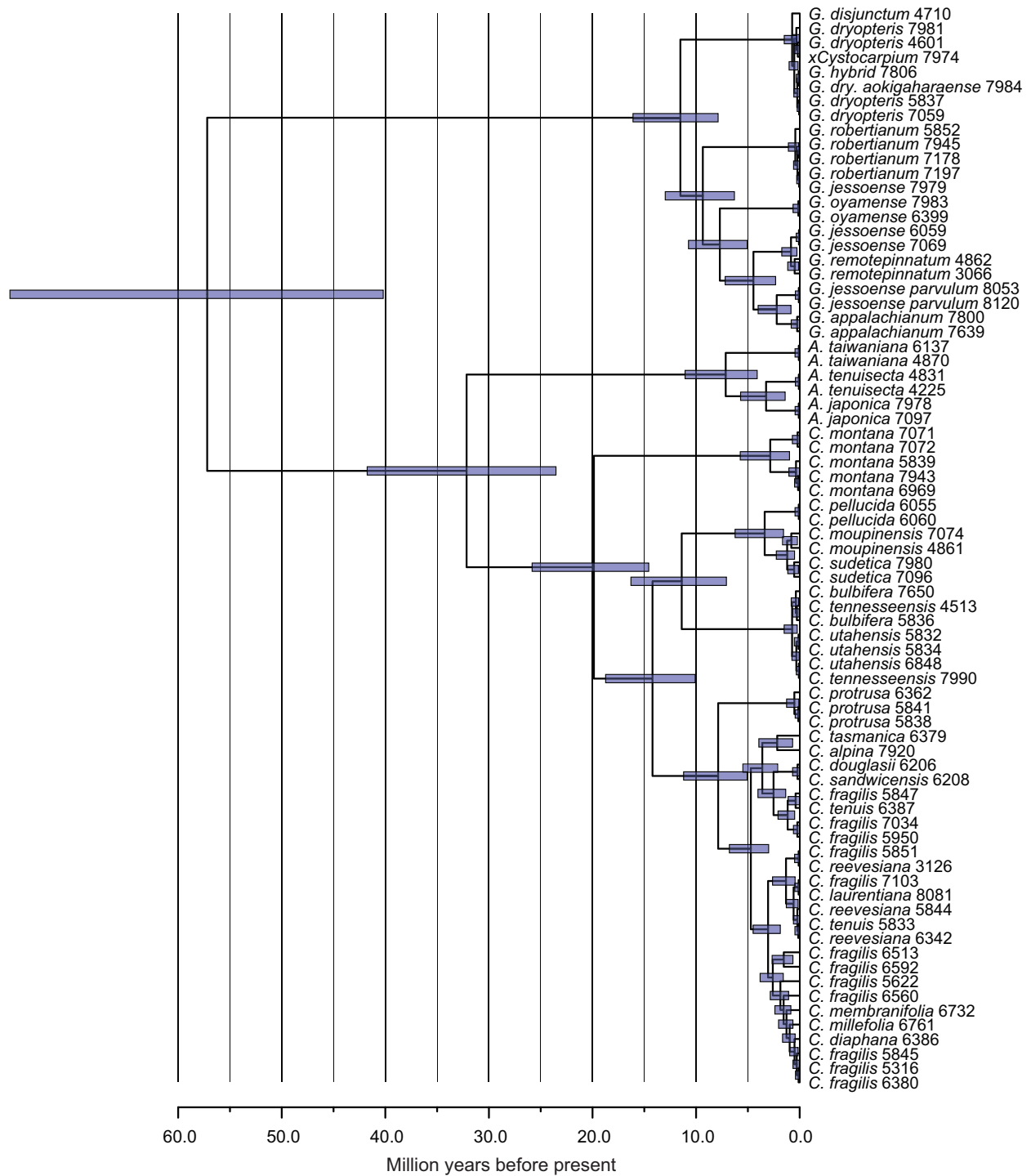


Figure A5: Chronogram of the Cystopteridaceae sample (*Gymnocarpium*, *Acystopteris*, and *Cystopteris* spp.) modified from Rothfels et al. (2013b), with tips labeled, corresponding to figure 1C. Node bars indicate 95% credibility intervals of divergence time. Numbers following the specific epithets are accession numbers from the Fern Lab Database (<http://fernlab.biology.duke.edu>).

Table A1. Voucher table for the *gapCp* data set, with GenBank numbers

DB#	Accession	Total clones	Total unique sequences	Alleles
7974 ^a	<i>×Cystocarpium roskamianum</i> Fraser-Jenk. Leiden 946515. Cult: originally, France (DUKE)	18	11 (KP099194 × 5; KP099195 × 3; KP099196 × 2; KP099186; KP099187; KP099188; KP099189; KP099190; KP099191; KP099192; KP099193)	4 (KP099186; KP099194; KP099195; KP099196)

Note: DB#: Fern Lab Database accession number (<http://fernlab.biology.duke.edu>). Numerals following GenBank numbers in the “Total unique sequences” column indicate the number of times that particular sequence was obtained

^aNewly published plastid sequences for *×Cystocarpium* have GenBank numbers KP099184 (*matK*) and KP099185 (*trnG-R*).

Table A2. Selected divergence-time calibrations

Divergence	Date (mya)
Dennstaedtiaceae + Pteridaceae + eupolypods	165.6
Aspleniaceae	57.7
Athyriaceae	78.4
Blechnaceae + Onocleaceae	77.8
<i>Ctenitis</i> + <i>Dryopteris</i>	77.2
<i>Dennstaedtia</i> + <i>Pteridium</i>	119.3
Eupolypods	116.7
<i>Hemidictyum</i> + Aspleniaceae	92.6
<i>Notholaena</i> + <i>Cryptogramma</i>	110.8
<i>Polypodium</i> + <i>Tectaria</i>	66.1
<i>Phegopteris</i> + <i>Pseudophegopteris</i> + <i>Macrothelypteris</i>	45.9
Thelypteridaceae	68.5
Eupolypods I	98.9
Eupolypods II	103.1

Note: Calibrations are from Schuettpelz and Pryer (2009) and are expressed as millions of years ago (mya). The calibrated nodes are indicated by black circles in figure 1B.

Table A3. Best-fitting lognormal and gamma distributions of the posterior age distributions of seven nodes from the relaxed-clock analysis of the Rothfels et al. (2012a) eupolypod II data set

Divergence	Best-fitting lognormal distribution			Best-fitting gamma distribution		
	Mean	SD	AIC	Shape	Rate	AIC
<i>Acystopteris</i>	2.10638	.37808	91,921	7.16640	.81188	92,321
<i>Acystopteris</i> + <i>Cystopteris</i>	3.50731	.21383	121,843	21.93603	.64265	122,060
Cystopteridaceae	4.10518	.19248	139,584	27.38896	.44333	139,544
<i>Cystopteris</i>	2.95370	.24544	106,874	16.76503	.84829	107,057
<i>Cystopteris fragilis</i> + <i>Cystopteris moupinensis</i>	2.50170	.30000	97,826	11.36187	.89042	97,955
<i>Gymnocarpium oyamense</i> + <i>Gymnocarpium remotepinnatum</i>	2.31657	.32355	93,881	9.73248	.91084	94,163
<i>Gymnocarpium</i>	2.64916	.27671	100,226	13.17442	.89647	100,527

Note: The best-fitting distributions for each node (indicated by bold AIC scores) were used as time-to-most-recent-ancestor priors for the relaxed-clock analysis of the Cystopteridaceae (Rothfels et al. 2013b). The calibrated nodes are indicated by black circles in figure 1C. Time units are in million years. AIC = Akaike information criterion.