Autopolyploid origin of the octoploid Turkish parsley fern (*Cryptogramma bithynica* Jessen, Lehmann, and Bujnoch)

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INTRODUCTION

The parsley ferns (*Cryptogramma*) are small ferns in the family Pteridaceae. Nine species are recognized worldwide (Fig. 1). These ferns are characterized by dimorphic leaves and false indusia. They grow almost exclusively on rocky surfaces. The diploid chromosome counts (2n) known within the genus were 60 and 120 until recently. The newly discovered Turkish parsley fern *Cryptogramma bithynica* is an octoploid with a 2n = 240 chromosome count (Jessen, Lehmann and Bujnoch 2012). This is an exciting new addition to our current knowledge of the genus *Cryptogramma*. We analyzed plastid and nuclear markers to determine this species progenitors and if it resulted from autopolyploidy or allopolyploidy.

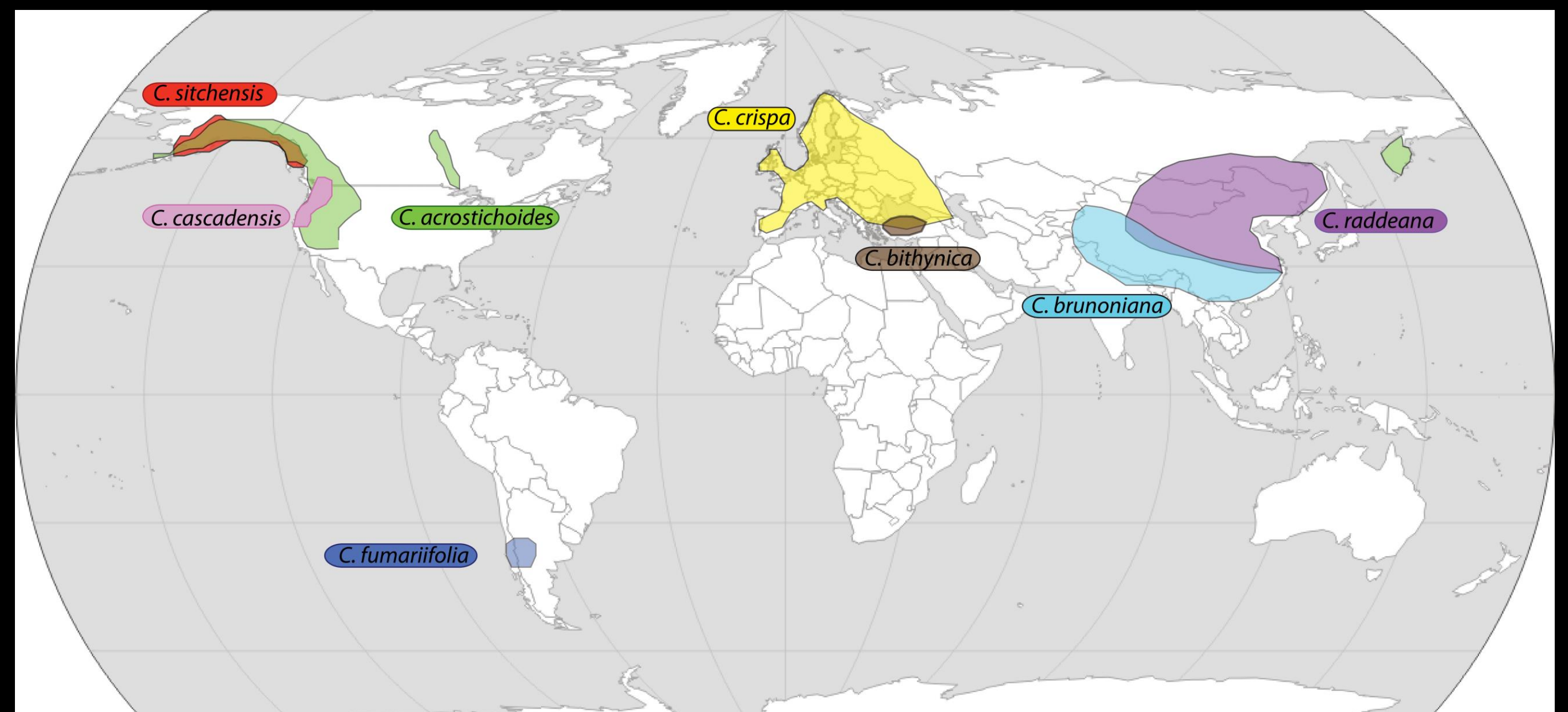


Fig. 1. Distribution of *Cryptogramma* sect. *Cryptogramma* species.

MATERIALS AND METHODS

Samples: 39 accessions representing 8 species

Matrix:

Six maternally inherited plastid loci = 6827bp

rbcL, rbcL-accD, atpB-rbcL, rps4, trnP-petG, trnGR

One biparentally inherited nuclear locus = 500 bp

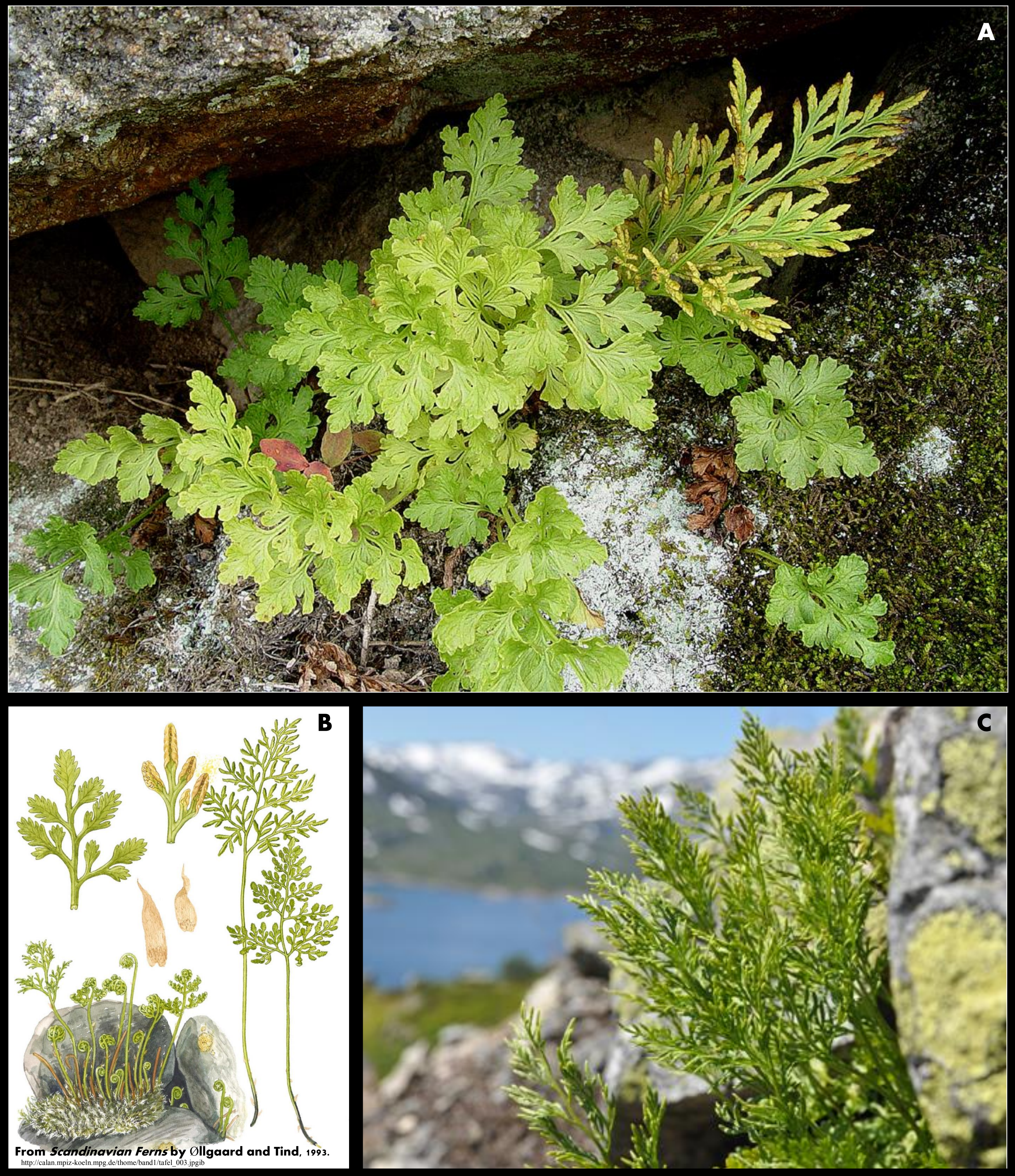
gapCp

Phylogenetic Analysis:

Maximum Parsimony with 1000 replicates; 500 BS reps with 10 searches each (PAUP*)

Maximum likelihood with 100 reps, GTR (GaRLi); 1000 BS reps, GTRGAMMA (RaxML v.7.2.1)

Bayesian inference ran for 10m generations, 25% burn-in (MrBayes v.3.1.2)



From *Scandinavian Ferns* by Øllgaard and Tind, 1993.
http://calan.mpiz-koeln.mpg.de/thome/band1/tafel_003.jpgib

Fig. 2A. *Cryptogramma bithynica* in northwestern Turkey. (Photo by Stefan Jessen.) Fig. 2B. Diagram of *C. crispa* morphology. Fig. 2C. *Cryptogramma crispa* in S Norway. (Photo by Stina Weststrand.)

C. bithynica is smaller in overall plant and habitat size than *C. crispa,* and has pointed rather than rounded tips on leaf segments. *Crytogramma bithynica* also has larger spores.

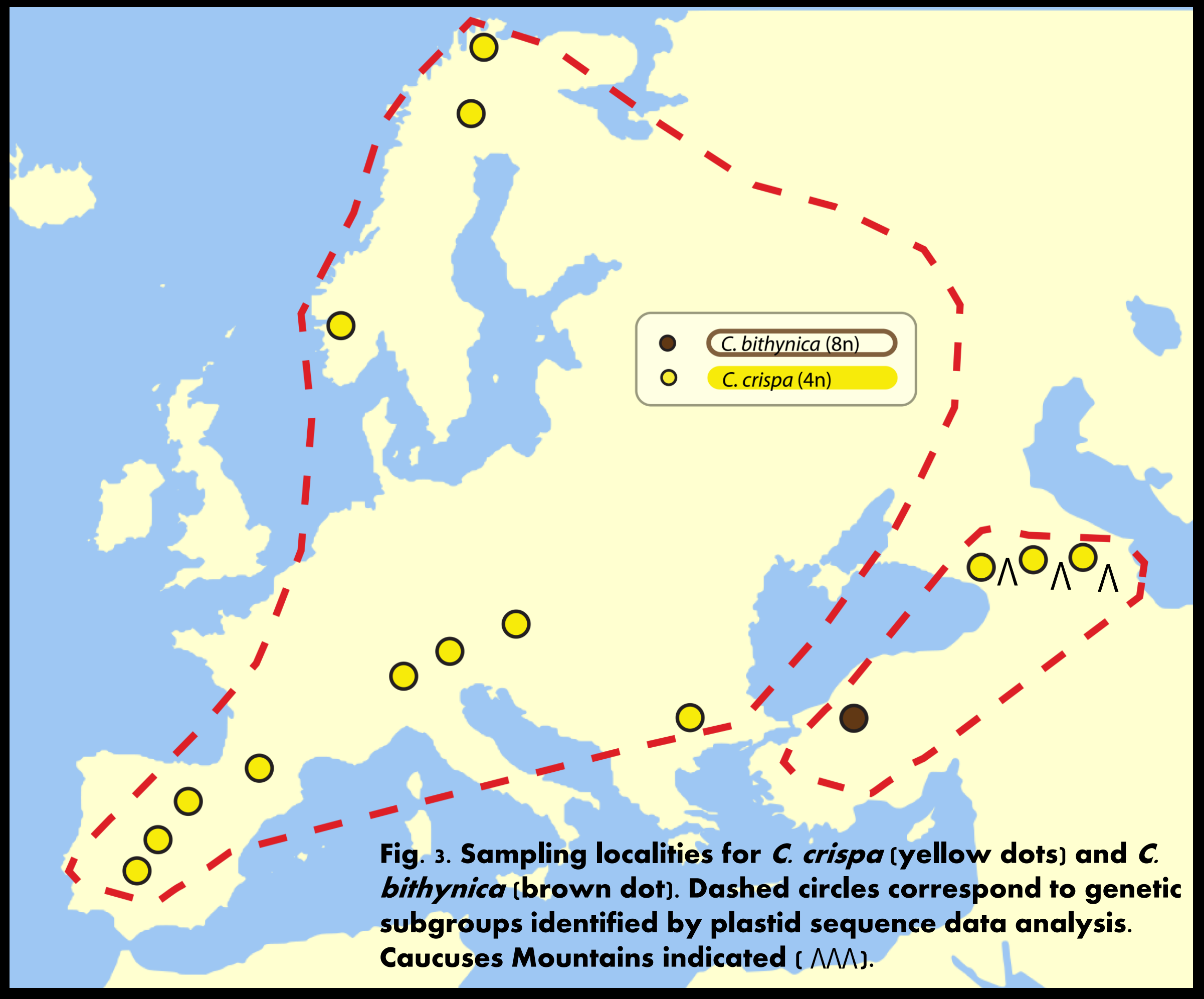


Fig. 3. Sampling localities for *C. crispa* (yellow dots) and *C. bithynica* (brown dot). Dashed circles correspond to genetic subgroups identified by plastid sequence data analysis. Caucuses Mountains indicated (ΛΛΛ).

RESULTS/ DISCUSSION

Our analysis of maternally inherited plastid data confirmed that *Cryptogramma crispa* is the maternal parent of *C. bithynica* (Fig. 4). The biparentally inherited nuclear *gapCp* recovered all *C. bithynica* alleles in a single clade (Fig. 5), meaning that it was formed as an autopolyploid. Thus, *C. bithynica* is an autopolyploid with *C. crispa* being the sole parent. Our plastid analysis also revealed a genetic division within *C. crispa*. The Caucasus Mountains populations of *C. crispa* and *C. bithynica* form a separate clade from other *C. crispa* samples (Fig. 3). This indicates that the Caucasus Mountains were a barrier during the Last Glacial Maximum, isolating these populations from the rest of *C. crispa* and creating genetic divergence.

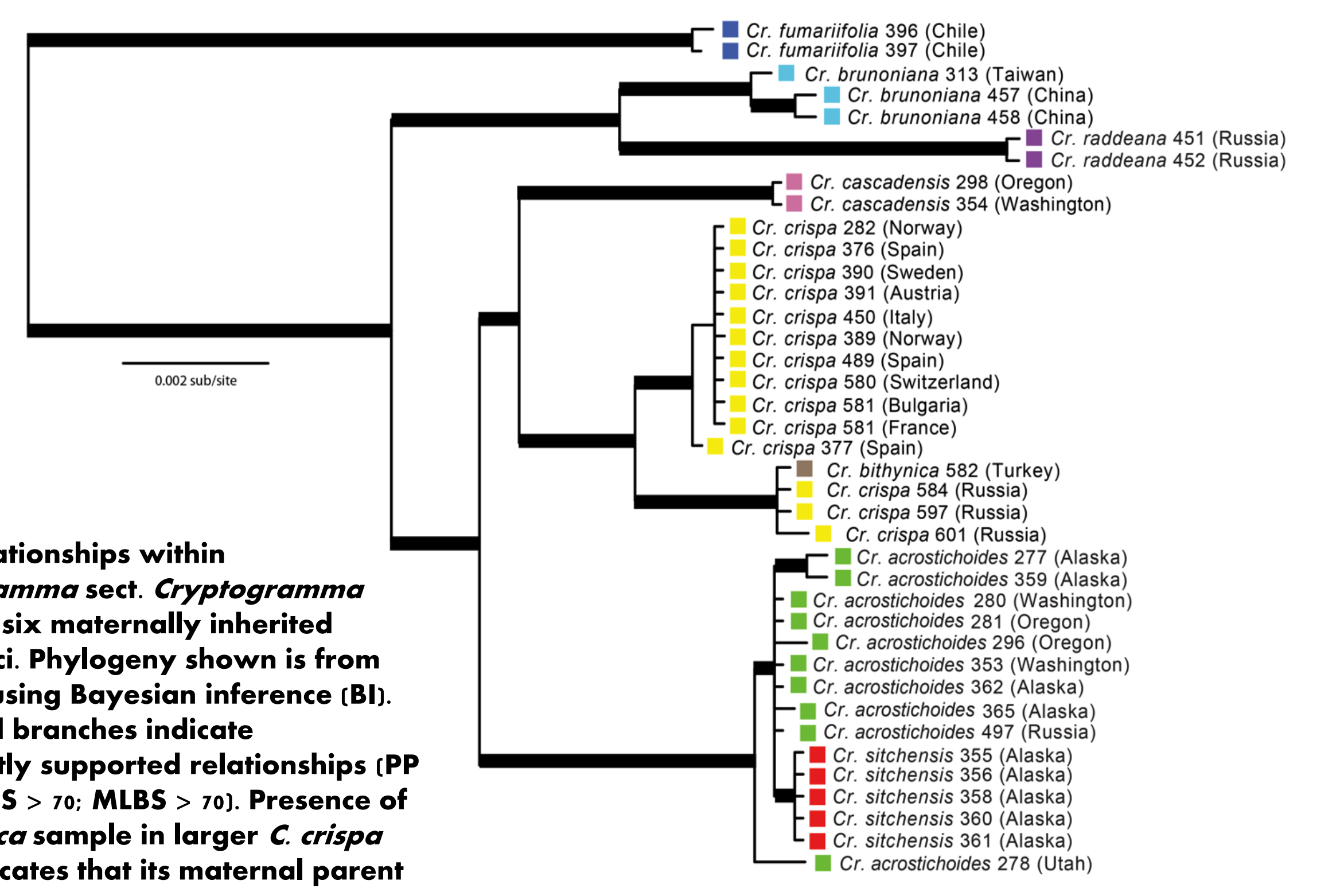


Fig. 4. Relationships within *Cryptogramma* sect. *Cryptogramma* based on six maternally inherited plastid loci. Phylogeny shown is from analysis using Bayesian inference (BI). Thickened branches indicate significantly supported relationships (PP > .95; MPBS > 70; MLBS > 70). Presence of *C. bithynica* sample in larger *C. crispa* clade indicates that its maternal parent is *C. crispa*.

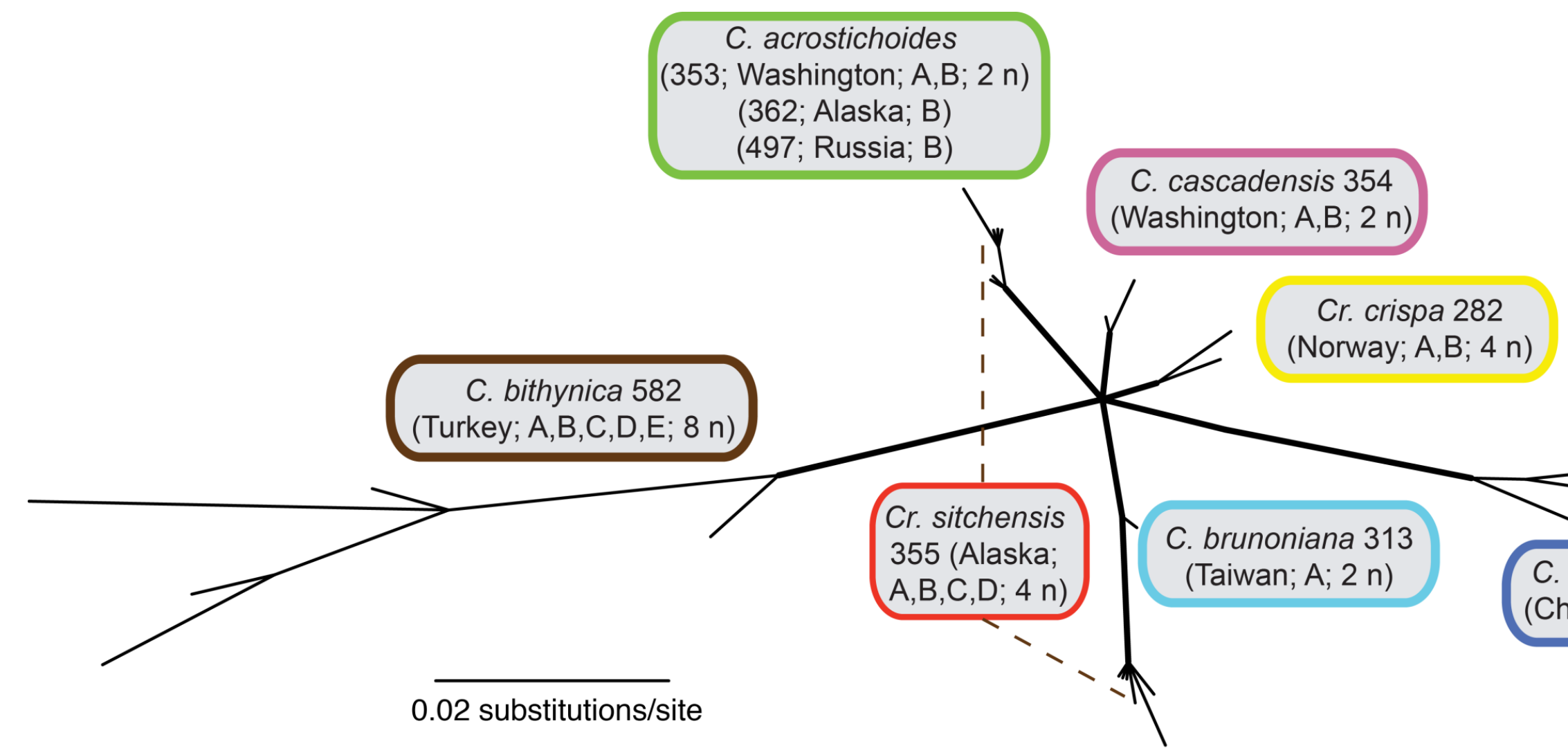


Fig. 5. Unrooted phylogeny based on BI analysis of *gapCp* sequence data. The single clade of *C. bithynica* alleles implies autopolyploid origin.

Thickened branches are significantly supported. Letters correspond to individual alleles recovered from each sample.

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LITERATURE CITED: Jessen, S., Lehmann, L., Bujnoch, W., 2012. *Cryptogramma bithynica* sp. nov. (Pteridaceae, Pteridophyta)—a new fern species from northwestern Anatolia/Turkey. Fern Gaz. 19:47–54.
Metzgar, J.S., Alverson, E.R., Chen, S., Vaganov, A.V., Ickert-Bond, S.M. 2013. Diversification and reticulation in the circumboreal fern genus *Cryptogramma*. Mol. Phyl. Evol. 67:589-599.