Molecular Phylogenetics and Evolution 67 (2013) 589–599

Contents lists available at SciVerse ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Diversification and reticulation in the circumboreal fern genus *Cryptogramma*

Jordan S. Metzgar [a,*], Edward R. Alverson [b], Sinian Chen [c], Alexey V. Vaganov [d], Stefanie M. Ickert-Bond [a]

[a] Herbarium (ALA), University of Alaska Museum of the North and Dept. of Biology and Wildlife, University of Alaska, Fairbanks, Fairbanks, AK 99775, USA
[b] Herbarium (OSC), Dept. of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331, USA
[c] West Valley High School, Fairbanks, AK 99709, USA
[d] Altai State University, Lenina St., 61, 656049 Barnaul, Russia

ARTICLE INFO

Article history:
Received 14 August 2012
Revised 3 February 2013
Accepted 15 February 2013
Available online 26 February 2013

Keywords:
Cryptogramma
Pteridaceae
gapCp
Introgression
Polyploidy
Coniogramme

ABSTRACT

We investigated the evolutionary complexity that resulted from cryptic diversification and polyploidy in parsley ferns (*Cryptogramma*). A total of 14 species were included in our data set, with six outgroup species and eight *Cryptogramma* species. DNA sequence data from six plastid loci (*rbcL*, *rbcL–accD*, *rbcL–atpB*, *rps4–trnS*, *trnG–trnR* and *trnP–petG*) were analyzed using maximum likelihood and Bayesian methods to provide the first rigorous assessment of diversification in the genus, including testing the monophyly of the genus and sections. *Cryptogramma* and *Coniogramme* are recovered as reciprocally monophyletic sister genera. We established the monophyly of both sections within *Cryptogramma*. Furthermore, our sequence data reveal that described species reflect mostly allopatric reciprocally monophyletic lineages that are independent evolutionary trajectories. Using sequence data from the nuclear locus (*gapCp*) we find that the European *C. crispa* is an autotetraploid with a partially diploidized genome, while the North American tetraploid *Cryptogramma sitchensis* is an allopolyploid derived from *C. acrostichoides* and *C. raddeana*. Subsequent backcrossing between *C. sitchensis* and *C. acrostichoides* has allowed the introgression of *C. raddeana* alleles into northern populations of *C. acrostichoides*.

1. Introduction

The leptosporangiate fern genus *Cryptogramma* R.Br. is comprised of nine species in two sections. Referred to as parsley ferns for the resemblance of their foliage to that of parsley, they combine to have a circumboreal distribution with a bipolar disjunction as one taxon is present in southern South America (Alverson, 1989a; Raven, 1963). All members of the genus possess dimorphic leaves, with erect fertile leaves and shorter more finely divided sterile leaves. Fertile leaves possess false indusia and the chromosome base number for the genus is 30 (Tryon and Tryon, 1990).

Cryptogramma stelleri (S.G. Gmel.) Prantl is the only representative of section *Homopteris* (Rupr.) C. Chr. Found in the northern regions of North America, Asia and extreme northeastern Europe, it has a creeping rhizome, leaves with a membraneous texture and is often a calciphile (Alverson, 1993; Hultén and Fries, 1986). Authors have been in wide agreement on the taxonomy of this section (Alverson, 1993; Hultén, 1968; Lellinger, 1985; Tryon and Tryon, 1990; Vaganov et al., 2010), while previous treatments of sect. *Cryptogramma* Prantl have ranged from one species (Hultén, 1968; Tryon and Tryon, 1990) to ten (Alverson, 1989a; Lellinger, 1985; Vaganov et al., 2010). Found across temperate and boreal regions of North America, Asia and Europe, one taxon is also found in southern South America (Tryon and Tryon, 1990). Species in sect. *Cryptogramma* have erect rhizomes, leaves with a more coriaceous texture and generally prefer acidic, rocky habitats (Alverson, 1989a).

Previous research has shown *Cryptogramma* to be closely related to *Coniogramme* Fée, a genus of approximately 30 species in the Old World tropics, and the monotypic Mexican genus *Llavea* Lag. (Prado et al., 2007; Schuettpelz et al., 2007; Zhang et al., 2005). These cryptogrammoid ferns possess an important phylogenetic position as the basal clade in the Pteridaceae (Gastony and Rollo, 1995; Prado et al., 2007; Schuettpelz and Pryer, 2007; Schuettpelz et al., 2007), comprising the subfamily Cryptogrammoideae (*sensu* Smith et al., 2006). This family is closely related to the large eupolypod clade (Schuettpelz and Pryer, 2007). With many active research inquiries into other subfamilies of the Pteridaceae, particularly the cheilanthoid ferns (e.g., Beck et al., 2011; Grusz et al., 2009; Kirkpatrick, 2007; Rothfels et al., 2008; Schuettpelz et al., 2008; Sigel et al., 2011) and the emerging model system *Ceratopteris* (e.g., McGrath and Hickok, 1999; Nakazato et al., 2007; Scott et al., 2007), there is an urgent need for a better understanding of the relationships and characteristics of the basal cryptogrammoid clade.

* Corresponding author.
E-mail addresses: jsmetzgar@alaska.edu (J.S. Metzgar), ealverson@tnc.org (E.R. Alverson), schen88@jhu.edu (S. Chen), vaganov_vav@mail.ru (A.V. Vaganov), smickertbond@alaska.edu (S.M. Ickert-Bond).

1055-7903/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ympev.2013.02.020

No study has previously analyzed the evolution or taxonomy of the genus as a whole, with studies that are regional in scope predominating. Recent studies of the Chinese taxa reduced *Cryptogramma emiensis* Ching and K.H. Shing and *Cr. shensiensis* Ching to synonymy with *Cr. brunoniana* Hook. & Grev. (Zhang and Zhang, 2003). Disagreement regarding the specific status of the Asian *Cr. raddeana* Fomin has emerged, with some authors treating it as a variety (Zhang and Zhang, 2003) or subspecies (Fraser-Jenkins, 2008) of *Cr. brunoniana,* while others maintain *Cr. raddeana* at the species level (Zhang et al., in press). Although it was unknown if it was of allopolyploid or autoployploid origin, the European tetraploid *Cr. crispa* (L.) R.Br. has been studied more extensively using breeding system evidence that suggests that its genome has been effectively diploidized (Pajarón et al., 1999). The North American members of sect. *Cryptogramma* were studied using a biosystematic approach (Alverson, 1989a, 1989b), which yielded the description of a new diploid species (*Cr. cascadensis* E.R. Alverson; Alverson, 1989b). Allozyme and morphological evidence established that *Cr. sitchensis* (Rupr.) Moore is an allotetraploid (Alverson, 1988, 1989a) with *Cr. acrostichoides* R.Br. serving as one parent and the other hypothesized to be the Asian *Cr. raddeana*. In addition, these studies discovered that triploid hybrids between *Cr. acrostichoides* and *Cr. sitchensis* are common and caused much of the taxonomic confusion regarding these species (Alverson, 1988). However, a comprehensive molecular study of the group is wholly lacking, with no previous molecular evidence supporting the monophyly of the genus, sections or species.

Cryptogramma represents an excellent system to further our understanding of fern evolution, polyploidy, introgression and chromosome number evolution. Our study will be the first comprehensive examination of the genus, using multiple molecular data sets to test generic and subgeneric circumscriptions and evaluating correspondence between described species and molecular differentiation. Subtle morphological variation between putative species in sect. *Cryptogramma* has led some authors to recognize only one species (Hultén, 1968; Tryon and Tryon, 1990) and others to recognize up to 10 (Alverson, 1989a; Lellinger, 1985; Vaganov et al., 2010). We will test these species circumscriptions using molecular data to determine if these data support the existence of one nearly worldwide species or a series of partially geographically isolated species that are in reciprocally monophyletic lineages with slight but consistent distinguishing morphological traits. Chromosome number evolution will also be reconstructed using an explicit likelihood framework to determine the number and location of polyploidization events. The combination of an explicit chromosome evolution hypothesis in combination with the molecular data sets will permit a detailed examination of both the tetraploid *Cr. crispa* and the North American allotetraploid *Cr. sitchensis*. We will deduce origins of both tetraploid species and examine introgression of *gapCp* alleles into *Cr. acrostichoides* following the formation of *Cr. sitchensis*.

2. Materials and methods

2.1. Taxon sampling

A total of 14 species were included in our data set, with *Llavea cordifolia*, five *Coniogramme* species and eight *Cryptogramma* species. Many species were represented by samples from multiple populations in an attempt to encompass potential geographic variation, with a total of 40 samples included in the data set (Table 1). An extensive effort was made to include material for as many species and populations as possible; however, no sequence data could be obtained for the recently described *Cr. gorovoii* A. Vaganov & Schmakov from the Russian Far East and Japan (*sensu Cr. crispa* var. *japonica*; Vaganov and Shmakov, 2007) or for the newly described *Cr. bithynica* S. Jess, L. Lehm. & Bujnoch (Jessen et al., 2012) from northwestern Turkey. *Llavea* accessions were used as the outgroup based on previously established relationships (Prado et al., 2007; Schuettpelz et al., 2007; Zhang et al., 2005).

2.2. DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from 15 to 20 mg of silica-dried leaf tissue per sample using the Qiagen DNeasy Plant Mini Kit (Qiagen, Valencia, California, USA). Six plastid loci (*rbcL*, *rbcL–accD*, *rbcL–atpB*, *rps4–trnS*, *trnG–trnR* and *trnP–petG*) were amplified and sequenced according to existing protocols (Table 1; Grusz et al., 2009; Korall et al., 2007).

For seven species of *Cryptogramma*, one exemplar accession was amplified and sequenced for the nuclear locus (*gapCp*) and three specimens of *Cr. acrostichoides* were amplified and sequenced to characterize potential introgression. Amplification protocols for this marker followed Schuettpelz et al. (2008) and PCR products were cloned using the Invitrogen TOPO TA Cloning kit (Invitrogen, Carlsbad, California, USA). Clones were amplified using Invitrogen's M13 primer pair and the same *gapCp* thermalcycler conditions as mentioned earlier. Alleles corresponding to the "short" copy and "long" copy of the *gapCp* locus were recovered (Schuettpelz et al., 2008). We did not use the *gapCp* "long" alleles due to a lack of taxonomic coverage (copies were recovered for only five taxa) and due to previously reported homology issues with distinguishing these alleles from the *gapC* locus (Grusz et al., 2009; Schuettpelz et al., 2008).

All plastid and nuclear sequencing chromatograms were corrected by eye using Sequencher version 4.10.1 (Gene Codes Corporation, Ann Arbor, Michigan, USA). For *gapCp* sequences, separate projects were used containing all of the sequences for a given individual. Mutations and indels were then charted through the length of the consensus sequence, allowing for the identification of separate alleles. PCR artifacts such as chimeric sequences were detected and removed during this step (Mason-Gamer et al., 2008). This study generated a combined total of 240 plastid and nuclear sequences and all were deposited in GenBank (Table 2).

2.3. Sequence alignments

For each locus, sequences were aligned using ClustalX 2.1 (Larkin et al., 2007) and the resulting alignments were then refined by eye. The aligned data matrix for the protein-coding *rbcL* locus lacked any insertions or deletions (indels). All of the alignments for the remaining loci included indels. Ambiguously aligned regions and indels were excluded from the final analyses, with excluded bases totaling 122 bp in *rbcL–accD,* 68 bp in *rbcL–atpB*, 324 bp in *rps4–trnS*, 219 bp in *trnG–trnR*, 249 bp in *trnP–petG* and 85 bp in the *gapCp* "short" copy. Much of the non-coding portion of the *rps4–trnS* matrix was excluded, as the alignment in this region was extremely ambiguous between the different sections of *Cryptogramma* and between *Cryptogramma* and the other genera. We did not use any gap-coding method.

2.4. Plastid data set combinability

Bayesian Markov chain Monte Carlo (B/MCMC) analyses were implemented in MrBayes version 3.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003; Ronquist et al., 2012) for each single locus data set following the protocol used for the combined data matrix analysis (see Section 2.5). Majority-rule consensus topologies were calculated for each locus. These topologies were manually inspected for topological conflicts (Mason-Gamer and Kellogg, 1996) using a minimum threshold of 0.95 posterior

Table 1
Voucher information with sampling localities, specimen voucher and GenBank accession numbers. Herbarium acronyms in "Collector/No." field follow Index Herbariorum.

DNA Ext. No.	Taxon	Locality	Collector/No.	GenBank Accession No.						
				rbcL	rbcL–accD	rbcL–atpB	rps4–trnS	trnG–trnR	trnP–petG	*gapCp* "short"
279	*Llavea cordifolia*	Mexico: Hidalgo: Municipio Nicolas Flores. On road to Nicolas Flores from Cardonal	Rothfels 3025 (ALA, DUKE, MEXU)	KC700108	KC700148	KC700186	KC700225	KC700263	KC700299	–
312	*Llavea cordifolia*	Mexico: Hidalgo: Municipio Zimapán, Los Mármoles gorge	Ledesma 2113 (HGOM)	KC700109	KC700150	KC700187	KC700226	KC700264	KC700300	–
388	*Llavea cordifolia*	Mexico: Guerrero: On outskirts of Fila De Caballos	Dyer 61 (MEXU, BM)	KC700110	KC700149	KC700188	KC700227	KC700265	KC700301	–
292	*Coniogramme japonica*	USA: North Carolina: Plant in cultivation at Juniper Level Botanic Garden	Schuettpelz 386 (DUKE)	KC700111	KC700151	KC700189	KC700228	KC700266	KC700302	–
293	*Coniogramme fraxinea*	Malaysia: Pahang: Cameron Highlands: Robinson Falls	Schuettpelz 836 (DUKE, KEP)	KC700112	–	KC700190	KC700229	KC700267	KC700303	–
294	*Coniogramme intermedia*	Taiwan: Nantou County: Mei-Feng Experimental Farm Reserve	Schuettpelz 1052A (DUKE, TAIF, BM)	KC700113	–	KC700191	KC700230	KC700268	KC700304	–
295	*Coniogramme* sp.	China: Guizhou: Shuicheng, Yushe	Zhang 246 (MO)	KC700114	KC700152	KC700192	KC700231	KC700269	KC700305	–
453	*Coniogramme wilsonii*	Vietnam: Bac Kan Province, Cho Don District, Ban Thi Community, Phia Khao Village	Hieu CPC1240 (ALA)	KC700115	KC700153	–	KC700232	KC700270	KC700306	–
277	*Cryptogramma acrostichoides*	USA: Alaska: Kodiak, near the transient boat harbor	Studebaker 09-473 (ALA)	KC700093	KC700133	KC700171	KC700210	KC700248	KC700284	–
278	*Cryptogramma acrostichoides*	USA: Utah: Salt Lake County, Little Cottonwood Canyon	Rothfels 2979 (ALA, DUKE, NHIC)	KC700094	KC700134	KC700172	KC700211	KC700249	KC700285	–
280	*Cryptogramma acrostichoides*	USA: Washington: Mason County: N of Lake Cushman along the Mt. Ellinor trail in the Olympic Mtns.	Windham 3624 (DUKE, UT)	KC700095	KC700135	KC700173	KC700212	KC700250	KC700286	–
281	*Cryptogramma acrostichoides*	USA: Oregon: Linn Co., Horse Rock Ridge, SW of Crawfordsville	Pryer 06-04 (DUKE)	KC700096	KC700136	KC700174	KC700213	KC700251	KC700287	–
296	*Cryptogramma acrostichoides*	USA: Oregon: Lane County: Trail to Proxy Falls	Alverson s.n. (ALA)	KC700097	KC700137	KC700175	KC700214	KC700252	KC700288	–
353	*Cryptogramma acrostichoides*	USA: Washington: King County: Source Lake Lookout Trail, above Source Lake, Cascade Range	Zika 25403 (ALA)	KC700098	KC700138	KC700176	KC700215	KC700253	KC700289	KC700066 , KC700071
359	*Cryptogramma acrostichoides*	USA: Alaska: Seward: Kenai Fjords National Park: Harding Icefield Trail	Metzgar 247 (ALA)	KC700099	KC700139	KC700177	KC700216	KC700254	KC700290	–
362	*Cryptogramma acrostichoides*	USA: Alaska: Southeast Alaska: 10 miles northwest of Juneau, Mendenhall Lake, behind Mendenhall Glacier Visitor Center	Anderson 745 (ALA)	KC700100	KC700140	KC700178	KC700217	KC700255	KC700291	KC700070 , KC700058
365	*Cryptogramma acrostichoides*	USA: Alaska: Sitkalidak Island: Sitkalida Lagoon, cliffs along east side of lagoon	Studebaker 10-61 (ALA)	KC700101	KC700141	KC700179	KC700218	KC700256	KC700292	–
497	*Cryptogramma acrostichoides*	Russia: Kamchatka, north of Kamchatka peninsula, near Karaginskij	Chernyagina s.n. (ALA)	KC700102	KC700142	KC700180	KC700219	KC700257	KC700293	KC700059 , KC700067
313	*Cryptogramma brunoniana*	Taiwan: NanTou County, Mt. ShihMen	Kuo 455 (TAIF)	KC700081	KC700121	KC700159	KC700198	KC700238	KC700273	KC700061
457	*Cryptogramma brunoniana*	China: Xizang (Tibet) Province: Baxoi Xian: Anjiu La (pass), N of Rawu (Raog)	Boufford 29733 (GH)	KC700082	KC700122	KC700160	KC700199	KC700239	KC700274	–
458	*Cryptogramma brunoniana*	China: Gansu Province: Wen Xian: Motianling Shan, Baishui Jiang Nature Reserve	Boufford 37747 (GH)	KC700083	KC700123	KC700161	KC700200	KC700240	KC700275	–
298	*Cryptogramma cascadensis*	USA: Oregon: Deschutes/Linn County boundary: McKenzie Pass	Alverson s.n. (ALA)	KC700086	KC700126	KC700164	KC700203	KC700241	KC700277	–
354	*Cryptogramma cascadensis*	USA: Washington: King County: Source Lake Lookout Trail, above Source Lake	Zika 25404 (ALA)	KC700087	KC700127	KC700165	KC700204	KC700242	KC700278	KC700064, KC700065
282	*Cryptogramma crispa*	Norway: Hordaland: Bergen	Reeb VR4-VIII-02/11 (DUKE)	KC700088	KC700128	KC700166	KC700205	KC700243	KC700279	KC700062 , KC700063
376	*Cryptogramma crispa*	Spain: Madrid Province, Sierra de Guadarrama, Siete Picos	Pajarón s.n. (ALA)	KC700089	KC700129	KC700167	KC700206	KC700244	KC700280	–

(*continued on next page*)

Table 1 (*continued*)

DNA Ext. No.	Taxon	Locality	Collector/No.	GenBank Accession No.						
				rbcL	rbcL–accD	rbcL–atpB	rps4–trnS	trnG–trnR	trnP–petG	*gapCp* "short"
390	*Cryptogramma crispa*	Sweden: Norrbotten Gällivare County. Dundret, Gällivare	Larsson 333 (DUKE, UPS)	KC700090	KC700130	KC700168	KC700207	KC700245	KC700281	–
391	*Cryptogramma crispa*	Austria: Steiermark, Niedere Tauern/Seckauer Alpen: Maierangerkogel – Vorwitzsattel	Pflugbeil 111847 (ALA)	KC700091	KC700131	KC700169	KC700208	KC700246	KC700282	–
450	*Cryptogramma crispa*	Italy: northwest of Brunico, Astnerberg	Shmakov s.n. (ALTB)	KC700092	KC700132	KC700170	KC700209	KC700247	KC700283	–
396	*Cryptogramma fumariifolia*	Chile: Provincia de Ñuble: Comuna de Pinto, Shangri-La	Larraín 34009 (ALA, CONC)	KC700079	KC700119	KC700157	KC700196	KC700236	KC700271	KC700073, KC700074 , KC700075
397	*Cryptogramma fumariifolia*	Chile: Provincia de Ñuble: Comuna de Pinto, Shangri-La	Larraín 34010 (ALA, CONC)	KC700080	KC700120	KC700158	KC700197	KC700237	KC700272	–
451	*Cryptogramma raddeana*	Russia: Republic of Buryatia, Severo-Muisky range, Samokuya	Naumov 1989 (NS)	KC700084	KC700124	KC700162	KC700201	–	–	KC70005
452	*Cryptogramma raddeana*	Russia: Khabarovsky krai, 30 km north of Sofiysk	Netchaev s.n. (NS)	KC700085	KC700125	KC700163	KC700202	–	KC700276	–
355	*Cryptogramma sitchensis*	USA: Alaska: between Portage and Whittier: Bering Glacier	Metzgar 248 (ALA)	KC700103	KC700143	KC700181	KC700220	KC700258	KC700294	KC700057 , KC700056 , KC700060, KC700068
356	*Cryptogramma sitchensis*	USA: Alaska: Taku Glacier	Bass s.n. (ALA)	KC700104	KC700144	KC700182	KC700221	KC700259	KC700295	–
358	*Cryptogramma sitchensis*	USA: Alaska: Seward: Kenai Fjords National Park: Harding Icefield Trail	Metzgar 246 (ALA)	KC700105	KC700145	KC700183	KC700222	KC700260	KC700296	–
360	*Cryptogramma sitchensis*	USA: Alaska: Palmer: Hatcher Pass	Metzgar 249 (ALA)	KC700106	KC700146	KC700184	KC700223	KC700261	KC700297	–
361	*Cryptogramma sitchensis*	USA: Alaska: Valdez: Thompson Lake	Metzgar 257 (ALA)	KC700107	KC700147	KC700185	KC700224	KC700262	KC700298	–
314	*Cryptogramma stelleri*	Taiwan: NanTou County, Hohuan Shelter	Kuo 492 (TAIF)	KC700076	KC700116	KC700154	KC700193	KC700233	–	–
375	*Cryptogramma stelleri*	USA: Alaska: Alexander Archipelago: Prince of Wales Island	Johnson 20104 (ALA)	KC700077	KC700117	KC700155	KC700194	KC700234	–	KC700072
386	*Cryptogramma stelleri*	Canada: Ontario. Thunder Bay District: Talbot Island	Oldham 23697 (OAC, BAB, DUKE)	KC700078	KC700118	KC700156	KC700195	KC700235	–	–

Table 2
Primer names and sequences used.

Locus	Primer name	Sequence (5′–3′)	References
rbcL	ESRBCL1F[a]	ATG TCA CCA CAA ACG GAG ACT AAA GC	Korall et al. (2006)
rbcL	ESRBCL645F	AGA YCG TTT CYT ATT YGT AGC AGA AGC	Korall et al. (2006)
rbcL	ESRBCL663R	TAC RAA TAR GAA ACG RTC TCT CCA ACG	Korall et al. (2006)
rbcL	ESRBCL1361R[a]	TCA GGA CTC CAC TTA CTA GCT TCA CG	Korall et al. (2006)
rbcL–accD	RBCL1187F[a]	GGA ACY TTG GGA CAT CCT TGG	Korall et al. (2007)
rbcL–accD	ACCDHIF4	GAA GAT AAA CGA AAA TTG GGT GG	Ebihara et al. (2003)
rbcL–accD	ACCD887R	TTA TCA CAB CGM GCC CAT AAT CC	Korall et al. (2007)
rbcL–accD	ACCD816R[a]	CCA TGA TCG AAT AAA GAT TCA GC	Ebihara et al. (2003)
rbcL–atpB	ESRBCL26R[a]	GCT TTA GTC TCC GTT TGT GGT GAC AT	Korall et al. (2007)
rbcL–atpB	ATPB609R[a]	TCR TTD CCT TCR CGT GTA CGT TC	Pryer et al. (2004)
rbcL–atpB	ATPBSPACER703R	CCA ATG ATC TGA GTA ATS TAT CC	Korall et al. (2007)
rps4–trnS^{GGA}	trnS^{GGAa}	TTA CCG AGG GTT CGA ATC CCT C	Shaw et al. (2005)
rps4–trnS^{GGA}	rps4.5′[a]	ATG TCS CGT TAY CGA GGA CCT	Souza-Chies et al. (1997)
trnG–trnR	TRNGR1F[a]	GCG GGT ATA GTT TAG TGG TAA	Nagalingum et al. (2007)
trnG–trnR	TRNGR353F	TTG CTT MTA YGA CTC GGT G	Korall et al. (2007)
trnG–trnR	TRNG63R	GCG GGA ATC GAA CCC GCA TCA	Nagalingum et al. (2007)
trnG–trnR	TRNR22R[a]	CTA TCC ATT AGA CGA TGG ACG	Nagalingum et al. (2007)
trnP^{UGG}*–petG*	trnP^{UGGa}	TGT AGC GCA GCY YGG TAG CG	Small et al. (2005)
trnP^{UGG}*–petG*	petG2[a]	CAA TAY CGA CGK GGY GAT CAA TT	Small et al. (2005)
gapCp	ESGAPCP8F1[a]	ATY CCA AGY TCA ACT GGT GCT GC	Schuettpelz et al. (2008)
gapCp	ESGAPCP11R1[a]	GTA TCC CCA YTC RTT GTC RTA CC	Schuettpelz et al. (2008)

[a] Primer used for both amplification and sequencing.

probability. No topological conflict among data sets was observed so all six plastid loci data sets were combined into a single data set. The combined plastid locus matrix had a length of 7143 base pairs.

2.5. Phylogenetic analyses

MrModeltest 2.3 (Nylander et al., 2004) was used to determine the optimal model of sequence evolution for each ML and B/MCMC analysis based on Akaike Information Criterion scores (Table 3).

Analyses for both the combined plastid data set and the nuclear data sets used the same procedures. Maximum parsimony (MP) searches were implemented in PAUP* 4.0b10 (Swofford, 2002), with heuristic searches of 1000 random addition sequence (RAS) replicates using tree-bisection-reconnection (TBR) branch swapping to determine the most parsimonious tree(s). Subsequent MP bootstrap analyses used 500 replicates, with 10 random-addition-sequence replicates each, TBR branch swapping and the nchuck option set to 100 trees to allow the bootstrap replicates to run to completion.

Maximum likelihood (ML) analyses and ML bootstrap analyses were run using Garli 2.0 (Zwickl, 2006) and implemented on the CIPRES Science Gateway computational portal (Miller et al., 2010). To avoid introducing potential biases in sequence evolution parameter values by combining loci (Winkworth et al., 2008), we instead partitioned the data set by locus and assigned each locus its own model of sequence evolution, with parameters determined by the optimal Akaike Information Criterion scores in MrModeltest 2.3 (Table 3). Each ML analysis was performed twice, once using random starting trees and once using stepwise addition starting trees. All ML analyses used eight replicates. ML bootstrap analyses included 100 bootstrap replicates each.

The combined plastid dataset and nuclear datasets were analyzed separately using a B/MCMC approach in MrBayes version 3.2 with each of the plastid loci and the nuclear locus receiving its own model of sequence evolution as determined using the Akaike Information Criterion scores in MrModeltest 2.3 (Table 3). Each analysis used four runs, with four chains each, for 10 million generations. Default priors were used with a sampling frequency of 1000 generations. Likelihood and generation scores were plotted to check for stationarity using Tracer v1.5 (Rambaut and Drummond, 2007). We conservatively discarded the first 2.5 million generations as the burn-in period. The remaining 30,000 trees were pooled to calculate the majority-rule consensus tree with average branch lengths and posterior probabilities.

Table 3
Models of DNA sequence evolution used in maximum likelihood (ML) and Bayesian inference (B/MCMC) analyses as selected by Akaike Information Criterion (AIC) scores in MrModeltest (Nylander et al., 2004).

Data set	ML model	B/MCMC model
rbcL	SYM + G	SYM + G
rbcL–accD	GTR + G	GTR + G
rbcL–atpB	GTR + G	GTR + G
rps4–trnS^{GGA}	HKY + I	HKY + I
trnG–trnR	GTR + G	GTR + G
trnP^{UGG}*–petG*	GTR + I	GTR + I
Combined plastid	GTR + G	GTR + G
gapCp "short"	GTR + I	GTR + I

2.6. chromEvol analysis

We also reconstructed genome evolution in the cryptogrammoid clade to test for ancient genome duplication events that could complicate interpretation of the nuclear gene results. The evolution of chromosome numbers in *Cryptogramma* was evaluated using the program chromEvol (Mayrose et al., 2010), which infers polyploidization events, chromosome gain/loss events and demi-polyploidization at ancestral nodes using a series of likelihood models. In order to detect any possible biases introduced by the analytical method or process used to generate the input reference tree topology required by chromEvol, four separate topologies were used (Mayrose, pers. comm.). Two of the topologies were generated by pruning accessions from our combined plastid data matrix until each taxon was represented by a single specimen. This data set was then analyzed using the ML and B/MCMC analytical procedures outlined in Section 2.5. The two resulting topologies were used as reference trees for the chromsome number evolution reconstruction. The other two topologies were obtained by modifying the optimal ML and B/MCMC topologies inferred for the full, 40-accession combined plastid data matrix in Section 2.5. These topologies had the same excess accessions pruned so that only one sample per species remained. Branch lengths for the ML and B/MCMC topolgies were then re-estimated for that data set in

PAUP* 4.0b10 (Swofford, 2002) and MrBayes version 3.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003; Ronquist et al., 2012), respectively.

Chromosome number counts were compiled from the literature and species with unknown counts were coded as missing data (Table 4). *Coniogramme intermedia* Hieron. and *Co. fraxinea* (D. Don) Fée each have published counts of both $n = 30$ and $n = 60$, so they were coded as polymorphic with both counts having a 50% occurrence rate.

Chromosome number reconstruction analyses were then run in chromEvol v1.3 (Mayrose et al., 2010; http://www.zoology.ubc.ca/~mayrose/cp/chromEvol/) on the University of Alaska Fairbanks Life Science Informatics Computational Portal for all four topologies. All eight models of chromosome transition properties were evaluated for each run. Default parameters were used.

3. Results

3.1. Phylogenetic analyses of the combined plastid data set

The MP, ML and B/MCMC analyses all returned well-resolved, robust and congruent topologies (Fig. 1). The MP analysis found a single island of 300 most parsimonious trees (length = 1307 steps) on all heuristic replicates. ML analyses using random and stepwise starting trees in GARLi (Zwickl, 2006) returned identical topologies and likelihood scores. After achieving stationarity, the remaining 7.5 million generations of B/MCMC topologies all achieved convergence.

Coniogramme and *Cryptogramma* were retrieved as reciprocally monophyletic sister taxa, as were both sections within *Cryptogramma* (Fig. 1). All intergeneric and interspecific relationships were well-supported (MPBS $\geqslant$ 91%; MLBS $\geqslant$ 95%; B/MCMC PP = 1.00). Accessions of the allotetraploid *Cr. sitchensis* formed a moderately supported monophyletic group (MPBS = 61%; MLBS = 95%; B/MCMC PP = 0.97) within the *Cr. acrostichoides* clade, while all other species were strongly supported as monophyletic lineages (Fig. 1). Two intraspecific relationships were significantly supported by MPBS, MLBS and B/MCMC, with two *Cr. acrostichoides* accessions (277 and 359) consistently supported as sister taxa and the two mainland Chinese *Cr. brunoniana* accessions retrieved as sister taxa (accessions 457 and 458).

3.2. Phylogenetic analyses of the gapCp data set

The *gapCp* "short" copy data set yielded MP, ML and B/MCMC topologies that were mostly congruent with one another and with the combined plastid loci data set analyses (Fig. 2). Alleles from most species formed supported, reciprocally monophyletic clades (MPBS $\geqslant$ 70%; MLBS $\geqslant$ 100%; B/MCMC PP = 0.99). The *Cr. raddeana* clade contained alleles from *Cr. raddeana*, *Cr. sitchensis* and *Cr. acrostichoides* (MPBS = 99%, MLBS = 100% and B/MCMC PP = 1.00). Additionally, the ML analysis recovered *Cr. cascadensis* and *Cr. acrostichoides* alleles as sister clades with 100% MLBS support while MP and B/MCMC did not recover this node.

3.3. chromEvol analysis

All four permutations of the chromEvol analysis (using topologies generated by ML and B/MCMC analyses of a reduced data set with one examplar accession per species and using topologies generating by pruning excess accessions from the optimal consensus from the ML and B/MCMC analyses and reestimating the branch lengths) had an optimal AIC score for the "Constant rate" model of chromosome evolution. All four analyses inferred a base chromosome number of 30 for the *Llavea*/*Coniogramme*/*Cryptogramma* clade (Fig. 1). One chromosome loss event was inferred; this was the loss of a single chromosome on the branch leading to *Llavea*. Three genome duplication events were inferred by all four chromEvol analyses: a duplication was inferred on each of the branches leading to the tetraploid *Cr. crispa* and *Cr. sitchensis* (Fig. 1) and a genome duplication event was inferred on the branch leading to *Coniogramme*, which was inferred to have a base number of n = 60.

4. Discussion

4.1. Intergeneric phylogenetic relationships

We have demonstrated the monophyly of both *Coniogramme* and *Cryptogramma* for the first time (Fig. 1). Although their circumscription has not generated controversy previously, their relationship as sister taxa was not suggested until the advent of molecular research (Prado et al., 2007; Schuettpelz et al., 2007; Zhang et al., 2005; but see Gastony and Rollo, 1995, for the first suggestion that *Llavea* and *Coniogramme* are closely related). Little molecular differentiation was found among accessions of *Llavea cordifolia*; however, our sampling was restricted to central Mexico and does not include possible variation across its geographic range (Table 1). Sampling within *Coniogramme* was limited and relationships and species boundaries within the genus remain enigmatic. The inferred base chromosome number of $n = 30$ for the cryptogrammoid clade is consistent with general patterns within Pteridaceae (Tryon and Tryon, 1990), but the inferred base number of $n = 60$ for *Coniogramme* may be an artifact of limited sampling in that genus. The inferred base chromosome number of 30 for the

Table 4
Haploid (n) chromosome numbers used in chromosome number evolution analysis.

Taxon	Chromosome number	Reference
Llavea cordifolia	29	Mickle et al. (1966), Knobloch (1967)
Co. fraxinea	30,60[a]	Singh and Roy (1988), Matsumoto and Nakaike (1990), Kato et al. (1992)
Co. intermedia	30,60[a]	Matsumoto and Nakaike (1990), Kato et al. (1992)
Co. japonica	60	Weng and Qiu (1988)
Co. wilsonii	X[b]	N/A
Cr. acrostichoides	30	Taylor and Lang (1963), Löve and Löve (1976), Alverson (1989a)
Cr. brunoniana	30	Khullar et al. (1988)
Cr. cascadensis	30	Alverson (1989a)
Cr. crispa	60	Manton (1950), Löve (1970), Löve et al. (1971), Pajarón et al. (1999)
Cr. fumariifolia	X[b]	N/A
Cr. raddeana	X[b]	N/A
Cr. sitchensis	60	Alverson (1989a)
Cr. stelleri	30	Wagner (1963), Britton (1964), Gervais et al. (1999)

[a] See Section 2 for details on coding taxa with conflicting counts.
[b] No published chromosome count available.

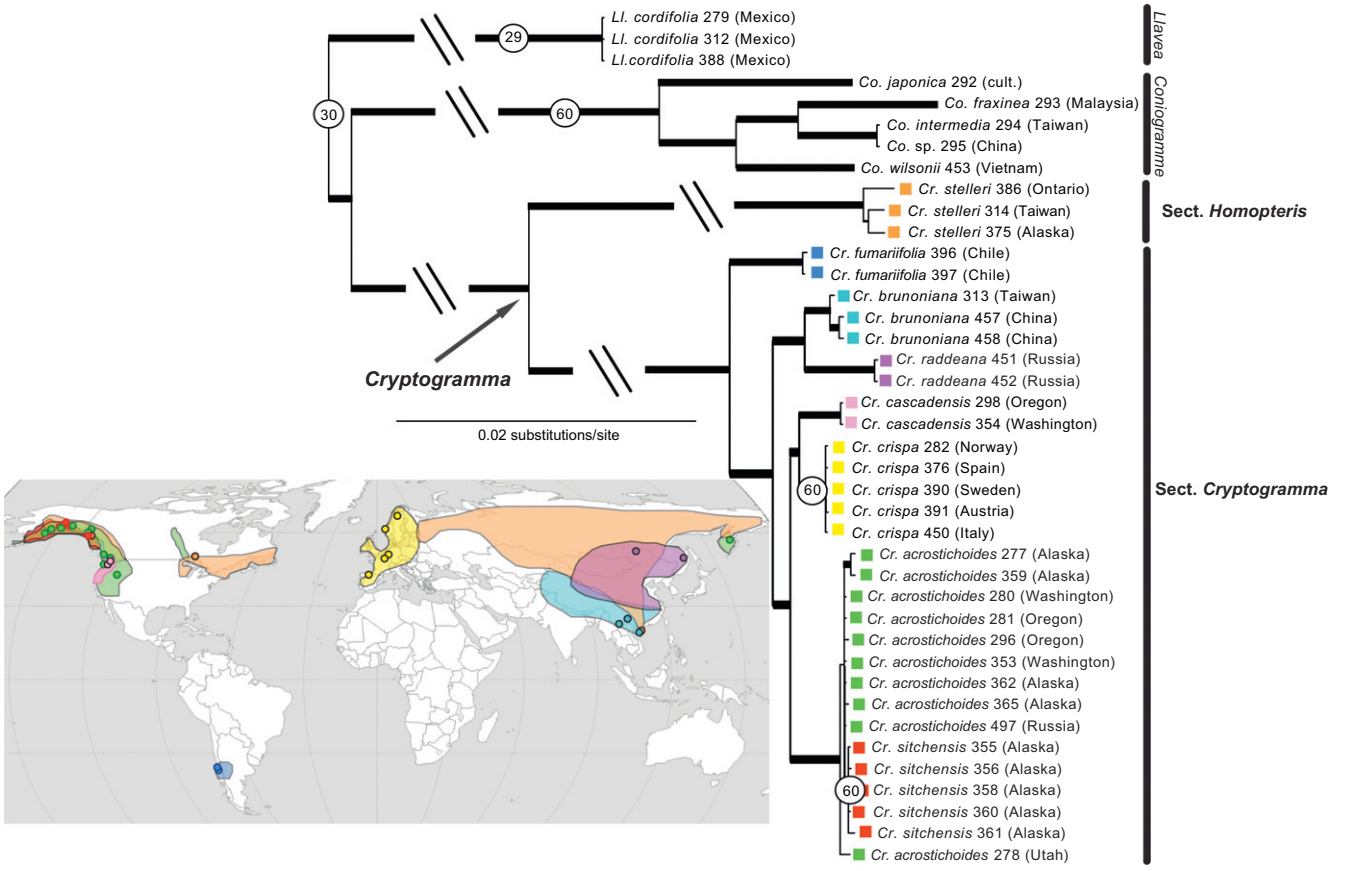

Fig. 1. Plastid phylogeny of cryptogrammoid ferns. Consensus phylogeny generated using a combined, six-locus plastid data set analyzed by Bayesian Markov chain Monte Carlo (B/MCMC). Thickened branches indicate relationships significantly supported by B/MCMC, maximum likelihood (ML) and maximum parsimony (MP) analyses (B/MCMC PP = 1.00; MLBS ⩾ 95%; MPBS ⩾ 91%). Branches shortened for clarity are indicated by hash marks. Inferred haploid chromosome numbers for the ancestral node and branches with chromosome transitions are indicated by circled numbers. Distribution map shows approximate range of each *Cryptogramma* species (colors correspond to those used in phylogeny) with sampled localities indicated with colored circles (Table 1). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Llavea/*Coniogramme*/*Cryptogramma* clade is a meaningful insight, but does not shed light on whether the ancestral chromosome number for Pteridaceae is $n = 29$ or $n = 30$.

4.2. Phylogenetic relationships within Cryptogramma

Our study recovers both sections within *Cryptogramma* as monophyletic in accordance with previous classification systems (Alverson, 1989a; Hultén, 1968; Lellinger, 1985; Tryon and Tryon, 1990; Vaganov et al., 2010) and the numerous morphological characters distinguishing the sections. Within monotypic section *Homopteris*, populations of *Cr. stelleri* from Taiwan and Alaska were found to be more closely related to one another than to a population from Ontario. However, this relationship was only well-supported in the maximum likelihood analysis and potential divergence within this taxon should be investigated with more thorough geographic sampling.

Within section *Cryptogramma*, the disjunct South American taxon *Cr. fumariifolia* (Phil.) Christ. is the earliest diverging member of the lineage although it is unclear whether this has resulted from a recent or ancient dispersal event. Most relationships are geographically unsurprising, with the Asian taxa *Cr. raddeana* and *Cr. brunoniana* being closely related and the western North American taxa *Cr. acrostichoides* and *Cr. sitchensis* forming a paraphyletic grade. One exception is the close relationship of the western North American *Cr. cascadensis* and the European *Cr. crispa*.

Most published species included in the data set are found to be reciprocally monophyletic. The lone exception is *Cr. acrostichoides*, which is polyphyletic due to the inclusion of allotetraploid *Cr. sitchensis* accessions. Thus, we support the recognition of all eight published *Cryptogramma* taxa in our data set at the species level (Fig. 1), including both tetraploid taxa as they represent reproductively autonomous, discrete genetic lineages and can be diagnosed morphologically (Barrington et al., 1989). Two taxa were not assessed in this study due to a lack of suitable material; the Turkish *Cr. bithynica* and the Russian Far Eastern and Japanese *Cr. gorovoii* (formerly *Cr. crispa* var. *japonica*) remain in need of inclusion in future molecular studies.

4.3. Deciphering the history of tetraploid Cr. crispa

Long established as a tetraploid (Manton, 1950; Löve, 1970; Löve et al., 1971; Pajarón et al., 1999), little else has been known regarding the history or formation of the European *Cr. crispa*. Our plastid and nuclear genetic data both indicate that this species is most closely related to only *Cr. cascadensis*, from northwestern North America (Fig. 1; Fig. 2). While geographically distant, *Cr. cascadensis* and *Cr. crispa* do share morphological synapomorphies such as deciduous fronds with a thin lamina (Alverson, 1989b). We hypothesize that *Cr. crispa* is an autotetraploid whose progenitor was the now extinct or undiscovered European diploid sister species of *Cr. cascadensis*. This is supported by the geographic distance separating tetraploid *Cr. crispa* and diploid *Cr. cascadensis*, as

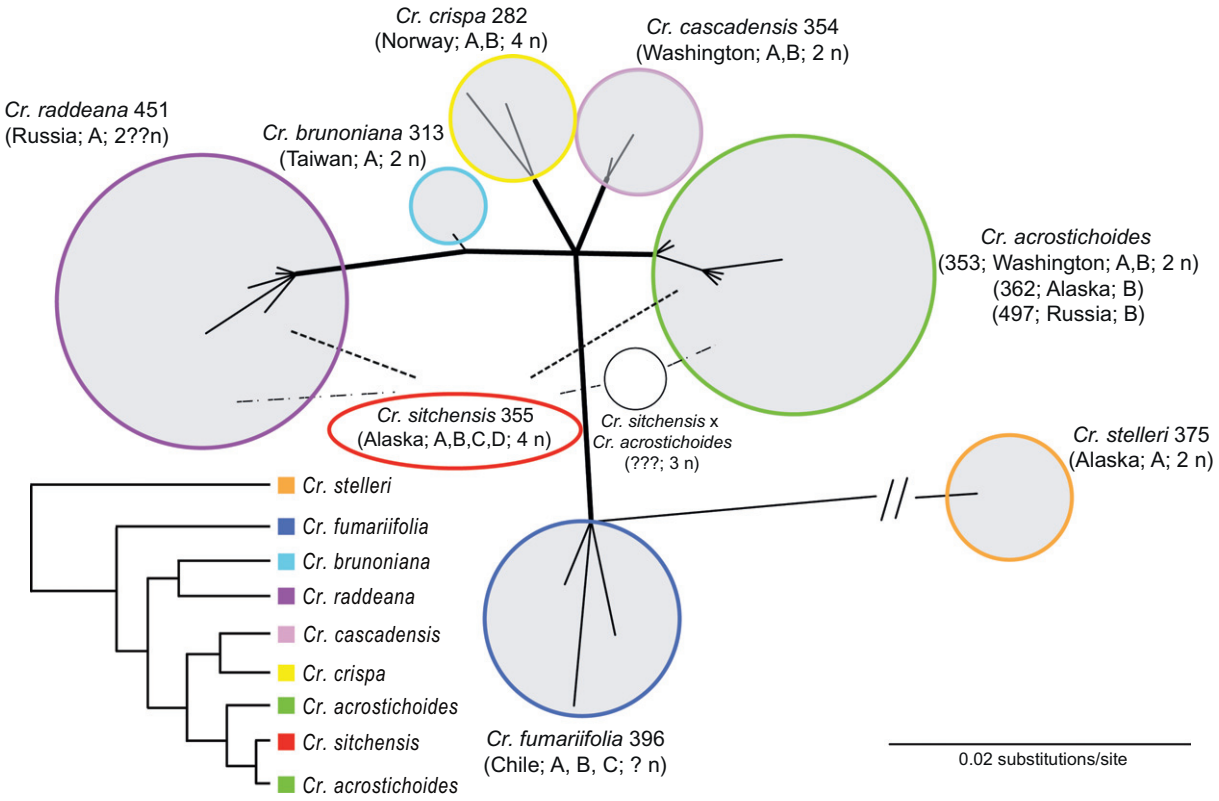

Fig. 2. Nuclear *gapCp* phylogeny of *Cryptogramma* species. Consensus phylogeny generated from *gapCp* "short" data set analyzed by Bayesian Markov chain Monte Carlo (B/MCMC). Thickened branches indicate relationships significantly supported by B/MCMC, maximum likelihood (ML) and maximum parsimony (MP) analyses (B/MCMC PP = 0.99; MLBS ⩾ 100%; MPBS ⩾ 70%). The branch leading to *Cr. stelleri* is shortened for clarity, as indicated by hash marks. Bubbles containing alleles represent taxa and are labeled with taxon name, DNA extraction number (Table 1), allele identifier and ploidy level. Alleles from allotetraploid *Cr. sitchensis* are found in multiple clades, indicated by dashed lines leading to *Cr. sitchensis*. Hypothesized position and role of the unsampled triploid hybrid between *Cr. sitchensis* and *Cr. acrostichoides* is depicted to illustrate its potential role in facilitating introgression (Fig. 3). A simplified cladogram depicts major relationships inferred from combined plastid loci analysis (Fig. 1).

well as several known genetic and mating system characteristics of *Cr. crispa*.

Previous research on the mating system and allozyme diversity within *Cr. crispa* reveal that its genome has been partially diploidized, as the species has an outcrossing mating system and greater genetic variability than associated with self-crossing polyploid taxa (Pajarón et al., 1999; Pangua et al., 1999). Branch lengths separating *Cr. crispa* and *Cr. cascadensis* are more similar in length to those separating other diploid *Cryptogramma* species, implying an older origin of this tetraploid or, minimally, of the unknown diploid that formed *Cr. crispa* (Figs. 1 and 2). Although dating the origin of polyploid taxa is notoriously difficult (Doyle and Egan, 2010), the unique, easily distinguished haplotypes and alleles of *Cr. crispa* (Figs. 1 and 2) also lead us to conclude that it is a paleopolyploid with an unknown ancestor (Stein et al., 2010). Although presumed extinct, this ancestral diploid could still be undetected within the known range of *Cr. crispa*, as evidenced by the recent discovery of an octoploid *Cryptogramma* from Turkey (Jessen et al., 2012). The current inability to disentangle the effects of evolutionary rates and time within *Cryptogramma* illustrates the need for a robust, fossil-calibrated estimate of divergence times for key parsley fern nodes.

4.4. Deciphering the history of tetraploid Cr. sitchensis

Previous research using morphology, spore measurement and allozyme data indicated that *Cr. sitchensis* is an allotetraploid and one of its progenitors was *Cr. acrostichoides* (Alverson, 1989b). The other progenitor was hypothesized to be the Russian *Cr. raddeana* based on morphology (Alverson, 1989a). Our plastid results (Fig. 1) confirm the role of *Cr. acrostichoides* as the maternal parent in this cross, as the plastid is maternally inherited in Pteridaceae (Gastony and Yatskievych, 1992) and the nuclear data (Fig. 2) confirm the morphological hypothesis that *Cr. raddeana* is the other progenitor. Interestingly, all sampled accessions of *Cr. sitchensis* form a single clade in the plastid data analysis (Fig. 1), implying a single origin of this taxon as opposed to the multiple origins typically observed in polyploid taxa (Soltis and Soltis, 2000).

A recent origin of this allopolyploid could be implied by the short branch lengths in the *Cr. sitchensis* clade (Fig. 1), although estimating polyploid lineage ages is fraught with difficulty (Doyle and Egan, 2010). The Beringian distribution of allotetraploid *Cr. sitchensis* and one diploid progenitor (*Cr. acrostichoides*) combined with the proximity of the other diploid progenitor (*Cr. raddeana*) suggests that *Cr. sitchensis* is another polyploid taxon formed during glacial maxima or subsequent recolonization (Consaul et al., 2010; Garcia-Jacas et al., 2009; Schmickl et al., 2010). Increased population-level sampling coupled with divergence time estimates and ecological niche modeling will refine this hypothesis (Metzgar, in progress).

4.5. Introgression within the Cr. acrostichoides complex

Our *gapCp* analysis identified a clade of *Cr. acrostichoides* alleles and a clade of *Cr. raddeana*, with alleles from the allotetraploid *Cr. sitchensis* occurring in both clades (Fig. 2). However, the *Cr. raddeana* clade also contains alleles from some populations of

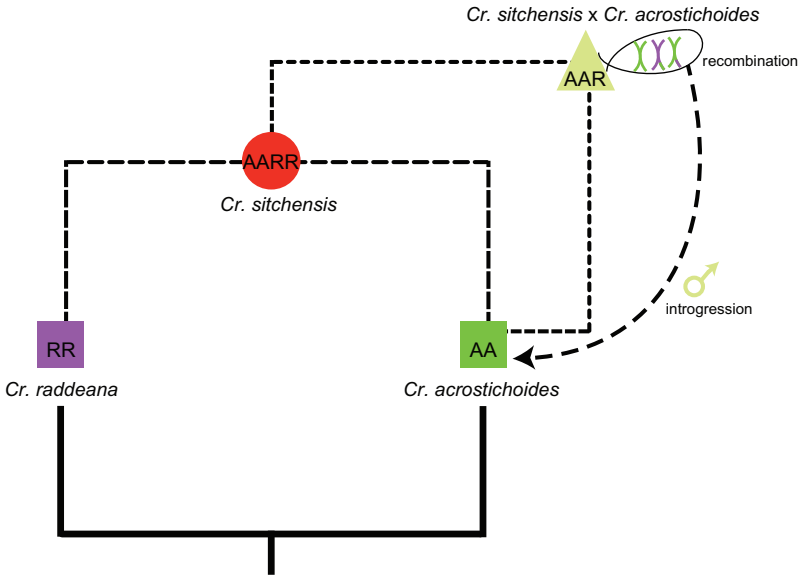

Fig. 3. Simplified phyloreticulogram demonstrating hypothesized introgression pathway in the *Cr. acrostichoides* complex. Taxa are labeled with genomic constitution, with diploid taxa depicted as squares; triploid taxa as triangles and tetraploid taxa as circles. A simplified recombination event in triploid hybrid is shown. Known hybridization events are depicted as dashed lines and hypothesized backcrossing between triploid hybrid and diploid *Cr. acrostichoides* is depicted with a dashed arrow.

Cr. acrostichoides. Lineage sorting is unlikely, as a gene duplication event would have had to occur deep in the history of section *Cryptogramma* (Fig. 1) and would require an unparsimonious number of losses in various taxa and populations and an unreasonably slow coalescence time (Brokaw and Hufford, 2010). Our chromosome evolution reconstruction also rejects a whole genome duplication event predating extant *Cryptogramma* diversity (Fig. 1). We hypothesize that the presence of *gapCp* alleles from two populations of *Cr. acrostichoides* in a clade with *Cr. raddeana* and *Cr. sitchensis* alleles (Fig. 2) results from the introgression of *Cr. raddeana* alleles into some *Cr. acrostichoides* populations via backcrossing between *Cr. sitchensis* and *Cr. acrostichoides* (Fig. 3). One *Cr. acrostichoides* population that does not demonstrate any evidence of introgression is from Washington State, USA (Fig. 2; Fig. 3) and its allopatry with respect to *Cr. sitchensis* would preclude any chance of backcrossing. A triploid hybrid bridge could be the mechanism for backcrossing between *Cr. sitchensis* and diploid *Cr. acrostichoides*, as the two species frequently form a triploid hybrid which produces some spores that appear normal and could produce viable sperm (DeBenedictis, 1969; Husband, 2004). Introgression has also been found to occur at the *gapCp* locus in other fern taxa (Nitta et al., 2011; Yatabe et al., 2009).

5. Conclusions

We have established the monophyly of *Coniogramme* and *Cryptogramma*, as well as both sections within *Cryptogramma*. Additionally, all included *Cryptogramma* species in our study represent monophyletic lineages (*Cr. acrostichoides* is monophyletic only when allotetraploid *Cr. sitchensis* is not considered). We support the recognition of these eight taxa at the species level. We have also confirmed or identified the parentage of allotetraploid *Cr. sitchensis* and autotetraploid *Cr. crispa* for the first time.

This study serves as the first rigorous molecular assessment of relationships within *Cryptogramma*. By independently analyzing monophyly and reticulation, we have identified numerous areas of interest or need for future study. The timing and direction of dispersal events and genome duplications in the genus are in need of more rigorous estimation, as well as possible correlations with climatic fluctuations such as interglacial cycles. Additionally, several *Cryptogramma* species would make ideal candidates for detailed molecular and ecological reconstructions of survival strategies used by free-sporing monilophytes during the Last Glacial Maximum in North America.

Acknowledgments

We thank Ellen Anderson, Polly Bass, David Boufford (collecting with support from NSF DEB-0321846), David Gernandt, Li-Yaung Kuo, Juan Larraín, Manuel González Ledesma, Phan Ke Loc, Alicia Marticorena, Emily Metzgar, Santiago Pajarón, Emilia Pangua, Georg Pflugbeil, Kathleen Pryer, Richard Ree, Carl Rothfels, Erin Sigel, A. Shmakov, Mary Stensvold, Stacy Studebaker, Andreas Tribsch, Mike Windham, Valentin Yakubov and Peter Zika for providing specimens. The U.S. National Park Service is thanked for granting a research collection permit. We also thank Mac Campbell, Itay Mayrose, Carl Rothfels, Eric Schuettpelz and Diana Wolf for laboratory advice or technical expertise. We thank the two anonymous reviewers for useful and instructive comments on the manuscript. This research was funded in part by a UAF Center for Global Change Student Research Grant with funds from the UAF Center for Global Change. Partial funding was also provided by the Alaska EPSCoR Landscape Genetics Student Research Award, Alaska Quaternary Center Hopkins Fellowship, American Society of Plant Taxonomists Graduate Student Research Award, and University of Alaska Ted McHenry Biology Field Research Award.

References

Alverson, E.R., 1988. Biosystematics of North American parsley ferns, *Cryptogramma* (Adiantaceae). Am. J. Bot. 75, 136–137.
Alverson, E.R., 1989a. Biosystematics of Parsley-ferns, *Cryptogramma* R. Br., in Western North America. Master's Thesis, Oregon State University.
Alverson, E.R., 1989b. *Cryptogramma cascadensis*, a new parsley fern from Western North America. Am. Fern. J. 79, 95–102.
Alverson, E.R., 1993. *Cryptogramma*. In: Flora of North America Editorial Committee (Eds.), The Flora of North America, vol. 2. Oxford University Press, New York, NY, pp. 137–139.
Barrington, D.S., Haufler, C.H., Werth, C.R., 1989. Hybridization, reticulation and species concepts in the ferns. Am. Fern J. 79, 55–64.
Beck, J.B., Windham, M.D., Pryer, K.M., 2011. Do asexual lineages lead short evolutionary lives? A case-study from the fern genus *Astrolepis*. Evolution 65, 3217–3229.

Britton, D.M., 1964. Chromosome numbers of ferns in Ontario. Can. J. Bot. 42, 1349–1354.
Brokaw, J.M., Hufford, L., 2010. Origins and introgression of polyploid species in *Mentzelia* section *Trachyphytum* (Loasaceae). Am. J. Bot. 97, 1457–1473.
Consaul, L.L., Gillespie, L.J., Waterway, M.J., 2010. Evolution and polyploid origins in North American Arctic *Puccinellia* (Poaceae) based on nuclear ribosomal spacer and chloroplast DNA sequences. Am. J. Bot. 97, 324–336.
DeBenedictis, V.M.M., 1969. Apomixis in Ferns with Special Reference to Sterile Hybrids. Ph.D. Dissertation, University of Michigan.
Doyle, J.J., Egan, A.N., 2010. Dating the origins of polyploidy events. New Phytol. 186, 73–85.
Ebihara, A., Iwatsuki, K., Ohsawa, T.A., Ito, M., 2003. *Hymenophyllum paniense* (Hymenophyllaceae), a new species of filmy fern from New Caledonia. Syst. Bot. 28, 228–235.
Fraser-Jenkins, C.R., 2008. Taxonomic Revision of Three Hundred Indian Subcontinental Pteridophytes with a Revised Census-List: A New Picture of Fern-Taxonomy and Nomenclature in the Indian Subcontinent. Bishen Singh Mahendra Pal Singh, Dehra Dun, India.
Garcia-Jacas, N., Soltis, P.S., Font, M., Soltis, D.E., Vilatersana, R., Susanna, A., 2009. The polyploid series of *Centaurea toletana*: glacial migrations and introgression revealed by nrDNA and cpDNA sequences analyzes. Mol. Phylogenet. Evol. 52, 377–394.
Gastony, G.J., Rollo, D.R., 1995. Phylogeny and generic circumscriptions of cheilanthoid ferns (Pteridaceae: Cheilanthoideae) inferred from *rbcL* nucleotide sequences. Am. Fern J. 85, 341–360.
Gastony, G.J., Yatskievych, G., 1992. Maternal inheritance of the chloroplast and mitochondrial genomes in cheilanthoid ferns. Am. J. Bot. 79, 716–722.
Gervais, C., Trahan, R., Gagnon, J., 1999. IOPB chromosome data 14. Newslett. Int. Organ. Pl. Biosyst. (Oslo) 30, 10–15.
Grusz, A.L., Windham, M.D., Pryer, K.M., 2009. Deciphering the origins of apomictic polyploids in the *Cheilanthes yavapensis* complex (Pteridaceae). Am. J. Bot. 96, 1636–1645.
Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogeny. Bioinformatics 17, 754–755.
Hultén, E., Fries, M., 1986. Atlas of North European Vascular Plants: North of the Tropic of Cancer, vol. 1–3. Königstein, Koeltz.
Hultén, E., 1968. Flora of Alaska and Neighboring Territories. Stanford University Press, Stanford, CA.
Husband, B.C., 2004. The role of triploid hybrids in the evolutionary dynamics of mixed-ploidy populations. Biol. J. Linn. Soc. 82, 537–546.
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.
Kato, M., Nakato, N., Cheng, X., Iwatsuki, K., 1992. Cytotaxonomic study of ferns of Yunnan, southwestern China. Bot. Mag. (Tokyo) 105, 105–124.
Kirkpatrick, R.E.B., 2007. Investigating the monophyly of *Pellaea* (Pteridaceae) in the context of a phylogenetic analysis of cheilanthoid ferns. Syst. Bot. 32, 504–518.
Khullar, S.P., Sharma, S.S., Verma, S.C., 1988. SOCGI plant chromosome number reports – VI. J. Cytol. Genet. 23, 38–52.
Knobloch, I.W., 1967. Chromosome Numbers in *Cheilanthes*, *Notholaena*, *Llavea* and *Polypodium*. Am. J. Bot. 54, 461–464.
Korall, P., Pryer, K.M., Metzgar, J.S., Schneider, H., Conant, D.S., 2006. Tree ferns: monophyletic groups and their relationships as revealed by four protein-coding plastid loci. Mol. Phylogenet. Evol. 39, 830–845.
Korall, P., Conant, D.S., Metzgar, J.S., Schneider, H., Pryer, K.M., 2007. A molecular phylogeny of scaly tree ferns (Cyatheaceae). Am. J. Bot. 94, 873–886.
Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J., Higgins, D.G., 2007. Clustal W and Clustal X version 2.0. Bioinformatics 23, 2947–2948.
Lellinger, D.B., 1985. A Field Manual of the Ferns & Fern-Allies of the United States & Canada. Smithsonian Institution Press, Washington, DC.
Löve, A., 1970. Islenzk ferdaflora. Almenna Bokfelagid, Reykjavik.
Löve, A., Löve, D., Kapoor, B.M., 1971. Cytotaxonomy of a century of Rocky Mountain orophytes. Arctic Alpine Res. 3, 139–165.
Löve, A., Löve, D., 1976. In IOPB chromosome number reports LIII. Taxon 25, 483–500.
Manton, I., 1950. Problems of Cytology and Evolution in the Pteridophyta. Cambridge University Press, London.
Mason-Gamer, R.J., Burns, M.M., Naum, M., 2008. Allohexaploidy, introgression, and the complex phylogenetic history of *Elymus repens* (Poaceae). Mol. Phylogenet. Evol. 47, 598–611.
Mason-Gamer, R.J., Kellogg, E.A., 1996. Testing for phylogenetic conflict among molecular data sets in the tribe Triticeae (Gramineae). Syst. Biol. 45, 524–545.
Matsumoto, S., Nakaike, T., 1990. Cytological observations of some ferns in Nepal (1). On the related taxa in Japan. In: M. Watanabe & S. B. Malla (editors). Cryptog. Himalayas 2, 163–178.
Mayrose, I., Barker, M.S., Otto, S.P., 2010. Probabilistic models of chromosome number evolution and the inference of polyploidy. Syst. Biol. 59, 132–144.
McGrath, J.M., Hickok, L.G., 1999. Multiple ribosomal RNA gene loci in the genome of the homosporous fern *Ceratopteris richardii*. Can. J. Bot. 77, 1199–1202.
Mickle, J.T., Wagner, W.H., Chen, K.L., 1966. Chromosome observations on the ferns of Mexico. Caryologia 19, 85–94.
Miller, M.A., Pfeiffer, W., Schwartz, T., 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computing Environments Workshop (GCE), 14 November 2010, New Orleans, LA, pp. 1–8.
Nagalingum, N.S., Schneider, H., Pryer, K.M., 2007. Molecular phylogenetic relationships and morphological evolution in the heterosporous fern genus *Marsilea*. Syst. Bot. 32, 16–25.
Nakazato, T., Jung, M.-K., Housworth, E.A., Rieseberg, L.H., Gastony, G.J., 2007. A genome-wide study of reproductive barriers between allopatric populations of a homosporous fern, *Ceratopteris richardii*. Genetics 177, 1–10.
Nitta, J.H., Ebihara, A., Ito, M., 2011. Reticulate evolution in the *Crepidomanes minutum* species complex (Hymenophyllaceae). Am. J. Bot. 98, 1782–1800.
Nylander, J.A.A., Ronquist, F., Huelsenbeck, J.P., Nieves Aldrey, J.L., 2004. Bayesian phylogenetic analysis of combined data. Syst. Biol. 53, 47–67.
Pajarón, S., Pangua, E., García-Álvarez, L., 1999. Sexual expression and genetic diversity in populations of *Cryptogramma crispa* (Pteridaceae). Am. J. Bot. 86, 964–973.
Pangua, E., García-Álvarez, L., Pajarón, S., 1999. Studies on *Cryptogramma crispa* Spore Germination. Am. Fern J. 89, 159–170.
Prado, J., Rodrigues, C.D.N., Salatino, A., Salatino, M.L.F., 2007. Phylogenetic relationships among Pteridaceae, including Brazilian species, inferred from *rbcL* sequences. Taxon 56, 355–368.
Pryer, K.M., Schuettpelz, E., Wolf, P.G., Schneider, H., Smith, A.R., Cranfill, R., 2004. Phylogeny and evolution of ferns (monilophytes) with a focus on the early leptosporangiate divergences. Am. J. Bot. 91, 1582–1598.
Rambaut, A., Drummond, A.J., 2007. Tracer v1.4. <http://beast.bio.ed.ac.uk/Tracer>.
Raven, P.H., 1963. Amphitropical relationships in the floras of North and South America. Q. Rev. Biol. 38, 151–177.
Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.
Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A., Huelsenbeck, J.P., 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61, 539–542.
Rothfels, C.J., Windham, M.D., Grusz, A.L., Gastony, G.J., Pryer, K.M., 2008. Toward a monophyletic *Notholaena* (Pteridaceae): resolving patterns of evolutionary convergence in xeric-adapted ferns. Taxon 57, 712–724.
Schmickl, R., Jørgensen, M.H., Brysting, A.K., Koch, M.A., 2010. The evolutionary history of the *Arabidopsis lyrata* complex: a hybrid in the amphi-Beringian area closes a large distribution gap and builds up a genetic barrier. BMC Evol. Biol. 10, 98.
Schuettpelz, E., Pryer, K.M., 2007. Fern phylogeny inferred from 400 leptosporangiate species and three plastid genes. Taxon 56, 1037–1050.
Schuettpelz, E., Schneider, H., Huiet, L., Windham, M.D., Pryer, K.M., 2007. A molecular phylogeny of the fern family Pteridaceae: assessing overall relationships and the affinities of previously unsampled genera. Mol. Phylogenet. Evol. 44, 1172–1185.
Schuettpelz, E., Grusz, A.L., Windham, M.D., Pryer, K.M., 2008. The utility of nuclear *gapCp* in resolving polyploid fern origins. Syst. Bot. 33, 621–629.
Scott, R.J., Gastony, G.J., Weatherford, J.W., Nakazato, T., 2007. Characterization of four members of the alpha-tubulin gene family in *Ceratopteris richardii*. Am. Fern J. 97, 47–65.
Shaw, J., Lickey, E.B., Beck, J.T., Farmer, S.B., Liu, W., Miller, J., Siripun, K.C., Winder, C.T., Schilling, E.E., Small, R.L., 2005. The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. Am. J. Bot. 92, 142–166.
Sigel, E.M., Windham, M.D., Huiet, L., Yatskievych, G., Pryer, K.M., 2011. Species relationships and farina evolution in the cheilanthoid fern genus *Argyrochosma* (Pteridaceae). Syst. Bot. 36, 554–564.
Singh, V.P., Roy, S.K., 1988. Cytology of forty four species from Sikkim, Himalaya. Indian Fern J. 5, 162–169.
Small, R.L., Lickey, E.B., Shaw, J., Hauk, W.D., 2005. Amplification of noncoding chloroplast DNA for phylogenetic studies in lycophytes and monilophytes with a comparative example of relative phylogenetic utility from Ophioglossaceae. Mol. Phylogenet. Evol. 36, 509–522.
Smith, A.R., Pryer, K.M., Schuettpelz, E., Korall, P., Schneider, H., Wolf, P.G., 2006. A classification for extant ferns. Taxon 55, 705–731.
Soltis, P.S., Soltis, D.E., 2000. The role of genetic and genomic attributes in the success of polyploids. Proc. Natl. Acad. Sci. USA 97, 7051–7057.
Souza-Chies, T.T., Bittar, G., Nadot, S., Carter, L., Besin, E., Lejeune, B., 1997. Phylogenetic analysis of Iridaceae with parsimony and distance methods using the plastid gene rps4. Plant Sys. Evol. 204, 109–123.
Stein, D.B., Hutton, C., Conant, D.S., Haufler, C.H., Werth, C.R., 2010. Reconstructing *Dryopteris "semicristata"* (Dryopteridaceae): molecular profiles of tetraploids verify their undiscovered diploid ancestor. Am. J. Bot. 97, 998–1004.
Swofford, D.L., 2002. PAUP*: phylogenetic analysis using parsimony (*and other models), v. 4.0 beta 10. Sunderland, Sinauer Associates.
Taylor, T.M.C., Lang, F., 1963. Chromosome counts in some British Columbia Ferns. Am. Fern J. 53, 123–126.
Tryon, R.M., Tryon, A.F., 1990. Pteridaceae. In: Kramer, K.U., Green, P.S., (Eds.), The Families and Genera of Vascular Plants, vol. I, Pteridophytes and Gymnosperms. Springer-Verlag, Berlin, pp. 230–256.
Vaganov, A., Shmakov, A., 2007. New species of *Cryptogramma* (Cryptogrammaceae) from the Far East. Turcz. 10, 5–8.
Vaganov, A.V., Shmakov, A.I., Kuznetsov, A.A., Gureeva, I.I., 2010. Spore morphology of *Cryptogramma* R. Br. ex Richards species (Cryptogrammaceae). Turcz 13, 50–58.
Wagner, W.H., 1963. A biosystematic study of United States ferns, A preliminary abstract. Am. Fern J. 56, 1–16.

Weng, R.F., Qiu, S.P., 1988. Chromosome counts of some ferns from Zhejiang. Invest. Stud. Nat. 8, 43–52.
Winkworth, R.C., Bell, C.D., Donoghue, M.J., 2008. Mitochondrial sequence data and Dipsacales phylogeny: mixed models, partitioned Bayesian analyses, and model selection. Mol. Phylogenet. Evol. 46, 830–843.
Yatabe, Y., Tsutsumi, C., Hirayama, Y., Mori, K., Murakami, N., Kato, M., 2009. Genetic population structure of *Osmunda japonica*, rheophilous *Osmunda lancea* and their hybrids. J. Plant Res. 122, 585–595.
Zhang, G.M., Alverson, E.R., Metzgar, J. S., in press. *Cryptogramma* R. Brown. In: Wu, Z.Y., Raven, P.H., Hong, D.Y. (Eds.), Flora of China Volume 2–3 (Lycopodiaceae through Polypodiaceae). Science Press, Beijing, and Missouri Botanical Garden Press, St. Louis.
Zhang, G.-M., Zhang, X.-C., 2003. Taxonomic revision of the genus *Cryptogramma* R.Br. from China. Acta Phyto. Sin. 41, 475–482.
Zhang, G., Zhang, X., Chen, Z., 2005. Phylogeny of cryptogrammoid ferns and related taxa based on *rbcL* sequences. Nord. J. Bot. 23, 485–493.
Zwickl, D. J., 2006. Genetic Algorithm Approaches for the Phylogenetic Analysis of Large Biological Sequence Datasets Under the Maximum Likelihood Criterion. Ph.D. Dissertation, University of Texas at Austin.