RESEARCH ARTICLE

Phylogeny, character evolution and biogeography of the genus *Sclerophylax* (Solanaceae)

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Abstract The genus *Sclerophylax* (Atropina clade, Solanaceae) has 14 species recognized to date, all except two endemic to Argentina. To clarify its phylogenetic relationships, molecular sequence data from the *trnL-trnF* and the *rpl32F-trnL* intergenic spacer regions, the *waxy* gene and the ITS region were employed in maximum likelihood and Bayesian inference analyses. Divergence times were estimated for the combined dataset using secondary calibrations. Ancestral distribution areas were reconstructed by Bayesian binary analyses and seven morphological traits (one continuous, six discrete) were traced. *Sclerophylax* is monophyletic and its sister clade is *Lycium* + *Nolana*. *Sclerophylax* is divided into *S.* sect. *Caducifructus* (1 sp.) and the monophyletic sect. *Sclerophylax* (11 spp.). *Sclerophylax* sect. *Sclerophylax* includes five well-supported clades that are also supported by morphological synapomorphies; circumscription of some species remains uncertain, probably as a result of rapid diversifications, but four lineages are recognized by a combination of morphological traits. The ancestor of *Sclerophylax* was an annual, procumbent/decumbent plant with rhomboidal leaves and sessile, persistent, 2–3-seeded fruits. The most variable traits were leaf shape and life form, while flower size was shown to be highly informative in diagnosing clades within *Sclerophylax*. The area of origin for *Sclerophylax* was the Prepuna biogeographical province, from which it has spread in recent geological times. A total of 12 species are recognized in *Sclerophylax*, with an updated synonymy, lectotypifications, and re-circumscriptions provided.

Keywords ancestral state reconstruction; Argentina; Atropina clade; biogeography; divergence times; morphological synapomorphies

Supporting Information may be found online in the Supporting Information section at the end of the article.

■ INTRODUCTION

The nightshade family, Solanaceae Juss., is a cosmopolitan family assigned to the order Solanales (Stevens, 2001–). Solanaceae is a monophyletic group with approximately 99 genera and 2400–3000 species (Olmstead & al., 2008; Särkinen & al., 2013; Barboza & al., 2016). Many of these species, such as potato, tobacco, tomato and eggplant, have remarkable economic importance worldwide. Since 1990, the phylogenetic relationships within Solanaceae have been exhaustively examined using molecular characters from nuclear DNA regions, chloroplast regions or both (e.g., Bohs & Olmstead, 1999; Bohs, 2005; Olmstead & al., 2008). The most recent phylogenetic hypothesis (Särkinen & al., 2013; Ng & Smith, 2014; De-Silva & al., 2017) includes nearly 50% of the Solanaceae species, although the most recent systematic proposal for the entire family is based on Olmstead & al. (2008). In addition to these advances on molecular phylogenies, important taxonomic changes within Solanaceae have comprised rearrangements of its circumscription; Solanaceae now includes the genera *Nolana* L. ex L.f. and *Sclerophylax* Miers (Olmstead & Palmer, 1992; Olmstead & al., 2008), which were previously placed in other families (Berchtold & Presl, 1820; Miers, 1848). Even with a clear picture of the circumscription of the family and with most of the generic relationships resolved, ca. 50% of its species have not been phylogenetically studied; these are scattered among the different clades of the family, including the Atropina clade.

In their molecular phylogenetic study of the Solanaceae, Olmstead & al. (2008) defined an unranked informal clade, the Atropina (or Atropina clade), which encompasses the tribes Lycieae (consisting only of *Lycium*, 92 spp.) and Hyoscyameae (43 spp.) as well as the genera *Jaborosa* (23 spp.), *Latua* (1 sp.), *Nolana* (±80 spp.), and *Sclerophylax*. In total, the Atropina clade comprises 13 genera, including the monophyletic *Lycium* L. and *Nolana*, which are two of the largest genera in the family after *Solanum* L. (±1400 spp.), *Cestrum* L. (240 spp.), *Lycianthes* (Dunal) Hassl. (±200 spp.) and *Physalis* L. (±95 spp.). *Lycium* and *Nolana* have been extensively studied from morphological, cytogenetic and phylogenetic

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points of view (e.g., Levin & Miller, 2005; Tu & al., 2008; Stiefkens & al., 2010; Lujea & Chiarini, 2017) and together form a clade sister to *Sclerophylax*. This latter is a small genus with 14 currently recognized species endemic to dry regions of central and western Argentina. Only two have ranges extending beyond Argentina: *S. spinescens* Miers., which grows in the Paraguayan Chaco, and *S. lorentzianus* O.Hoffm., which reaches Uruguay. Its centre of diversity is probably in the Argentinian province of Catamarca, where 6 out of the 14 species are found. *Sclerophylax* species are succulent, mostly annual herbs; their flowers are bisexual, axillary, solitary, sessile or shortly pedicellate, with asymmetrical calyces and slightly zygomorphic corollas (Barboza, 2013) (Fig. 1). The fruit of *Sclerophylax* (Fig. 1) is dry, indehiscent, and embedded in the stem and crowned by the calyx that usually becomes spiny (Di Fulvio, 1961). These are features that are rare within Solanaceae and led to the placement of *Sclerophylax* in its own family. The species of *Sclerophylax* are all morphologically similar, and some of them have been described only relatively recently (Del Vitto & Petenatti, 2001). The taxonomic position of this genus has been troublesome: first it was placed in a monotypic family (Sclerophylacaceae) but later it was transferred to Boraginaceae and then to Hydrophyllaceae (Di Fulvio, 1961). With the advent of molecular phylogenies, Sclerophylacaceae was revealed as paraphyletic and nested within Solanaceae (Olmstead & al., 2008; Särkinen & al., 2013; Moré & al., 2015). However, previous molecular phylogenetic studies only considered three species at most (Levin & Miller, 2005; Olmstead & al., 2008), and the interspecific relationships within the genus remain unresolved.

According to molecular phylogenies and fossil evidence, the family Solanaceae would have evolved early in South America when it was still part of the Gondwanan supercontinent (Dupin & al., 2017; Wilf & al., 2017), and short-range movements would explain most of the spread of Solanaceae within the Americas, but at least 20 transoceanic long-distance dispersals have been proposed to account for range expansions to the Old World (Olmstead, 2013; Dupin & al., 2017). One of these long-distance dispersals occurred within the Atropina clade (Tu & al., 2010), so a more accurate phylogeny of *Sclerophylax* and its relatives could help in completing the biogeographical picture of the family. According to Särkinen & al. (2013), *Sclerophylax* split from its sister clade (*Nolana* + *Lycium*) at ca. 12 Ma, and species diversification occurred after 4.4 Ma.

In the present study, we aim to clarify the evolutionary history of *Sclerophylax*. There are near-complete published phylogenies for *Lycium* (Levin & Miller, 2005; Levin & al., 2011), *Nolana* (Dillon & al., 2007; Tu & al., 2008), the tribe Hyoscyameae (Tu & al., 2010; Sanchez-Puerta & Abbona, 2014) and *Jaborosa* (Moré & al., 2015), but a comprehensive molecular study of *Sclerophylax* is still lacking. The main goals of this study are to clarify the phylogenetic relationships within the genus *Sclerophylax* and its allies and to infer evolutionary patterns in several biological features (fruit, life form, floral size). Therefore, we aim to (i) determine the phylogenetic relationships of the species currently recognized within *Sclerophylax*, (ii) examine patterns of morphological trait evolution in the genus, and (iii) examine its biogeographical patterns in an evolutionary context.

■ MATERIALS AND METHODS

Sampling. — The ingroup comprised 33 samples belonging to 13 out of 14 currently recognized species of *Sclerophylax* (Barboza, 2013); only *S. tenuicaulis* Di Fulvio was not represented since it was not possible to get material for DNA extraction. Several accessions were sampled for 10 taxa, especially for those with unclear morphological circumscriptions or widespread ranges with intraspecific morphological variation (*S. arnottii*, *S. kurtzii*, *S. spinescens*). *Nicotiana attenuata* of subfamily Nicotianoideae was included and designated as the outgroup in all analyses. We also included 21 taxa, mostly representatives of the Atropina clade according to Särkinen & al. (2013). Available sequences for the outgroups were retrieved from GenBank, and new sequences were generated when possible. A list of all samples with their GenBank accession numbers is provided in Appendix 1.

DNA extraction, PCR amplification and sequencing. — Total genomic DNA was extracted from fresh, silica gel-dried material using the DNeasy plant mini extraction kit (Qiagen, Valencia, California, U.S.A.). Primer sequences are provided in suppl. Table S1. Two non-coding regions of the chloroplast genome, *trnL-trnF* and *rpl32F-trnL*, were amplified following standard procedures described in Taberlet & al. (1991), Bohs (2004) and Shaw & al. (2007). When possible, *trnL-trnF* was amplified as a single fragment using primers a and f (Taberlet & al., 1991) and following PCR conditions of Bohs & Olmstead (2001) or, alternatively, overlapping fragments were amplified and assembled using combinations of primers a, c and e with b, d and f. The ITS region was amplified as a single fragment using primers ITS-leu1 (Bohs & Olmstead, 2001) and ITS4 (White & al., 1990) using PCR conditions described in Bohs & Olmstead (2001). *Waxy* was amplified as a single fragment using PCR conditions and primers waxyF and waxy2R, according to Levin & al. (2006). When necessary, overlapping fragments were amplified and assembled using primers waxyF with 1171R, 1058F with 2R, and Ex4F with Ex4R or waxy3nr (Peralta & Spooner, 2001; Stern & al., 2010; Walsh & Hoot, 2001). PCR products were cleaned using the Promega Wizard SV PCR Clean-Up System (Promega, Madison, Wisconsin, U.S.A.). The University of Utah DNA Sequencing Core Facility performed sequencing on an ABI automated sequencer. Sequences were edited in MEGA 6 (Tamura & al., 2013) and all new sequences were submitted to GenBank (Appendix 1).

Phylogenetic analyses. — Sequence alignments (suppl. Appendix S1) were performed using the MUSCLE algorithm (Edgar, 2004) implemented in MEGA 6 (Tamura & al., 2013). Aligned sequences were analysed using jModelTest v.2.1.3

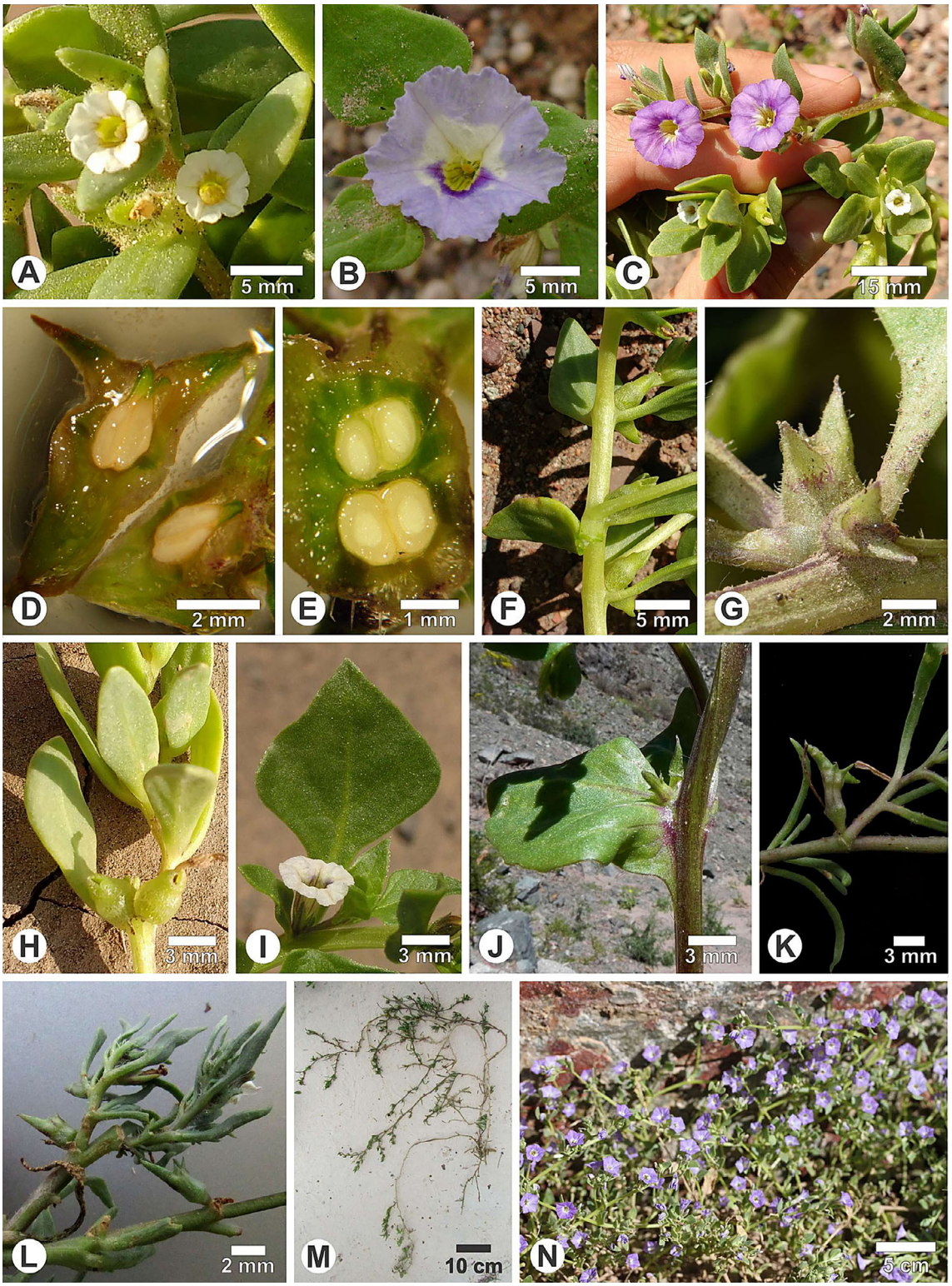

Fig. 1. **A**, *Sclerophylax caducifructus*, flowers (*Barboza & al. 4777*); **B**, *S. arnottii*, flower (*Barboza 1976*); **C**, *S. kurtzii* (left; *Barboza & al. 4784*) and *S. caducifructus* (right; *Barboza & al. 4780*); **D** & **E**, Fruits of *S. spinescens* in longitudinal and cross section, respectively (*Barboza 2320*); **F**, Branch of *S. caducifructus* with fruits (*Barboza & al. 4777*); **G**, *S. trispermus* (*Barboza & al. 5131*), detail of the fruits adnate with the leaf axil; **H**, Branch of *S. caducifructus* showing the ovate leaves and deciduous fruits (*Barboza & al. 4777*); **I**, *S. spinescens*, detail of flower and rhomboidal leaf (*Barboza 2320*); **J**, *S. adnatifolius*, detail of the leaf blade decurrent on the stem, with two adnate fruits in its axil (*Barboza & al. 2466*); **K**, *S. spinescens*, detail of the linear leaves and a pedicellate fruit (*Chiarini 1461*); **L**, *S. spinescens*, showing intermediate linear-rhombic leaves and adnate and pedicellate fruits in the same individual (*Chiarini 1451*); **M**, *S. spinescens*, prostrate habit (*Chiarini 1461*); **N**, *S. arnottii*, erect habit (*Urdampilleta 601*). — Photos: A–C, F, H & K–N by F. Chiarini; D, E, I & J by G. Barboza; G by S. Knapp. All vouchers at CORD.

(Darriba & al., 2012) to obtain the best-fit nucleotide substitution models for the data (Table 1) based on the Akaike information criterion (AIC; Posada & Crandall, 1998). Maximum likelihood (ML) analyses were conducted in RAxML v.8 (Stamatakis, 2014) using the CIPRES platform to reduce the execution time (Miller & al., 2010). To assess nodal support of the ML tree, a resampling of 1000 bootstrap (BS) inferences was done. We first estimated the phylogeny of *Sclerophylax* using each DNA region independently. Sequences leading to long branches were double-checked by blasting for sequences highly similar to the introns and exons in order to exclude contamination problems. Trees were then compared across genes to identify areas of hard incongruence (BS > 70%; Mason-Gamer & Kellogg, 1996).

Given the absence of hard incongruence, we conducted a ML analysis on the combined dataset (suppl. Appendix S1). The four matrices were concatenated with SequenceMatrix v.1.8 (Vaidya & al., 2011) and partitioned by gene before ML analysis. We also identified unstable tips based on the ML bootstrap analyses using the software RogueNaRok (Aberer & al., 2013). Two iterations of RogueNaRok were run with settings according to Särkinen & al. (2013), and rogue taxa were removed after each iteration, resulting in the pruning of 6 tips in total. The final combined matrix included 4232 bp of aligned sequence data for 54 accessions, including outgroups, and was analysed using RAxML partitioned by gene according to the parameters used for individual regions (see above). Phylogenetic trees were visualised in FigTree v.1.4.3 (Rambaut, 2016).

Morphological character evaluation and ancestral state reconstruction. — One continuous and six discrete traits were traced on the maximum clade credibility (MCC) tree (suppl. Tables S2, S3) obtained from the combined dataset using BEAST 2 (Bouckaert & al., 2014). These characters were selected according to their previous use in identification keys for *Sclerophylax*: fruit pedicel, fruit abscission, leaf shape, life form, habit, seeds per fruit (Di Fulvio, 1961; Barboza, 2013). Flower size (i.e., corolla length), the only continuous trait, was mapped onto the MCC tree using the ContMap function in the phytools package (Revell, 2012), in R v.3.4.2 (R Core Team, 2017). Ancestral character states were estimated through a ML-based procedure assuming that characters evolve under a Brownian motion mode. The six discrete traits were reconstructed using the ace function from the package APE (Paradis & al., 2004) and stochastic mapping using the make.simmap function from the package phytools (Revell, 2012), in R v.3.4.2 (R Core Team, 2017). We conducted a Bayesian stochastic mapping (Nielsen, 2001; Huelsenbeck & al., 2003), with 1000 simulations of character histories on the combined MCC tree. We estimated the median number of changes per transition, generally preferred over means for non-normal distributions (Deanna & al., 2018).

Divergence time estimates. — Both BI and divergence time estimates were performed for the combined dataset using BEAST 2 (Bouckaert & al., 2014) on the CIPRES platform (Miller & al., 2010). As fossils for *Sclerophylax*/Atropina clade are not available, we used three calibration points based on the estimates of Särkinen & al. (2013): the most recent common ancestor of tribe Hyoscyameae (11.8 ± 2.5 Ma), the most recent common ancestor of the genus *Lycium* (4.9 ± 1.25 Ma), and the most recent common ancestor of the clade Hyoscyameae + Atropina + *Sclerophylax* (16.94 ± 2.5 Ma). We assigned a uniform distribution to the node constraints, and site models were set up for each partition following the results of jModelTest (see above). Three independent BEAST analyses were run for 50 million generations with tree sampling every 5000 generations, using an uncorrelated, lognormal relaxed clock model to describe the branch-specific substitution rates (Drummond & al., 2006) and a birth-death prior. Convergence and stationarity of the parameters were inspected using Tracer v.1.7 (Rambaut & al., 2018), targeting minimum effective sample sizes (ESS) of 200. Trees were combined with burn-in set to 25% using LogCombiner as implemented in the BEAST package. The phylogenetic relationships were summarized in a MCC tree, and their posterior probabilities (PP), median age estimates, and 95% highest posterior density (HPD) intervals for all nodes were estimated using TreeAnotator v.2.4.7. The annotated tree was displayed in FigTree v.1.4.3 (Rambaut, 2016).

Ancestral areas reconstruction. — A Bayesian binary Markov chain Monte Carlo (BBM) analysis was performed in RASP v.3.2 (Yu & al., 2015) to reconstruct ancestral areas of geographic distribution, allowing a maximum of three areas per node, which is the maximum number of areas that our studied species inhabit (only two species of *Lycium* are in three areas). The distribution range of each sample was based

Table 1. Summary of sequence information and properties of the nuclear and chloroplast regions used to infer the phylogeny.

DNA region		ITS	*waxy*	*rpl32F-trnL*	*trnL-trnF*
Number of accessions with sequence data (% of total accessions sampled)	Ingroup	24 (72)	25 (76)	23 (70)	20 (61)
	Outgroup	11 (52)	18 (86)	12 (57)	12 (57)
Number of characters		621	1710	968	933
Number of constant characters (%)		238 (38.3)	971 (56.8)	484 (50)	645 (69.1)
Number of variable characters (%)		383 (61.7)	739 (43.2)	484 (50)	288 (30.9)
Number of parsimony-informative characters (%)		159 (25.6)	405 (23.7)	263 (27.2)	34 (3.6)
Best-fitting likelihood model		GTR+Γ	TPM1uf+Γ	GTR	GTR+Γ

on voucher locations housed at several herbaria (CORD, MASS, NL) and F.E. Chiarini's fieldwork. Seven areas were defined for biogeographical analysis: Old World, North America, Chile, and the biogeographic provinces of Cabrera & Willink (1973): Monte, Prepuna, Chaco, and Pampa + Espinal. Multiple states were scored for taxa with widespread distributions. The BBM analysis used 10,000 resampled trees generated during the BEAST analysis to incorporate phylogenetic uncertainty and was performed in parallel for five million generations with 10 chains under the Jukes-Cantor fixed-state frequencies model and among-site variation set to Gamma.

■ RESULTS

Molecular phylogeny. — The combined dataset contained 4232 characters, 2331 from nuclear regions and 1901 from the plastid regions (Table 1). ITS and *trnL-trnF* had the highest and lowest percentage of variable characters, respectively. ML and BI analyses of the combined dataset resulted in largely congruent topologies with high resolution at the generic level and moderate resolution within genera, with slightly lower branch supports in the ML tree (Fig. 2) compared to the BI tree (suppl. Fig. S1). The combined-dataset analysis is mostly driven by the patterns seen in the *waxy* dataset (suppl. Figs. S2–S5).

Sclerophylax is resolved as a monophyletic genus in every gene tree obtained (BS = 98% for ITS, 100% for *waxy*, 100% for *rpl32F-trnL* and 79% for *trnL-trnF*; suppl. Figs. S2–S5). Two highly to moderately supported clades were recovered within *Sclerophylax* in both phylogenetic analyses (suppl. Fig. S1, Fig. 2): a clade including the samples identified as *S. caducifructus* (Caducifructus clade hereafter, PP = 1, BS = 100%) and a clade including all the remaining species (PP = 1, BS = 76%; suppl. Fig. S1, Fig. 2). We refer to this latter clade as *S.* sect. *Sclerophylax* hereafter. Additionally, five

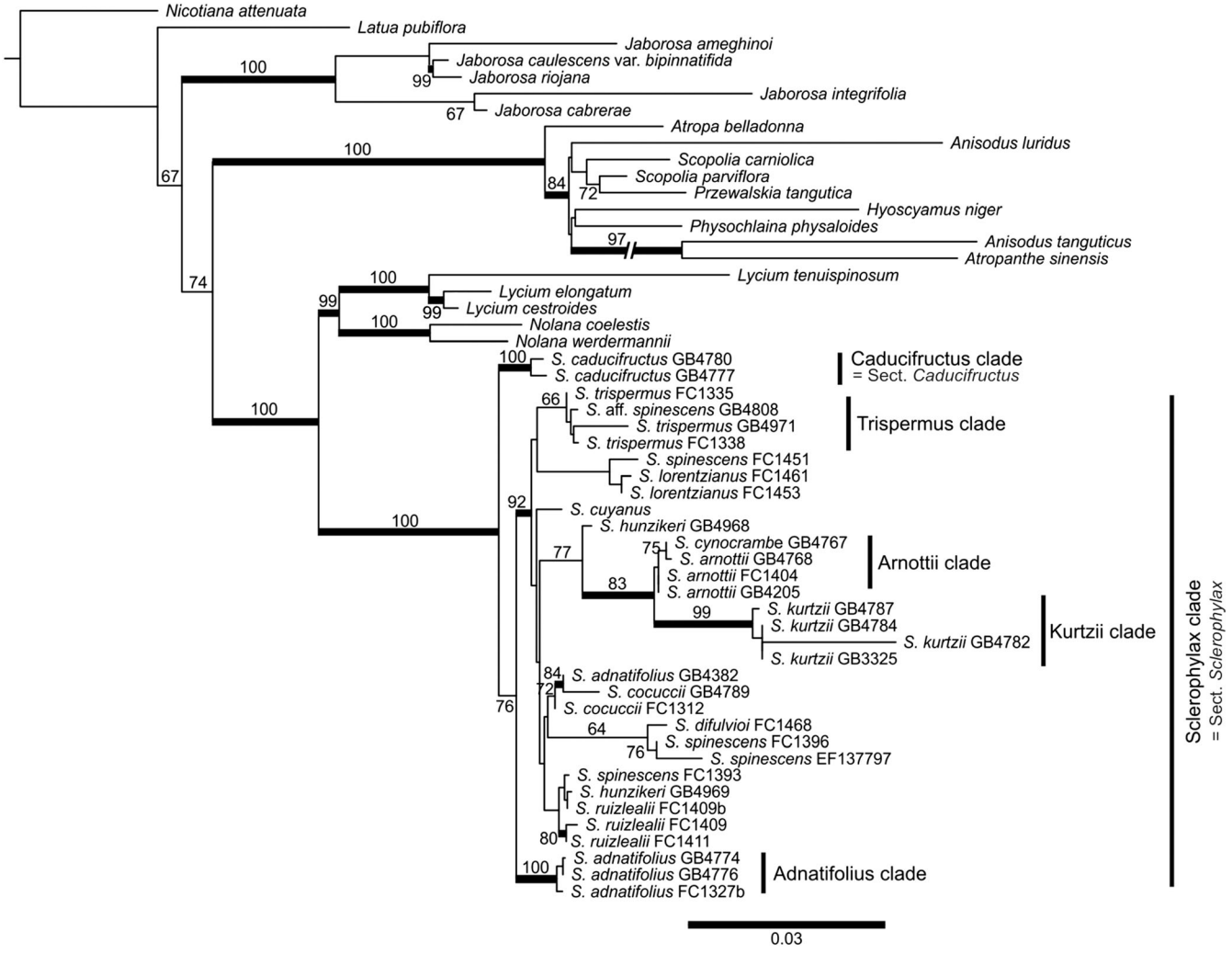

Fig. 2. Phylogenetic relationships of *Sclerophylax* based on a maximum likelihood analysis of the combined dataset of four markers (ITS, *waxy*, *rpl32F-trnL*, *trnL-trnF*). Bootstrap support (BS) values >60% are given above (or beneath) each branch, and bold branches indicate BS > 80%. Double slash indicates branch shortened to a quarter of its length.

sub-clades were resolved in both analyses (Fig. 2). These clades embrace groups of morphologically similar samples: the Trispermus clade (Fig. 2) including all the samples identified as *S. trispermus* and a sample morphologically similar to *S. spinescens* (PP = 0.88, BS = 66%); the Kurtzii clade (Fig. 2) including all the samples identified as *S. kurtzii* (PP = 1, BS = 99%); the Adnatifolius clade (Fig. 2) with three of the four samples identified as *S. adnatifolius* (PP = 1, BS = 100%); the Arnottii + Kurtzii clade (PP = 0.79, BS = 83%), and Arnottii + Kurtzii clade + 1 accession of *S. hunzikeri* (PP = 1, BS = 77%). The specimens identified as *S. spinescens* appeared interspersed within *S.* sect. *Sclerophylax* (Fig. 2).

Morphological characters evaluation and ancestral state reconstructions. — The ancestor of genus *Sclerophylax* was reconstructed as an annual and procumbent/decumbent plant with rhomboidal leaves and pedicellate, persistent fruits containing two or three seeds (Figs. 3–5, suppl. Tables S2, S3). We estimated only one change for the presence of fruit pedicel, fruit persistence, and seed number; the pedicel is present (i.e., fruit not sessile nor adnate with the axil) in the ancestor of *S.* sect. *Sclerophylax* (Fig. 5A) and also the fruit became deciduous (Fig. 3A). Deciduous fruits evolved in the Caducifructus clade from ancestors with persistent fruits, and pedicels were lost in members of *S.* sect. *Sclerophylax*. Seed number per fruit was reduced to 1–3 in the entire genus *Sclerophylax* from multi-seeded ancestors (Fig. 5B). In contrast to most species of the Atropina clade, which are perennials, the majority of *Sclerophylax* are annual, but four changes to perennial habit were identified within the genus. Regarding leaf shape, most *Sclerophylax* species have rhomboidal leaves, but the Kurtzii clade is characterized by lanceolate leaves, and other *Sclerophylax* species exhibit a variety of leaf shapes (lanceolate, rhomboidal and linear).

Across the Atropina clade, the habit shifted six times, resulting in synapomorphic habits for several clades and directional towards erect plants (four changes, Fig. 3B; suppl. Table S3). Habit was reconstructed as ancestrally procumbent/decumbent for most *Sclerophylax* species except for erect

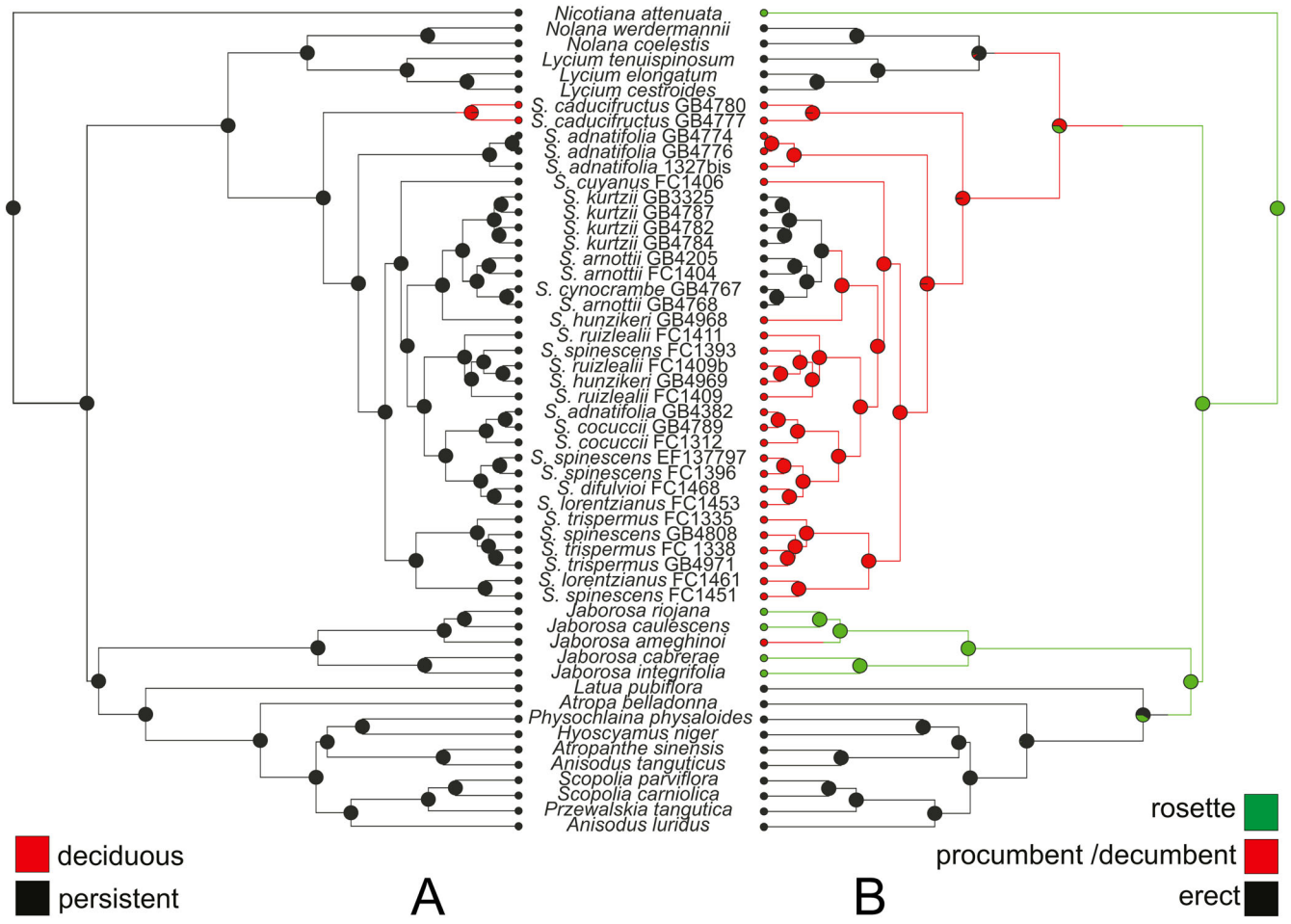

Fig. 3. Ancestral state reconstruction of morphological traits in *Sclerophylax* and its allies on the combined MCC tree, using stochastic mapping. Pies at nodes indicate frequencies of node states across 1000 simulations of character evolution and the colours of the tip labels represent tip states. **A**, Fruit abscission; **B**, Habit.

plants in the Kurtzii + Arnottii clade, paralleling habits in Hyoscyameae and *Nolana* + *Lycium*, and a rosette habit for most *Jaborosa*. The most variable traits analysed were leaf shape and life form, with 22 and 12 changes respectively (Fig. 4, suppl. Table S3). Flower size (i.e., corolla length) was highly informative in diagnosing clades within the genus *Sclerophylax* (Fig. 6) and also in the context of the Atropina clade (suppl. Figs. S6, S7). Species of the Kurtzii + Arnottii clade are differentiated from the rest of *Sclerophylax* by their larger flowers (Fig. 6), whereas the *Sclerophylax* + *Lycium* + *Nolana* clade was conspicuous in having smaller flowers than the rest of the Atropina clade (suppl. Figs. S6, S7).

Reconstruction of ancestral areas and divergence time estimates. — The Bayesian MCC analysis suggested 43 dispersals and 17 vicariant events to explain the current distribution of the sampled groups of Atropina, including one long-distance dispersal to Asia (Hyoscyameae clade). The reconstruction of the ancestral areas by the Bayesian MCC analyses and divergence-time estimate placed the putative ancestor of *Sclerophylax* + *Lycium* + *Nolana* in the Prepuna biogeographic province (probability P = 74%, Fig. 7) during the middle Miocene (12.40 [8.05–16.67] Ma, Fig. 7). The ancestor of genus *Sclerophylax* was likely distributed in the Prepuna (P = 95%, Fig. 7) during the late Miocene (8.33 Ma [4.42–12.38]), an area currently occupied by several species of the genus (e.g., species of the Caducifructus and Kurtzii clades). Ancestors of the monophyletic *S.* sect. *Caducifructus* Di Fulvio (= Caducifructus clade) and sect. *Sclerophylax* were both probably distributed in the Prepuna (P = 99% and 89%, respectively, Fig. 7), whereas the distribution reconstructed for the ancestor of *S.* sect. *Sclerophylax* excluding the Adnatifolius clade was ambiguous. The ancestor of the Arnottii clade was reconstructed in the Monte biogeographic province (P = 97%, Fig. 7) and that of the Trispermus clade in Chaco province (P = 99%, Fig. 7).

■ DISCUSSION

Phylogenetic relationships and generic circumscription. — Our study has considerably expanded the sampling of the genus *Sclerophylax*, from 4 accessions in previous studies

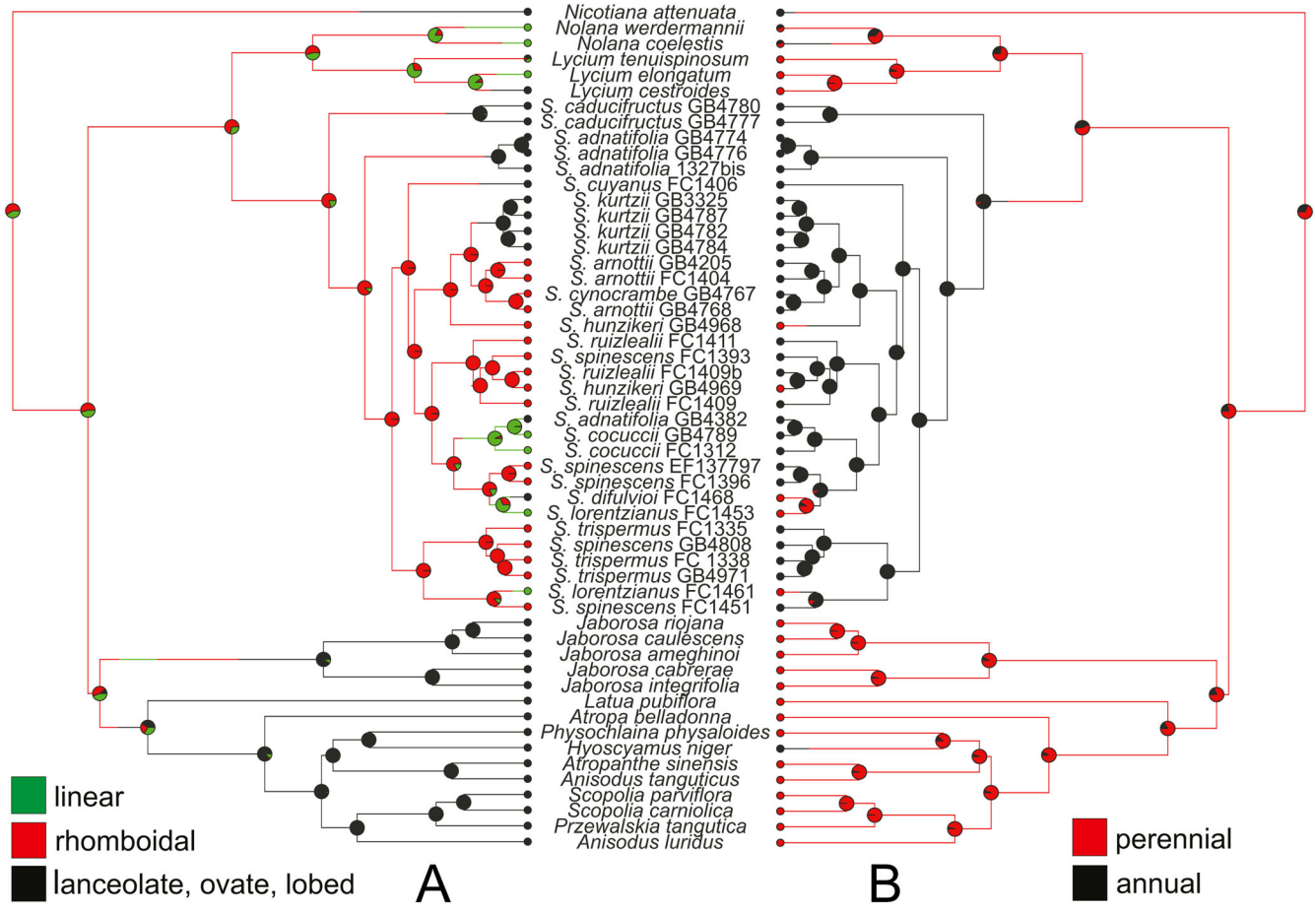

Fig. 4. Ancestral state reconstruction of morphological traits in *Sclerophylax* and its allies on the combined MCC tree, using stochastic mapping. Pies at nodes indicate frequencies of node states across 1000 simulations of character evolution and the colours of the tip labels represent tip states. **A**, Leaf shape; **B**, Life form.

(Särkinen & al., 2013) to 33. Thus, our results encompass almost all the taxonomic, morphological, and geographic variation within the genus. This study confirms that the genus *Sclerophylax* is monophyletic and is sister to the clade formed by *Lycium* + *Nolana* (Särkinen & al., 2013). Genus *Sclerophylax* can be distinguished from *Lycium* and *Nolana* by morphological synapomorphies, mainly its peculiar few-seeded and dry fruits that are crowned by the usually spiny calyx. Also, the data support the infrageneric arrangement of Di Fulvio (1961), in which the genus is divided into two sections, one including only *S. caducifructus* (sect. *Caducifructus*), and the other (sect. *Sclerophylax*) including all the remaining species. The phylogeny presented here is not fully resolved: relationships among some species of *S.* sect. *Sclerophylax* remain uncertain as well as the boundaries of some species. This situation may have two explanations: one relative to the nature of the markers employed, and the other concerning the species concept of the taxa recognized for the genus so far (according to the classical morphological Linnaean species concept). Concerning the markers, the granule-bound starch synthase I gene (*waxy*) has proven to be useful in resolving species relationships in several genera of Solanaceae (e.g., Peralta & Spooner, 2001). However, there are also examples in which the obtained trees have internal branches with low support (*Jaltomata*, Miller, R.J. & al., 2011; *Physalis*, Whitson & Manos, 2005; *Cestrum*, Laport & al., in prep.). It also did not seem like ITS, and particularly the plastid markers, *rpl32F-trnL* and *trnL-trnF*, had enough informative sites to resolve the phylogenetic relationships (Table 1). The most likely explanation is the lack of informative characters at different branching levels within the phylogeny. Given the recent diversification time of *Sclerophylax* and the lack of informative characters in the other gene regions examined, this is not unexpected. Also, in cases where the plastid sequences are identical, one plausible explanation is chloroplast capture (Tsitrone & al., 2003).

Assessment of diagnostic value of morphological characters. — Our findings remark the importance of studying morphological traits in Solanaceae in a phylogenetic framework (e.g., Knapp, 2002; Pabón-Mora & Litt, 2011) and highlight the frequency and speed with which these transitions occur among close relatives. Fruits and seeds provide solid characters for

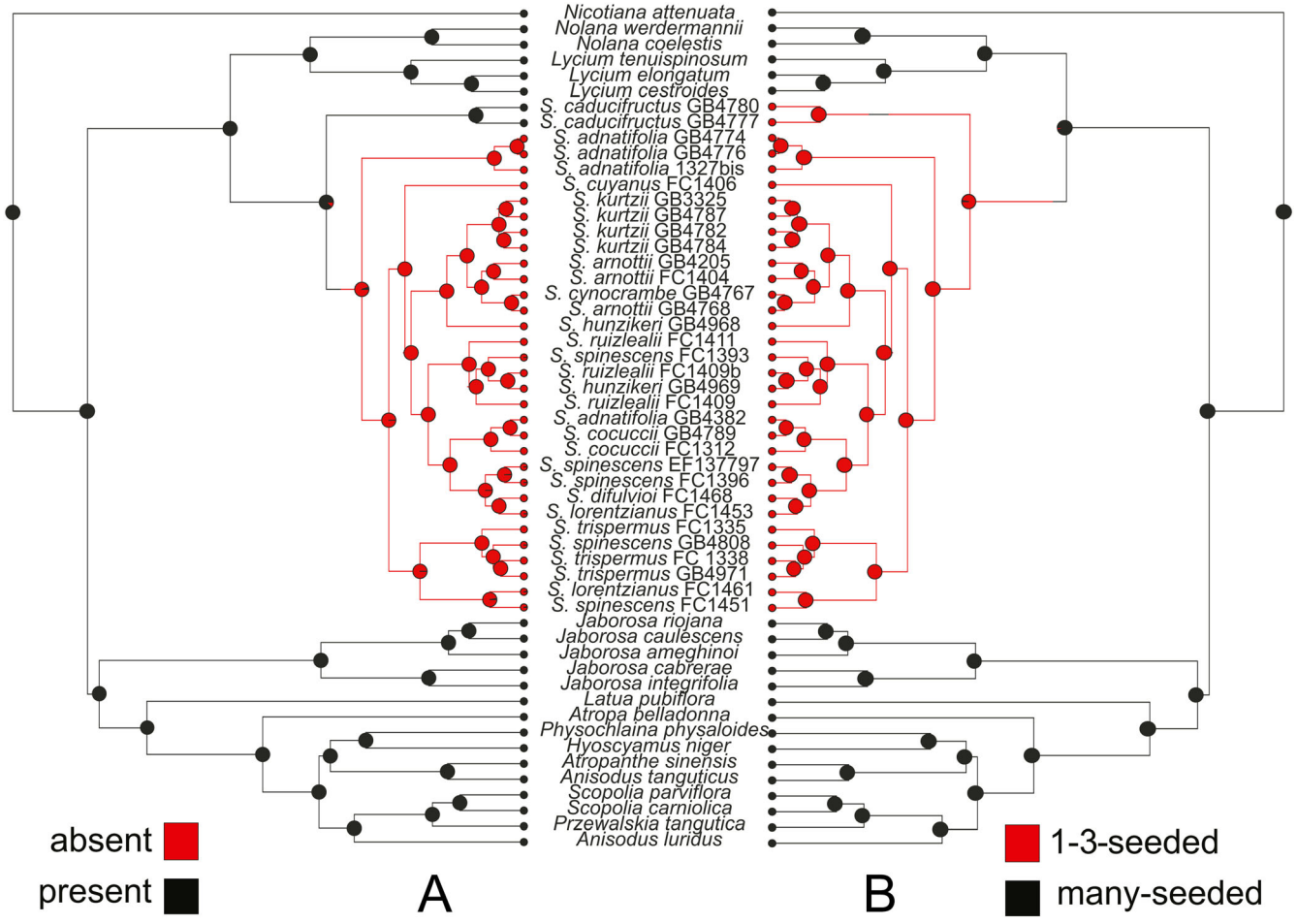

Fig. 5. Ancestral state reconstruction of morphological traits in *Sclerophylax* and its allies on the combined MCC tree, using stochastic mapping. Pies at nodes indicate frequencies of node states across 1000 simulations of character evolution and the colours of the tip labels represent tip states. **A**, Fruit pedicel; **B**, Number of seeds per fruit.

Sclerophylax that define the genus morphologically. A fruit with 2–3 seeds is a synapomorphy that characterizes the entire genus *Sclerophylax*; according to our ancestral state reconstructions (ASR), it has arisen from an ancestor with a multiseeded fruit. The fruit of *Sclerophylax* is probably an adaptation to the arid or semiarid areas where these species are found, often on stony slopes and sandy or saline soils. The hard covering of the fruit perhaps causes a physical dormancy that is broken when a disturbance happens in the seed bank, since the plants appear in quantity in disturbed or alluvial soils (F.E. Chiarini, pers. obs.). Also, in annual, erect species like *S. arnottii* and *S. kurtzii*, the whole plant dries after its life cycle is completed, retaining the fruits, suggesting a dispersal mode like that of the tumbleweeds (e.g., *Eryngium campestre* L. or *Salsola kali* L.). Type of fruit and its mode of dispersal may be related to the different pathways followed by other clades of Atropina. Long dispersals could have occurred in *Lycium* since its fruit is a berry that can be dispersed by birds, while fruits such those of *Nolana* or *Sclerophylax* have a more limited dispersal.

Other features proved useful to define sections: habit, corolla size and fruit abscission. ASR recovered a procumbent/decumbent habit as the ancestral state of the genus *Sclerophylax* (Fig. 3B). Erect habit is a synapomorphy of the clade formed by *S. arnottii* and *S. kurtzii*. This may be related to the dispersal mode and life cycle discussed above. Corolla size is a continuous character that clearly differs amongst clades, with an estimated value around 10 mm long for the ancestor of all *Sclerophylax* species (Fig. 6). The character has apparently evolved in two directions: on the one hand towards small flowers in the clades including samples of *S. adnatifolius*, *S. spinescens* and *S. cocucci* (homoplastic). These species with inconspicuous small flowers are probably autogamous (Ornduff, 1969; Cruden, 1977). On the other hand, larger flowers are a synapomorphy of the Arnottii + Kurtzii clade, suggesting a different floral biology and reproductive system. However, no studies dealing with these aspects are available for the genus. ASR recovered a persistent fruit for the ancestor to all species of the genus *Sclerophylax* (Fig. 3A), while deciduous fruits are an autapomorphy of *S. caducifructus* (Fig. 1). This feature probably enables a different dispersal mode, in contrast to the rest of species in the genus in which fruits are persistent and never detach from the plant. Dispersal studies are needed to clarify this matter.

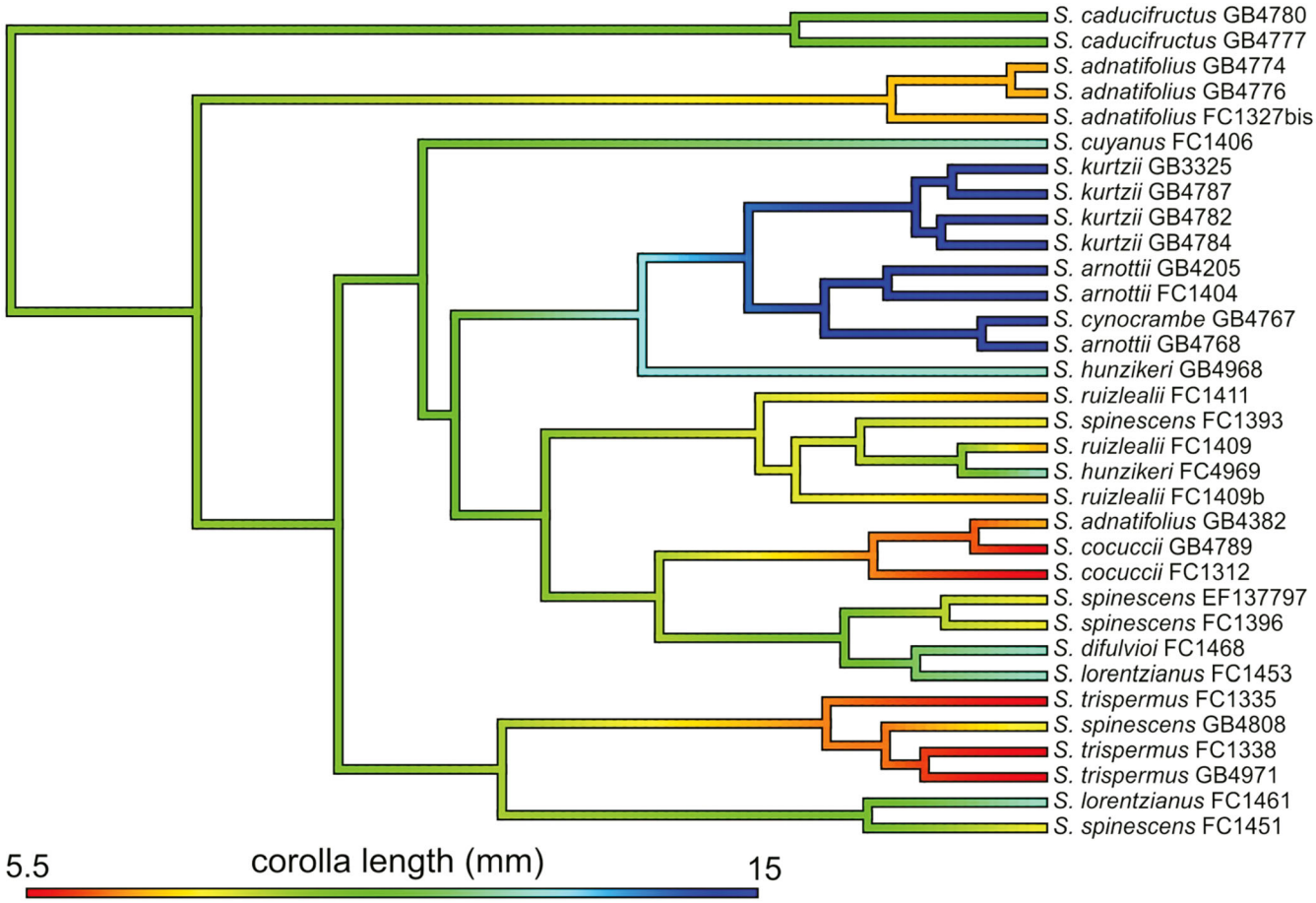

Fig. 6. Maximum likelihood reconstruction of corolla length for *Sclerophylax* (without outgroups). Phylogenetic signal lambda: 0.97; Bloombers's K: 1.13. The K-value is significantly different from zero (no phylogenetic signal, P = 1e-04) and not significantly different from one (signal expected under Brownian Motion, P = 0.7819).

Two morphological features resulted useful to define species: fruit pedicels and leaf shape. Pedicellate fruit is the ancestral state for the genus *Sclerophylax* (Fig. 5A), retained in the Caducifructus clade, while *S.* sect. *Sclerophylax* (i.e., the rest of the species in the genus) evolved a sessile fruit, adnate with the leaf axils and more or less embedded in the stem. This character state is not totally fixed in *S. spinescens*/*S. lorentzianus*, where both sessile and pedicellate fruits can be present in the same individual. Rhomboidal leaf blade was recovered as the state in the ancestor to all species of the genus *Sclerophylax* (Fig. 4A). Combined with other traits (presence/absence of petioles and decurrent leaf bases) leaf shape is useful in distinguishing some species. However, it is homoplastic, with linear and ovate/lanceolate leaves arising independently in different branches of the phylogeny.

Finally, a set of characters previously used to define species or sections are shown to be highly homoplastic, including raphides, pubescence, consistency of fruiting calyx and life cycle. The presence of raphides (needle-like calcium oxalate crystals) was considered a significant character, enough to separate species (Di Fulvio, 1961). In *Sclerophylax*, raphides are found in *S. arnottii*, *S. cynocrambe*, and *S. trispermus*, species that fall into to two separate clades. Raphides are a difficult character to observe in herbarium material and their presence or absence in other species of *Sclerophylax* needs confirmation. Like other calcium oxalate crystals, they are dependent either on environmental factors like herbivory (Ward & al., 1997) or on metabolic activity (Coley & al., 1985), but there are no studies in *Sclerophylax* that have evaluated how such factors affect the presence of crystals. For these reasons we have preferred not to use this character to delimit species.

The consistency of the fruiting calyx (foliaceous vs. spiny) has been used as a character to delimit species (Di Fulvio, 1961). Spiny calyces occur in *Sclerophylax spinescens*, the most troublesome species, but also in *S. trispermus* and in *S. hunzikeri*. The latter two species occur in widely separated branches of the molecular tree. In addition, calyx consistency is a difficult trait to assess, since there are different degrees of prickliness, and it also depends on the degree of desiccation of the calyces.

Pubescence is not a presence/absence type of character. It has been used in dichotomous keys as "pubescent" versus

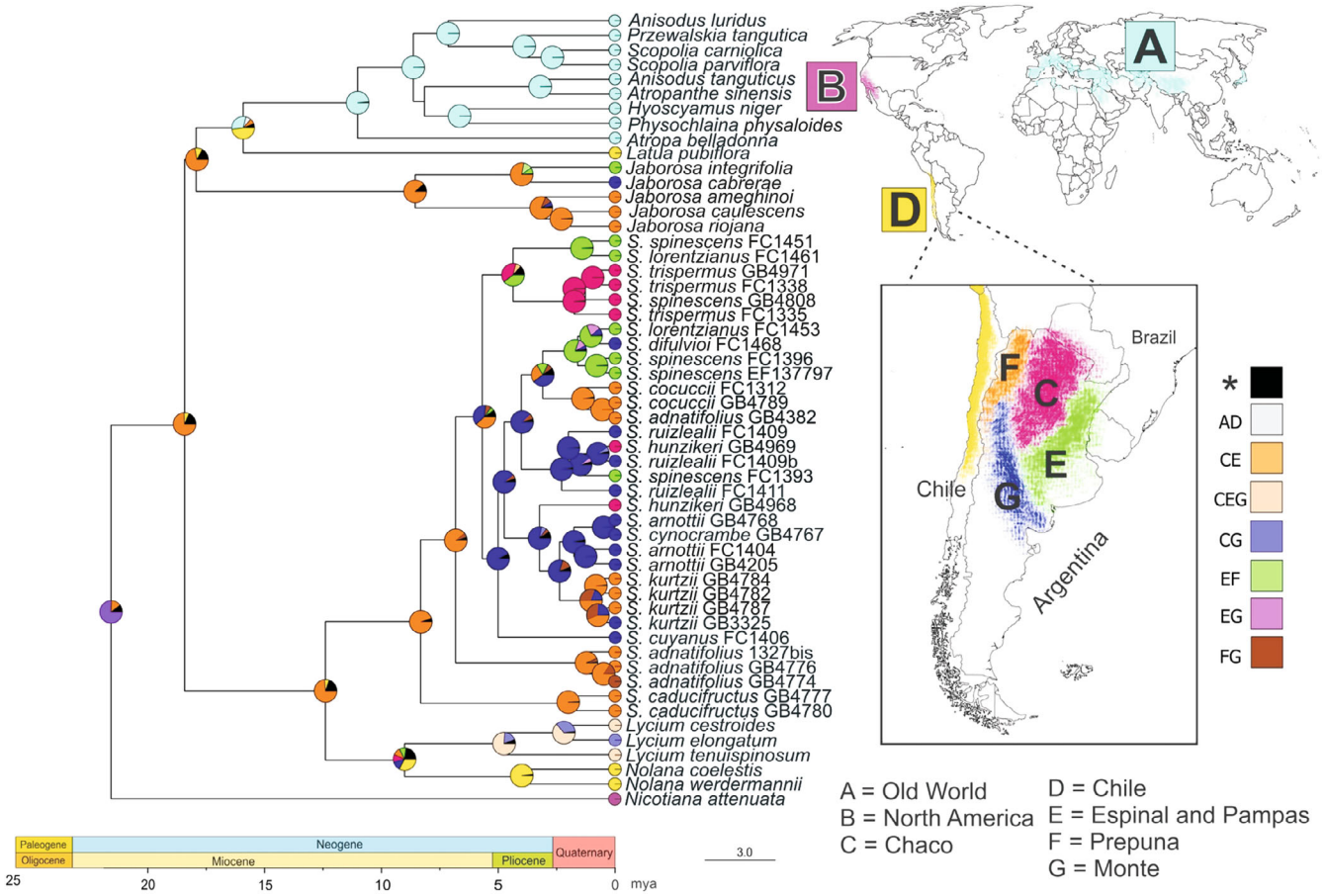

Fig. 7. Combined MCC chronogram based on four markers (ITS, *waxy*, *rpl32F-trnL*, *trnL-trnF*), obtained from a secondary calibration based on Särkinen & al. (2013) and ancestral area reconstruction of *Sclerophylax*. Pies at nodes show relative probability of alternative areas, with the colours representing geographical areas as coloured on the maps. Rectangles indicate colours of combined areas. Branch lengths are proportional to relative ages (in millions of years; see scale at bottom of tree). Posterior probabilities of each branch are indicated in suppl. Fig. S1.

"scarcely pubescent" (Di Fulvio, 1961, 1999), but the difference between the two states of the character is subjective, and there are many individuals from different species with intermediate characteristics, so we reject the use of pubescence in delimiting species.

Our ASR for life cycle recovered an annual habit in the ancestor of the entire genus *Sclerophylax* (Fig. 4B). Perennial habit appeared independently in the branches that include *S. difulvioi* and *S. hunzikeri*. Also *S. lorentzianus* (which we consider an extreme form of *S. spinescens*) shows a tendency to become perennial. Noteworthy are *S. kurtzii* and *S. arnottii*, which live in very dry areas of the Monte; they have a rapid life cycle, germinating, blooming and fruiting massively during the short period of time of humidity produced by the sporadic rains. In contrast, the species inhabiting milder, wetter areas (Pampas) have the chance of surviving more than one season, thus becoming short-lived perennials or perennials.

Biogeography. — Knowing the distribution of species and the associations between them is essential for evolutionary biogeography (Arana & al., 2021). That is why the biogeographical findings in *Sclerophylax* are applicable to not only the wider Solanaceae group but also across the wider Chilean and South American flora. Although most biogeographical events in Solanaceae comprise within-area speciation (Dupin & al., 2017), the clade Atropina is remarkable because transoceanic migrations have been suggested to occur in this group: Hyoscyameae and *Mandragora* to the Old World in the Miocene (Tu & al., 2010) and *Lycium* dispersal to Africa ca. 4 Ma and to East Asia ca. 1.21 Ma (Miller, J.S. & al., 2011). However, the discovery of new fossils in the family (Wilf & al., 2017; Deanna & al., 2020) will force all these dates to be recalculated. Also, within Atropina, early divergence of *Jaborosa* into two clades took place during the middle and late Miocene (ca. 17–10 Ma) and was related to three successive Atlantic marine transgressions, informally known as the "Paranean Sea" (Moré & al., 2020). On the other hand, diversification in *Sclerophylax* is more recent and it is probably related to the development of the South American Transition Zone, which is an area of overlap between the Neotropical and Andean regions. This zone embraces several districts (including the Puna, Prepuna and Monte provinces) and it can be considered a barrier both to the Neotropical biotic expansion in warmer periods and to the expansion of the Antarctic flora in colder periods, and where the xerophytic biota could develop and diversify. It has allowed a biotic mix of the xerophytic flora and its associated fauna, as a result of dispersal events from neighbouring regions starting from the Neogene (Morrone, 2015). *Sclerophylax*, like its sister genus *Nolana*, is entirely South American, with its origin in the Andes. In the case of *Sclerophylax*, we confirm that its area of origin is the Prepuna biogeographical province (probably in the Argentinian province of Catamarca), and from there it spread to the Chaco and Pampas in relatively recent geological times. This is a novel association since previously, only a relationship between Pampas, Chaco and Monte had been suggested (Cabrera & Willink, 1973). More precise calculations of the dates when this would have happened is subject to the study of the new fossils, as are the dates of diversification of the species of the genus with respect the geological events that shaped the current landscapes of South America. The genus would have expanded its distribution to the south, following the arid diagonal (Villagrán & Hinojosa, 2005), diversifying in the Monte biogeographic province, to the northeast with some species in the Chaco province, and to the east with one species in the Espinal and Pampas provinces, but always preferring habitats with dry, rocky, salty or clay soils.

Taxonomic treatment. — Our results suggest that some species of *Sclerophylax* are not monophyletic. In order to resolve some circumscriptions supported by molecular data and morphological traits, we propose the synonymy of two species; we consider *S. cynocrambe* to be a synonym of *S. arnottii* and *S. lorentzianus* to be a synonym of *S. spinescens*. A list of the currently accepted species, which includes new lectotypifications for two names, is presented below.

Sclerophylax Miers in London J. Bot. 7: 18. 1848 – Type: *S. spinescens* Miers.

= *Sterrhymenia* Griseb. in Abh. Königl. Ges. Wiss. Göttingen 19: 231, t. 2, fig. 5. 1874 – Type: *S. cynocrambe* Griseb.

Sclerophylax adnatifolius Di Fulvio in Kurtziana 1: 82, fig. 32E–I. 1961 ('*adnatifolia*') – Lectotype (designated by Barboza in Anton & Zuloaga, Fl. Argentina 13: 320. 2013): Argentina. La Rioja, Famatina Dept., "Ruta 40 (Km 633), yendo de Famatina a Tinogasta", 20 Mar 1960, *A.T. Hunziker, A.A. Cocucci & T.E. Di Fulvio 15162* (CORD barcode CORD00004330!; isolectotype: CORD barcode CORD00004331!).

Sclerophylax arnottii Miers in London J. Bot. 7: 20. 1848 – **Lectotype (designated here):** Argentina. Prov. San Juan, sine loc., *J. Gillies s.n.* (E barcode E00230375!; isolectotypes: BM barcode BM000992227!, E barcodes E00230373!, E00230374!, E00230385!, K barcode K000585478!).

= *Sclerophylax gilliesii* Miers in London J. Bot. 7: 21, t. 26. 1848 – **Lectotype (designated here):** Argentina. Prov. Mendoza, "on the South side of Río El Diamante, near el Fuerte San Rafael", *J. Gillies s.n.* (E barcode E00230372!; isolectotypes: BM barcode BM000992227!, K barcode K000585477!).

= *Sterrhymenia cynocrambe* Griseb. in Abh. Königl. Ges. Wiss. Göttingen 19: 231, t. 2, fig. 5. 1874 ≡ *Sclerophylax cynocrambe* (Griseb.) Griseb. in Abh. Königl. Ges. Wiss. Göttingen 24: 268. 1879 ≡ *Sclerophylax cynocrambe* (Griseb.) Kuntze, Revis. Gen. Pl. 3(2): 224. 1898, nom. illeg. (comb. superfl.) – Lectotype (designated by Hunziker in Bol. Acad. Nac. Ci. Republ. Argent. 41: 372. 1960): Argentina. Prov. Catamarca, "Kraut, nicht selten in d. Umgebung von Yakutula", Feb 1872, *P.G. Lorentz 656* (GOET barcode GOET009267!; isolectotype: CORD barcode CORD00004989!), **syn. nov.**

Comment. – The protologue of *Sclerophylax arnottii* lacks type designation. In its description, Miers indicates "San Juan — Prov. Argentin. *v. s. in Herb Hook.*" As there are several specimens from Hooker's herbarium in different institutions, we chose E00230375 because it is the best preserved and includes Miers's handwriting, which is indication of being original material according to Art. 9.4 of the *ICN* (Turland & al., 2018).

Likewise, multiple specimens exist of *Gillies s.n.*, the type of *Sclerophylax gilliesii* Miers. Since Miers did not designate a particular specimen as the holotype, we chose E00230372 as the lectotype because it is the best preserved and has observations in the author's handwriting.

Sclerophylax caducifructus Di Fulvio in Kurtziana 1: 91. 1961 – Holotype: Argentina. Prov. Catamarca [Tinogasta Dept.], "Ruta internacional 60, ± km 150, cerca de Loro Huasi, ± 2200 m alt", 03 Apr 1959, *A.T. Hunziker, A.A. Cocucci & T.E. Di Fulvio 14196* (CORD barcode CORD00004340!).

Sclerophylax cocuccii Di Fulvio in Kurtziana 1: 80. 1961 – Holotype: Argentina. Prov. Catamarca, [Tinogasta Dept.], "Ruta 40, entre Tinogasta y Cuesta de Zapata, rumbo a Londres", 21 Mar 1960, *A.T. Hunziker, A.A. Cocucci & T.E. Di Fulvio 15213* (CORD barcode CORD00005003!).

Sclerophylax cuyanus Di Fulvio in Kurtziana 27(1): 244. 1999 – Holotype: Argentina. Prov. San Juan, Dept. Pocito, "Ruta 40, Km 129, cerca del límite con el dpto. Sarmiento", 02 May 1997, *T.E. Di Fulvio 952* (CORD barcode CORD00005007!).

Sclerophylax difulvioi Del Vitto & Peten. in Kurtziana 29(1): 85, fig. 1. 2001 – Holotype: Argentina. Prov. San Luis, Dept. Belgrano, "Parque Nac. Sierra de Las Quijadas, El Mirador, 32°27′S, 66°53′W, 700 m alt.", 10 Mar 1995, *L. Del Vitto, E.M. Petenatti & M.E. Petenatti 8067* (UNSL; isotype: CORD barcode CORD00005014!).

Sclerophylax hunzikeri Di Fulvio in Kurtziana 1: 85. 1961 – Lectotype (designated by Barboza in Anton & Zuloaga, Fl. Argentina 13: 324. 2013): Argentina. Prov. La Rioja, Dept. General Ocampo, "Dique de Anzulón", 18 Feb 1959, *A.T. Hunziker, A.A. Cocucci & T.E. Di Fulvio 13922* (CORD barcode CORD00004388!; isolectotypes: BM barcode BM000992228!, CORD barcode CORD00004389!, K barcode K000585480!, LL barcode 00372868!).

Sclerophylax kurtzii Di Fulvio in Kurtziana 1: 88. 1961 – Lectotype (designated by Barboza in Anton & Zuloaga, Fl. Argentina 13: 324. 2013): Argentina. Prov. La Rioja, [Dept. Independencia], "Ruta 74, Cueva del Chacho, entre Patquía y Los Colorados", 19 Mar 1960, *A.T. Hunziker, A.A. Cocucci & T.E. Di Fulvio 15131* (CORD barcode CORD00004363!; isolectotypes: B barcode B 10 0248775!, BM barcode BM000992229!, CORD barcode CORD00004364!, K barcode K000585481!, LL barcode 00372869!, S No. S04-3083!).

Sclerophylax ruizlealii Di Fulvio in Kurtziana 1: 78. 1961 – Lectotype (designated by Barboza in Anton & Zuloaga, Fl. Argentina 13: 325. 2013): Argentina. Prov. Mendoza, Dept. Luján, "Cacheuta", 30 Jan 1959, *T.E. Di Fulvio & R. Subils 50* (CORD barcode CORD00005017!; isolectotype: CORD barcode CORD00005018!).

Sclerophylax spinescens Miers in London J. Bot. 7: 19. 1848 – Holotype: Argentina. Prov. Córdoba, [Dept. San Justo], "Arroyuelo San José, prov. Córdoba, in uliginosis salitrosis v.v.", *J. Miers 733* (BM barcode BM000992230!).

= *Sclerophylax lorentzianus* O.Hoffm. in Linnaea 43: 136. 1880 – Lectotype (designated by Di Fulvio in Kurtziana 1: 62. 1961): Argentina. Prov. Entre Ríos, Dept. Uruguay, "Concepción del Uruguay. Puntas del Arroyo Tala", Oct 1878, *P.G. Lorentz 1656* (CORD barcode CORD00005016!; isolectotype: B† [photo neg. in F No. 3016!]), **syn. nov.**

Sclerophylax tenuicaulis Di Fulvio in Kurtziana 1: 64, fig. 27A–D. 1961 – Holotype: Argentina. Prov. Catamarca, [Dept. Tinogasta], "La Estrechura, entre ruta nacional 60 (km 169 a 172), 2600 msm", 4 Apr 1959, *A.T. Hunziker, A.A. Cocucci & T.E. Di Fulvio 14259* (CORD barcode CORD00005026!).

Sclerophylax trispermus Di Fulvio in Kurtziana 1: 70. 1961 – Lectotype (designated by Barboza in Anton & Zuloaga, Fl. Argentina 13: 327. 2013): Argentina. Prov. La Rioja, [Dept. General Ocampo], "Ambil, Flor blanca", 18 Feb 1959, *A.T. Hunziker, A.A. Cocucci & T.E. Di Fulvio 13871* (CORD barcode CORD00005040!; isolectotype: CORD barcode CORD00005041!).

Species boundaries. — To date, 14 species of *Sclerophylax* are currently recognized (Di Fulvio, 1961; Del Vitto & Petenatti, 2001; Barboza, 2013) but we disagree with this count. The characters used to delimit them are sometimes imprecise or are continuous variables with overlapping ranges, so we decrease the number of species. From the molecular phylogeny and stochastic character mapping, some natural groups are recovered and some species circumscriptions are not supported. In the following section we discuss problematic species circumscriptions with respect to the molecular phylogeny.

Three accessions identified as *Sclerophylax adnatifolius* appear together in a strongly supported clade in molecular analyses. The species is distinguished mainly by an autapomorphy: the blade of the minor leaf of each pair is sessile, markedly asymmetrical, and decurrent on the stem. The adnate leaf is not always present in all the nodes of a single individual, especially in young plants that could be confused with *S. trispermus*. *Sclerophylax adnatifolius* is a species

belonging to the Prepuna biogeographical province. Another accession identified as *S. adnatifolius* does not appear on this clade but instead clusters with *S. cocuccii*. This specimen conforms morphologically to *S. adnatifolius* rather than *S. cocuccii*, so its position in this clade is puzzling. Such position is based mainly on ITS sequence, which is known to have pseudogenes and multiple copies in some groups (Álvarez & Wendel, 2003; Nieto Feliner & Rosselló, 2007), though this is not the case in our study. ITS of *S. adnatifolius* have neither polymorphic bases nor missing data. A plausible explanation could be that introgression/hybridization yields that single accession to be placed in a different clade.

Sclerophylax arnottii and *S. cynocrambe* are characterized by the unique combination of large corollas, annual life cycle, erect habit, and rhombic leaves (Figs. 1). Accessions of this clade inhabit the Monte biogeographic province (Fig. 7). *Sclerophylax arnottii* (Fig. 1B) and *S. cynocrambe* are supposedly differentiated by their calyx lobes: in the former, two lobes are longer than the rest, while in the latter only one lobe is longer (Di Fulvio, 1961). This feature is not very noticeable and can vary within species. Another difference is the more abundant glandular pubescence in *S. cynocrambe*. However, as Di Fulvio (1961) pointed out, there are individuals with intermediate characteristics, especially from Prov. La Rioja, Dept. Famatina. Di Fulvio (1961) suggests that these may be possible hybrids and introgressants with *S. arnottii*, since the ranges of the two species are not exclusive. However, we favour the hypothesis that the two species are not well-differentiated morphologically, geographically, or phylogenetically and we regard them as conspecific. Accordingly, we synonymize *S. cynocrambe* under *S. arnottii*.

The specimens identified as *Sclerophylax cocuccii* from Catamarca and Salta provinces, plus one identified as *S. adnatifolius*, are clustered together (Fig. 2). Despite the low support in the molecular analyses, the morphological characteristics that define *S. cocuccii* are very clear: the linear leaves and the small flowers. No intermediate individuals have been found concerning these features. It is a species of the Prepuna province (Fig. 7).

The only accession of *Sclerophylax cuyanus* sampled occupies a rather isolated position within *S.* sect. *Sclerophylax* in the molecular phylogeny. According to Di Fulvio (1999), it is related to *S. arnottii*, *S. cynocrambe*, and *S. trispermus* due to the presence of raphides in all four species, but the molecular phylogeny does not support the close relationship of these taxa. *Sclerophylax cuyanus* is a species endemic to the type locality, an area within the Monte biogeographical province (Fig. 7).

In this study, *Sclerophylax difulvioi* is represented by a single sample that groups with two accessions of *S. spinescens*. According to Del Vitto & Petenatti (2001), this species is related to *S. tenuicaulis* and *S. lorentzianus*. *Sclerophylax tenuicaulis* was not sampled, but *S. difulvioi* did not cluster with *S. lorentzianus*. It also does not group with *S. kurtzii*, a similar species from which it is distinguished by its perennial life cycle (*S. kurtzii* is an annual). It is a species endemic to the type locality in Prov. San Luis, Dept. Belgrano, an area within the Monte province (Fig. 7). It appears to be restricted to saline soils (Del Vitto & Petenatti, 2001).

Sclerophylax hunzikeri is characterized by being a perennial with creeping stems more than 50 cm long. Although this trait is obvious in many specimens of *S. hunzikeri*, young individuals without well-developed stems are difficult to categorize. In the original diagnosis, Di Fulvio (1961) assigned to this species materials that live on both sides of the Córdoba hills, in areas that belong to two different biogeographical provinces; towards the west of the hills it is Chaco and towards the east it is Pampas and Espinal provinces. In our study, *S. hunzikeri* resulted paraphyletic, one of the accessions (GB4968, *Barboza & al. 4968*, collected to the west of the hills, in the Chaco province, Figs. 2, 7) is grouped with the Arnotti + Kurtzii clade. The other accession (GB4969, *Barboza & al. 4969* collected also to the west of the hills) is in a clade with *S. ruizlealii* and one accession of *S. spinescens* (Fig. 2). *Sclerophylax ruizlealii* is a species found to the west of the Córdoba hills but occupying the Monte biogeographical province; Figs. 7). A possible explanation for the paraphyly of the accessions is missing data because different markers had to be used for the two accessions. We did not sample specimens identified as *S. hunzikeri* from the east of the mountains in our molecular phylogenies, but based on morphology we consider these materials to belong to *S. spinescens* and restrict the name *S. hunzikeri* for the collections that strictly agree with the two synapomorphies mentioned above and that inhabit the Chaco biogeographic province west of the Córdoba mountain ranges where the type locality is placed.

In molecular analyses, the materials identified as *Sclerophylax ruizlealii* from Mendoza are resolved together along with one accession each of *S. hunzikeri* and *S. spinescens*, but on a branch with low support, thus monophyly of the species cannot be absolutely ascertained. *Sclerophylax ruizlealii* can be characterized by rhomboidal leaves and white, medium-sized corollas. It is a species of the Monte biogeographical province (Fig. 7). The features that define it are not distinctive and some specimens can be confused with *S. spinescens*, from which it is distinguished in being more pubescent and having fruiting calices with foliaceous lobes. Consequently, we have decided to reserve the name *S. ruizlealii* for the materials that strictly agree with the aforementioned diagnostic morphological traits and that inhabit the Monte biogeographic province, where the type locality is (Prov. Mendoza, Dept. Luján).

Sclerophylax spinescens has the widest distribution of the genus and is also the most morphologically variable. Its range overlaps with those of several other species. Five accessions of *S. spinescens* were sampled in the molecular analyses; they did not form a monophyletic group and were scattered throughout *S.* sect. *Sclerophylax* on four poorly supported clades. Within the *S. spinescens* herbarium collections, there are individuals intermediate between *S. hunzikeri* and *S. ruizlealii*, and to a lesser extent with *S. trispermus*. These could represent interspecific hybrids, or polyploids, but these hypotheses

remain to be tested. They could also be interpreted as individuals of other species with extreme characteristics resembling *S. spinescens*. The molecular variation in the accessions sequenced stems from *waxy*. There is no signal of multiple divergent plastid copies in the two regions sequenced (this could be due to low variation in the two plastid markers), but ITS does not support the wide divergence between the *S. spinescens* accessions.

Sclerophylax lorentzianus was described from materials from the Argentinian province of Entre Rios and is distinguished by pedicellate fruits and linear leaves. However, as previously noted by Di Fulvio (1961), the presence of pedicellate fruits can be variable, as there are individuals with both sessile and pedicellate fruits. It is also possible to find intermediate states between the rhomboid leaves of *S. spinescens* and the linear leaves of *S. lorentzianus* throughout their distribution range (Fig. 1L). Thus, we consider that the description of *S. lorentzianus* was based on extreme individuals of *S. spinescens* appearing more frequently towards the east of its distribution range, and we regard *S. lorentzianus* as a synonym of *S. spinescens*.

Due to its morphological variability and disparate positions on the molecular tree, *Sclerophylax spinescens* deserves further study. Most accessions that morphologically conform to the type have been collected from the plains and in the biogeographical provinces of Pampas and Espinal. On the molecular tree, the accessions of *S. spinescens* are distributed on various branches within *S.* sect. *Sclerophylax*, always in poorly supported clades. Future collections and morphological and molecular work is needed to delimit this species and ascertain whether some individuals with intermediate characters represent hybrids or introgressants.

In the future, it is desirable to improve our understanding of the interspecific relationships in *Sclerophylax* by using more precise molecular markers, to assess the diagnostic value of other characters (morphological, cytological, physiological, etc.) and to gather information on floral and reproductive biology.

■ AUTHOR CONTRIBUTIONS

FEC and LB designed the research; FEC performed field trips, collected plant materials and performed all labwork; FEC and RD run all the analyses; LB was the supervisor of the present work and provided the lab resources; FEC and RD wrote the manuscript under the lead of LB. — FEC, https://orcid.org/0000-0002-6473-3129; RD, https://orcid.org/0000-0001-8753-7596; LB, https://orcid.org/0000-0003-2803-2656

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Appendix 1. List of the individuals sampled for the molecular analysis.

Species are listed in alphabetical order. The herbarium (when available) cited in brackets indicates where the voucher specimen is housed. GenBank accession numbers are included for each of the four genic regions used in the current study (*waxy*, ITS, *rpl32F-trnL*, *trnL-trnF*). An asterisk (*) indicates sequences newly generated in this study; an en-dash (–) indicates unsequenced regions.

OUTGROUPS: ***Anisodus luridus*** Link ex Spreng., NL accession 934750185, *Z.Y. Zhang 020* (NL), DQ069255, –, –, –; ***Anisodus tanguticus*** (Maxim.) Pascher, Data not provided, *R.G. Olmstead 2003-083b*/specimen_voucher = RGO2003-083b, –, –, KF951354, –; Data not provided, *M.T. Yu & al.*, –, EU239680.1, –, –; ***Atropanthe sinensis*** (Hemsl.) Pascher, NL accession 904750040, *Z.Y. Zhang 088* (NL), DQ069256, –, –, –; ***Atropa belladonna*** L., NL accession 974750054, *Z.Y. Zhang 054* (NL), DQ069251, –, –, –; U.S.A., Illinois, Du Page County, Downers Grove, *Wong Sui-Ming 17* (US 2918256), –, MK895642*, –, –; ***Hyoscyamus niger*** L., NL accession 924750033, *Z.Y. Zhang 017* (NL), DQ069257, –, –, –; South Korea, Bisan-ri, Soi-myeon, Eumseong-gun, Chungcheongbuk-do, N36°57′22.7″N E127°43′39.7″, TKM-1-000042, 2013.08.01, –, KM051464, –, –; ***Jaborosa ameghinoi*** (Speg.) Macloskie & Dusén, Argentina, Chubut Prov., Gaiman Dpt.: Km 103–Ruta n° 25 de Trelew a Las Chapas, *González 1048* (CORD), MT196376*, –, –, –; ***Jaborosa cabrerae*** Barboza, Argentina, Catamarca Prov., Belén Dpt.: Nacimientos de San Antonio, S26°52′17″ W66°43′57″, *G. Barboza & al. 3487* (CORD), KT013305, –, –, MT050475*; ***Jaborosa caulescens*** Gillies & Hook. var. ***bipinnatifida*** (Dunal) Reiche, Argentina, Mendoza Prov., Tupungato Dpt.: RP 94, S33°36′50″ W69°30′55″, *A.A. Cocucci 2265* (CORD), KT013306, –, –, –; Argentina, La Rioja Prov., Famatina Dpt.: Cueva de Pérez, S28°59′56″ W67°43′57″, *G. Barboza & al. 3186bis* (CORD), MT196385*, MT084396*, MT009221*, –; ***Jaborosa integrifolia*** Lam., Argentina, Entre Ríos Prov., Villaguay Dpt.: Villaguay, *A.A. Cocucci 4140* (CORD), KT187296, –, –, –; South America (cultivated at Botanic Garden, Genoa University, Italy), *S.0290* (BIRM), –,

Appendix 1. Continued.

AY028148, –, –; ***Jaborosa riojana*** Hunz. & Barboza, Argentina, La Rioja Prov., Vinchina Dpt.: Quebrada El Peñón, S28°27′21″ W68°50′36″, *G. Barboza & al. 4741* (CORD), MT196386*, MT084397*, –, MT050488*; ***Latua pubiflora*** (Griseb.) Baill., Chile, Valdivia Prov., Los Ríos Region: Parque Oncol, S39°44′29″ W73°21′02″, *J. Chiapella & al. 1774* (CORD), KT187308, KX752697, –, –; ***Lycium cestroides*** Schltdl., Argentina, Córdoba Prov., Tulumba Dpt.: Ruta 60, km 907, *G. Bernardello 878* (CORD), DQ124513, –, –, –; ***Lycium cestroides*** Schltdl., Argentina, Córdoba Prov., Capital Dpt.: Ciudad de Córdoba, Barrio Cofico, S31°24′14.69″, W64°11′00.11″, *F. Chiarini 1431* (CORD), –, MT084399*, –, –; ***Lycium elongatum*** Miers, Argentina, Córdoba Prov., Capital Dpt.: Barrio Cofico, S31°24′15″ W64°11′00″, *F. Chiarini 1430* (CORD), MT196388*, –, –, –; ***Lycium tenuispinosum*** S.B.Jones & W.Z.Faust, Argentina, Córdoba Prov., Tulumba Dpt.: R60, km 907, S30°16′17″ W63°48′24″, *G. Bernardello 892* (CORD), EF137795, DQ124633, –, –; ***Nicotiana attenuata*** Torr. ex S.Watson, Data not provided, Kew DNA bank 12610, –, KR083023, –, –; Data not provided, *T. Helgason & A. Monro 621* (BM), –, AJ492427, –, –; ***Nolana coelestis*** Miers ex Dunal, Chile, Location not provided., *J. Miller & al. 04-98* (MASS), EF137800, FJ439763, –, –; ***Nolana werdermannii*** I.M.Johnst., Chile, Location not provided., *J. Miller & al. 04-77* (MASS), EF137799, FJ439764, –, –; ***Przewalskia tangutica*** Maxim., Chenduo, Qinghai, China, *Y.F. Huang 01* (HNWP), DQ069261, –, –, –; ***Physochlaina physaloides*** (L.) G.Don, NL accession 924750021, *Z.Y. Zhang 032* (NL), DQ069260, –, –, –; ***Scopolia carniolica*** Jacq., NL accession 984750174, *Z.Y. Zhang 120* (NL), DQ069263, –, –, –; ***Scopolia parviflora*** Nakai, Mt. Cheonma, Korea (N37°40′34.85″ E127°15′33.00″), *J.H. Park 20150505-141* (NNIBR), –, –, KU900232, KU900232. — **INGROUPS:** ***Sclerophylax adnatifolius*** Di Fulvio, Argentina, Jujuy Prov., Tumbaya, *F. Chiarini 1327b* (CORD), –, MW798783*, MW801449*, –; Argentina, Jujuy Prov., Tumbaya Dpt.: Salinas Grandes, S23°46′18″ W65°56′51″, *G. Barboza & al. 4382* (CORD), –, MT084382*, –, –; Argentina, La Rioja Prov., Famatina Dpt.: S28°22′42″ W67°39′48″, *G. Barboza & al. 4774* (CORD), MT196374, MT084386*, –, MT050489*; Argentina, Catamarca Prov., Tinogasta Dpt.: km 1362 R60, S27°45′36″ W67°39′11″, *G. Barboza & al. 4776* (CORD), MT196377*, MT084387*, MT009215*, MT050481*; ***Sclerophylax arnottii*** Miers, Argentina, San Juan Prov., Sarmiento Dpt.: Ruta 40, Retamito, S32°08′25.5″ W68°29′14.9″, *F. Chiarini 1404* (CORD), –, MT084390*, –, MT050483*; Argentina, La Rioja Prov., Chilecito Dpt., from Nonogasta to Chilecito, S29°15′29″ W67°30′27″, *G. Barboza & al. 4205* (CORD), –, MT084395*, –, –; Argentina, La Rioja Prov., Independencia Dpt.: Los Colorados, S29°51′25″ W67°13′16″, *G. Barboza & al. 4768* (CORD), MT196371*, MT084384*, –, –; ***Sclerophylax caducifructus*** Di Fulvio, Argentina, Catamarca Prov., Tinogasta Dpt.: Fiambalá, S27°41′32″ W67′38′08″, *G. Barboza & al. 4780* (CORD), MT196383*, MT084392*, –, MT050486*; Argentina, Catamarca Prov., Tinogasta Dpt.: km 1362 R60, S27°45′36″ W67°39′11″, *G. Barboza & al. 4777* (CORD), MT196378*, –, MT009216*, MT050482*; ***Sclerophylax cocuccii*** Di Fulvio, Argentina, Salta Prov., Rosario de Lerma Dpt.: Arroyo Colorado, S24°42′08.3″ W65°45′24.9″, *F. Chiarini 1312* (CORD), –, MT084379*, MT009209*, –; Argentina, Catamarca Prov., Belén Dpt.: Río Los Nacimientos, *G. Barboza & al. 4789* (CORD), MT196370*, –, MT009211*, MT050478*; ***Sclerophylax cuyanus*** Di Fulvio, Argentina, San Juan Prov., Rivadavia Dpt.: Dique Ignacio de la Roza, S31°29′23.0″ W68°38′54.5″, *F. Chiarini 1406* (CORD), MT196382*, –, MT009220*, MT050485*; ***Sclerophylax cynocrambe*** (Griseb.) Griseb., Argentina, La Rioja Prov., Independencia Dpt.: Patquía, *G. Barboza & al. 4767* (CORD), MT196375*, –, MT009214*, –; ***Sclerophylax difulvioi*** Del Vitto & Peten., Argentina, San Luis Prov., Belgrano Dpt.: Parque Nac. Sierra de Las Quijadas, Potrero de la Aguada, S32°29′53.6″, W67°00′32.7″, *F. Chiarini 1468* (CORD), –, MT084378*, MT009206*, MT050474*; ***Sclerophylax hunzikeri*** Di Fulvio, Argentina, Córdoba Prov., Minas Dpt.: Casas Viejas, *G. Barboza & al. 4968* (CORD), MT196372*, –, –, –; Argentina, Córdoba Prov., Cruz del Eje Dpt.: Tuclame, *G. Barboza & al. 4969* (CORD), –, –, MT009213*, MT050479*; ***Sclerophylax kurtzii*** Di Fulvio, Argentina, Catamarca Prov., Tinogasta Dpt.: S27°39′52″ W67°45′46″, *G. Barboza & al. 4787* (CORD), MT196380*, MT084389*, MT009218*, –; Argentina, La Rioja Prov., Capital Dpt.: RN 60, Km 151, *G. Barboza & al. 3325* (CORD), –, MT084394*, –, –; Argentina, Catamarca Prov., Tinogasta Dpt.: S27°41′20″ W67°47′48″, *G. Barboza & al. 4782* (CORD), MT196384, MT084393*, –, MT050487*; Argentina, Catamarca Prov., Tinogasta Dpt.: from Fiambalá to Paso de San Francisco, S27°42′17″ W67°54′07″, *G. Barboza & al. 4784* (CORD), MT196379*, MT084388*, MT009217*, –; ***Sclerophylax lorentzianus*** O.Hoffm., Argentina, Corrientes Prov., Paso de los Libres Dpt.: RN 14, S26°56′23.2″, W57°38′51.8″, *F. Chiarini 1461* (CORD), MT196365*, MT084377*, MT009205*, MT050473*; Argentina, Corrientes Prov., Paso de los Libres / Mercedes Dpt., Miriñay River, S29°33′16.4″, W57°29′19.6″, *F. Chiarini 1453* (CORD), MT196364*, –, MT009204*, MT050472*; ***Sclerophylax ruizlealii*** Di Fulvio, Argentina, Mendoza Prov., Luján de Cuyo Dpt.: Embalse de Potrerillos, S32°57′49″ W69°11′12″, *F. Chiarini 1411* (CORD), MT196373*, MT084383*, –, MT050480*; Argentina, San Juan Prov., Ullum Dpt.: S30°59′13″ W68°48′10″, *F. Chiarini 1409b* (CORD), –, MT084381*, –, –; Argentina, San Juan Prov., Ullum Dpt.: S30°59′13″ W68°48′10″, *F. Chiarini 1409* (CORD), MT196367*, –, MT009207*, –; ***Sclerophylax spinescens*** Miers, Argentina, Entre Ríos Prov., Gualeguaychú Dpt.: Ñancay, S33°25′47″ W58°42′20″, *F. Chiarini 1396* (CORD), MT196387*, –, MT009223*, –; Argentina, Entre Ríos Prov., La Paz Dpt.: Paso Yunque, S30°23′19.6″, W59°15′36″, *F. Chiarini 1451* (CORD), MT196363*, MT084376*, MT009203*, MT050471*; Argentina, Entre Ríos Prov., Gualeguaychú Dpt.: Ruta 12, entre Médanos y Ceibas, *F. Chiarini 1393* (CORD), –, MT084398, MT009222*, –; Argentina, Santa Fe Prov., Vera Dpt.: Calchaquí, *J. Miller & al. 05-11* (MASS), EF137797, –, –, –; ***Sclerophylax*** aff. ***spinescens*** Miers, Argentina, Córdoba Prov., Tulumba Dpt.: Lucio V. Mansilla, *G. Barboza & al. 4808* (CORD), –, MT084385*, MT009212*, –; ***Sclerophylax trispermus*** Di Fulvio, Argentina, Tucuman Prov., Trancas Dpt.: S26°35′42.3″ W65°17′09.3″, *F. Chiarini 1335* (CORD), MT196381*, MT084391*, MT009219*, MT050484*; Argentina, Tucuman Prov., Graneros Dpt.: S27°48′17.7″ W65°12′16.4″, *F. Chiarini 1338* (CORD), MT196369*, MT084380*, MT009210*, MT050477*; Argentina, Córdoba Prov., Cruz del Eje Dpt.: Entre Paso Viejo y Villa de Soto, *G. Barboza & al. 4971* (CORD), MT196368*, MT196368*, MT009208*, MT050476*