True Black nightshades: Phylogeny and delimitation of the Morelloid clade of *Solanum*

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DOI http://dx.doi.org/10.12705/645.5

Abstract The Morelloid clade of *Solanum*, also known as the black nightshades or Maurella (Morella), is one of the 10 major clades within *Solanum*. The pantropical clade consists of ca. 76 non-spiny herbaceous and suffrutescent species with simple or branched hairs with or without glandular tips, with a centre of distribution in the tropical Andes. Although the core members of the clade have long been recognised, complete circumscription of the Morelloid clade has remained elusive. Here we provide the first detailed molecular study of the clade. Plastid (*trnT-F* intergenic spacers), nuclear ribosomal ITS, and low-copy nuclear (*waxy*) data show three of the four previously unplaced taxa loosely associated with the Morelloids to be placed outside (*S. anomalostemon*, *S. valdiviense*, *S. reductum*), whilst *S. salicifolium*, a species previously associated with the Dulcamaroid clade, is found to be nested within the group. Four monophyletic groups within the Morelloids can be recognised: (1) Black nightshade clade, including all members of *S.* sect. *Solanum*, *S.* sect. *Campanulisolanum*, branched-haired taxa, *S. salicifolium*, and *S. triflorum*; (2) Episarcophyllum clade, including most members of *S.* sect. *Episarcophyllum*; (3) Chamaesarachidium clade, including members of *S.* sect. *Chamaesarachidium*; and (4) Radicans clade, including four species from *S.* sect. *Parasolanum*. Incongruence between nuclear and plastid gene trees is discussed in relation to known ploidy level variation across the Morelloid clade, especially the African species often referred to as the *S. nigrum* complex. A new, revised circumscription of the Morelloid clade is provided together with morphological characterisations of the monophyletic clades recovered within it. A preliminary checklist of accepted names for Morelloids is also presented.

Keywords Andes; anther morphology; incongruence; molecular phylogenetics; plant systematics; Solanaceae; South America; unplaced taxa

Supplementary Material Electronic Supplement (Figs. S1–S6) and DNA sequence alignments are available in the Supplementary Data section of the online version of this article at http://www.ingentaconnect.com/content/iapt/tax

■ INTRODUCTION

Solanum L., with approximately 1400 species, is one of the largest genera of flowering plants (Frodin, 2004). The genus poses a taxonomic challenge not only due to its large size, but also due to the large number of published names, many of which are associated with the cultivated and widespread species of the genus, including the potato (*S. tuberosum* L.), tomato (*S. lycopersicum* L.), and the eggplant (*S. melongena* L.) (http://solanaceaesource.org/). Recent taxonomic and molecular systematic efforts (http://www.solanaceaesource.org) have helped to identify major clades within *Solanum* (e.g., Weese & Bohs, 2007), clarify relationships and the monophyly of previously recognised morphological sections (e.g., Stern & al., 2011), and to provide taxonomic revisions for major clades with keys for species identification (e.g., Knapp, 2013).

The Morelloid clade of *Solanum* is amongst the 10 robustly supported major clades within *Solanum* (Bohs, 2005; Weese & Bohs, 2007). The clade, also known as the black nightshades or Maurella (Morella), consists of ca. 76 non-spiny herbaceous and suffrutescent species with simple or branched hairs with or without glandular tips and inflorescences usually arising from the internodes (Fig. 1; Bohs, 2005). Ploidy level within the group varies from diploid to hexaploid (e.g., Edmonds & Chweya, 1997; Moyetta & al., 2013). The Morelloid clade includes five morphological groups traditionally recognised at the sectional level (Fig. 1): the Andean sections *Episarcophyllum* Bitter, *Campanulisolanum* Bitter, and *Chamaesarachidium* Bitter, the pantropical sect. *Solanum*, and a group comprising some of the members of sect. *Parasolanum* A.Child (Bohs, 2005). Section *Solanum* is the largest of these, and includes the seemingly intractable *S. nigrum* complex that comprises

Received: 14 Jul 2014 | returned for (first) revision: 7 Jan 2015 | (last) revision received: 11 May 2015 | accepted: 13 May 2015 || publication date(s): online fast track, 30 Sep 2015; in print and online issues, 28 Oct 2015 ||

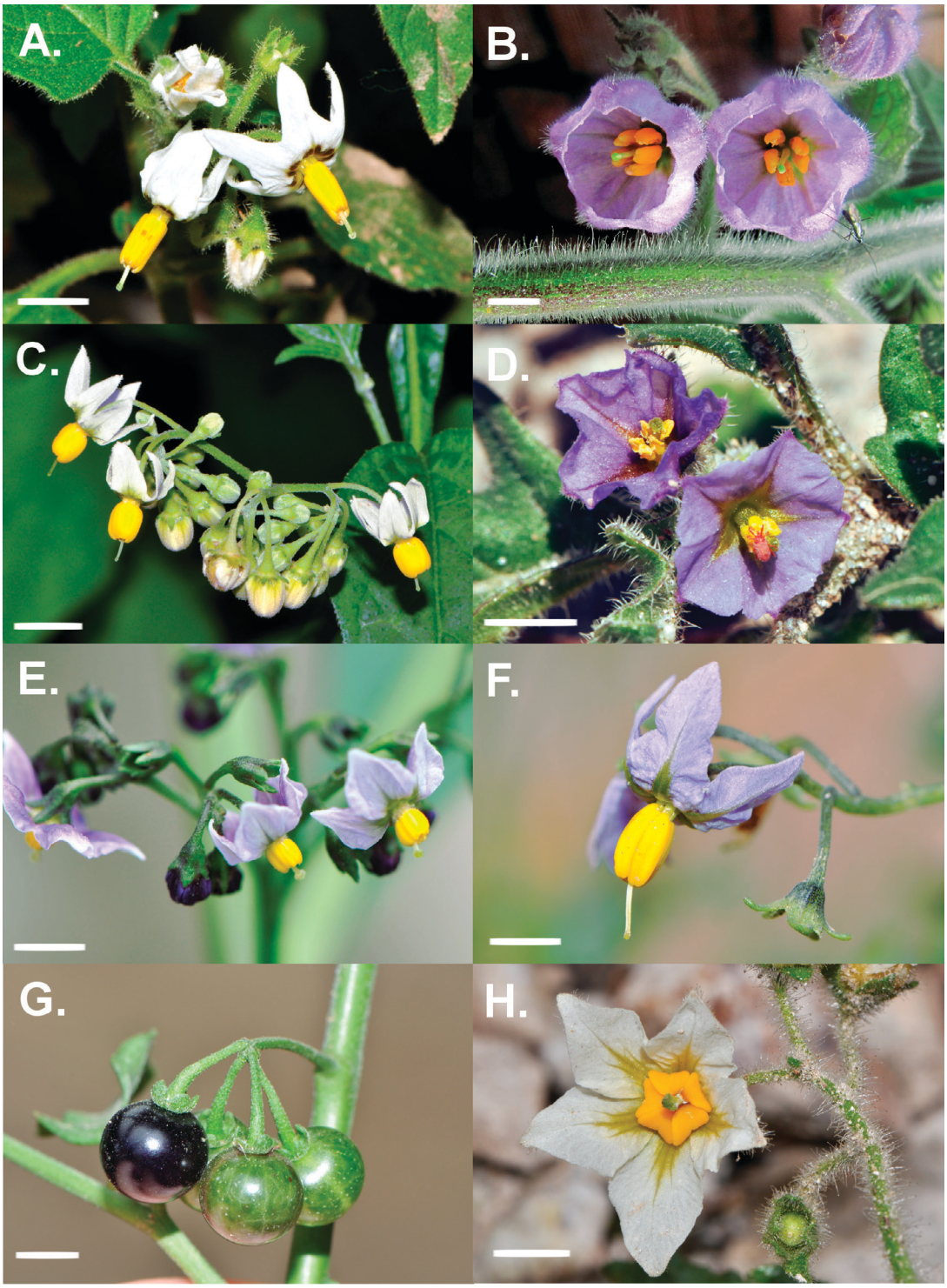

Fig. 1. Morphology of the Morelloid clade **(A–G)** and *Solanum anomalostemon* S.Knapp & M.Nee **(H)**, a postulated relative of the clade. **A,** Typical flowers of *S.* sect. *Solanum*, with stellate corollas and strongly reflexed lobes (*S. glandulosipilosum* Bitter, *Barboza & al. 3565*); **B,** campanulate corollas of *S.* sect. *Campanulisolanum* (*S. sinuatiexcisum* Bitter, *Barboza & al. 3664*); **C,** a typical, relatively condensed inflorescence of *S.* sect. *Solanum* (*S. zuloagae* Cabrera, *Barboza & al. 3569*); **D,** minute, rotate corollas of *S.* sect. *Chamaesarachidium* endemic to high-elevation Andes (*S. weddellii* Phil., *Barboza & al. 3475*); **E,** typical small flowers with capitate stigmas found in *S.* sect. *Parasolanum* (*S. corymbosum* Jacq., *Gonzáles & al. 2860*); **F,** flowers with slightly unequal and/or curved stamens typical of *S.* sect. *Episarcophyllum* endemic to high-elevation Andes (*S. echegarayi* Hieron, *Knapp & al. 10540*); **G,** typical fruits of the Morelloid clade, arising from relatively condensed, simple to furcate inflorescences positioned between leaf nodes, maturing green, purple, black, yellow or orange-red (*S. americanum* Mill., *Knapp & al. 10210*); **H,** flowers of *S. anomalostemon* showing the distinct stamen morphology unique within the genus (*Knapp & al. 10353*). — Scale bars = 5 mm. Photos by T. Särkinen, S. Knapp & G. Barboza.

a set of widespread and morphologically variable species native to the Old World, including the European black nightshade *S. nigrum* L. (type of *Solanum*), the American black nightshade *S. americanum* Mill., the southern European *S. villosum* Mill., and a set of cultivated species such as *S. retroflexum* Dunal and *S. scabrum* Mill. (Edmonds & Chweya, 1997).

Prior to recent molecular studies, the Morelloid clade was not recognised as a natural group. The name for the clade is derived from Dunal's (1852) infrageneric group Morella (Maurella) published as part of the first (and last) monographic treatment of the genus *Solanum*. Dunal's (1852) concept of Morella was narrow, however, where he recognised members of *S.* sect. *Solanum* only, and did not include members of the other sections later found related based on molecular phylogenetics (Bohs, 2005; Weese & Bohs, 2007). Within Morella, Dunal recognised two groups based on inflorescence position, Morellae spuriae (6 spp.) and Morellae verae (54 spp.). The concept of Morella remained obscure and loose during most of the last century, where many herbaceous non-spiny taxa where treated as members of the group, resulting in a total of 594 names associated with the Morelloid clade. Many of these names do not belong to species considered members of the clade as now recognised based on phylogenetic data (Bohs, 2005; Weese & Bohs, 2007). An extreme example of this is *S. stipuloideum* Rusby from the morphologically distinct Potato clade of *Solanum* (Spooner & Knapp, 2013), which was originally described as a member of *S.* sect. *Solanum*.

Whilst taxonomic revisions of the smaller sections within the Morelloids have recently been published (sect. *Episarcophyllum* by Del Vitto & Petenatti, 1999; sect. *Chamaesarachidium* by Barboza, 2003, and sect. *Campanulisolanum* by Barboza & Hunziker, 2005), the entire sect. *Solanum* has not been revised since Dunal (1852). General overviews of sect. *Solanum* taxonomy have been published (Edmonds, 1977, 1978, 1979), including geographically focused taxonomic treatments (Africa by Edmonds & Chweya, 1997; Olet, 2004; Manoko, 2007; Edmonds, 2012; North America by Schilling, 1981; South America by Edmonds, 1972) and detailed cytological and morphological studies (Edmonds, 1982, 1983, 1984; Venkateswarlu & Roo, 1972). These studies have greatly enhanced our understanding of the complex morphological and ploidy level variation present in the group, but much taxonomic work remains to be done within the Morelloids especially so in South America, where over half of the known Morelloid species are found (Barboza & al., 2013). As new species are being described (Manoko & al., 2012; Särkinen & al., 2013a, 2015a, b), a robust circumscription of the clade is needed in order to aid ongoing efforts for the production of a full monographic treatment of the group. Such a treatment will need to incorporate molecular systematic work to fully understand the origin of the polyploid species and species delimitation in complex taxonomic groups within the clade (e.g., Dehmer & Hammer, 2004; Manoko, 2007; Manoko & al., 2007; Poczai & Hyvönen, 2011b).

Here we aim to provide a first detailed molecular phylogenetic study of the Morelloid clade. The study aims to (1) confirm molecular circumscription of the Morelloid clade, (2) establish the position of previously unplaced taxa within clade I of *Solanum* (sensu Särkinen & al., 2013b), and (3) test for the monophyly of the traditionally recognised morphological sections within the Morelloid clade. Previous studies have sampled representatives of all five morphological sections of the Morelloid clade with nine species (Weese & Bohs, 2007). Here we sample 43 species of the total ca. 76 species (57 %) representing all five morphological sections for plastid (*trnT-F*), nuclear ribosomal (ITS) and low-copy nuclear (*waxy*) markers to test for the monophyly and internal relationships within the Morelloids. We include four species with uncertain phylogenetic placement (*S. reductum* C.V.Morton, *S. anomalostemon* S.Knapp & M.Nee, *S. salicifolium* Phil. [including its synonym *S. incisum* Griseb.], *S. valdiviense* Dunal) and a broad set of outgroups to fully explore the delimitation of the Morelloid clade. The plastid and nuclear topologies are compared to explore possible incongruences between gene trees due to potential hybrid origin of taxa and known ploidy level variation and allopolyploid species origins within the group (Edmonds, 1977). The phylogeny is used to establish a preliminary checklist of accepted species names for the clade, and to propose names and morphological characterisations for monophyletic groups within the Morelloids.

■ MATERIALS AND METHODS

Taxon sampling. — A total of 43 of the total ca. 76 species of the Morelloid clade of *Solanum* were sampled (Appendices 1 & 2). The samples included both species of *S.* sect. *Campanulisolanum*, all four species of *S.* sect. *Chamaesarachidium*, all four species of *S.* sect. *Episarcophyllum* (Barboza & al., 2013), and all five species of *S.* sect. *Parasolanum* that are known to lie within the Morelloid clade (*S. corymbosum* Jacq., *S. palitans* C.V.Morton, *S. radicans* L.f., *S. tripartitum* Dunal, *S. triflorum* Nutt.; Bohs, 2005; Appendices 1 & 2). A total of 34 of the 65 species (52%) from *S.* sect. *Solanum* were sampled, including eight species belonging to the African *S. nigrum* complex (Edmonds & Chweya, 1997), two of the three Asian species, and the two North American native species. A broad set of outgroups were included in order to allow for testing the placement of previously ambiguous taxa that have been suggested to have affinity to the Morelloid clade, including *S. salicifolium*, *S. anomalostemon*, *S. valdiviense*, and *S. reductum* (Appendix 2). Outgroups included 48 species representing all of the major clades of *Solanum* and the subclades of clade I of *Solanum* (as defined by Särkinen & al., 2013b, including Regmandra, Potato, African Non-Spiny, Normania, Archaesolanum, and Dulcamaroid; Appendices 3 & 4; Weese & Bohs, 2007). Sampling within Morelloids was designed to represent the morphological diversity present in the group across the entire geographic range, as well as within the traditionally recognised sections.

Molecular methods. — Total genomic DNA from silica dried leaves or herbarium material was isolated using a modified CTAB and DNeasy Plant Mini Kit (Qiagen, Venlo, Netherlands) extraction protocol designed to maximise DNA quality and quantity from poor quality templates (Särkinen

& al., 2012). We chose the intergenic plastid region *trnT-F*, nuclear ribosomal internal transcribed spacer (ITS) and the low-copy nuclear gene *waxy* (GBSSI), because these markers have been widely used in molecular phylogenetic studies of *Solanum* (Weese & Bohs, 2007), and a large number of outgroup samples for these regions are hence already available. The regions were amplified using published primers (Table 1). For highly degraded samples, *waxy* was amplified in four parts using primer combinations waxyF+EX4R, EX4F+waxyl171R, waxyl058F+waxy3F, and waxy3NR+waxy2R (Table 1). All PCR reactions were carried out in 25 µl reactions containing ~5–20 ng of DNA template, 1× Buffer, 1 µM of BSA, 1.5 mM of $MgCl_2$, 0.1 mM of each dNTP, 0.2 µM of each primer, and 0.08 U of *Taq* polymerase (Yorkshire Bioscience, Heslington, U.K.). PCR conditions were 94°C for 4 min, 30 cycles of 1 min at 94°C, 1 min at 50°C and 1 min at 72°C, followed by a final extension of 10 min at 72°C. Annealing temperature of 45°C was used for *trnT-F*. PCR products were purified using Promega Wizard SV Gel and PCR Clean-up system (Promega, Madison, Wisconsin, U.S.A.) and sequenced using the PCR primers following Big Dye chemistry on an ABI automated DNA sequencer at the Natural History Museum, London. Consensus sequences from the four strands were assembled using Sequencher v.4.5 (GeneCodes, Ann Arbor, Michigan, U.S.A.). Standard nucleotide ambiguity codes were used to identify all instances where more than one peak was apparent in the chromatogram. Segments with continuous double peaks were deleted and noted as missing data. Alignment was done using ClustalW with default settings as implemented in BioEdit Sequence Alignment Editor v.7.0.9 (Hall, 1999–2007) with manual adjustments. For *trnT-F*, a variable repeat region towards the 5′ end of the intergenic spacer was removed, because the region is known to include putative pseudogenic copies in some species of *Solanum*, although none are known for species within the Morelloid clade (Poczai & Hyvönen, 2011a).

Phylogenetic analyses. — Details of the newly generated sequences, including voucher details, locality information, and GenBank accession numbers, are listed in Appendices 2 & 3. The final alignments of the individual regions included a number of informative indels, but these were not used for the phylogenetic analyses. Bayesian analyses were conducted using MrBayes v.3.1.2 (Huelsenbeck & Ronquist, 2003; Ronquist & Huelsenbeck, 2005). The GTR+G+I nucleotide substitution model was chosen based on the Akaike information criterion as implemented in jModelTest v.2.1.5 (Darriba & al., 2012; Guindon & Gascuel, 2003). Each Markov chain was started from a random tree and ran for up to 10 million generations, with a sampling frequency of 1000. Two independent runs of four chains were run simultaneously with default priors. The initial one million generations were discarded as burn-in samples. Maximum likelihood analyses were conducted with RAxML v.7.8.127 using the GTRGAMMAI model. Bootstrapping was conducted with 1000 replicates to assess clade support. Ten independent runs were done for each dataset with random starting trees and topological robustness was investigated using 1000 non-parametric bootstrap replicates.

Matrix concatenation. — The ITS, *waxy*, and *trnT-F* datasets were analysed separately (Electr. Suppl.: Figs. S1–S6) prior to concatenating them into a combined matrix. Topological discrepancies were carefully analysed by comparing the position of known and suspected polyploids. In two groups well-supported incongruences with branch support > 0.8 in the Bayesian analysis were detected. These included a set of polyploid species (*S. nigrum*, *S. grandidentatum* Phil., *S. furcatum* Dunal, *S. fragile* Wedd., *S. scabrum*), and the Chamaesarachidium clade. In order to run a combined analysis to resolve the position of species where no incongruence was detected, only part of genes were used in these two cases. For the polyploid species, only *waxy* was included, where parts of sequences with consistent double peaks were removed, because *waxy*

Table 1. Details of all the primers used in the study.

Region	Primer	Direction	Primer sequence	Reference
trnT-F	tabA	Forward	CAT TAC AAA TGC GAT GCT CT	Taberlet & al., 1991
	tabC	Forward	CGA AAT CGG TAG ACG CTA CG	Taberlet & al., 1991
	tabD	Reverse	GGG GAT AGA GGG ACT TGA AC	Taberlet & al., 1991
	tabF	Reverse	ATT TGA ACT GGT GAC ACG AG	Taberlet & al., 1991
ITS	its-leu1	Forward	GTC CAC TGA ACC TTA TCA TTT AG	Vargas & al., 1998
	its4	Reverse	TCC TCC GCT TAT TGA TAT GC	White & al., 1990
waxy	waxyF	Forward	CGG GTA ATG ACA ATA TCC CC	Levin & al., 2005
	waxy1171R	Reverse	TCA TAC CCA TCA ATG AAA TC	Levin & al., 2005
	waxy1058F	Forward	ATT CCC TGC TAC TTG AAG TC	Levin & al., 2005
	waxy2R	Reverse	GTT CCA TAT CGC ATA GCA TG	Levin & al., 2005
	waxy3F	Forward	GAT ACC CAA GAG TGG AAC CC	Tepe & al., 2011
	waxy3NR	Reverse	GCC ATT CAC AAT CCC AGT TAT GC	Tepe & al., 2011
	EX4F	Forward	CTA TGG CCC CAA AGC TGG AC	Tepe & al., 2011
	EX4R	Reverse	CAC AAC CTG AAC CTA AG	Tepe & al., 2011

provided the strongest phylogenetic signal within the Morelloids (see below), and had the highest taxon sampling. Because the polyploid species in question are tetra- or hexaploids, both plastid and nuclear data will have issues in terms of multiple parentages, and detailed studies will be needed to fully resolve the origin of these complex polyploids. Sequences of the rest of the polyploids from the *S. nigrum* complex were kept as there was no indication of topological incongruence between markers. For the members of the Chamaesarachidium clade, we kept only the plastid data to reflect the maternal position of the species in the combined analysis.

■ RESULTS

Sequence divergence. — A total of 797 parsimony-informative (PI) characters were recovered for the Morelloids in the concatenated aligned matrix (4515 bp, 18%), of which 99 were from *waxy* (53%), 55 from ITS (29%), and 34 from *trnT-F* (18%). Sequence divergence was highest in ITS in terms of percentage of PI per aligned length of the region (55 PI characters in 716 bp, 8%), compared to *waxy* (99 PI characters in 1879 bp, 2%) and *trnT-F* (34 PI characters in 1920 bp, 2%). Resolution within the Morelloids is highest in the combined tree with four major clades resolved with high branch support (Fig. 2), while individual gene trees, ITS in particular, show poor support (Fig. 3). Because of incongruence issues, and presence of polyploids, we discuss these differences in detail below.

Gene tree incongruence. — Consistent double peaks were observed in parts of *waxy* sequences in 11 accessions, of which 10 are known polyploids (*S. grandidentatum*, *S. florulentum* Bitter, *S. fragile*, *S. furcatum*, *S. nigrum*, *S. scabrum*, *S. opacum* A.Braun & C.D.Bouché, *S. retroflexum* Dunal, *S. tarderemotum* Bitter, *S. umalilaense* Manoko, and *S. villosum* Mill.; Appendix 1; Edmonds & Chweya, 1997; Manoko & al., 2013). No count is available for *S. hirtulum* Steud. ex A.Rich. but the *waxy* sequences strongly suggest the species to be polyploid similar to other members of the *S. nigrum* complex in Africa. Many of the known polyploid species have allopolyploid origins (Edmonds, 1979), meaning that the species would be expected to show incongruent gene tree topologies between the maternally inherited plastid and the biparentally inherited nuclear markers. The consistent double peaks in the bi-parentally inherited *waxy* support the allopolyploid origin of some species, and hence we included only one of these markers in the combined analyses (*waxy*) for the known and suspected allopolyploids to eliminate the effect of gene tree incongruence in the analysis. For these taxa, we kept only *waxy* sequences due to higher level of sequence divergence that would allow us to deliver a better-resolved species-level topology for sect. *Solanum*, where regions with double peaks were excluded prior to analyses. The position of clades/species is carefully discussed below.

Position of uncertain taxa. — The Morelloids are resolved as a monophyletic clade in the combined phylogeny with high branch support (posterior probability [PP] 1.0, bootstrap [BS] 72), with one of the four species with previous noted affinities to the Morelloid clade, *S. salicifolium*, nested within *S.* sect. *Solanum* (Fig. 2). The remaining three species are placed clearly outside the clade. *Solanum valdiviense*, which was treated by Knapp (2013) as a member of the Dulcamaroid clade, is positioned as sister to a clade composed of African Non-Spiny, Normania and Archaesolanum clades within clade I of Särkinen & al. (2013b) with high branch support (PP 1.0, BS 76; Fig. 2). *Solanum reductum* is found to be closely related to members of the Geminata clade in clade II (sensu Särkinen & al., 2013b) with high branch support (PP 1.0, BS 99; Fig. 2). *Solanum anomalostemon* is placed as sister to *S. clandestinum* Bohs and *S. mapiriense* Bitter, two unplaced taxa that have been found to form their own clade at the base of clade II in previous large analyses of *Solanum* (PP 1.0, BS 96; Fig. 2; Weese & Bohs, 2007; Särkinen & al., 2013b).

Relationships within the Morelloid clade. — Due to the presence of known allopolyploids, individual gene trees are presented in Fig. 3. The individual analyses indicate differential placement of the polyploid species *S. nigrum*, *S. grandidentatum*, *S. furcatum*, *S. fragile*, and *S. scabrum* in the nuclear, nuclear ribosomal, and plastid topologies (Fig. 3). For example, *S. scabrum* is placed within the African polyploid clade in the ITS and plastid trees (Fig. 3B & C), but remains unresolved in the *waxy* topology (Fig. 3A). These incongruences are not supported with high branch support values of both Bayesian and maximum likelihood analyses, indicating that further sequence data and cloning will be required. We hence focus on describing only broad-scale results of the major clades below, leaving the detailed analyses of species-level relationships for a further study.

Four major clades can be identified within the Morelloid clade in the combined tree, including the Radicans clade (PP 1.0, BS 100), the Episarcophyllum clade (PP 1.0, BS 98), the Chamaesarachidium clade (PP 1.0, BS 100), and the Black nightshade clade (PP 1.0, BS 97; Fig. 2). The first of these, the Radicans clade, includes four species, *S. corymbosum*, *S. palitans*, *S. radicans* and *S. tripartitum* (Fig. 2). The Episarcophyllum clade corresponds to the core members of *S.* sect. *Episarcophyllum*, but excludes *S. triflorum* and *S. caesium* Griseb. (Fig. 2). The Chamaesarachidium clade includes the core members of sect. *Chamaesarachidium* (*S. gilioides* Rusby and *S. weddellii* Phil. [= *S. chamaesarachidium* Bitter]), while the third postulated member of sect. *Chamaesarachidium*, *S. annuum* C.V.Morton, falls outside the clade, and is nested within the Black nightshade clade (Fig. 2). There is a noted incongruence, however, in the position of the Charamaesarachidium clade between the individual gene trees, where the *waxy* and ITS topologies resolve the clade as nested within the Black nightshade clade (Fig. 3A & B), while the plastid phylogeny resolves it as the first branching clade within the Morelloids (Fig. 3C). This hard incongruence with high branch support indicates a possible differential origin of the organellar and nuclear genomes in the members of the Chamaesarachidium clade, and will require further investigation. Here, the combined analysis reflects plastid data only where nuclear data was removed prior to concatenation in order to provide an overview of the Morelloid clade and relationships within it.

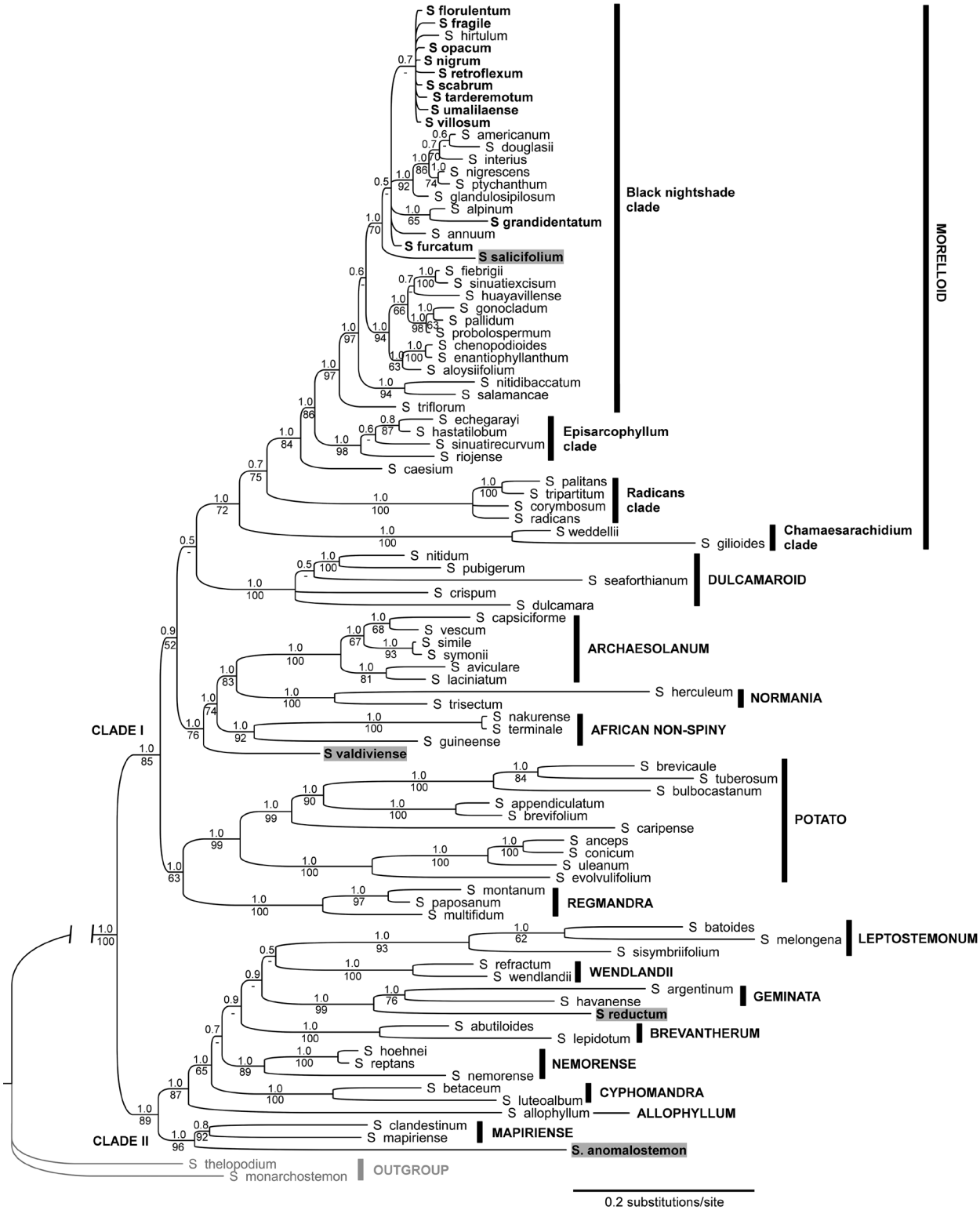

Fig. 2. Bayesian 50% majority-rule tree of the Morelloid clade of *Solanum* with broad outgroup selection including representatives from all major clades of *Solanum* from Bayesian analysis of the combined ITS, *trnT-F*, and *waxy* dataset. Bayesian posterior probability support values are indicated above and maximum likelihood bootstrap values below branches. Major clades of *Solanum* are highlighted by bars on the right, including clades I and II sensu Särkinen & al. (2013b) and major well-supported clades within the Morelloid clade. Previously unplaced taxa are indicated in bold with grey background. All known polyploid species within the Morelloid clade are shown in bold. The combined analysis included only *waxy* data for the polyploids and species of sect. *Chamaesarachidium* due to topological incongruence detected in individual analyses (see text for details).

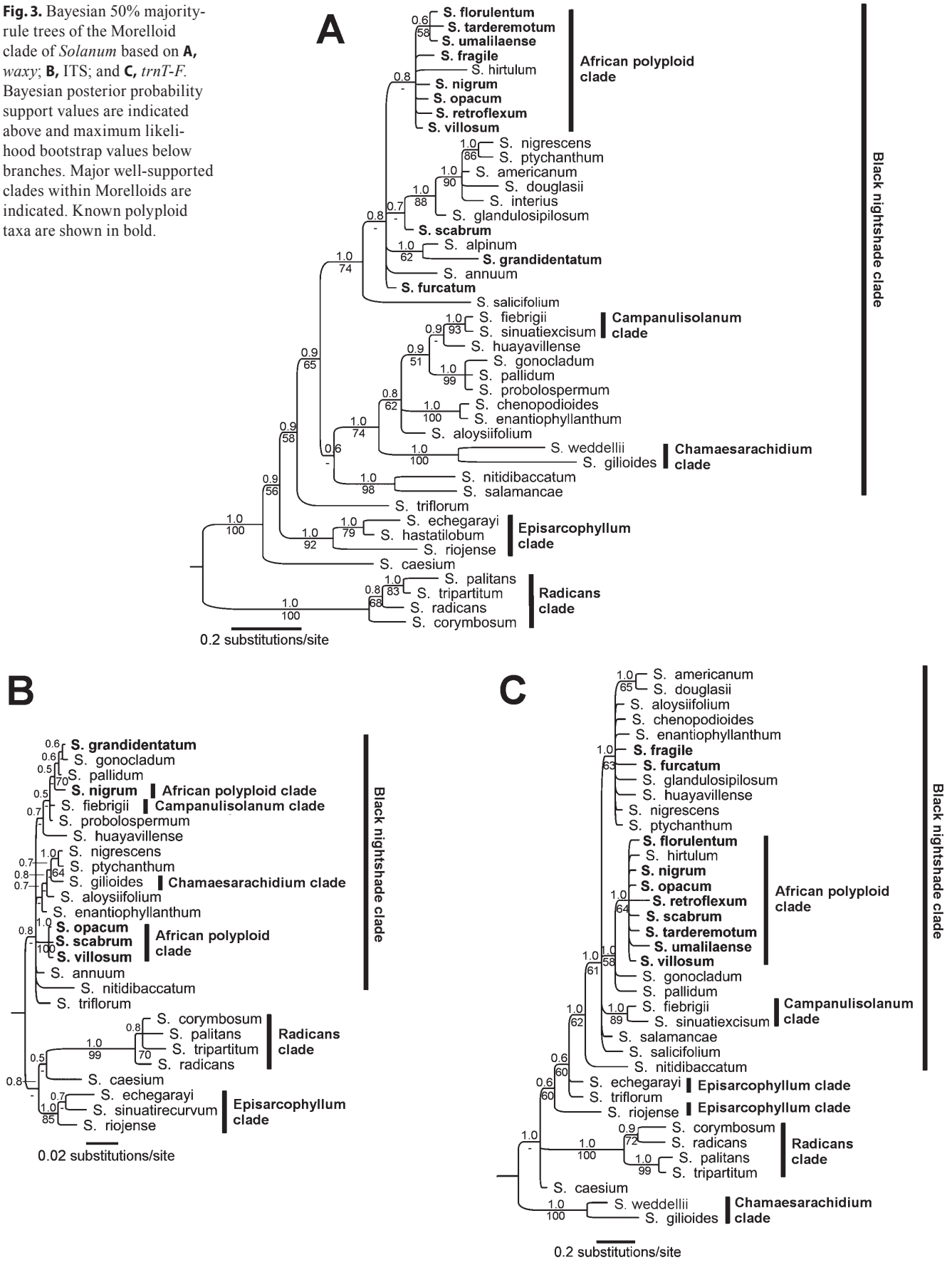

Fig. 3. Bayesian 50% majority-rule trees of the Morelloid clade of *Solanum* based on **A,** *waxy*; **B,** ITS; and **C,** *trnT-F*. Bayesian posterior probability support values are indicated above and maximum likelihood bootstrap values below branches. Major well-supported clades within Morelloids are indicated. Known polyploid taxa are shown in bold.

The fourth major clade, the Black nightshade clade, includes most of the species diversity within the Morelloids (Fig. 2). The clade includes all members of *S.* sect. *Solanum*, *S. salicifolium*, *S. triflorum*, the monophyletic sect. *Campanulisolanum*, and the Chamaesarachidium clade in the *waxy* and ITS topologies (see above; Figs. 2 & 3). A subclade is resolved within the Black nightshade clade in the plastid (PP 1.0, BS 64) and *waxy* topologies (PP 0.8, BS –), consisting of most of the known African polyploid species as well as some suspected African polyploid species for which ploidy counts are still lacking, here named as the African polyploid clade (Fig. 3A & C). ITS topology does not resolve the clade as monophyletic due to lack of resolution (Fig. 3B). All diploid species present in Africa such as *S. americanum* and *S. chenopodioides* Lam. fall outside the clade, with closer affinities with the South American species (Fig. 2). The position of the known polyploid species from South America sampled here (*S. furcatum*, *S. fragile*, *S. grandidentatum*) remains uncertain due to incongruence between the plastid and nuclear datasets (*S. fragile*, *S. furcatum*), or lack of sampling in both trees (*S. grandidentatum*).

■ DISCUSSION

Circumscription of the Morelloid clade. — The Morelloid clade was first recognised as monophyletic by Bohs (2005). Our phylogenetic analyses confirm the monophyly of the clade based on a broader species sampling, and add *S. salicifolium* as a new member to the Morelloids (see Discussion below). A set of morphological characters can be used to define the clade, including herbaceous or shrubby growth form, indumentum with simple, glandular, or branched hairs, simple to deeply lobed leaves with entire, serrulate or toothed margins, simple to bi-(multi-)furcating mostly relatively condensed inflorescences borne along leaf internodes, fruits maturing green, yellow, orange-red, purple or black, and presence of stone cell aggregates (i.e., sclerosomes) in the fruits of many species.

Although the name Morelloid refers to Dunal's subsection Morella (1852) that corresponds to sect. *Solanum* as traditionally defined (e.g., Child & Lester, 2001), the clade also includes a set of species from sect. *Campanulisolanum*, sect. *Chamaesarachidium*, sect. *Episarcophyllum*, and some members of sect. *Parasolanum*. The detailed circumscription of the Morelloid clade and the monophyly of these smaller sections have remained unclear due to lack of detailed species-level taxonomic work. Recent new species findings (Manoko & al., 2013; Särkinen & al., 2013a) combined with ongoing efforts of providing a full taxonomic monograph of the group have provided the much-needed understanding of morphological diversity present within the group that helps to establish robust molecular studies where identification of voucher material is crucial. Our current preliminary checklist of the Morelloid clade now includes 76 accepted species names (Appendix 1).

Phylogenetic position of the uncertain taxa. — In order to fully circumscribe the Morelloid clade, we tested the phylogenetic position of four previously unplaced taxa which have been loosely associated with the Morelloids based on some morphological characteristics. Of these, our analyses show one of them, *S. salicifolium*, to be clearly nested within the Morelloid clade, whilst three are placed in different parts of the *Solanum* phylogeny (see below). *Solanum salicifolium* has previously been included in the Dulcamaroid clade based on morphology (Knapp, 2013), but was allied with *S. triflorum* by Morton (1976; as *S. incisum*). The species was included in the Dulcamaroid monograph based on the presence of a cup-shaped pedicel base found in all members of the Dulcamaroid clade (Knapp, 2013). The distinct pedicel base has been amongst the few morphological characters available to separate members of Dulcamaroids from the Morelloid clade. Other morphological characteristics exist however, including the predominantly vining habit in the Dulcamaroids and presence of larger, more open and branching inflorescences compared to predominantly herbaceous or suffrutescent Morelloid species that have smaller, simple to bifurcate, mostly umbellate to sub-umbellate inflorescences. *Solanum salicifolium*, with a suffrutescent habit and simple or furcate inflorescences fits well within the Morelloids morphologically, with the exception of having a distinct pedicel base like that of most Dulcamaroids.

Solanum valdiviense has been morphologically associated with the Dulcamaroid clade (Knapp, 2013), but our analyses show here for the first time that this southern South American species is sister to the exclusively Old World clades, including the African Non-Spiny, Normania and the Archaesolanum clades. Morphologically *S. valdiviense* belongs clearly with the Dulcaramoid clade due to its growth form and the presence of the distinctive pedicel base, and the placement of the species as sister to the Old World clades away from the Dulcamaroids is somewhat surprising, especially in terms of geography. The Old World clades include the Archaesolanum clade (semi-woody shrubs with highly variable leaf morphology, plurifoliate sympodial units, rotate corollas with abundant interpetalar tissue, anthers with long filaments, and fruits with numerous and conspicuous stone cell aggregates), the Normania clade (herbs or weak shrubs with zygomorphic corollas, foliaceous and accrescent calyces, unequal stamens with anthers dehiscent by both apical pores and longitudinal slits, presence of horned anthers, dry fruits or fruits with sparse pulp, large seeds and radially expanded seed coat cell walls), and the relatively poorly understood African Non-Spiny clade (shrubs or climbers with simple or branched hairs, and purple to white corollas). *Solanum valdiviense* has regular, relatively short anthers with short filaments and its morphological association with members of the Old World clade should be studied further to fully understand potential synapomorphies of the clade as a whole. In order to recognise the distinct phylogenetic position of *S. valdiviense* within *Solanum*, we propose *S. valdiviense* as its own clade within clade I (sensu Särkinen & al., 2013b) of *Solanum*.

Since its description in 2009, *S. anomalostemon* has remained a poorly understood species from the Peruvian inter-Andean dry valley of Río Apúrimac near Cusco (Knapp & Nee, 2009). This narrowly endemic species is morphologically one of the most distinct species within *Solanum*. The glandular-haired, suffrutescent species lacks spines, stellate hairs or pungent vegetative odors, and has unique anther morphology

with short (ca. 2 mm long) cordate anthers that curve towards the middle, forming a central heart-shaped anther column. The species was originally postulated to be closely affiliated to the Morelloid clade due to its herbaceous growth habit, presence of glandular hairs, simple inflorescence, and the unique anther morphology that loosely resembles the anther spurs found in *S. weddellii* of sect. *Chamaesarachidium* (Knapp & Nee, 2009). Our molecular analysis shows that *S. anomalostemon* is not related to the Morelloids but instead to *S. mapiriense* and *S. clandestinum* at the base of clade II (sensu Särkinen & al., 2013b) of *Solanum* where the three species form a clade although long branches within the group indicate that these species have been separated for quite some time. The results are surprising in placing the species in clade II with species that are mostly shrubs and trees often with lepidote or stellate hairs, rather than clade I where many less tree-like species with simple, glandular or branched hairs are found. The major division within *Solanum* into clade I and II corresponds not only to growth habit and indumentum, but most intriguingly also to anther morphology. Clade I consists of mainly herbs, shrubs and vines with predominantly oblong anthers with relatively large terminal pores and later longitudinal dehiscence, while clade II contains the large monophyletic spiny solanums (the Leptostemonum clade) with attenuate anthers with small terminal pores that do not later open longitudinally and the Geminata and Cyphomandra clades which are shrubs and trees with oblong anthers with relatively large terminal pores that later "unzip" longitudinally or somewhat attenuate anthers that open by small terminal pores (Knapp & Nee, 2009; Särkinen & al., 2013b). Based on these morphological characters, *S. anomalostemon* might be expected to fall within clade I, and any obvious synapomorphies with the two currently unplaced taxa *S. mapiriense* and *S. clandestinum* are lacking. Both these species are endemic to Andean Bolivia, but differ morphologically: for example, *S. clandestinum* has relatively broad, blunt anthers with pores opening into longitudinal slits, whilst those of *S. mapiriense* are strongly tapered and dehisce by small terminal pores (Nee & al., 2006). Further morphological, cytological, and anatomical studies are needed to fully understand potential synapomorphies that unite these three unusual species. What is interesting to note is that strong stamen heteromorphism is present in the first branching taxa in the Thelopodium clade (Knapp, 2000; Weese & Bohs, 2007), indicating perhaps that progenitors had more variation in stamen morphology as compared to most of the surviving present-day members of *Solanum*.

Solanum reductum, a small shrub endemic to northern Argentina, has remained a species with uncertain taxonomic position since its original description by Morton (1976). The species was originally described as a member of the *S.* subsect. *Monadelphoidea* Bitter (now considered part of the Geminata clade, see Knapp, 2008), but has been suggested as closely allied to the Morelloids (Nee, 1999) or *Cyphomandropsis* Bitter by others (Barboza & al., 2013). Morton (1976) described *S. reductum* as most morphologically closely related to *S. delitescens* C.V.Morton, another endemic species from northern Argentina (Morton, 1976). Our study shows the placement of *S. reductum* as sister to the well-established Geminata clade (Knapp, 2008). We add *S. reductum* to the circumscription of the Geminata clade by previous authors (Bohs, 2005; Weese & Bohs, 2007; Knapp, 2008), and informally name *S. reductum* and *S. delitescens* as the sole two members of a new "Solanum reductum subclade".

Monophyletic groups within the Morelloids. — The increased taxon sampling used here reveals the need for revising the circumscriptions of the morphological groups used for guiding species identification within the Morelloids. Below we provide a new circumscription for each of the monophyletic groups identified based on the molecular results summarised in Table 2, with a list of species and associated names in Appendix 1.

Section *Episarcophyllum* was originally described to include eight species restricted to high montane habitats of Argentina and Chile (Bitter, 1912a; Del Vitto & Petenatti, 1999). Nee (1999) added *S. triflorum* and *S. caesium* to the section on morphological grounds. Our phylogenetic analyses support the narrower concept of the section, where *S. triflorum* and *S. caesium* are excluded. The Episarcophyllum clade, as here circumscribed, includes four succulent-leaved, perennial herbs with underground rhizomes and fruits with stone cell aggregates from dry high-elevation Andes of Chile and Argentina. These species can be easily recognised as a natural group within the Morelloids and *Solanum* as a whole based on their growth form with herbaceous stems from woody subterreanean rootstocks and succulent, simple to deeply toothed leaves. While Del Vitto & Petenatti (1999) split the group into eight species, the most recent treatment recognises only four more morphologically variable species (Barboza & al., 2013). *Solanum caesium*, a species previously treated as part of the sect. *Episarcophyllum*, is here kept as unplaced within the Morelloids.

A strongly supported monophyletic clade here referred to as the Radicans clade was found in all gene topologies. This group of four species has often been incorrectly referred to as *S.* sect. *Parasolanum* (e.g., Knapp, 2013), but the name sect. *Parasolanum* as formally described by Child (1984) is typified with *S. triflorum* and should only be applied to that species. Nee (1999) recognised the four species as his "Sect. *Dulcamara* Subsect. 1", but this position is not supported by our analyses. We refrain from assigning a new sectional name to the Radicans clade until sectional classification is revised across *Solanum* as a whole in order to avoid further confusion. The Radicans clade, as here recognised, includes four erect to prostrate herbs with simple to mostly lobed leaves, small corollas, short anthers, capitate stigmas, and pale yellow to orange-red coloured fruits from dry montane habitats from Andean Peru, Bolivia and Argentina with *S. corymbosum* also occurring in Mexico, possibly as the result of an introduction (Nee, 1999).

Section *Chamaesarachidium* as originally described by Bitter (1917) included two weakly stemmed annual herbs from high-elevation habitats of Peru, Bolivia and northern Argentina, *S. weddellii* (as *S. chamaesarachidium*) and *S. gilioides*. One further species (*S. annuum*) was later added into the section based on morphological characters (Barboza, 2003). Our phylogenetic analyses show clearly that *S. annuum* is not

closely related to the two core members of the group, and is nested within members of sect. *Solanum* in the Black nightshade clade. The results imply that the tuberculate seed coat and accrescent fruiting calyxes used to define sect. *Chamaesarachidium* (Barboza, 2003) have evolved multiple times within the Morelloids. Accrescent fruiting calyces are also found in several other species of sect. *Solanum*, such as *S. salamancae* Barboza and *S. tweedianum* Gillies ex Nees (see Barboza & al., 2013). Members of the Chamaesarachidium clade as here circumscribed are small annual herbs with procumbent habit with dentate to pinnatifid or pinnatisect leaf blades, rotate to moderately campanulate corollas, capitate stigmas, accrescent fruiting calyces, and absence of stone cell aggregates in fruit from high-elevation dry montane habitats of Peru, Bolivia and northern Argentina.

In relation to *S.* sect. *Solanum*, or the Black nightshade clade as here defined, the phylogenetic results support a wider concept compared to some previous authors (e.g., Edmonds, 1972) that includes species with simple, glandular and/or branched hairs, and species with stellate to campanulate corollas. The circumscription of the section has varied widely in the past, where species of sect. *Campanulisolanum* with campanulate corollas (*S. fiebrigii* Bitter, *S. sinuatiexcisum* Bitter) have been variously treated as members of sect. *Solanum* (D'Arcy, 1972; Edmonds, 1972, 1977, 1978; Edmonds & Chweya, 1997), differentiated as sect. *Campanulisolanum* (Bitter, 1912b; Morton, 1976; Barboza & Hunziker, 2005), or recognised as a subsection within sect. *Solanum* (Child, 1998; Nee, 1999). The single species with branched hairs, *S. pallidum* Rusby, has similarly been either included or excluded from the section, or treated as a member of his subsection *Campanulisolanum* by Child (1998). Our results support a wider circumscription of *S.* sect. *Solanum* with a total of 65 species, including sect. *Campanulisolanum*, species with branched hairs (*S. pallidum*), and two species previously placed in other sections/clades (*S. triflorum*, *S. salicifolium*). The centre of diversity for the section is clearly in the Andes, with a total of 46 species native to South America, 11 species native to Africa (*S. nigrum* complex, see below), 5 species native to North America and Mexico, and 3 from Asia and the Pacific. Based on the current phylogenetic results, these geographic groups do not form clades within sect. *Solanum*.

Polyploidy and species-level relationships. — Fourteen species of the Morelloid clade are known to be tetra- or hexaploids based on chromosome counts, and all of these are found

Table 2. Revised morphological groups belonging to the Morelloid clade of *Solanum* based on study results.

Clade	No. of species	Morphology	Distribution	Notes
Chamaesarachidium clade	2	Annual montane herbs, hairs simple to glandular, leaves (deeply) toothed, membranous, corollas rotate, anthers 1.1–2.5 mm, stigmas capitate, fruits maturing green with inflated strongly nerved calyces	Andean Peru, Bolivia and Argentina; from high-elevation dry montane habitats	Includes two species only. Section *Chamaesarachidium* Bitter defined similar to the clade here, but including all species with tuberculate seed coat texture, which is now shown to be a homoplastic trait.
Episarcophyllum clade	4	Prostrate herbaceous perennials, hairs simple, leaves toothed, succulent, corollas stellate to rotate, anthers 2–5 mm long, stigmas capitate-bilobed, fruits maturing green	Andean Chile and Argentina; from high-elevation dry montane habitats	Excludes *S. caesium* Griseb., a species included in sect. *Episarcophyllum* Bitter. The exclusion does not change the morphological circumscription of the group.
Radicans clade	4	Erect to prostrate herbs, hairs simple, leaves semi-succulent to membranous, simple or lobed, corollas stellate, white or with purple colouration, anthers 1–2.5 mm long, stigmas capitate, fruits maturing green, yellow, or orange red	Andean Peru, Bolivia and Argentina; from low- to high-elevation montane habitats	Includes four of the total five species included in sect. *Parasolanum* by Child (1984; type *S. triflorum* Nutt.). The four species in the Radicans clade were previously identified as an infraspecific group "sect. *Dulcamara* subsect. 1" by Nee (1999) without a formal sectional name.
Black nightshade clade	65	Herbs and subshrubs, erect to prostrate, hairs simple, glandular or branched, leaves membranous to semi-succulent, serrulate to shallowly toothed, corollas stellate to campanulate, white, purple or pale yellow, anthers 1–5.5 mm long, stigmas bilobed to capitate, fruits maturing green, purple, black, or orange with inflated calyces in some species	Centre of diversity in Andean South America, 11 species native to Africa, 5 to North and Central America, and 3 from Asia; from low to high elevation	Includes sect. *Campanulisolanum* Bitter, thus expanding the circumscription to include species with campanulate corollas. Also includes species with branched hairs (*S. pallidum* Bitter), *S. triflorum*, and *S. salicifolium* Phil. previously placed within the Dulcamaroid clade.
Unplaced	1			*S. caesium*

within the Black nightshade clade (Appendix 1). Our molecular results show that most of the African polyploid species form a monophyletic clade, corresponding to the core members of the *S. nigrum* complex as previously referred to in literature, here named as the African polyploid clade. The clade also includes suspected polyploids from Africa for which chromosome counts are still lacking. For example, our study indicates the African *S. hirtulum* (a member of the *S. nigrum* complex) is likely also to be polyploid based on multiple double peaks recovered in *waxy* sequences. Suggested diploid parents of many of the allopolyploids, such as *S. americanum* (= *S. nodiflorum* Jacq. sensu Edmonds, 1979), *S. chenopodioides* and *S. sarrachoides* Sendtn. native to the Americas, and the Australasian diploid species, are not included in this clade. Of the putative diploid parents, *S. sarrachoides* was not sampled here but is unlikely to fall within the African polyploid clade. The position of the South American polyploids, such as *S. fragile*, *S. furcatum*, and *S. grandidentatum*, remains uncertain based on our results, and should be further investigated. Because some of these polyploids originated from not only two but several parents, resolving the origin of these species is likely to require considerable amounts of molecular data.

The known complex genomic history of the allopolyploid species within the Black nightshade clade, involving up to three parents in the hexaploids (Edmonds, 1979, 1986), proposes real challenges for constructing further robust species-level relationships in the group. Our results indicate that homoploid hybrid origin might be involved in the origin of the Chamaesarachidium clade based on the hard incongruence between plastid and nuclear topologies resolving the position of the clade within the Morelloids. Detailed analyses involving sequencing of multiple plastid markers, and cloning of multiple nuclear low copy regions similar to *waxy* will be required to establish the exact genomic origin of problematic taxa. *Waxy* has been successfully used to establish the genomic origin of allopolyploids in *Solanum* (Spooner & al., 2008), but more markers will be needed in the Morelloids where *waxy* provides inadequate sequence variation to fully resolve species-level relationships of the closely related taxa in sect. *Solanum*.

■ ACKNOWLEDGEMENTS

We thank Lynn Bohs, Stephen Stern, Frank Farrugia, Karena Leo, Gerard van der Weerden and Celestina Mariani for sharing laboratory protocols, DNA material, specimen vouchers, and sequences used in this study, Xavier Aubriot for DNA extraction and sequencing of *S. anomalostemon*, Michael Nee for his invaluable nomenclatural notes of the Morelloids, Morvah George, Samantha Murphy and Pawel Ficinski for databasing and curational help, and the Peruvian authorities of the Ministerio de Agricultura, Dirección General Forestal y de Fauna Silvestre, for granting our collection permit No. 084-2012-AG-DGFFS/DGEFFS and the contract for genetic resources No. 008-2014-MINAGRI-DGFFS/DGEFFS under which this study was executed. This work was supported by NSF grant DEB-0316614 "PBI Solanum: A worldwide treatment" to SK, and by CONICET and FONCyT grants to GEB.

■ LITERATURE CITED

Acosta, M.C., Bernardello, G., Guerra, M. & Moscone, E.A. 2005. Karyotype analysis in several South American species of *Solanum* and *Lycianthes rantonnei* (Solanaceae). *Taxon* 54: 713–723. http://dx.doi.org/10.2307/25065428

Barboza, G.E. 2003. Taxonomic revision of *Solanum* sect. *Chamaesarachidium* (Solaneae, Solanaceae). *Nordic J. Bot.* 23: 155–168. http://dx.doi.org/10.1111/j.1756-1051.2003.tb00377.x

Barboza, G.E. & Hunziker, A.T. 2005. Revision of *Solanum fiebrigii* and *Solanum sinuatiexcisum*, and their inclusion in section *Campanulisolanum*. Pp. 51–67 in: Keating, R., Hollowell, V.C. & Croat, T.B. (eds.), *A festschrift for William G. D'Arcy: The legacy of a taxonomist*. St. Louis: Missouri Botanical Garden Press.

Barboza, G.E., Knapp, S. & Särkinen, T. 2013. *Solanum* Grupo VII. Moreloide. Pp. 231–264 in: Barboza, G.E. (coord.), *Flora Argentina: Flora vascular de la Republica Argentina*, vol. 13, *Dicotyledoneae: Solanaceae*. San Isidro: Instituto de Botanica Darwinion.

Bhiravamurty, P.V. & Rethy, P. 1983. Taximetric studies of the *Solanum nigrum* L. complex. *Proc. Indian Natl. Sci. Acad., B* 49: 661–666.

Bitter, G. 1912a. Solana nova vel minus cognita. IV. *Repert. Spec. Nov. Regni Veg.* 11: 241–247.

Bitter, G. 1912b. Solana nova vel minus cognita. III. *Repert. Spec. Nov. Regni Veg.* 11: 202–237.

Bitter, G. 1917. Solana nova vel minus cognita. XVI. *Repert. Spec. Nov. Regni Veg.* 15: 93–96.

Bohs, L. 2005. Major clades in *Solanum* based on *ndhF* sequence data. Pp. 27–49 in: Keating, R., Hollowell, V.C. & Croat, T.B. (eds.), *A festschrift for William G. D'Arcy: The legacy of a taxonomist*. St. Louis: Missouri Botanical Garden Press.

Child, A. 1984. Taxonomic studies in *Solanum* L. 2. Two new infrageneric taxa for the subgenus *Solanum*. *Feddes Repert.* 95: 141–150.

Child, A. 1998. Studies in *Solanum* and related genera (6). New infrageneric taxa for the genus *Solanum* L. (Solanaceae). *Feddes Repert.* 109: 407–427. http://dx.doi.org/10.1002/fedr.19981090512

Child, A. & Lester, R.N. 2001. Synopsis of the genus *Solanum* L. and its infrageneric taxa. Pp. 39–52 in: Van den Berg, R.G., Barendse, G.W.M., Van der Weerden, G.M. & Mariani, C. (eds.), *Solanaceae V: Advances in taxonomy and utilization*. Nijmegen: Nijmegen University Press.

D'Arcy, W.G. 1972. Solanaceae studies II: Typification of subdivisions of *Solanum*. *Ann. Missouri Bot. Gard.* 59: 262–278. http://dx.doi.org/10.2307/2394758

Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. 2012. jModelTest 2: More models, new heuristics and parallel computing. *Nature, Meth.* 9: 772. http://dx.doi.org/10.1038/nmeth.2109

Dehmer, K.J. & Hammer, K. 2004. Taxonomic status and geographic provenance of germplasm accessions in the *Solanum nigrum* L. complex: AFLP data. *Genet. Resources Crop Evol.* 51: 551–558. http://dx.doi.org/10.1023/B:GRES.0000024163.86762.fc

Del Vitto, L.A. & Petenatti, E.M. 1999. Notas en *Solanum* (Solanaceae) de Argentina II. Aportes al conocimiento de la sect. *Episarcophyllum*. *Kurtziana* 27: 319–326.

Dunal, M.-F. 1852. Solanaceae. Pp. 1–690 in: Candolle, A.P. de (ed.), *Prodromus systematis naturalis regni vegetabilis*, vol. 13(1). Parisiis [Paris]: sumptibus Victoris Masson. http://dx.doi.org/10.5962/bhl.title.286

Edmonds, J.M. 1972. A synopsis of the taxonomy of *Solanum* sect. *Solanum* (Maurella) in South America. *Kew Bull.* 27: 95–114. http://dx.doi.org/10.2307/4117874

Edmonds, J.M. 1977. Taxonomic studies on *Solanum* section *Solanum* (Maurella). *Bot. J. Linn. Soc.* 75: 141–178. http://dx.doi.org/10.1111/j.1095-8339.1977.tb01482.x

Edmonds, J.M. 1978. Numerical taxonomic studies on *Solanum* L. section *Solanum* (Maurella). *Bot. J. Linn. Soc.* 76: 27–51. http://dx.doi.org/10.1111/j.1095-8339.1978.tb01497.x

Edmonds, J.M. 1979. Biosystematics of *Solanum* L., section *Solanum* (Maurella). Pp. 529–548 in: Hawkes, J.G., Lester, R.N. & Skeldig, A.D. (eds.), *The biology and taxonomy of the Solanaceae*. London: Linnean Socety of London; Academic Press.

Edmonds, J.M. 1982. Epidermal hair morphology in *Solanum* L. section *Solanum*. *Bot. J. Linn. Soc.* 85: 153–167. http://dx.doi.org/10.1111/j.1095-8339.1982.tb02583.x

Edmonds, J.M. 1983. Seed coat structure and development in *Solanum* L. section *Solanum* (Solanaceae). *Bot. J. Linn. Soc.* 87: 229–246. http://dx.doi.org/10.1111/j.1095-8339.1983.tb00992.x

Edmonds, J.M. 1984. Pollen morphology of *Solanum* L. section *Solanum*. *Bot. J. Linn. Soc.* 88: 237–251. http://dx.doi.org/10.1111/j.1095-8339.1984.tb01573.x

Edmonds, J.M. 1986. Biosystematics of *Solanum sarrachoides* Sendtner and *S. physalifolium* Rusby (*S. nitidibaccatum* Bitter). *Bot. J. Linn. Soc.* 92: 1–38. http://dx.doi.org/10.1111/j.1095-8339.1986.tb01425.x

Edmonds, J.M. 2012. *Solanaceae*. Pp. 124–144 in: Beentje, H. (ed.), *Flora of tropical East Africa*. Richmond: Royal Botanic Garden, Kew.

Edmonds, J.M. & Chweya, J.A. 1997. *Black nightshades – Solanum nigrum L. and related species*. Promoting the conservation and use of underutilized and neglected crops 15. Gatersleben: International Plant Genetic Resources Institute. http://pdf.usaid.gov/pdf_docs/Pnach882.pdf

Fernández Casas, F.J. 1982. Dos nuevas Dicapetaláceas sudamericanas. *Fontqueria* 2: 15–18.

Frodin, D.G. 2004. History and concepts of big plant genera. *Taxon* 53: 753–776. http://dx.doi.org/10.2307/4135449

Guindon, S. & Gascuel, O. 2003. A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. *Syst. Biol.* 52: 696–704. http://dx.doi.org/10.1080/10635150390235520

Hall, T. 1999–2007. BioEdit Sequence Alignment Editor. Carlsbad, California: Ibis Biosciences.

Huelsenbeck, J.P. & Ronquist, F. 2003. MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* 17: 754–755. http://dx.doi.org/10.1093/bioinformatics/17.8.754

Knapp, S. 2000. A revision of *Solanum thelopodium* species group (section *Anthoresis* sensu Seithe, pro parte): Solanaceae. *Bull. Nat. Hist. Mus. London, Bot.* 30: 13–30.

Knapp, S. 2008. A revision of the *Solanum havanense* species group and new taxonomic additions to the Geminata clade (*Solanum*, Solanaceae). *Ann. Missouri Bot. Gard.* 95: 405–458. http://dx.doi.org/10.3417/2006159

Knapp, S. 2013. A revision of the Dulcamaroid clade of *Solanum* L. (Solanaceae). *PhytoKeys* 22: 1–428. http://dx.doi.org/10.3897/phytokeys.22.4041

Knapp, S. & Nee, M. 2009. *Solanum anomalostemon* (Solanaceae), an endangered new species from southern Peru with unusual anther morphology. *Novon* 19: 178–181. http://dx.doi.org/10.3417/2007108

Levin, R.A., Watson, K. & Bohs, L. 2005. A four-gene study of evolutionary relationships in *Solanum* section *Acanthophora*. *Amer. J. Bot.* 92: 603–612. http://dx.doi.org/10.3732/ajb.92.4.603

Manoko, M.L.K. 2007. *A systematic study of African Solanum L. section Solanum (Solanaceae).* Ph.D. thesis, Radboud University, Nijmegen, The Netherlands.

Manoko, M.L.K., Van den Berg, R.G., Feron, R.M.C., Van der Weerden, G.M. & Mariani, C. 2007. AFLP markers support separation of *Solanum nodiflorum* from *S. americanum* sensu stricto (Solanaceae). *Pl. Syst. Evol.* 267: 1–11. http://dx.doi.org/10.1007/s00606-007-0531-4

Manoko, M.L.K, Van der Weerden, G.M., Van den Berg, R.G. & Mariani, C. 2012. A new tetraploid species of *Solanum* L. sect. *Solanum* (Solanaceae) from Tanzania. *PhytoKeys* 16: 65–74. http://dx.doi.org/10.3897/phytokeys.16.2884

Morton, C.V. 1976. *A revision of the Argentine species of Solanum*. Cordoba, Argentina: Academia Nacional de Ciencias.

Morton, J.K. 1993. Chromosome numbers and polyploidy in the flora of Cameroon Mountain. *Opera Bot.* 121: 159–172.

Moscone, E.A. 1992. Estudios de cromosomas meióticos en Solanaceae de Argentina. *Darwiniana* 31: 261–297.

Moyetta, N.R., Stiefkensa, L.B. & Bernardello, G. 2013. Karyotypes of South American species of the Morelloid and Dulcamaroid clades (*Solanum*, Solanaceae). *Caryologia* 66: 333–345. http://dx.doi.org/10.1080/00087114.2013.855389

Nee, M. 1999. Synopsis of *Solanum* in the New World. Pp. 285–333 in: Nee, M., Symon, D.E., Lester, R.N. & Jessop, J.P. (eds.), *Solanaceae IV: Advances in biology and utilization*. Richmond: Royal Botanic Gardens, Kew.

Nee, M., Bohs, L. & Knapp, S. 2006. New species of *Solanum* and *Capsicum* (Solanaceae) from Bolivia, with clarification of nomenclature in some Bolivian *Solanum*. *Brittonia* 58: 322–356. http://dx.doi.org/10.1663/0007-196X(2006)58[322:NSOSAC]2.0.CO;2

Olet, E.A. 2004. *Taxonomy of Solanum L. section Solanum in Uganda*. Ph.D. thesis, Agricultural University of Norway, Ås.

Olet, E.A., Lye, K.A. & Stedje, B. 2015. Crossing relationships and chromosome numbers of *Solanum* section *Solanum* in Uganda. *Nordic J. Bot.* 33: 472–483. http://dx.doi.org.10.1111/njb.00592

Poczai, P. & Hyvönen, J. 2011a. Identification and characterization of plastid *trnF*(GAA) pseudogenes in four species of *Solanum* (Solanaceae). *Biotechnol. Lett.* 33: 2317–2323. http://dx.doi.org/10.1007/s10529-011-0701-x

Poczai, P. & Hyvönen, J. 2011b. On the origin of *Solanum nigrum*: Can networks help? *Molec. Biol. Reporter* 38: 1171–1185. http://dx.doi.org/10.1007/s11033-010-0215-y

Ronquist, F. & Huelsenbeck, J.P. 2005. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574. http://dx.doi.org/10.1093/bioinformatics/btg180

Särkinen, T., Staats, M., Richardson, J.E., Cowan, R.S. & Bakker, F.T. 2012. How to open the treasure chest? Optimising DNA extraction from herbarium specimens. *PLoS ONE* 7(8): e43808. http://dx.doi.org/10.1371/journal.pone.0043808

Särkinen, T.S., Gonzáles, P. & Knapp, S. 2013a. Distribution models and species discovery: The story of a new *Solanum* species from the Peruvian Andes. *PhytoKeys* 16: 1–20. http://dx.doi.org/10.3897/phytokeys.31.6312

Särkinen, T.S., Bohs, L., Olmstead, R.G. & Knapp, S. 2013b. A phylogenetic framework for evolutionary study of the nightshades (Solanaceae): A dated 1000-tip tree. *B. M. C. Evol. Biol.* 13: 214. http://dx.doi/org/10.1186/1471-2148-13-214

Särkinen, T.S., Knapp, S. & Nee, M. 2015a. Two new non-spiny *Solanum* species from the Bolivian Andes (Morelloid clade). *PhytoKeys* 47: 97–109. http://dx.doi.org/10.3897/phytokeys.47.4423

Särkinen, T.S., Gonzáles, P. & Knapp, S. 2015b. Four new non-spiny *Solanum* (Solanaceae) species from South America. *PhytoKeys* 44: 39–64. http://dx.doi.org/10.3897/phytokeys.44.8693

Schilling, E.E. 1981. Systematics of *Solanum* sect. *Solanum* (Solanaceae) in North America. *Syst. Bot.* 6: 172–185. http://dx.doi.org/10.2307/2418547

Schilling, E.E. & Heiser, C.B. 1979. Crossing relationships among diploid species of the *Solanum nigrum* complex in North America. *Amer. J. Bot.* 66: 709–716. http://www.jstor.org/stable/2442416

Spooner, D.M. & Knapp, S. 2013. *Solanum stipuloideum* Rusby, the correct name for *Solanum circaeifolium* Bitter. *Amer. J. Potato Res.* 90(4): 301–305. http://dx.doi.org/10.1007/s12230-013-9304-5

Spooner, D.M., Rodriguez, F., Polgar, Z., Ballard, H.E. & Jansky, H. 2008. Genomic origins of potato polyploids: GBSSI gene sequencing data. *Crop Sci.* 48: S27–S36. http://dx.doi.org/10.2135/cropsci2007.09.0504tpg

Stern, S., Agra, M.F. & Bohs, L. 2011. Molecular delimitation of clades within New World species of the "spiny solanums" (*Solanum* subg. *Leptostemonum*). *Taxon* 60: 1429–1441.

Taberlet, P., Gielly, L., Pautou, G. & Bouvet, J. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Pl. Molec. Biol.* 17: 1105–1110. http://dx.doi.org/10.1007/BF00037152

Tepe, E.J., Farruggia, F.T. & Bohs, L. 2011. A 10-gene phylogeny of *Solanum* section *Herpystichum* (Solanaceae) and a comparison of phylogenetic methods. *Amer. J. Bot.* 98: 1356–1365. http://dx.doi.org/10.3732/ajb.1000516

Vargas, P., Baldwin, B.G. & Constance, L. 1998. Nuclear ribosomal DNA evidence for a western North American origin of Hawaiian and South American species of *Sanicula* (Apiaceae). *Proc. Natl. Acad. Sci. U.S.A.* 95: 235–240. http://dx.doi.org/10.1073/pnas.95.1.235

Venkateswarlu, J. & Roo, M.K. 1972. Breeding system, crossability relationships and isolating mechanisms in the *Solanum nigrum* complex. *Cytologia* 37: 317–326. http://dx.doi.org/10.1508/cytologia.37.317

Weese, T.L. & Bohs, L. 2007. A three-gene phylogeny of the genus *Solanum* (Solanaceae). *Syst. Bot.* 32: 445–463. http://dx.doi.org/10.1600/036364407781179671

White, T.J., Bruns, T., Lee, S. & Taylor, J.W. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315–322 in: Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J. (eds.), *PCR protocols: A guide to methods and applications*. San Diego: Academic Press. http://dx.doi.org/10.1016/b978-0-12-372180-8.50042-1

Appendix 1. A preliminary checklist of currently accepted species names within the Morelloid clade of *Solanum*, ordered in the newly circumscribed informal groups as delimited here based on molecular phylogenetic results. Species not sampled in the phylogeny are shown without bold font, and have been placed within the informal groups based on morphology, as overviewed in Table 2. Chromosome counts for species for which data is available are indicated based on literature (Schilling & Heiser, 1979; Fernández Casas, 1982; Edmonds, 1982, 1983, 1986; Bhiravamurty & Rethy, 1983; Moscone, 1992; Morton, 1993; Edmonds & Chweya, 1997; Acosta & al., 2005; Olet, 2004; Manoko, 2007; Manoko & al., 2012; Moyetta & al., 2013; Olet & al., 2015).

MORELLOID CLADE OF *SOLANUM*

Chamaesarachidium clade
- ***S. gilioides*** Rusby, 2*n* = 24
- ***S. weddellii*** Phil.

Episarcophyllum clade
- ***S. echegarayi*** Hieron., 2*n* = 24
- ***S. hastatilobum*** Bitter, 2*n* = 24
- ***S. riojense*** Bitter
- ***S. sinuatirecurvum*** Bitter, 2*n* = 24

Radicans clade
- ***S. corymbosum*** Jacq.
- ***S. palitans*** C.V.Morton, 2*n* = 24
- ***S. radicans*** L.f., 2*n* = 24
- ***S. tripartitum*** Dunal, 2*n* = 24

Black nightshade clade
- *S. albescens* (Britton ex Rusby) Hunz.
- *S. alliariifolium* M.Nee & Särkinen
- ***S. aloysiifolium*** Dunal, 2*n* = 24
- ***S. alpinum*** Zoll. & Moritzi
- ***S. americanum*** Mill., 2*n* = 24
- ***S. annuum*** C.V.Morton
- *S. antisuyo* Särkinen & S.Knapp
- *S. arenicola* Särkinen & P.Gonzáles
- *S. atriplicifolium* Gillies ex Nees, 2*n* = 24
- ***S. chenopodioides*** Lam., 2*n* = 24
- *S. concarense* Hunz., 2*n* = 24
- *S. dasyadenium* Bitter subsp. *potosanum* Bitter
- *S. dianthum* Rusby
- ***S. douglasii*** Dunal, 2*n* = 24
- ***S. enantiophyllanthum*** Bitter, 2*n* = 24
- ***S. fiebrigii*** Bitter, 2*n* = 24
- ***S. florulentum*** Bitter, 2*n* = 48
- ***S. fragile*** Wedd., 2*n* = 48
- ***S. furcatum*** Dunal, 2*n* = 72
- ***S. glandulosipilosum*** Bitter, 2*n* = 24
- ***S. gonocladum*** Dunal
- ***S. grandidentatum*** Phil., 2*n* = 48
- ***S. hirtulum*** Steud. ex A.Rich., 2*n* > 24?
- ***S. huayavillense*** Del Vitto & Peten.
- *S. interandinum* Bitter, 2*n* = 48
- ***S. interius*** Rydb., 2*n* = 24
- *S. juninense* Bitter
- *S. leonii* Heiser
- *S. leptocaulon* Van Heurck & Müll.Arg.
- *S. longifilamentum* Särkinen & P.Gonzáles
- *S. macrotonum* Bitter, 2*n* = 72
- *S. memphiticum* J.F.Gmel., 2*n* = 72
- ***S. nigrescens*** M.Martens & Galeotti, 2*n* = 24
- ***S. nigrum*** L., 2*n* = 72
- ***S. nitidibaccatum*** Bitter, 2*n* = 24
- ***S. opacum*** A.Braun & C.D.Bouché, 2*n* = 72
- ***S. pallidum*** Rusby
- *S. paucidens* Bitter
- *S. pentlandii* Dunal, 2*n* = 24
- *S. physalifolium* Rusby
- *S. pilcomayense* Morong, 2*n* = 24
- *S. polytrichostylum* Bitter
- ***S. probolospermum*** Bitter, 2*n* = 24
- *S. profusum* C.V.Morton
- *S. pseudoamericanum* Särkinen, Gonzáles & S.Knapp
- *S. pseudogracile* Heiser, 2*n* = 24
- *S. pseudospinosum* C.H.Wright, 2*n* = 48
- ***S. ptychanthum*** Dunal, 2*n* = 24
- *S. pygmaeum* Cav., 2*n* = 24
- ***S. retroflexum*** Dunal, 2*n* = 48
- *S. rhizomatum* Särkinen & M.Nee
- ***S. salamancae*** Hunz. & Barboza
- ***S. salicifolium*** Phil., 2*n* = 24
- *S. sarrachoides* Sendtn., 2*n* = 24
- ***S. scabrum*** Mill., 2*n* = 72
- *S. sinaicum* Boiss., 2*n* = 24
- *S. sinuatiexcisum* Bitter, 2*n* = 24
- *S. subtusviolaceum* Bitter
- ***S. tarderemotum*** Bitter, 2*n* = 48
- ***S. triflorum*** Nutt., 2*n* = 24
- *S. tweedianum* Hook., 2*n* = 24
- ***S. umalilaense*** Manoko, 2*n* = 48
- ***S. villosum*** Mill., 2*n* = 48
- *S. zahlbruckneri* Bitter, 2*n* = 24
- *S. zuloagae* Cabrera, 2*n* = 24

Unplaced
- ***S. caesium*** Griseb.

Appendix 2. Accession and voucher details of the ingroup *Solanum* sequences generated as part of the study. Seed bank accession numbers are shown for vouchers linked to genebank collections. Dash (–) indicates missing sequence.

Taxon, nrITS, *trnL-F*, *waxy*, voucher specimen (herbarium code or seedbank: NIJ = Experimental Garden and Genebank, Radboud University, Nijmegen, Netherlands; USDA = U.S. Department of Agriculture), collection country, major area (if available), DNA code (if applicable). Polyploid species are incicated with an asterisk.

S. aloysiifolium Dunal, –, KT820802, KT820890, *Nee 51846* (NY), Bolivia, Cochabamba, LB847. ***S. alpinum*** Zoll. & Moritzi, –, –, KT820844, *Kostermans 6278* (L), Indonesia, Java. ***S. americanum*** Mill. (= *S. nodiflorum* Jacq.), –, KT820803, KT820845, *Bohs 3095* (UT), U.S.A., Utah, LB1015. ***S. annuum*** C.V.Morton, KT820891, –, KT820846, *Barboza 2176* (CORD), Argentina, Tucumán. ***S. anomalostemon*** S.Knapp & M.Nee, KT820892, –, –, *Sanchez & al. 5109* (NY), Peru, Cusco, LB1927. ***S. anomalostemon*** S.Knapp & M.Nee, –, KT820801, KT820847, *Knapp & al. 10353* (BM), Peru, Apurímac. ***S. caesium*** Griseb., KT820893, KT820804, KT820848, *Bohs 2815* (UT), Bolivia, Santa Cruz, LB341. ***S. chenopodioides*** Lam., –, KT820805, KT820849, *Manoko A95/185* (NIJ), Australia. ***S. corymbosum*** Jacq., –, KT820806, KT820850, *Bohs 3109* (UT), Peru, Ancash, LB727. ***S. douglasii*** Dunal, –, KT820807, KT820851, *Wicklund 34* (BM), U.S.A., Arizona. ***S. echegarayi*** Hieron., KT820894, KT820808, –, *Cabrera 29559* (K), Argentina, San Juan, LB698. ***S. echegarayi*** Hieron., –, –, KT820852, *Cabrera 30142* (K), Argentina, San Juan, K38633. ***S. enantiophyllanthum*** Bitter (= *S. itatiaiae* Glaz. ex Edmonds), KT820895, KT820809, KT820853, *Flinte s.n.* (UT), Brazil, Rio de Janeiro, LB2152. ***S. fiebrigii*** Bitter, KT820896, KT820810, KT820854, *Bohs 2784* (UT), Bolivia, Santa Cruz, LB313. ***S. florulentum**** Bitter, –, KT820811, KT820855, *Manoko A1/164a* (NIJ), Kenya. ***S. fragile**** Wedd., –, KT820812, KT820856, *Beck 11788* (K), Bolivia, Oruro, K38595. ***S. furcatum**** Dunal, –, KT820813, KT820857, *Gardner & al. 174* (BM), Chile, Region VII. ***S. gilioides*** Rusby, –, KT820814, KT820858, *Wood & al. 22689* (K), Bolivia, LB2004. ***S. glandulosipilosum*** Bitter, –, KT820815, KT820859, Nee & *Bohs 50793* (NY), Argentina, Jujuy, LB593. ***S. gonocladum*** Dunal, KT820897, KT820816, KT820860, *Nee 51815* (NY), Bolivia, La Paz, LB862. ***S. grandidentatum**** Phil. (= *S. excisirhombeum* Bitter), KT820898, –, KT820861, *Tepe 2291* (NY), Peru, Pasco, LB3056. ***S. hastatilobum*** Bitter, –, –, KT820862, *Barboza & al. 2962* (CORD), Argentina, Catamarca. ***S. hirtulum**** Steud. ex A.Rich., –, KT820817, KT820863, *Friis & al. 11912* (K), Ethiopia, Amhara, K38608. ***S. huayavillense*** Del Vitto & Peten., KT820899, KT820818, KT820864, *Bohs 2786* (UT), Bolivia, Santa Cruz, LB336. ***S. interius*** Rydb., –, –, KT820865, *Bohs 2464* (UT), U.S.A., Nebraska, LB111. ***S. nigrescens*** M.Martens & Galeotti, –, KT820819, KT820866, *Bohs 2400* (UT), Costa Rica, La Selva, LB70. ***S. nigrum**** L., KT820900, KT820820, –, *Bohs 2534* (UT), Australia, LB141. ***S. nigrum**** L., –, –, KT820867, *Bohs 2698* (UT), Italia, Sicily, LB223. ***S. nitidibaccatum*** Bitter, KT820901, KT820821, KT820868, *Bohs 2467* (UT), Bolivia, Colorado, LB72. ***S. opacum**** A.Braun & C.D.Bouché, KT820902, KT820822, KT820869, *Bohs 2459* (UT), Australia, New South Wales, LB106. ***S. palitans*** C.V.Morton, –, KT820823, KT820872, *Bohs 2449* (UT), Australia, New South Wales, LB65. ***S. pallidum*** Rusby (= *S. planifurcum* Bitter), KT820903, KT820824, KT820873, *Nee 51759* (NY), Bolivia, La Paz, LB787. ***S. probolospermum*** Bitter, KT820904, –, KT820874, *Nee & Wen 53930* (NY), Bolivia, La Paz, LB3057. ***S. ptychanthum*** Dunal, –, KT820825, KT820875, *Bohs 3652* (UT), U.S.A., Florida, LB3058. ***S. radicans*** L.f., –, KT820826, KT820876, *Bohs 3148* (UT), Ecuador, Pinchincha, LB1638. ***S. reductum*** C.V.Morton, KT820905, –, KT820877, *Krapovickas & al. 27854* (MO), Argentina, Tucumán, LB898. ***S. retroflexum**** Dunal, –, KT820827, KT820878, *Manoko A1/025b* (NIJ), South Africa. ***S. riojense*** Bitter, –, KT820828, KT820879, *Nee & Bohs 50843* (NY), Argentina, Jujuy, LB544. ***S. salamancae*** Hunz. & Barboza, –, KT820829, KT820880, *Barboza 2191* (CORD), Argentina, Salta. ***S. salicifolium*** Phil., –, KT820830, KT820881, *Nee & Bohs 50848* (NY), Argentina, Jujuy, LB545. ***S. scabrum**** Mill., –, KT820831, KT820882, *Manoko A1/031a* (NIJ), Uganda. ***S. scabrum**** Mill., KT820906, KT820832, KT820883, *Bohs 2729* (UT [= NIJ 884750172]), unknown, LB257. ***S. sinuatiexcisum*** Bitter, –, KT820833, KT820884, *Nee 51766* (BM), Bolivia, La Paz, LB811. ***S. sinuatirecurvum*** Bitter, KT820907, –, KT820885, *Johns 8348* (BM), Bolivia, Oruro, LB699. ***S. tarderemotum*** Bitter, –, KT820834, KT820886, *Manoko A1/167b* (NIJ), Kenya. ***S. triflorum*** Nutt., –, KT820835, KT820887, *Bohs 3062* (UT), U.S.A., Utah, LB861. ***S. tripartitum*** Dunal, KT820908, KT820836, KT820888, *Bohs 2465* (UT [= NIJ 904750148]), origin unknown, LB112. ***S. umalilaense**** Manoko, –, KT820837, KT820889, *Manoko A1/133* (NIJ), Tanzania. ***S. valdiviense*** Dunal, KT820909, KT820838, KT820843, *Knapp 10105* (BM), Argentina, Neuquén, LB2499. ***S. villosum**** Mill., KT820910, KT820839, KT820870, *Bohs 2553* (UT [= USDA PI 304600]), Iran, LB150. ***S. weddellii*** Phil. (= *S. chamaesarachidium* Bitter), –, KT820840, KT820871, *Villavicencio 318* (LPB), Bolivia, La Paz, LB796.

Appendix 3. Accession and voucher details for the outgroup sequences of *Solanum* generated in this study. Dash (–) indicates missing sequence.

Taxon, nrITS, *trnL-F*, voucher specimen (herbarium code; NIJ = Experimental Garden and Genebank, Radboud University, Nijmegen, Netherlands), collection country, major area (if available), DNA code (if applicable).

S. crispum Ruíz & Pav.: KT820911, –, BIRM S.0486 (NIJ), cultivated (unknown origin), RGO112. ***S. monarchostemon*** S.Knapp: KT820912, – , *Stern 290* (UT), Ecuador, Sucumbios, LB2379. ***S. multifidum*** Lam.: – , KT820841, *Stern & al. 85* (UT), Peru, Lima, LB2141. ***S. pubigerum*** Dunal: KT820913, –, *Bohs 3477* (UT) and BIRM/S.0729 (NIJ), cultivated (origin unknown), LB633. ***S. seaforthianum*** Andrews: KT820914, –, BIRM S.0051 (NIJ), Zimbabwe, RGO300. ***S. terminale*** Forssk.: – , KT820842, *Vorontsova & al. 93* (UT), Kenya, LB2493.

Appendix 4. Details of the *Solanum* sequences downloaded from GenBank used as outgroups in the study. Dash (–) indicates missing sequence.

Taxon: nrITS, *trnL-F*, *waxy*.

S. abutiloides (Griseb.) Bitter & Lillo: AF244716, HM006829, AY562948; ***S. allophyllum*** (Miers) Standl.: AF244732, DQ180422, AY996379; ***S. anceps*** Ruíz & Pav.: GQ221541, GQ221568, GQ221593; ***S. appendiculatum*** Dunal: AF244746, DQ180461, DQ169018; ***S. argentinum*** Bitter & Lillo: AF244718, DQ180425, AY996382; ***S. aviculare*** G.Forst.: AF244743, AY559238, AY562952; ***S. batoides*** D'Arcy & Rakot.: KF720741, KF720766, –; ***S. betaceum*** Cav.: AY523873, HM006830, AY996387; ***S. brevicaule*** Bitter: AY875826, DQ180443, DQ169019; ***S. brevifolium*** Dunal: GQ221562, GQ221589, GQ221614; ***S. bulbocastanum*** Dunal: AY875758, DQ180444, AY875562; ***S. capsiciforme*** (Domin) G.T.S.Baylis: KF720742, HM006859, –; ***S. caripense*** Dunal: GQ221563, GQ221590, GQ221615; ***S. clandestinum*** Bohs: KF720743, DQ180462, DQ169023; ***S. conicum*** Ruíz & Pav.: GQ221549, GQ221576, GQ221601; ***S. crispum*** Ruíz & Pav.: –, DQ169024, DQ169024; ***S. dulcamara*** L.: AY875753, HM006840, KC469866; ***S. evolvulifolium*** Greenm.: HQ856116, HQ856056, HQ856211; ***S. guineense*** L.: KF720740, DQ180460, DQ169014; ***S. havanense*** Jacq.: –, DQ180431, DQ169030; ***S. herculeum*** Bohs: AF244734, DQ180466, DQ169031; ***S. hoehnei*** C.V.Morton: AY996519, DQ180484, AY996426; ***S. laciniatum*** Aiton: AF244744, DQ180467, AY996431; ***S. lepidotum*** Dunal: JN542590, DQ180486, DQ169035; ***S. luteoalbum*** Pers.: AF244715, DQ180433, AY562957; ***S. mapiriense*** Bitter: AY996527, DQ180434, AY996439; ***S. melongena*** L.: EU176120, HM006827, EU176136; ***S. monarchostemon*** S.Knapp: –, KF720767, KF720779; ***S. montanum*** L.: AY996531, DQ180468, AY996443; ***S. multifidum*** Lam.: KF720744, –, KF720780; ***S. nakurense*** C.H.Wright: KF720745, KF720768, –; ***S. nemorense*** Dunal: AY996536, DQ180488, AY996447; ***S. nitidum*** Ruíz & Pav.: AF244740, DQ180451, DQ169039; ***S. paposanum*** Phil.: KF720746, KF720769, KF720781; ***S. pubigerum*** Dunal: –, DQ180455, DQ169043; ***S. refractum*** Hook. & Arn.: AY996547, HQ457408, AY996460; ***S. reptans*** Bunbury: AY996548, –, –; ***S. seaforthianum*** Andrews: –, DQ180438, DQ169048; ***S. simile*** F. Muell.: KF720747, HM006856, KF720783; ***S. sisymbriifolium*** Lam.: KC539158, KC539180, KC539182; ***S. symonii*** H.Eichler: KF720748, HM006858, KF720784; ***S. terminale***: KF720749, KF720770, –; ***S. thelopodium*** Sendtn.: AY996556, DQ180470, AY996471; ***S. trisectum*** Dunal: AY996475, DQ180471, AY996475; ***S. tuberosum*** L.: AY875827, FJ490824, AF274513; ***S. uleanum*** Bitter: GQ221561, GQ221588, GQ221613; ***S. vescum*** F.Muell.: KF720751, HM006848, –; ***S. wendlandii*** Hook.f.: AF244731, DQ180440, AY562974.