RESEARCH ARTICLE

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Greatly reduced phylogenetic structure in the cultivated potato clade (*Solanum* section *Petota* pro parte)

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PREMISE OF THE STUDY: The species boundaries of wild and cultivated potatoes are controversial, with most of the taxonomic problems in the cultivated potato clade. We here provide the first in-depth phylogenetic study of the cultivated potato clade to explore possible causes of these problems.

METHODS: We examined 131 diploid accessions, using 12 nuclear orthologs, producing an aligned data set of 14,072 DNA characters, 2171 of which are parsimony-informative. We analyzed the data to produce phylogenies and perform concordance analysis and goodness-of-fit tests.

KEY RESULTS: There is good phylogenetic structure in clades traditionally referred to as clade 1+2 (North and Central American diploid potatoes exclusive of *Solanum verrucosum*), clade 3, and a newly discovered basal clade, but drastically reduced phylogenetic structure in clade 4, the cultivated potato clade. The results highlight a clade of species in South America not shown before, 'neocardenasii', sister to clade 1+2, that possesses key morphological traits typical of diploids in Mexico and Central America. Goodness-of-fit tests suggest potential hybridization between some species of the cultivated potato clade. However, we do not have enough phylogenetic signal with the data at hand to explicitly estimate such hybridization events with species networks methods.

CONCLUSIONS: We document the close relationships of many of the species in the cultivated potato clade, provide insight into the cause of their taxonomic problems, and support the recent reduction of species in this clade. The discovery of the neocardenasii clade forces a reevaluation of a hypothesis that section *Petota* originated in Mexico and Central America.

KEY WORDS conserved nuclear orthologs; goodness of fit tests; hybridization; phylogeny; potato; Solanaceae; taxonomy.

Solanum section *Petota*, which includes the potato and its wild relatives, is a difficult taxonomic group complicated by interspecific hybridization, introgression, allopolyploidy, a mixture of sexual and asexual reproduction, and possible recent species divergence (Spooner and van den Berg, 1992a; Spooner, 2009). Every taxonomist who has worked on the section has provided different hypotheses of the number of species, their interrelationships, and the hybrid origins of various taxa. A recent taxonomic conspectus (Spooner et al., 2014) recognized 107 species in section *Petota*, less than half as many as a treatment by Hawkes (1990) that recognized 232 species.

Plastid DNA restriction site studies (Spooner et al., 1991; Spooner and Sytsma, 1992; Rodríguez and Spooner, 1997; Spooner and Castillo, 1997) recognized four main clades in section *Petota*, referred to as clades 1–4. Subsequent studies using nuclear orthologs (Spooner et al., 2008b; Rodríguez and Spooner, 2009; Cai et al., 2012) recovered three main clades, with similar interspecific relationships except they combined clades 1 and 2 with the nuclear clades then referred to as 1+2, 3, and 4. In-depth studies of these individual clades have been conducted with morphological and molecular data in clade 1+2 (Rodríguez and Spooner, 1997; Lara-Cabrera and Spooner, 2004, 2005) and clade 3 (Ames et al., 2008; Ames and Spooner, 2010).

Putatively related species complexes within the cultivated potato clade (clade 4) have been studied with DNA markers and morphological data, but never with a broad-scale phylogenetic study using multiple nuclear orthologs. According to the taxonomy of Spooner et al. (2014), the cultivated potato clade contains 34 predominately diploid species (some with rare triploid or tetraploid populations), two exclusively tetraploid species, and one species with diploid, tetraploid, and hexaploid populations. An additional 17 allopolyploid species share alleles with clades 1 or 3 and clade 4 (the cultivated potato clade) and are not studied here. The best-studied species complex in

the cultivated potato clade is the *Solanum brevicaule* complex. Using the prior taxonomy of Hawkes (1990) and Ochoa (1990, 1999), the *Solanum brevicaule* complex contains about 20 morphologically similar wild taxa; Spooner (2014) reduced these to two taxa, *S. candolleanum* and *S. brevicaule*. They are difficult to distinguish from cultivated potatoes (Correll, 1962; Ugent, 1970; Grun, 1990). Some members of this complex, all endemic to central Peru, Bolivia, and northern Argentina, were considered ancestors of potato landraces (Ugent, 1970). They share pinnately dissected leaves, round fruits, rotate to rotate-pentagonal corollas, and are largely sexually compatible with each other and with cultivated potatoes (Hawkes, 1958; Hawkes and Hjerting, 1969, 1989; Ochoa, 1990, 1999; Spooner and van den Berg, 1992a). The complex includes diploids, tetraploids, and hexaploids, with traditionally recognized species possessing multiple ploidy levels (e.g., previously recognized *S. gourlayi* with diploids and tetraploids, and previously recognized *S. oplocense* with diploids, tetraploids, and hexaploids). Members of the complex have been so difficult to distinguish from each other that even experienced potato taxonomists Hawkes and Hjerting (1989) and Ochoa (1990) provided different independent identifications for identical collection numbers of the *Solanum brevicaule* complex in fully 38% of the cases (Spooner and van den Berg, 1992a).

Field collections in Peru (Spooner et al., 1999; Salas et al., 2001), Bolivia (Spooner et al., 1994), and Argentina (Spooner and Clausen, 1993); phenetic analyses of morphological data in the Netherlands (van den Berg et al., 1996), the United States (van den Berg et al., 1998), and Peru (Alvarez et al., 2008) single- to low-copy nuclear restriction fragment length polymorphism (nRFLPs), and random amplified fragment length (RAPD) data (Miller and Spooner, 1999), and amplified fragment length polymorphism (AFLP) data (Spooner et al., 2005) failed to clearly differentiate many wild species in the *S. brevicaule* complex, but defined two geographic subsets: (1) the central to southern Peruvian populations initially referred to as the *S. brevicaule* "north" group, and the Bolivian and Argentinean populations referred to as the *S. brevicaule* "south" group. However, even these two species groups could be distinguished only by computer-assisted statistical analyses of widely overlapping morphological character states, not by species-specific characters. A subsequent monographic study (Spooner et al., 2016) synonymized most of the names in the *S. brevicaule* north group under *S. candolleanum* and in the south group under *S. brevicaule*. Other investigated difficult species complexes in the cultivated potato clade are the *S. berthaultii* complex (Spooner and van den Berg, 1992b; Spooner et al., 2007), *S. boliviense* complex (Giannattasio and Spooner, 1994a, b; Spooner et al., 1997), and the *S. medians* complex (Spooner et al., 2008a).

The focus of the present study is to use multiple representative accessions of the cultivated potato clade and 12 nuclear orthologs to provide the first broad-scale DNA sequence study to investigate the species boundaries and interrelationships of representative members of this clade.

MATERIALS AND METHODS

Plants

We analyzed a single individual from each of 131 diploid accessions: four from the outgroup (*Solanum etuberosum* Lindl. and *S. fernandezianum* Phil.; Table 1), 10 from clade 1+2, six from a new clade neocardenasii discovered here (see Results), eight from clade 3, and 103 from the cultivated potato clade. In total, we examined 42 species (two from the outgroup, six from clade 1+2, two from the neocardenasii clade, six from clade 3, and 26 from the cultivated potato clade), following the taxonomy of Spooner et al. (2014), or 67 species relative to the taxonomy of Hawkes (1990) or Ochoa (1990, 1999). Table 1, Fig. 1, and Appendices S1 and S2 list both old and new names (Spooner et al., 2014). Our paper is designed to examine the cladistic structure of section *Petota* and to use this structure to comment on new taxonomic changes that are in many cases quite drastic through synonymy. Hence, we provide both the old and new (Spooner et al., 2014) names to make these changes clear. We make these names clear in the figures by placing the old names in parentheses and in the few cases where an old name is used in the text this is made clear by citing it as an old name.

Marker selection

We examined 12 conserved nuclear orthologs (Table 2), five of which (C2At1g13380, C2At5g14320, C2At1g32130, C2At1g20050, C2At2g38020; Table 2) were initially screened by Rodriguez et al. (2009). We selected these markers based on single band amplification and length of the PCR product (mostly between 600–1200 bp and with more than 60% intron content). Five of these loci were examined in a few of the diploid species of section *Petota* by Rodriguez et al. (2009), Ames and Spooner (2010), Fajardo and Spooner (2011), and Cai et al. (2012). Markers C2At1g13380, C2At5g14320, C2At1g32130, C2At1g20050, C2At2g38020 were initially amplified and sequenced following Rodríguez et al. (2009). Subsequently, we examined seven additional markers, C2At1g44760, C2At1G77470, C2At2G24090, C2At2G37500, C2At5G64730, C2At4g02680, C2At 2g06530, amplified and sequenced using single-strand confirmation polymorphism (SSCP), that circumvents the need for cloning, following Rodríguez et al. (2011), except all SSCP gels ran for 72 h (Table 2).

DNA extraction, sequencing, and alignment

DNA from freeze-dried leaf tissue was extracted using a CTAB method (Ghislain, 1999) and Sanger sequenced using an ABI 3730xl capillary-based automated DNA sequencer (Applied Biosystems) with 50 cm POP-7 polymer capillaries at the Biotechnology Center of the University of Wisconsin-Madison. For markers C2At1g13380, C2At5g14320, C2At1g32130, C2At1g20050, C2At2g38020, when a faint second band appeared or when the sequence was unreadable, the PCR product was cloned and five positive colonies were sequenced. For the remaining seven markers using SSCP, cloning was not needed. Sequences were edited using the Staden Package version 1.7.0 (Staden, 1996). Gene sequences were aligned using MUSCLE version 3.8.31 (Edgar, 2004). The aligned sequences were manually corrected to minimize gaps using Mesquite version 3.03 (Maddison and Maddison, 2015). Because we amplified multiple nuclear orthologs from different genomic regions, we were not able to align different alleles in concatenated analyses. Hence, alleles were randomly assigned as either "A" or "B" and were concatenated into two files based on allele designation, and we analyzed these as two separate trees. The allelic variants were not a problem in species tree construction however where we could combine both alleles into a single file. Genes were annotated as a character set in the final nexus file. The aligned database of the A and B files are deposited in the TreeBase repository (http://purl.org/phylo/treebase/phylows/study/TB2:S21303).

TABLE 1. Accessions examined in this study.

Taxon (Spooner et al., 2014)	Taxon (Hawkes, 1990; Ochoa, 1990, 1999)[a]	Accession[b]	Location[c]
Outgroup			
Solanum etuberosum Lindl.		PI 498311	Chile. Bio-Bio
S. fernandezianum Phil.		PI 320270	Chile. Juan Fernández
S. fernandezianum		PI 473463	Chile. Juan Fernández
S. fernandezianum		PI 566752	Chile. Juan Fernández
Clade 1+2			
S. bulbocastanum Dunal		PI 347757	Mexico. Michoacán
S. polyadenium Greenm.		PI 161728	Mexico. Michoacán
S. ×sambucinum Rydb.		PI 595478	Mexico. Querétaro
S. stenophyllidium Bitter	*S. brachistotrichium* (Bitter) Rydb.	PI 255527	Mexico. Aguascalientes
S. stenophyllidium	*S. brachistotrichium*	PI 320265	Mexico. Chihuahua
S. stenophyllidium	*S. brachistotrichium*	PI 558460	Mexico. Jalisco
S. tarnii Hawkes and Hjert.		PI 498043	Mexico. Hidalgo
S. tarnii		PI 498047	Mexico. Hidalgo
S. tarnii		PI 545742	Mexico. Veracruz
S. trifidum Correll		PI 255536	Mexico. Michoacán
New clade neocardenasii			
S. neocardenasii Hawkes and Hjert.		PI 498129	Bolivia. Santa Cruz
S. neocardenasii		PI 502642	Bolivia. Santa Cruz
S. stipuloideum Rusby	*S. capsicibaccatum* Cárd.	PI 205560	Bolivia. Cochabamba
S. stipuloideum	*S. soestii* Hawkes and Hjert.	PI 639276	Bolivia. Cochabamba
S. stipuloideum	*S. circaeifolium* Bitter	PI 498117	Bolivia. Cochabamba
S. stipuloideum	*S. circaeifolium*	PI 545974	Bolivia. La Paz
Clade 3			
S. acroscopicum Ochoa		PI 365314	Peru. Arequipa
S. andreanum Baker		PI 247360	Columbia. Nariño
S. andreanum		PI 561658	Ecuador. Bolívar
S. chomatophilum Bitter		CIP 762569	Peru. Cajamarca
S. chomatophilum		PI 365339	Peru. Junín
S. contumazaense Ochoa		CIP 762118	Peru. Cajamarca
S. minutifoliolum Correll		PI 583298	Ecuador. Tungurahua
S. multiinterruptum Bitter		PI 498266	Peru. Ancash
Clade 4 (cultivated potato clade)			
S. berthaultii Hawkes		PI 265857	Bolivia. Cochabamba
S. berthaultii	*S. tarijense* Hawkes	PI 442689	Argentina. Salta
S. boliviense Dunal	*S. sanctae-rosae* Hawkes	PI 230464	Argentina. Tucumán
S. boliviense	*S. sanctae-rosae*	PI 320324	Argentina. Tucumán
S. boliviense	*S. toralapanum* Cárd. and Hawkes	PI 472804	Argentina. Salta
S. boliviense	*S. sanctae-rosae*	PI 473200	Argentina. Tucumán
S. boliviense	*S. megistacrolobum* Bitter	PI 473360	Bolivia. La Paz
S. boliviense	*S. megistacrolobum*	PI 500029	Argentina. Salta
S. boliviense	*S. astleyi* Hawkes and Hjert.	PI 545848	Bolivia. Potosí
S. boliviense	*S. astleyi*	PI 545959	Bolivia. Potosí
S. boliviense	*S. toralapanum*	PI 546009	Bolivia. Cochabamba
S. brevicaule Bitter	*Solanum* spp.	PI 320341	Bolivia. Cochabamba
S. brevicaule	*Solanum* spp.	PI 473204	Argentina. Jujuy
S. brevicaule	*Solanum* spp.	PI 473215	Argentina. Salta
S. brevicaule	*Solanum* spp.	PI 558119	Argentina. Jujuy
S. brevicaule	*S. sparsipilum* (Bitter) Juz. and Bukasov	PI 246536	Peru. Cuzco
S. brevicaule	*S. spegazzinii* Bitter	PI 472988	Argentina. Tucumán
S. brevicaule	*S. gourlayi* Hawkes subsp. *vidaurrei* (Cárd.) Hawkes and Hjert.	PI 472991	Argentina. Jujuy
S. brevicaule	*S. incamayoense* K.A. Okada and A.M. Clausen	PI 473060	Argentina. Salta
S. brevicaule	*S. incamayoense*	PI 473067	Argentina. Salta
S. brevicaule	*S. leptophyes* Bitter	PI 473077	Argentina. Salta
S. brevicaule	*S. hondelmannii* Hawkes and Hjert.	PI 473365	Bolivia. Chuquisaca
S. brevicaule	*S. alandiae* Cárd.	PI 498085	Bolivia. Cochabamba
S. brevicaule	*S. alandiae*	PI 498086	Bolivia. Santa Cruz
S. brevicaule	*S. avilesii* Hawkes and Hjert.	PI 498091	Bolivia. Santa Cruz
S. brevicaule	*S. avilesii*	PI 498092	Bolivia. Santa Cruz
S. brevicaule	*S. sparsipilum*	PI 498285	Bolivia. Potosí

TABLE 1. (Continued)

Taxon (Spooner et al., 2014)	Taxon (Hawkes, 1990; Ochoa, 1990, 1999)[a]	Accession[b]	Location[c]
S. brevicaule	*S. gourlayi*	PI 537026	Bolivia. Potosí
S. brevicaule	*S. leptophyes*	PI 545865	Bolivia. Potosí
S. brevicaule	*S. hondelmannii*	PI 545879	Bolivia. Cochabamba
S. brevicaule		PI 545968	Bolivia. Cochabamba
S. brevicaule		PI 545970	Bolivia. La Paz
S. brevicaule	*S. leptophyes*	PI 545978	Bolivia. Chuquisaca
S. brevicaule	*S. leptophyes*	PI 545997	Bolivia. Potosí
S. candolleanum P.Berthault[d]	*S. blanco-galdosii*	PI 442701	Peru. Cajamarca
S. candolleanum	*S. brevicaule*	PI 473451	Peru. Ayacucho
S. candolleanum	*S. multiinterruptum* Bitter	PI 275272	Peru. Cuzco
S. candolleanum	*S. ambosinum* Ochoa	PI 365362	Peru. Ancash
S. candolleanum	*S. pampasense* Hawkes	PI 442697	Peru. Apurímac
S. candolleanum	*S. marinasense* Vargas	PI 458380	Peru. Cuzco
S. candolleanum	*S. pampasense*	PI 458381	Peru. Ayacucho
S. candolleanum	*S. bukasovii* Juz.	PI 458403	Peru. Apurímac
S. candolleanum	*S. bukasovii*	PI 458404	Peru. Apurímac
S. candolleanum	*S. multidissectum* Hawkes	PI 473352	Peru. Puno
S. candolleanum	*S. canasense* Hawkes	PI 473355	Peru. Ayacucho
S. candolleanum	*S. ambosinum*	PI 473492	Peru. Huancavelica
S. candolleanum	*S. ambosinum*	PI 498213	Peru. Ancash
S. candolleanum		PI 498227	Bolivia. La Paz
S. candolleanum	*S. marinasense*	PI 498254	Peru. Cuzco
S. candolleanum		PI 545972	Bolivia. La Paz
S. candolleanum	*S. achacachense* Cárd.	PI 558032	Bolivia
S. candolleanum	*S. bukasovii*	PI 568954	Peru. Puno
S. candolleanum	*S. orophilum* Correll	PI 590894	Peru. Ancash
S. candolleanum		PI 607890	Peru. Apurímac
S. amayanum	*S. candolleanum*	PI 607896	Peru. Apurímac
S. chacoense Bitter	*S. yungasense* Hawkes	PI 614703	Bolivia. La Paz
S. chacoense		PI 472830	Argentina. La Rioja
S. chacoense		PI 500020	Argentina. Jujuy
S. chacoense	*S. yungasense*	PI 614713	Bolivia. La Paz
S. ×*doddsii* Correll		PI 442690	Bolivia. Cochabamba
S. ×*doddsii*		PI 545854	Bolivia. Cochabamba
S. ×*doddsii*		PI 545861	Bolivia. Cochabamba
S. gandarillasii Cárd.		PI 265866	Bolivia. Cochabamba
S. infundibuliforme Phil.		PI 472857	Argentina. Jujuy
S. kurtzianum Bitter and Wittm.		PI 472924	Argentina. Mendoza
S. kurtzianum		PI 472952	Argentina. Catamarca
S. laxissimum Bitter		PI 498252	Peru. Junín
S. lignicaule Vargas		PI 473351	Peru. Cuzco
S. limbaniense Ochoa		CIP 762824	Peru. Puno
S. maglia Schltdl.		PI 558316	Chile. Valparaiso
S. malmeanum Bitter		PI 458318	Argentina. Corrientes
S. malmeanum		PI 472841	Argentina. Corrientes
S. malmeanum		PI 498416	Argentina. Santa Fé
S. medians Bitter		PI 210045	Peru. Lima
S. medians		PI 320260	Peru. Lima
S. medians		PI 458402	Peru. Lima
S. medians		PI 473496	Peru. Huánuco
S. medians		PI 607894	Peru. Arequipa
S. medians	*S. sandemanii* Hawkes	PI 607895	Peru. Arequipa
S. microdontum Bitter		PI 545884	Bolivia. Cochabamba
S. neorossii Hawkes and Hjert.		PI 473201	Argentina. Salta
S. neorossii		PI 473529	Argentina
S. okadae Hawkes and Hjert.		PI 458368	Argentina. Salta
S. okadae		PI 498064	Bolivia. La Paz
S. okadae		PI 498065	Bolivia. La Paz
S. okadae		PI 500061	Argentina. Salta
S. raphanifolium Cárd. and Hawkes		PI 265862	Peru. Cuzco
S. ×*rechei* Hawkes and Hjert.		PI 558227	Argentina. La Rioja

TABLE 1. (Continued)

Taxon (Spooner et al., 2014)	Taxon (Hawkes, 1990; Ochoa, 1990, 1999)[a]	Accession[b]	Location[c]
S. ×*rechei*		PI 587112	Argentina. La Rioja
S. tuberosum L. *Andigenum* group		PI 195188	Peru. Ayacucho
S. tuberosum L. *Andigenum* group		PI 230512	Peru. Amazonas
S. tuberosum L. *Andigenum* group		PI 230513	Peru. Loreto
S. tuberosum L. *Andigenum* group		PI 234011	Bolivia
S. tuberosum L. *Andigenum* group		PI 458393	Bolivia. La Paz
S. velardei Ochoa		PI 619114	Peru. Apurímac
S. venturii Hawkes and Hjert.		PI 558224	Argentina. Catamarca
S. vernei Bitter and Wittm.		PI 320332	Argentina. Catamarca
S. vernei		PI 320333	Argentina. Jujuy
S. vernei		PI 500070	Argentina. Salta
S. vernei		PI 558150	Argentina. Jujuy
S. verrucosum Schltdl.		PI 161173	Mexico. Michoacán
S. verrucosum		PI 275256	Mexico. Michoacán
S. violaceimarmoratum Bitter		CIP 760331	Bolivia. La Paz

[a]No entry in this column indicates this accession was identified the same by these different taxonomic treatments.
[b]The 6-digit Plant Introduction (PI) and CIP numbers are permanent numbers assigned to germplasm accessions in the National Plant Germplasm System (NPGS) and the International Potato Center, respectively.
[c]More complete locality data for the NPGS accessions can be found at the Germplasm Resources Information System (GRIN) website at http://www.ars-grin.gov/) and for the CIP accessions at https://cipotato.org/genebank/.
[d]Ochoa (1999) indicated that this accession, originally introduced by him to the U.S. Potato Genebank as *S. blanco-galdosii* Ochoa, is *S. chaucha* Juz. and Bukasov, a triploid potato cultivar. It groups in our results with *S. candolleanum*, and the voucher appears as this species.

Phylogeny reconstruction

We rooted our trees with *S. etuberosum* and *S. fernandezianum* as did Spooner et al. (1993). Question marks and blank spaces were treated as missing data and gaps, respectively. For maximum parsimony (MP) using the program PAUP* version 4.0a147 (Swofford, 2002), all characters were treated as unordered and weighted equally (Fitch, 1971). The most parsimonious trees were found using a heuristic search (Farris, 1970) by generating 100,000 random-addition sequence replicates and one tree held for each replicate. Branch swapping used tree–bisection–reconnection (TBR) retaining all most parsimonious trees. Then, we ran a final heuristic search of the most equally parsimonious trees from this analysis using TBR and MULPARS. Bootstrap values (Felsenstein, 1985) were estimated using 1000 replicates setting maxtrees at 1000 and using TBR and MULPARS.

Species trees combine different accessions of what you assign as a species into a single terminal. To obtain species trees, we used a method implemented in the software SVDquartets (Chifman and Kubatko, 2014), which was executed in PAUP* version 4.0a147 (Swofford, 2002). This method assumes each SNP has its own genealogical history and relationships among quartets of taxa are inferred under the coalescent model (Rannala and Yang, 2003). To conduct these analyses, we evaluated all possible quartets including 100 nonparametric bootstrap replicates. The resulting trees were viewed in FigTree version 1.4.0. (http://tree.bio.ed.ac.uk/software/figtree/). We applied the Tree Incongruence Checking in R (TICR) goodness-of-fit test (Stenz et al., 2015) to the species tree estimated by SVDQuartets to infer the cause of low taxonomic resolution and discordance in the cultivated potato clade. Due to computational burden, we reduced the data set to 89 diploid species accessions in 24 species to maximize potentially hybridizing species in the cultivated species clade (1 from clade 1+2, 1 from clade 3, and 22 from the cultivated potato clade). First, for each gene, we estimated the single-gene posterior distribution using MrBayes version 3.2.2 (Ronquist and Huelsenbeck, 2003). We used a HKY+I+G model as suggested by jModelTest 2.1.10, three independent runs, and each run with three chains with the heated chain at temperature 0.40 and swapping attempts every 10 generations. Five million generations were used with 25% burnin, sampled every 2500 generations. Runs were checked to reach the targeted standard deviation of split frequencies less than 0.05. Bayesian concordance analysis (Ané et al., 2007) was used to estimate the concordance factors of each of the 11,716,640 four-taxon subsets for the 131 taxa. This analysis was conducted in BUCKy 1.4.4 (Larget et al., 2010) with 1 million post-burnin generation and alpha=1, default values on the TICR pipeline (Stenz et al., 2015). Third, we estimated branch lengths in coalescent units in the species tree with maximum pseudolikelihood estimation (Solís-Lemus and Ané, 2016). Finally, we performed the TICR test (Stenz et al., 2015) to evaluate whether the observed quartet concordance factors match the expectations generated under the species tree model. We used the outlier quartets (quartets whose concordance factors do not match the species tree model) to identify species whose relationship could be better explained by hybridization. Finally, we used two species networks methods to explicitly estimate hybridization events: SNaQ (Solís-Lemus and Ané, 2016) and HyDe (Kubatko and Chifman, 2015).

RESULTS

Phylogenetic analyses

The aligned length of alleles A and B aligned one by one together varied from 462 (primer C2At1g32130) to 2647 (primer C2At2g38020), and average fragment alignment length was 1220 (Table 2). The aligned length of data set allele A was 14,072 characters and of allele set B 14,092 characters. Maximum parsimony analyses of allele set A produced 64 equally parsimonious 10,118-character trees with 2171 parsimony informative characters, consistency index 0.4925 and 0.3563, with and without autapomorphies, respectively, retention index of 0.6570 and rescaled consistency index of 0.3236. Maximum parsimony analyses of allele set B produced 72 equally

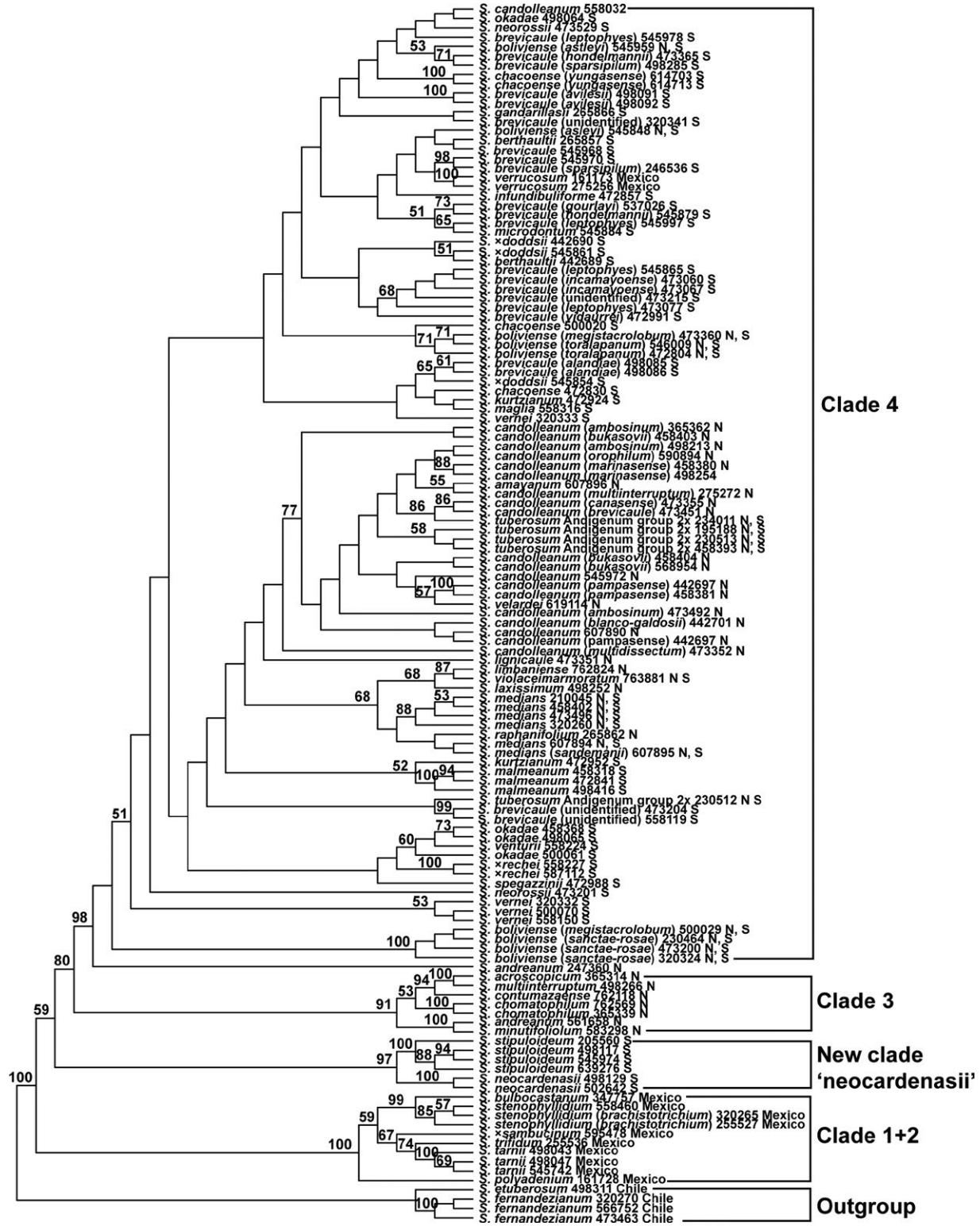


FIGURE 1. Maximum parsimony cladogram of the 131 diploid species accessions examined using allele set A with bootstrap values over 50% overlaid over the branches, with the major clades indicated. The first name of the terminals represents the taxonomy of Spooner et al. (2014); the epithet in parentheses represents the name they synonymize relative to Hawkes (1990) or Ochoa (1999). The terms Mexico, Chile, N, and S represent distributions of the species, with N representing areas in South America from Peru northward, and S representing areas in South America from Bolivia southward. Some widespread species occur in both northern and southern areas (*S. boliviense*, *S. tuberosum* Andigenum group, *S. violaceimarmoratum*), and individual accessions are listed in both areas to highlight this, but Table 1 lists their specific locality data.

TABLE 2. Primers for the 12 conserved orthologous sequences examined in this study.

Marker	Lab ID	Forward 5′→3′	Reverse 5′→3′	Asymmetric PCR primer (SSCP)	Aligned length of alleles A and B aligned together
C2At1g13380	1C	AGGTGCTTTCTTGTTTCTTCTTTC	AGAGCATATCACGATACTTGGTGTG	No SSCP	979
C2At5g14320	11A	TTCTCTTTCCCTTATCTGCAACAC	TCCTTCAATCATGTACTTAGAGACTTC	No SSCP	754
C2At1g32130	3A	TCAACAAGAGTACACGGTTTGAAGAC	TTGCTCTAGCCCTGGCCCTAAC	No SSCP	462
C2At1g20050	3C	ATGATCTAAAATTGCCTGGTTTTG	AATAGCCCTCAAGGACCATGTGG	No SSCP	1254
C2At2g38020	9A	TGCAGCTTTGCTTTATGATGCC	AAAGGCTTGGCCGTAGCTTGC	No SSCP	2647
C2At1g44760	dom11	GGATTTAACAATATGTGTGGAGGAG	TTTTTCTGACCCAAGACTAACACAG	Reverse	1295
C2At1G77470	dom15	GGCGGAGACAATCTCGTTAAT	AATAGTACTAGCTTCACCGCTGACT	Forward	764
C2At2G24090	dom16	AGCTTCAATGACTCTAACAATGGC	AGCTCTCCTCCTCATTATCTTCC	Forward	1221
C2At2G37500	dom19	TATATCAATGTCAAAAGAGGCATCA	GATATGGCATCTACATCACAAGTGA	Reverse	1243
C2At5G64730	dom37	GGACAATTCAAAACTTTGTTCATGT	TTCCATCAACACTTCCAGCAATTAT	Forward	1925
C2At4g02680	dom43	CCATGAGGAGTTTACAGTTGGC	GCCTTCTCAATCAGTTTGGTCA	Reverse	1076
C2At2g06530	f20	AAGGTGTCTCCCTCAGAATTCAG	ATCTGTCCCATTGCCTTTGTAAC	Reverse	1016

parsimonious 10,038-length trees with 2159 parsimony informative characters, consistency index 0.5018 and 0.3610, with and without autapomorphies, respectively, retention index of 0.6610 and rescaled consistency index of 0.3320. The strict consensus tree of allele set A is shown in Fig. 1 and allele set B in Appendix S1 (see Supplemental Data with this article). Both trees are nearly identical in 1) recovering the same three clades containing species as in prior nuclear ortholog results (1+2, 3, and the cultivated potato clade), but recovering a new clade neocardenasii (containing the species *S. neocardenasii* and *S. stipuloideum*) situated as sister to clades 3+4; 2) placing one accession of *S. andreanum*, PI 247360, outside clade 3, sister to clade 4 in the A tree or nested within the cultivated potato clade in the B tree and, and 3) having an identical topology and good phylogenetic structure (as assessed by high bootstrap values and well-resolved branches) in clades 1+2, neocardenasii and 3, but drastically reduced phylogenetic structure (as assessed by polytomies, low bootstrap support, and the failure of all species to resolve in species-specific clades) in the internal branches of the cultivated potato clade.

There are many points of topological incongruence between the trees from alleles A and B within the cultivated potato clade (clade 4; Fig. 1, Appendix S1). Considering the low support (bootstrap values) in this clade, this is essentially soft incongruence. However, there are some interesting similarities between the A and B trees: (1) *Solanum candolleanum* and *S. brevicaule*, described above as largely confined in a "northern" area from central to southern Peru and "southern" area in Bolivia and Argentina, respectively, are separated in different clades. However, some widespread species occur in both northern and southern areas (*S. boliviense*, *S. tuberosum* Andigenum group, *S. violaceimarmoratum*), but individual accessions are listed in both areas to highlight this; Table 1 lists their specific locality data. New to this study, however, is showing that this north–south geographic separation also extends to the majority of the species in the cultivated potato clade, not just *S. brevicaule* and *S. candolleanum*. (2) All accessions of *S. candolleanum* form a clade, along with four of the five accessions of the cultivated species *S. tuberosum*. (3) Most of the accessions of *S. brevicaule* resolve into the southern portion of clade 4, but are sometimes intermixed with other species. (4) Both accessions of the Mexican species *S. verrucosum* resolve with the southern members of the cultivated potato clade.

The species trees constructed with SVD quartets using all accessions and all sequences and using the new names (Spooner et al., 2014; Fig. 2) and old names (Appendix S2) recovered the same four clades as the MP analyses, but placed *S. andreanum* sister to the cultivated potato clade, making clade 3 a grade. Like the MP analyses, the exclusively southern members form a clade (except for those species distributed across the north and south boundary), but the northern do not. Bootstrap support values of the species trees are increased relative to the MP analyses.

To study whether hybridization is the cause of the taxonomic problems in the cultivated potato clade, we performed a goodness-of-fit test on the species tree (taxonomy of Spooner et al., 2014) estimated by SVDQuartets. The TICR test (Stenz et al., 2015) is a nonparametric test that evaluates whether the species tree adequately explains the observed quartet concordance factors, estimated with BUCKy (Larget et al., 2010). The null hypothesis is rejected if too many quartet concordance factors do not follow the pattern expected by incomplete lineage sorting (ILS). In this case, potential hybridization events could explain the relationships between species. Some of the branch lengths in the cultivated potato clade were estimated being close to zero, which suggests that polytomies could fit the data better than the fully resolved species tree. In particular, branches leading to species *S. candolleanum*, *S. laxissimum*, *S. limbaniense*, *S. raphanifolium*, *S. tuberosum*, and *S. violaceimarmoratum* were estimated to be close to zero, so a polytomy of these species fits the observed concordance factors better.

The null hypothesis of the TICR test was rejected with *P* value of 0.00007, meaning that the species tree model (ILS) does not fully explain the observed quartet concordance factors, suggesting the presence of hybridization. Species were identified as potentially involved in hybridization if they belonged to the outlier quartets (quartets whose concordance factors do not match the expectations within the species tree model). The outlier analysis identified several species in the cultivated potato clade, such as *S. boliviense*, *S. lignicaule*, *S. medians*, *S. microdontum*, *S. tuberosum*, and *S. verrucosum*, as potentially involved in hybridization events.

To explicitly estimate the hybridization events, we used SNaQ (Solís-Lemus and Ané, 2016), which is a species network estimation method. Given the computational complexity of network methods, we further reduced the data set to 12 species in the cultivated potato clade: *S. berthaultii*, *S. boliviense*, *S. brevicaule*, *S. chacoense*, *S. doddsii*, *S. gandarillasii*, *S. infundibuliforme*, *S. lignicaule*, *S. medians*, *S. microdontum*, *S. neorosii*, and *S. verrucosum* and estimated a network with one hybridization event. We did not find enough signal in the 12 nuclear orthologs to estimate the hybridization event, which is not surprising, based on the simulations of Solís-Lemus and Ané (2016).

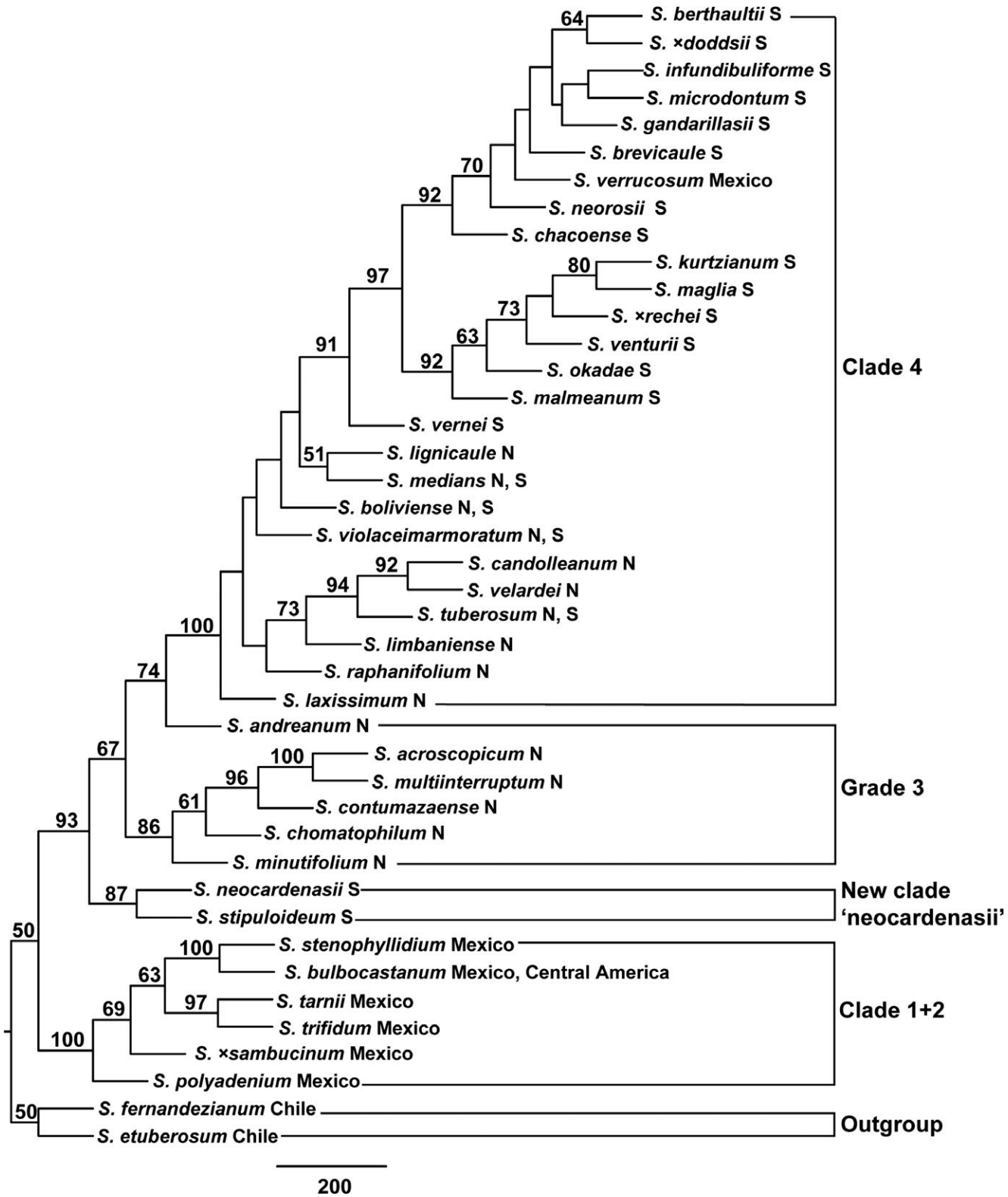


FIGURE 2. Species tree constructed using SVD quartets using both alleles A and B of the 131 accessions of 42 species using the taxonomy of Spooner et al. (2014), with bootstrap values overlaid over the branches, with the major clades indicated. The terms Mexico, Chile, N and S represent distributions of the species, with N representing areas in South America from Peru northward, and S representing areas in South America from Bolivia southward. Some widespread species occur in both northern and southern areas (*S. boliviense*, *S. tuberosum* Andigenum group, *S. violaceimarmoratum*), and individual accessions are listed in both areas to highlight this, but Table 1 lists their specific locality data.

Alternatively, we used HyDe (Kubatko and Chifman, 2015) on the full data set (131 diploid species accessions in 42 species). This program identifies which taxa could have arisen via hybridization and which are their potential parental taxa. However, we again did not find any evidence of hybrid origin for any of the sequences using *S. etuberosum* as outgroup, at a level of 0.05, corrected by Bonferroni as 0.00000381971.

In conclusion, the SVDQuartets species tree (ILS assumption) is rejected by the TICR test, which suggests potential hybridization between species in the group. However, we do not have enough signal with the data at hand to explicitly estimate such hybridization events with species networks methods such as SNaQ or HyDe.

DISCUSSION

Bootstrap supports

There is a noticeable discrepancy in the bootstrap support of the internal nodes between the concatenation analyses (Fig. 1) in clade 4 and the species tree analyses with SVDQuartets (Fig. 2), with most clades in the species tree having higher support. The reason is that concatenation analyses ignore ILS, so low support values could actually represent the presence of ILS. On the contrary, SVDquartets accounts for ILS in the model, so the bootstrap support values have taken into account ILS, and low values can reflect low support instead of uncertainty due to ILS.

Taxonomic problems in section Petota

The assumption that ILS solely explains the gene tree discordance on the species tree estimated by SVDQuartets (Chifman and Kubatko, 2014) is rejected by the TICR goodness-of-fit test (Stenz et al., 2015). This result suggests that the evolutionary history of the group involves some potential hybridization events. However, it is known that species network estimation demands more informative data than does species tree estimation (see Solís-Lemus and Ané, 2016), and currently, we do not have enough signal with the 12 nuclear orthologs to explicitly estimate the hybridization events.

The taxonomy of section *Petota* has been notoriously difficult and fraught with problems of species delimitation and varying hypotheses of interspecific relationships. Experimental studies document the ease of artificial interspecific hybridization in section *Petota* through hundreds of successful artificial interspecific hybrids (e.g., Bukasov and Kameraz, 1959). Introgression and interspecific hybridization not leading to speciation have been believed to be common in the section (Hawkes, 1962). Hawkes and Hjerting (1969) interpreted 9.5% of the wild potato specimens they examined for the flora of Argentina, Brazil, Paraguay, and Uruguay to be interspecific hybrids, and Hawkes and Hjerting (1989) and Ochoa (1999) provided extensive lists of natural and artificial interspecific hybrids for Bolivia. Rabinowitz et al. (1990) tested hypotheses of gene flow between the diploid wild species *S. candolleanum* (as *S. sparsipilum*) and the cultivated diploid *S. stenotomum* (=*S. tuberosum* Andigenum group). By use of isozyme markers specific to these populations, they were able to document high levels of gene flow in experimental field plots in the Andes. They used these data to speculate that extensive gene flow occurs among other cultivated and wild species. Clausen and Spooner (1998) reexamined the putative hybrid origin of *S.* ×*rechei*, hypothesized by Hawkes and Hjerting (1969) and Okada and Hawkes (1978) to be of hybrid origin between *S. kurtzianum* and *S. microdontum*. *Solanum* ×*rechei* occurs in the overlap zone of its two parents and has reduced fertility in comparison to natural and artificially constructed hybrids of these species. Additive profiles of nuclear RFLPs strongly support its hybrid origin. Celis et al. (2004) documented, with AFLP markers, the possibility of gene flow from cultivated species to diverse wild species occurring in the Andes. If such hybrid populations would be able to survive in the wild, they provide support to hypotheses of Ugent (1970) who proposed that the cultivated species were formed and genetically enriched subsequent to formation by gene flow from the wild species.

Some studies support an over-description of species in section *Petota*, with the majority of these in the cultivated potato clade. In large-scale AFLP studies, Jacobs et al. (2008, 2011) examined hundreds of accessions throughout the section, and they concluded that many of the species were poorly defined. Similarly, Camadro et al. (2012, p. 542) concluded on the basis of literature surveys, "Hybridization and subsequent gene flow and introgression in sympatric populations [of section *Petota*], within and between ploidy levels, often results in exceedingly complicated patterns of variation." Such hybridization has resulted in a complex aggregation of genotypes, and Mayr's (1969) biological concept of a species is impossible to apply in the section, especially among members of the cultivated potato clade. The cause of these taxonomic problems, whether incomplete lineage sorting or hybridization, at least at the diploid level investigated here is unclear. Like our study, Pease et al. (2016) found evidence for both hybridization and incomplete lineage sorting in the sister clade to section *Petota*, *Solanum* section *Lycopersicum* (wild and cultivated tomatoes). Whatever the ultimate cause of these taxonomic problems in section *Petota*, the majority of these problems occur in the cultivated potato clade, and our results provide evidence that there is greatly reduced phylogenetic structure in this clade, supporting decisions of synonymy made by Spooner et al. (2014).

Hawkes biogeography and the newly discovered neocardenasii clade

Potato species have a strong biological isolating mechanism governed by endosperm breakdown. They have been assigned endosperm balance numbers (EBN) based solely on their ability to hybridize with each other (Hanneman, 1994). Successful hybridization is expected when male and female gametes have matching EBN, regardless of ploidy. Viable seeds will be produced from crosses between plants with matching EBN values, irrespective of ploidy, as long as other hybridization barriers are absent. EBN numbers are empirically determined through artificial interspecific crosses and cytological examinations with EBN testers of known ploidy and EBN, and evidenced by endosperm death in EBN incompatible crosses. Ploidy(EBN) combinations in potato include 2*x*(1EBN), 2*x*(2EBN), 4*x*(2EBN), 4*x*(4EBN), and 6*x*(4EBN). All of the diploid members of clade 1+2 are 2*x*(1EBN) with white stellate corollas. Different species of clade 3 are 2*x*(1EBN) and others 2*x*(2EBN) with a variety of corolla shapes and colors (a few stellate and white), and most of the diploid members of the cultivated potato clade are 2*x*(2EBN) with corollas white to blue to purple, but most species with rotate to rotate-pentagonal corollas.

Hawkes (1988, 1990) proposed that wild potato species originated in Mexico as 2*x*(1EBN), with stellate corollas, migrated to South America, and evolved to species with pentagonal to rotate

corollas and 2*x*(2EBN). *Solanum neocardenasii* and *S. stipuloideum* of the neocardenasii clade, unlike the other species in clade 1+2 are from South America, not from North or Central America. Like these species, they both possess white stellate corollas; *S. stipuloideum* is 2*x*(1EBN) and *S. neocardenasii* is 2*x* but of unknown EBN. The neocardenasii clade is sister to clade 1+2. Interestingly, both of its species are members of the cultivated potato clade based on plastid data, showing big discrepancy of the present nuclear data and suggesting a history of chloroplast capture (Spooner and Castillo, 1997).

One interpretation of these phylogenetic, EBN, and morphological results is that *S. neocardenasii* and *S. stipuloideum* represent the species, or their closest extant relatives, most closely related to these Mexican and Central American migrants, gained the plastid of a member of the cultivated potato clade through plastid capture event. Further tests of this interpretation, and of the synonymy proposed by Spooner et al. (2014) relative to prior taxonomic treatments, will come from examination of more accessions and additional data from next generation sequencing data such as genotyping by sequencing (GBS) or resequencing data.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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