



Re-evaluation of the generic status of *Athenaea* and *Aureliana* (Withaniinae, Solanaceae) based on molecular phylogeny and morphology of the calyx

PRISCILLA M. ZAMBERLAN^{1†}, IZABELLA M. C. RODRIGUES^{2†}, GERALDO MÄDER^{1†}, LUANA CASTRO¹, JOÃO R. STEHMANN², SANDRO L. BONATTO³ and LORETA B. FREITAS^{1*}

¹Laboratory of Molecular Evolution, Department of Genetics, Universidade Federal do Rio Grande do Sul, PO Box 15053, 91501-970 Porto Alegre, RS, Brazil

²Laboratory of Vegetal Systematics, Department of Botany, Universidade Federal de Minas Gerais, Avenida Antônio Carlos 6627, 31270-901 Belo Horizonte, MG, Brazil

³Laboratory of Genomic and Molecular Biology, Pontifícia Universidade Católica do Rio Grande do Sul, Avenida Ipiranga 6681, 90610-001 Porto Alegre, RS, Brazil

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Subtribe Withaniinae (Solanaceae) comprises seven genera and c. 40 species, with an almost cosmopolitan distribution. *Athenaea* and *Aureliana* are exclusively South American, with diversity centres in the Brazilian Atlantic Rainforest. The generic status of *Athenaea* and *Aureliana* was investigated using molecular phylogenetic analysis of five plastid regions (*ndhF* gene, *trnL* intron and *trnL-trnF*, *psaI-accD* and *trnC-ycf6* intergenic spacers), nuclear internal transcribed spacers (ITS) and morphometric analysis of the calyx. Divergence time estimates were also performed. Withaniinae was recovered as monophyletic. The diversification time estimated for Withaniinae was 6.3 Myr, and the estimated diversification time for the *Athenaea* and *Aureliana* clades was 2.3 Myr. *Athenaea* and *Aureliana* species formed a strongly supported clade. However, the genera were not monophyletic, and support for internal relationships was moderate to weak. The morphometric analysis of the increasing size of the fruit calyx that included all species of the genera showed a cline that did not allow us to conclude that the species could be separated into two genera. Because the accrescent calyx is the only morphological character that distinguishes them, we recognize *Athenaea* as a synonym of *Aureliana* and propose five new combinations. The list of accepted species is presented. © 2015 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2015, 177, 322–334.

ADDITIONAL KEYWORDS: Atlantic Rainforest – calyx accrescence – ITS – phylogenetic analysis – plastid markers.

INTRODUCTION

The taxonomic knowledge of Solanaceae has been greatly improved in recent years. First, Hunziker (2001) published a seminal work that revised the family based on morphological characteristics. More recently, molecular phylogenetic analyses of the

family (Olmstead *et al.*, 2008; Särkinen *et al.*, 2013) have clarified many generic relationships, but with major differences from the classification proposed by Hunziker (2001). *Athenaea* Sendtn. and *Aureliana* Sendtn. are two genera with relationships that are markedly different in these two classifications.

Hunziker (2001) proposed that *Athenaea* and *Aureliana* should be included in subtribe Capsicinae (Solanaceae, Solanoideae) with *Capsicum* L., *Darcyanthus* Hunz., *Eriolarynx* (Hunz.) Hunz., *Vassobia* Rusby,

*Corresponding author. E-mail: loreta.freitas@ufrgs.br

†These authors contributed equally to this work.

Larnax Miers, *Dunalia* Kunth and *Withania* Pauq. Olmstead *et al.* (2008) established that both genera are related to *Withania* and form a newly circumscribed and monophyletic subtribe, Withaniinae, with other genera treated by Hunziker (2001) as members of a number of different tribes (see also Olmstead & Bohs, 2007); Särkinen *et al.* (2013) confirmed this result using a much larger dataset. Withaniinae includes *c.* 40 species distributed among seven genera and has an almost worldwide distribution. Representatives of the group are found in South America (*Aureliana* and *Athenaea*), some Atlantic oceanic islands (*Withania* from the Canary Islands, *Melissia* Hook. endemic to St. Helena), in Africa (*Withania*, *Discopodium* Hochst.), Eurasia (*Withania*), Asia [*Tubocapsicum* (Wettst.) Makino] and the Hawaiian Archipelago (the endemic genus *Nothocestrum* A.Gray).

Solanaceae has been postulated to have a post-Gondwanan origin (Antonelli & Sanmartín, 2011; Olmstead, 2013). The stem ages for the family range between 62 Myr (54–70 Myr, Paape *et al.*, 2008) and 49.2 Myr (46.2–53.7 Myr, Särkinen *et al.*, 2013). Särkinen *et al.* (2013) conducted a review of all fossils of the group. The authors used a densely sampled molecular phylogenetic tree with 1075 terminals calibrated with two selected fossils. The previously oldest known fossil taxon attributed to the family (*Cantisolanium daturoides* Reid & Chandler) was shown not to belong to the family, and the oldest fossil securely identified as Solanaceae was the fossil taxon *Solanispermum reniforme* Chandler.

The genus *Athenaea* is composed of seven species; all are endemic to the Atlantic Rainforest, which is one of the most threatened environments in the world (Myers *et al.*, 2000; Ribeiro *et al.*, 2009). *Aureliana* contains eight species, which occur mainly in the Atlantic Rainforest, and there are some records of its most widespread species, *Aureliana fasciculata* (Vell.) Sendtn., in the Atlantic Rainforest and the surrounding areas of Argentina, Bolivia, Paraguay and Peru. Both genera include shrubby plants with small white and green or purple axillary flowers (Barboza & Hunziker, 1989; Hunziker & Barboza, 1990). The only morphological difference between the genera is the accrescent calyx that envelops the mature fruit in *Athenaea* (see Fig. 1 for examples of *Athenaea* and *Aureliana* species).

In this article, we re-evaluate the generic status of *Aureliana* and *Athenaea* by reconstructing the phylogenetic tree of the genera using five plastid genome regions, the nuclear internal transcribed spacers (ITS) and morphometric analysis of mature calyx lobes. The divergence times in the subtribe were also estimated to compare with previous analyses of the family as a whole.

MATERIAL AND METHODS

PLANT MATERIAL

Six *Athenaea* spp. and nine *Aureliana* spp. were included in the phylogenetic analysis. Eight species that belong to five other genera of Withaniinae were also added to the dataset. *Capsicum hunzikerianum* Barboza & Bianch., *Solanum trachytrichium* Bitter and *Vassobia breviflora* (Sendtn.) Hunz. were used as outgroups. The voucher and source information is given in Appendix 1. It should be noted that the species names in Appendix 1 correspond to those considered to be valid and the new combinations elaborated here. To access the terminals displayed in the phylogenetic analysis (Appendix 3), please consult the table of equivalence in Appendix 2. Silica gel-preserved samples of leaf tissue from field collections and material provided by other researchers were used for DNA extraction.

DNA EXTRACTION, POLYMERASE CHAIN REACTION (PCR) AMPLIFICATION AND SEQUENCING

Genomic DNA was extracted from young leaves using the Nucleo Spin Plant II kit (Macherey-Nagel, Düren, Germany). One nuclear region and five plastid regions were amplified through PCR. The *trnL-trnF* intergenic spacer and the *trnL* intron were amplified together using 0.2 μ M *c* and *f* primers (Taberlet *et al.*, 1991), 0.2 mM deoxynucleoside triphosphates (dNTPs), 2 mM MgCl₂, 1 unit of Taq DNA polymerase (Invitrogen, Carlsbad, CA, USA), and its 1 \times reaction buffer using the following parameters: 3 min of initial denaturation at 94 °C, 35 cycles of 1 min at 94 °C, 1 min at 55 °C and 1 min at 72 °C, followed by a final extension at 72 °C for 10 min. Internal primers *d* and *e* (Taberlet *et al.*, 1991) were used for sequencing.

The *ndhF* gene region was amplified using the primers of Olmstead & Sweere (1994). Two PCRs were conducted for each sample. The first reaction used primers 1F and 1318R, and the second reaction used primers 972F and 2110R, at 0.16 μ M each, 0.2 mM dNTPs, 2 mM MgCl₂, 1 unit Taq DNA polymerase and 1 \times reaction buffer. Additional internal primers (described by the same authors) were used to sequence the complete amplified region.

Plastid intergenic spacers *ycf6-trnC* and *psaI-accD* were amplified using the primers described in the literature [Shaw *et al.* (2005) and Shaw *et al.* (2007), respectively]. Both fragments were amplified using 0.16 μ M of each specific primer, 0.2 mM dNTPs, 2 mM MgCl₂, 1 unit Taq DNA polymerase and 1 \times reaction buffer according to the following conditions: 3 min of initial denaturation at 94 °C, 30 cycles of 1 min at 94 °C, 1 min at 50 °C and 1 min (for *psaI-accD*) or 1 min 30 s (for *trnC-ycf6*) at 72 °C, followed by a final extension at 72 °C for 10 min.

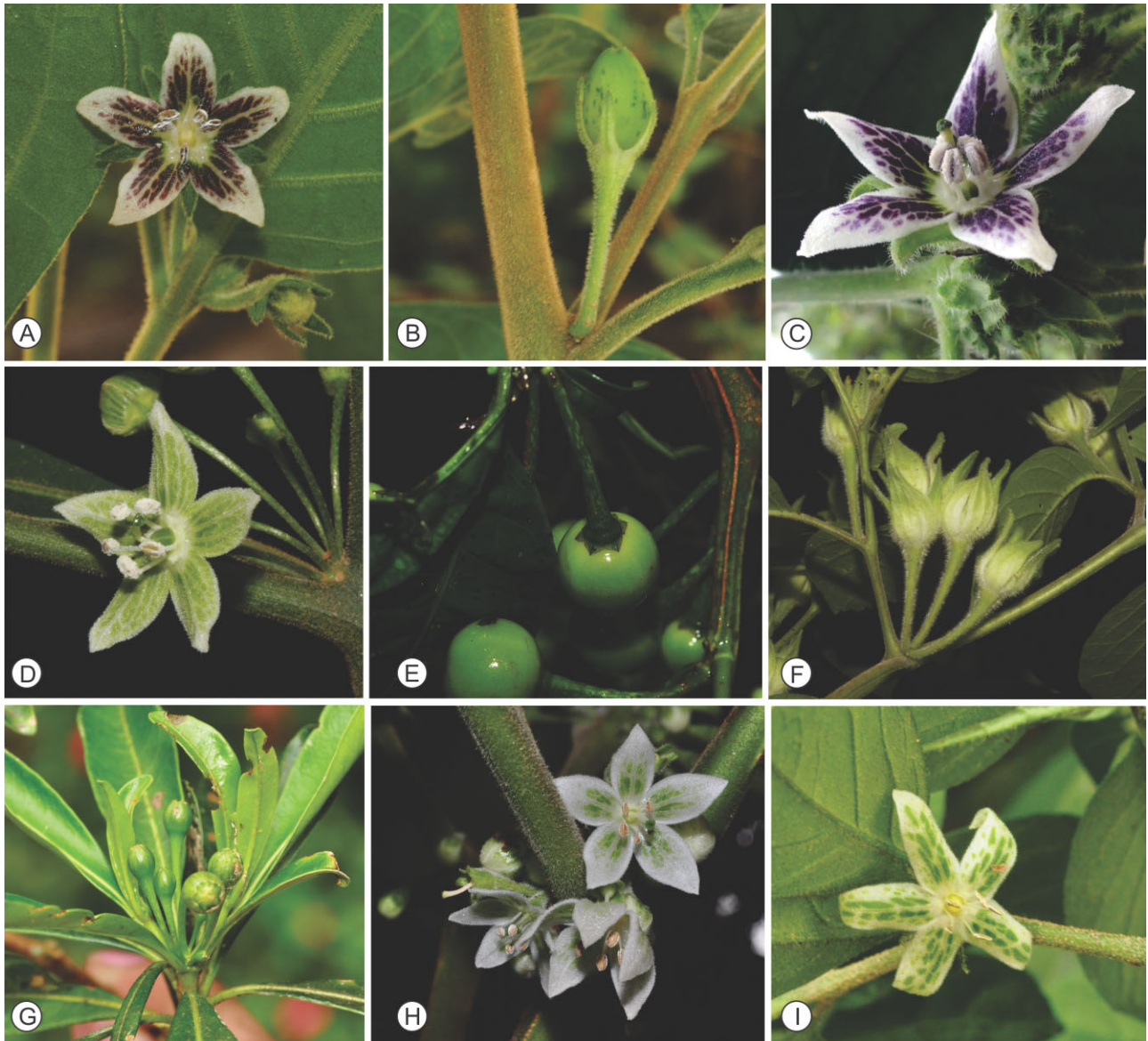


Figure 1. *Athenaea* and *Aureliana* representatives. A, B, *Athenaea anonacea* (***Aureliana anonacea***); C, *Athenaea cuspidata* (***Aureliana cuspidata***); D, E, *Aureliana fasciculata*; F, *Athenaea pogogena* (***Aureliana pogogena***); G, *Aureliana sellowiana*; H, *Aureliana velutina*; I, *Aureliana wettsteiniana*. The names in parentheses refer to new combinations proposed in this study. Photographs by C. N. Fraga (D), I. M. C. Rodrigues (C, H) and J. R. Stehmann.

The ITS region was amplified using 0.2 μM of each specific primer (Desfeux & Lejeune, 1996), 0.2 mM dNTPs, 2 mM MgCl_2 , 1 unit Taq DNA polymerase, 10% dimethyl sulphoxide (DMSO) and 1 \times reaction buffer according to the following conditions: 5 min of initial denaturation at 94 $^{\circ}\text{C}$, 35 cycles of 1 min at 94 $^{\circ}\text{C}$, 1 min at 55 $^{\circ}\text{C}$ and 2 min at 72 $^{\circ}\text{C}$, followed by a final extension at 72 $^{\circ}\text{C}$ for 10 min. All PCR products were purified using the polyethyleneglycol precipitation method (Dunn & Blattner, 1987). The sequencing reaction was performed using an ET terminators kit

(GE Healthcare Biosciences, Pittsburgh, PA, USA) on a MegaBACE 1000 (GE Healthcare Biosciences) automatic machine following the manufacturer's protocols.

PHYLOGENETIC ANALYSIS

Forward and reverse reads were analysed for all sequences generated in this study using Chromas 2.33 (Technelysium Pty Ltd, Brisbane, Australia). For ITS, ambiguous (heterozygous) sites were treated as missing data according to Mäder *et al.* (2010). Seven

ndhF, two ITS and six *trnL* intron and *trnL-trnF* sequences of species of Withaniinae available in GenBank (<http://www.ncbi.nlm.nih.gov>) were downloaded and included in the analysis. (See Appendix 3 for the GenBank accession numbers for all the sequences employed in the present study.) The final plastid dataset was composed of *psaI-accD* and *ycf6-trnC* intergenic spacer sequences generated for all the sampled groups, plus *ndhF* and *trnL-trnF* sequences obtained from *Aureliana* spp. and *Athenaea* spp., and those downloaded from GenBank. The *trnL-trnF* region was divided into *trnL* intron and *trnL-trnF* intergenic spacer for alignment and sequence characterization. The nuclear dataset was composed of ITS sequences generated for *Athenaea* and *Aureliana*, in addition to outgroup sequences downloaded from GenBank. The sequences were aligned using ClustalW (Thompson, Higgins & Gibson, 1994) implemented on Mega 6 (Tamura *et al.*, 2013). All the alignments were manually adjusted.

Bayesian phylogenetic relationships and divergence time estimates were conducted through Bayesian analysis in the BEAST package version 1.8 (Drummond *et al.*, 2012). The divergence time estimates were obtained adopting a relaxed molecular clock approach (Drummond *et al.*, 2006) and a GTR + G substitution model. A normal prior (mean value, 6.3; standard deviation, 2.1) was set as the crown age of Withaniinae according to Särkinen *et al.* (2013). Two independent BEAST analyses were performed for 100 million iterations, and samples were taken for each 10 000 chains. The initial 10% of the chains were discarded as burn-in. TRACER 1.5 (available at <http://beast.bio.ed.ac.uk/Tracer>) was used to visually inspect the runs. The results were combined using LogCombiner as implemented on the BEAST package. The phylogenetic relationships were summarized in a maximum clade credibility tree, and their 95% highest posterior densities (HPDs) were estimated using TreeAnnotator v.1.8. Maximum likelihood (ML) phylogenetic analysis was performed with the online version of PhyML 3.0 (Guindon *et al.*, 2010, <http://www.atgc-montpellier.fr/phyml/>). An ML phylogenetic model was first estimated using simultaneous Nearest Neighbor Interchanges moves to improve a BIONJ (Gascuel, 1997) starting tree (default option) under the GTR substitution model with 1000 bootstrap replications. The plastid and nuclear sequences were analysed independently and then combined. Because no differences were observed between the dataset results, only the combined results are presented and discussed.

CALYX ACCRESCENCE

The ratio of the length of the calyx lobes in the fruit to the length of the calyx lobes in the flower was

chosen to represent the increase in the calyx. We used dried material from various herbaria to analyse calyx accrescence, and the specimens used in the morphometric analysis are detailed in Appendix 1. To make the measurements, the flowers and developed fruits were rehydrated. Analysis of variance (ANOVA) was performed to evaluate the influence of the calyx characters that have been used previously to differentiate the genera. The total number of specimens measured was 70 (35 *Athenaea* and 35 *Aureliana*), and all *Athenaea* spp. and *Aureliana* spp. were sampled. Statistical analysis was performed using PAST 1.79 software (Hammer, Harper & Ryan, 2001). Box-plots were built with the software IBM SPSS Statistics 20.0 to depict the variation of the calyx accrescence in the taxa analysed.

TAXONOMY

The taxonomic and nomenclatural decisions were based on a comprehensive study that includes a survey of the original sources for all names (synonyms and basonyms) related to *Athenaea* spp. and *Aureliana* spp., extensive field work and the revision of the type and non-type material kept in the following herbaria: A, B, BM, BR, BHC, CESJ, CORD, G, FI, M, MO, P, R, RB, S, SP, W and WU (acronyms according to Thiers, 2013).

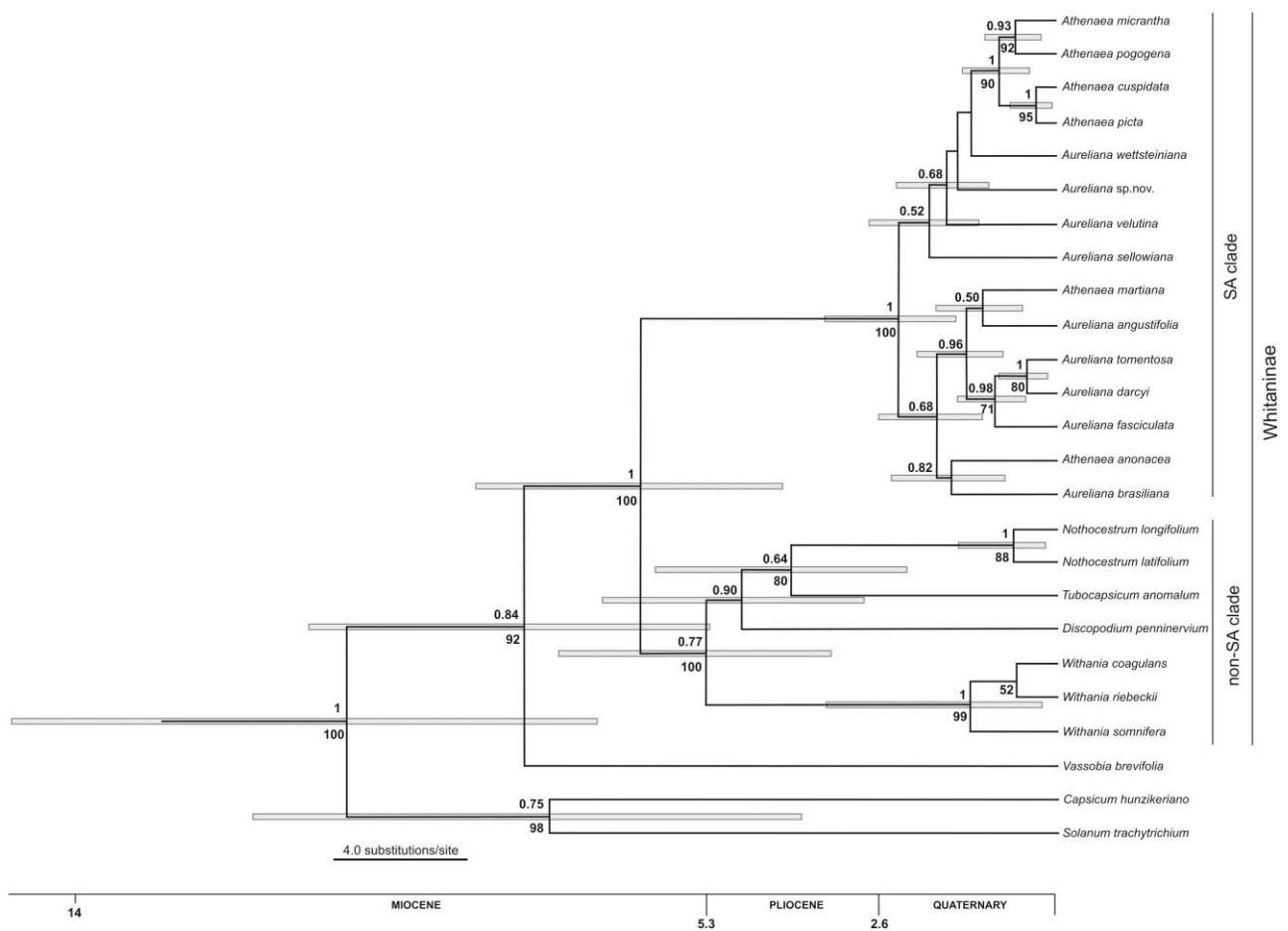
RESULTS

The detailed alignment characteristics are displayed in Table 1. Missing data corresponded to 9.6% of the dataset. Insertion/deletions (indels) of several sizes occurred in all the markers. The most informative of these is in the *trnL* intron, where two indels were detected in the three *Withania* spp. and one was found in all *Athenaea* and *Aureliana* specimens. Regions of alignment uncertainty were excluded from the analyses. The combined final dataset was 5112 base pairs (bp) long.

The Bayesian maximum clade credibility phylogenetic tree is shown in Figure 2 with the ML bootstrap values. As in previous analyses, subtribe Withaniinae was recovered as monophyletic. Within Withaniinae, two major clades were detected: one group composed of only taxa that occur outside South America (non-SA clade) and a clade named South America group (SA clade) that comprised species of *Aureliana* and *Athenaea* which are endemic to South America and predominantly from the Atlantic Rainforest. The first group, with moderate support, was formed by two clades: one that includes the three *Withania* spp. and another composed of the *Nothoestrum* spp. plus *Discopodium penninervium* Hochst. and *Tubocapsicum anomalum* (Frach & Sav.) Makino. *Withania* and

Table 1. Alignment characteristics of the plastid regions (*ndhF* gene, *trnL* intron and *trnL-trnF*, *psaI-accD* and *trnC-ycf6* intergenic spacers) and the nuclear internal transcribed spacers (ITS)

Character	<i>ndhF</i>	<i>trnL</i> intron	<i>trnL-trnF</i>	<i>psaI-accD</i>	<i>trnC-ycf6</i>	ITS
Length range (bp)						
Ingroup only	2054–2060	489–502	218–395	714–780	566–605	643–649
Outgroup included	2054–2060	489–523	218–400	714–780	566–605	634–649
Aligned length (bp)						
Ingroup only	2060	502	412	782	644	650
Outgroup included	2060	523	412	829	660	655
Variable characters (%)						
Ingroup only	49 (2.38)	11 (2.19)	21 (5.1)	22 (2.81)	12 (1.86)	66 (10.15)
Outgroup included	105 (5.1)	20 (3.82)	41 (9.95)	43 (5.19)	35 (5.3)	157 (23.97)

**Figure 2.** Maximum clade credibility phylogenetic tree showing the relationships among species of subtribe Withaniinae with an emphasis on *Athenaea* and *Aureliana*. The values above the branches correspond to the posterior probabilities, and the values below the branches indicate the maximum likelihood bootstraps. The geological time scale is shown at the bottom, and the values indicate million years. The horizontal bars indicate the 95% highest posterior age intervals based on BEAST analyses.

Nothoestrum were strongly supported. In the SA clade, the individual genera *Athenaea* and *Aureliana* were not supported as monophyletic, but resulted in a strongly supported monophyletic group. The estimated

crown mean age of subtribe Withaniinae diversification was *c.* 6.3 Myr, and the estimated crown mean age of the *Athenaea* plus *Aureliana* clade was 2.3 Myr. (See Table 2 for the estimated mean ages and their

respective 95% HPDs for the major clades recovered in the phylogenetic tree.)

The ANOVA performed with the calyx character showed significant differences in the average between the genera ($P < 0.05$), but none from each species among themselves (Tukey: $P > 0.05$). Figure 3 illustrates the variation in the length of the calyx lobes at fruiting and the clinal and overlapping distribution of accrescence. The calyx increase in fruit development is maximized in *Athenaea pogogena*, which enlarged

1.5–5.0 (15.0) times from its size in the flower. Most *Aureliana* spp., as traditionally defined, have calyx lobes that expand to approximately twice the length at flowering at the fruiting stage.

DISCUSSION

PHYLOGENETIC RELATIONSHIPS AND TAXONOMIC IMPLICATIONS

Our results, which included additional species of the group, supported the monophyly of Withaniinae in concordance with previous molecular phylogenetic studies (Olmstead *et al.*, 2008; Särkinen *et al.*, 2013). The grouping of *Athenaea* with *Aureliana* in a strongly supported clade and the clade position as a sister group to the rest of Withaniinae, which have been suggested by others (Olmstead & Bohs, 2007; Olmstead *et al.*, 2008; Särkinen *et al.*, 2013), were also corroborated here, with a much more robust taxonomic sampling of these two genera. The strong support for the *Athenaea* plus *Aureliana* clade is associated with its long stem branch, which might have been caused by different events, such as a long

Table 2. Estimated mean ages (million years) with 95% highest posterior densities (HPDs) for the major clades of Withaniinae. The groups refer to Figure 2

Group	Stem age (95% HPD)	Crown age (95% HPD)
Withaniinae	8.1 (5.3–11.3)	6.3 (4.2–8.8)
SA (<i>Athenaea</i> + <i>Aureliana</i>) clade	6.3 (4.2–8.8)	2.3 (1.6–3.5)
Non-SA clade	6.3 (4.2–8.8)	5.3 (3.4–7.5)

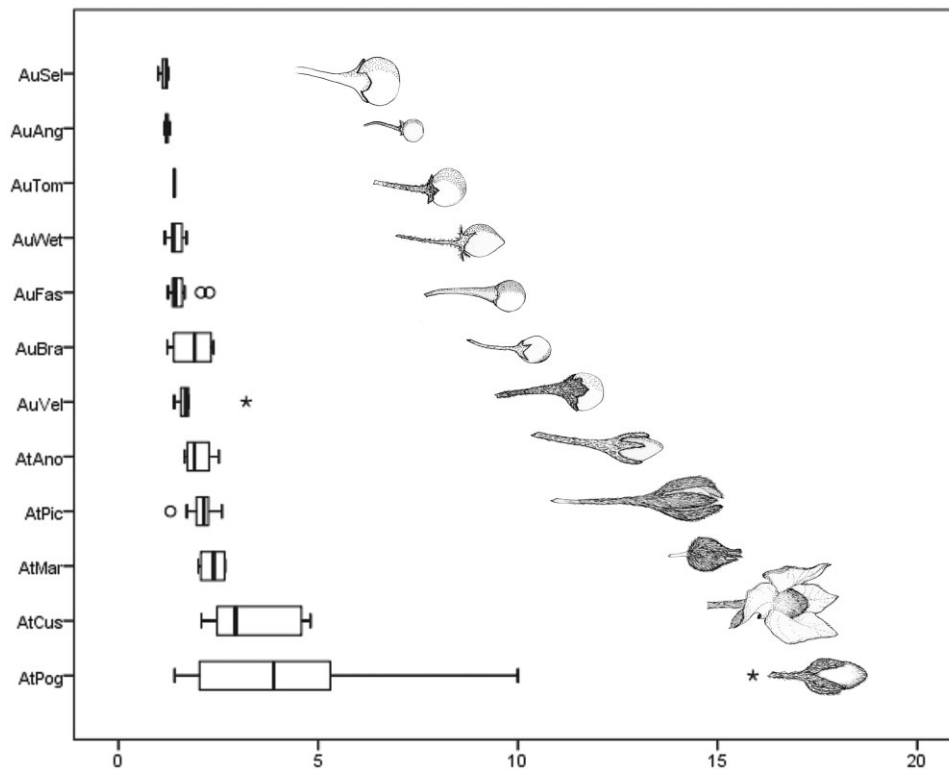


Figure 3. Variation of the calyx accrescence in *Athenaea* and *Aureliana* indicated by the ratio between the length of the calyx lobes in the fruits and the length of the calyx lobes in the flowers. The morphology of the corresponding calyx in the fruit is illustrated. AuSel, *Aureliana sellowiana*; AuAng, *Aureliana angustifolia*; AuTom, *Aureliana tomentosa*; AuWet, *Aureliana wettsteiniana*; AuFas, *Aureliana fasciculata*; AuBra, *Aureliana brasiliiana*; AuVel, *Aureliana velutina*; AtAno, *Athenaea anonacea*; AtPic, *Athenaea picta*; AtMar, *Athenaea martiana*; AtCus, *Athenaea cuspidata*; AtPog, *Athenaea pogogena*.

period of isolation followed by a recent radiation, and the extinction of other members of the clade as a result of climate change since the Miocene. Neither *Athenaea* nor *Aureliana* was monophyletic, but the moderate to weak support for most of the internal groups does not preclude a conclusive decision regarding the generic status of both genera. *Athenaea* and *Aureliana* should be recognized as a single genus, and this is morphologically supported by the overlapping nature of calyx accrescence found in the fruits of both species.

The generally weak support of the internal groups of the *Athenaea* and *Aureliana* clade only enables a few careful considerations to be raised with regard to species taxonomy. The presence of a strongly supported group that includes *Athenaea pogogena* (Moric.) Sendtn., *Athenaea micrantha* Sendtn., *Athenaea cuspidata* Witasek and *Athenaea picta* (Mart.) Sendtn. supports the synonymization of *A. micrantha* with *A. pogogena*. The clade composed of *Aureliana tomentosa* Sendtn., *Aureliana darcyi* Carvalho & Bovini and *Aureliana fasciculata* (Vell.) Sendtn. was also strongly supported. At the present moment, the basic data concerning micro- and macromorphology and related to geographical distribution support the synonymization of *A. darcyi* with *A. fasciculata* (I. M. C. Rodrigues & J. R. Stehmann, unpubl. data), but not *A. tomentosa*. Additional collection efforts would make detailed morphological studies that should address the specific status of *A. tomentosa* possible.

DIVERSIFICATION AGES

The crown age of Withaniinae obtained using molecular rates is 6.3 (4.2–8.8) Myr [stem age, 8.1 (5.3–11.3) Myr], which corresponds to the Messinian age of the Miocene epoch, when the global climate was warmer in comparison with the current climate (Dowsett *et al.*, 1994; Sloan, Crowley & Pollard, 1996). Särkinen *et al.* (2013) used a broader sample of the tribe and obtained older ages for the group [stem age, 10.41 (8.3–13.0) Myr; crown age, 6.3 (4.2–8.4) Myr]. Despite differences in the estimated dates, which are most probably a result of the number of genera sampled, both results support the hypothesis that the ancestor of this group was South American, as suggested by Olmstead *et al.* (2008). The continent is the centre of diversity of Solanaceae (Hunziker, 2001) at both the generic and specific levels (Knapp *et al.*, 2004). As indicated by the data from vertebrate palaeontology and phytoliths, South America had a more humid and warmer climate during the Messinian (Salzmann *et al.*, 2011). *Athenaea* and *Aureliana* seem to be descendants of the ancestor that remained in South America. The estimated age of the clade is 2.3 Myr [crown age, 1.6–3.5 Myr; stem age, 5.3 (4.2–

8.8) Myr], which corresponds to the Pleistocene. Several studies have indicated that climate changes during this period had a great influence on the Atlantic Rainforest dynamics (Carnaval & Moritz, 2008; Carnaval *et al.*, 2014). However, it is noteworthy that a broader sample of Withaniinae, including especially representatives of *Withania*, and a fully resolved phylogenetic tree are necessary to address properly questions about the intriguing biogeographical pattern displayed by the group.

CALYX ACCRESCENCE

‘Accrescence’ in an organ is the ability of an organ to carry on growing after its formation (Font Quer, 2001). Several authors have demonstrated that mutations in regulatory sequences of flower development genes and plant–hormone interactions are associated with the evolution of calyx accrescence in Solanaceae (He & Saedler, 2005; Khan *et al.*, 2009; Khan, Hu & He, 2012; Zhang *et al.*, 2012). When species of *Aureliana* and *Athenaea* are analysed together, the accrescence of the calyx lobe is observed in all species as a gradual enlargement. The species with smaller lobes represent *Aureliana*, and those with larger lobes are mostly in *Athenaea*. However, there is no clear distinction into two morphological groups that could represent the genera. In Solanaceae, the accrescent calyx occurs in several phylogenetically unrelated groups that arose in distinct lineages from an ancestor with an unenlarged fruiting calyx (Knapp, 2002). Gradation in calyx enlargement can also be observed in *Nothoecstrum*, which belongs to Withaniinae (Olmstead & Bohs, 2007). Because the main difference between *Aureliana* and *Athenaea* was focused on the accrescent calyx, because of the knowledge of the developmental rules that guide this character in Solanaceae and because of the plesiomorphic nature of the inflated calyx (Hu & Saedler, 2007), we considered this not to be a useful diagnostic character. Thus, we accept *Aureliana* and *Athenaea* as a single genus with a continuum of levels of calyx lobe accrescence.

TAXONOMY

Our results indicate that *Athenaea* and *Aureliana* form a strongly supported monophyletic clade, the diversification of which started *c.* 2.3 Myr ago, and that the genera as traditionally circumscribed are not monophyletic. These results support the combination of *Athenaea* and *Aureliana* into a single genus. Because *Athenaea* and *Aureliana* were published in the same work, we elect to use the generic name *Aureliana* because fewer new combinations are required. A list of the currently accepted species, which includes their distribution, is presented below.

- Aureliana* Sendtn., Fl. Bras. (Martius) 10(6): 138. 1846 – Type species: *Aureliana velutina* Sendtn.
= *Athenaea* Sendtn., Fl. Bras. (Martius) 10(6): 133. 1846 – Type species: *Athenaea picta* (Mart.) Sendtn.
- Aureliana angustifolia* Almeida-Lafetá, Novon 10: 187–189. 2000 – Type: Brazil. Minas Gerais: Juiz de Fora, Reserva Biológica Santa Cândida, 29.v.1996, R.C. Almeida-Lafetá & F.A.P.L. Costa 95 (holotype, CESJ30673; isotypes, MO, R, RB) – Distribution: Brazil (Minas Gerais).
 - Aureliana anonacea* (Sendtn.) I.M.C.Rodrigues & Stehmann, **comb. nov.** ≡ *Athenaea anonacea* Sendtn. in Mart., Fl. Bras. 10(6): 137. 1846 ≡ *Witheringia anonacea* (Sendtn.) Miers, Ann. Mag. Nat. Hist. Ser. II, 3 (14): 146. 1849 ≡ *Withania anonacea* (Sendtn.) Dunal in DC., Prodr. 13 (1): 463. 1852 ≡ *Bassovia anonacea* (Sendtn.) Miers, Ann. Mag. Nat. Hist. Ser. 2, 11(62): 94. 1853 – Type: Brazil. ‘Brasilia australiore’, Sellow s.n. – Distribution: Brazil (Minas Gerais and Rio de Janeiro).
 - Aureliana brasiliiana* (Hunz.) Barboza & Hunz., Darwiniana 30: 105. 1990 ≡ *Athenaea brasiliiana* Hunz., Kurtziana 5: 397, f.2. 1969 – Type: Brazil. São Paulo: Pindamonhangaba, 23.ix.1961, E.L.M. Kuhlmanns.n. (holotype, SP; isotype, CORD) – Distribution: Brazil (Minas Gerais, Rio de Janeiro and São Paulo).
 - Aureliana cuspidata* (Witasek) I.M.C.Rodrigues & Stehmann, **comb. nov.** ≡ *Athenaea cuspidata* Witasek, Deutsche Akad. Wiss. Wien 79 (2): 318–319, table 29, f.2a–c, 5. 1931 – Type: Brazil. São Paulo: ‘Prope Raiz da Serra’, 20–50 m.s.m., vi.1901, Wettstein et Schiffners.n. (holotype, WU0062927) – Distribution: Brazil (Espírito Santo, Minas Gerais and São Paulo).
 - Aureliana fasciculata* (Vell.) Sendtn. in Mart., Fl. Bras. 10(6): 140, 1846 ≡ *Solanum fasciculatum* Vell., Fl. Flumin. 2, table 106. 1829 ≡ *Bassovia fasciculata* (Vell.) Dunal in DC., Prodr. 13 (1): 408. 1852 ≡ *Capsicum fasciculatum* (Vell.) Kuntze, Rev. Gen. Pl. 2: 450. 1891 – Distribution: Argentina, Bolivia, Brazil, Paraguay and Peru.
= *Aureliana darcyi* Carvalho & Bovini, in Novon 5: 257–258, 1995 – Type: Brazil. Rio de Janeiro: Paraty, Apa-Cairuçu, Orla da Ilha das Almas, 15.iii.1989, A.L. Almeida & B. Kurtz et al. 7 (holotype, RB; isotype, MO). **Syn. nov.**
 - Aureliana martiana* (Sendtn.) I.M.C.Rodrigues & Stehmann, **comb. nov.** ≡ *Athenaea martiana* Sendtn. in Mart., Fl. Bras. 10(6): 136. 1846 ≡ *Witheringia martiana* (Sendtn.) Miers, Ann. Mag. Nat. Hist. Ser. 2, 3 (14): 146. 1849 ≡ *Withania martiana* (Sendtn.) Dunal in DC., Prodr. 13 (1): 462. 1852 ≡ *Bassovia martiana* (Sendtn.) Miers, Ann. Mag. Nat. Hist. Ser. 2, 11(62): 94. 1853 – Type: Brazil. Minas Gerais: ‘In prov. Minarum ad Sobradinho, Chapeo d’Uvas et Caveiras’, Pohl s.n. (lectotype, M0171588, designated by Barboza & Hunziker, 1989) – Distribution: Brazil (Espírito Santo and Minas Gerais).
 - Aureliana picta* (Mart.) I.M.C.Rodrigues & Stehmann, **comb. nov.** ≡ *Witheringia picta* Mart., Nov. Gen. Sp. Pl. 3: 74, t.227. 1829 ≡ *Athenaea picta* (Mart.) Sendtn. in Mart., Fl. Bras. 10(6): 134, 1846 ≡ *Withania picta* (Mart.) Dunal in DC., Prodr. 13 (1): 458. 1852 ≡ *Bassovia picta* (Mart.) Miers, Ann. Mag. Nat. Hist. Ser. 2, 11(62): 94. 1853 – Type: Brazil. Minas Gerais: ‘São João Del Rey, In vale Prope de Cheftas praedium, Sept,’ Martius 94 (lectotype, M0171583, designated by Barboza & Hunziker, 1989) – Distribution: Brazil (Minas Gerais, Rio de Janeiro and São Paulo).
 - Aureliana pogogena* (Moric.) I.M.C.Rodrigues & Stehmann, **comb. nov.** ≡ *Solanum pogogenum* Moric., Plantes Nouv. D’Amerique 25, t.17. 1837 ≡ *Athenaea pogogena* (Moric.) Sendtn. in Mart., Fl. Bras. 10(6): 134. 1846 ≡ *Witheringia pogogena* (Moric.) Miers, Ann. Mag. Nat. Hist. Ser. II, 3 (14): 145. 1849 ≡ *Withania pogogena* (Moric.) Dunal in DC., Prodr. 13 (1): 459. 1852 ≡ *Bassovia pogogena* (Moric.) Miers, Ann. Mag. Nat. Hist. ser. 2, 11(62): 94. 1853 – Type: Brazil. Bahia, Ilhéus, 1898, Blanchet 2110 (lectotype, G00227986, designated by Barboza & Hunziker, 1989; isolectotypes, BM, FI, P, W) – Distribution: Brazil (Espírito Santo, Minas Gerais, Rio de Janeiro and São Paulo).
= *Athenaea micrantha* Sendtn., in Mart., Fl. Bras. 10(6): 135, 1846 ≡ *Witheringia micrantha* Miers in Ann. Mag. Nat. Hist. Ser. II, 3 (14): 145. 1849 ≡ *Withania micrantha* Dunal in DC., Prodr. 13 (1): 460. 1852 *Bassovia micrantha* Miers in Ann. Mag. Nat. Hist. Ser. 2, 11(62): 94, 1853 – Type: Brazil. Bahia. **Syn. nov.**
 - Aureliana sellowiana* (Sendtn.) Barboza & Stehmann, Systematic Botany 35(2): 420–424, 2010 ≡ *Witheringia sellowiana* Sendtn. in Mart., Fl. Bras. 10(6): 123. 1846 – Type: Brazil. São Paulo: ‘Cubatao’, Sellow s.n. (holotype, B, destroyed; lectotype P ex B designated by Barboza et al., 2010; isolectotype K). Brazil. São Paulo: São Paulo distrito Parelheiros, a 300 m de la Estrada Ponte Seca rumo a Marsilac, 23°53’52”S, 46°43’31”W, 605 m, 15.iv.2008, G. E. Barboza, M. V. Romero and G. Bertone 2024 (epitype SP001621, designated by Barboza et al., 2010; isoeotypes: BHCB, CORD) – Distribution: Brazil (São Paulo).
 - Aureliana tomentosa* Sendtn. in Mart., Fl. Bras. 10(6): 140, 1846 ≡ *Bassovia tomentosa* (Sendtn.) Dunal in DC., Prodr. 13 (1): 409.

- 1852 = *Capsicum tomentosum* (Sendtn.) Kuntze, Rev. Gen. Pl. 2: 450. 1891 – Type: Brazil. ‘Brasilia australiore,’ *Sellow s.n.* [holotype, B, destroyed (F neg. 2882)] – Distribution: Brazil (Espírito Santo and Minas Gerais).
11. ***Aureliana velutina*** Sendtn. in Mart., Fl. Bras. 10(6): 140, 1846 = *Bassovia velutina* (Sendtn.) Dunal in DC., Prodr. 13 (1): 410. 1852 = *Capsicum velutinum* Kuntze, Rev. Gen. Pl. 2: 448. 1891 = *Athenaea velutina* (Sendtn.) D’Arcy, Solanaceae Newsletter 2 (4): 16. 1986 – Type: Brazil. Goiás: ‘Ad praedium Faz. S. Cruz da Donna Tereza, Província Goyazana’, *Pohl s.n.* – Distribution: Brazil (Bahia, Goiás and Minas Gerais).
 12. ***Aureliana wettsteiniana*** (Witasek) Hunz. & Barboza, Darwiniana 30: 107. 1990 = *Bassovia wettsteiniana* Witasek, Deutsche Akad. Wiss. Wien 79 (2): 323–324, f-6a–d. 1910 – Type: Brazil. São Paulo: ‘Prope Campo Grande inter Santos et urbem São Paulo’, 750 m.s.m., vii.1901, *Wettstein et Schiffners. n.* (holotype, WU0037952) – Distribution: Brazil (Paraná, Rio Grande do Sul, São Paulo and Santa Catarina).
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APPENDIX 1

Complete list of the specimens included in the present study, which indicates whether they were employed in the phylogenetic or morphometric analysis, the collection site, and the voucher information. All the samples were collected in Brazil unless indicated otherwise. The abbreviations correspond to the Brazilian states as follows: BA, Bahia; DF, Distrito Federal; ES, Espírito Santo; MG, Minas Gerais; RJ, Rio de Janeiro; SC, Santa Catarina; SP, São Paulo. The herbaria acronyms refer to the Universidade Federal de Minas Gerais (BHCB) and the Bishop Museum (BISH). The collection numbers were provided when voucher information was not available. The specimens marked with an asterisk were kindly provided as DNA samples by Dr R. G. Olmstead (University of Washington, Seattle, WA, USA). NA, information not available.

Taxon	Collection site	Phylogenetic analysis	Morphometric analysis	Voucher
<i>Aureliana angustifolia</i> R.C.Almeida-Lafetá	Juiz de Fora, MG	X		BHCB134380
<i>Aureliana angustifolia</i>	Juiz de Fora, MG		X	BHCB134381
<i>Aureliana angustifolia</i>	Juiz de Fora, MG		X	RB366021
<i>Aureliana angustifolia</i>	Juiz de Fora, MG		X	MO5313956

Appendix 1 *Continued*

Taxon	Collection site	Phylogenetic analysis	Morphometric analysis	Voucher
<i>Aureliana anonacea</i> (Sendtn.) I.M.C.Rodrigues & Stehmann	Caraça Sanctuary, MG	X	X	BHCB142292
<i>Aureliana anonacea</i>	Rio Preto Farm, MG		X	K000788447
<i>Aureliana anonacea</i>	Caraça Sanctuary, MG		X	K000788450
<i>Aureliana anonacea</i>	Resende, Serra da Mantiqueira, RJ		X	K000788446
<i>Aureliana brasiliana</i> (A.T.Hunz.) Barboza & A.T.Hunz.	Itatiaia National Park, RJ	X	X	BHCB145043
<i>Aureliana brasiliana</i>	Itatiaia National Park, RJ		X	BHCB145056
<i>Aureliana brasiliana</i>	Rio de Janeiro, RJ		X	K000788437
<i>Aureliana brasiliana</i>	Mont Serrat, Itatiaia National Park, RJ		X	S128280
<i>Aureliana cuspidata</i> (Witasek) I.M.C.Rodrigues & Stehmann	Conservation Area Boracéia, SP	X		BHCB115951
<i>Aureliana cuspidata</i>	Muniz Freire, ES		X	MO5311797
<i>Aureliana cuspidata</i>	Santo André, Paranapiacaba, SP		X	MO5311817
<i>Aureliana cuspidata</i>	Caparaó National Park, MG		X	BHCB30956
<i>Aureliana cuspidata</i>	Caraguatatuba, SP		X	SP102184
<i>Aureliana cuspidata</i>	Biritiba Mirim, SP		X	BHCB38701
<i>Aureliana fasciculata</i> (Vell.) Sendtn.	Conservation Area Japi, Jundiá, SP	X (sp1)		BHCB115929
<i>Aureliana fasciculata</i>	Trindade, Paraty, RJ	X (sp2)		BHCB115994
<i>Aureliana fasciculata</i>	Serra do Caracol, SP		X	S128391
<i>Aureliana fasciculata</i>	Serra dos Paramirim, Piraquara, PR		X	G00189912
<i>Aureliana fasciculata</i>	Macaé de Cima, Nova Friburgo, RJ		X	G00189914
<i>Aureliana fasciculata</i>	Alcobaça, BA		X	K000788443
<i>Aureliana fasciculata</i>	San Pedro, Misiones, Argentina		X	S128405
<i>Aureliana fasciculata</i>	Jacareí, SP		X	S128401
<i>Aureliana fasciculata</i>	Jacarepaguá, RJ		X	M-0183107
<i>Aureliana fasciculata</i>	São Paulo, Jaraguá, SP		X	MO5769826
<i>Aureliana fasciculata</i>	Morretes, PR		X	G00189984
<i>Aureliana fasciculata</i>	Campo dos Afonsos Air-Base, RJ		X	G00189917
<i>Aureliana fasciculata</i>	Ponta Grossa, PR		X	S128410
<i>Aureliana fasciculata</i>	NA		X	S128411
<i>Aureliana fasciculata</i>	Nova Santa Rita, RS		X	B100448815
<i>Aureliana martiana</i> (Sendtn.) I.M.C.Rodrigues & Stehmann	Juiz de Fora, MG	X	X	BHCB134379
<i>Aureliana martiana</i>	Chapeo D'Uva, MG		X	BM000099841
<i>Aureliana martiana</i>	MG		X	K000788448
<i>Aureliana martiana</i>	Nova Lombardia Biological Reserve, Santa Teresa, ES		X	MO3311633
<i>Aureliana picta</i> (Mart.) I.M.C.Rodrigues & Stehmann	Bananal, SP	X		BHCB144999
<i>Aureliana picta</i>	Tunas do Paraná, Pacas, PR		X	G00189928
<i>Aureliana picta</i>	Tijuca, Rio de Janeiro, RJ		X	BM001016005
<i>Aureliana picta</i>	Roça Nova, PR		X	S128264
<i>Aureliana picta</i>	Putuna River, Bocaiuva do Sul, PR		X	MO2034500
<i>Aureliana picta</i>	RJ		X	G00189937
<i>Aureliana picta</i>	Recanto das Araucárias, Curitiba, PR		X	S128278
<i>Aureliana picta</i>	Corcovado, RJ		X	G00189940
<i>Aureliana picta</i>	Santana do Itararé, PR		X	S128270
<i>Aureliana picta</i>	Caminho dos Macacos, RJ		X	S128255
<i>Aureliana picta</i>	Nova Friburgo, RJ		X	G00189941
<i>Aureliana pogogena</i> (Moric.) I.M.C.Rodrigues & Stehmann	Road São José – Una, BA	X		BHCB126140
<i>Aureliana pogogena</i>	Conservation Area Serra Bonita, Camacan, BA	X		BHCB126160

Appendix 1 *Continued*

Taxon	Collection site	Phylogenetic analysis	Morphometric analysis	Voucher
<i>Aureliana pogogena</i>	Galo River, Domingos Martins, ES		X	BR571849
<i>Aureliana pogogena</i>	Road São José – Una, BA		X	MO2668212
<i>Aureliana pogogena</i>	Santa Cruz de Cabrália, BA		X	MO5596664
<i>Aureliana pogogena</i>	Ilhéus, BA		X	BM000617954
<i>Aureliana pogogena</i>	Luzitania Farm, Arataca, BA		X	BM001016008
<i>Aureliana pogogena</i>	Porto Seguro, Frei Calixto, BA		X	M0183114
<i>Aureliana pogogena</i>	Nova Friburgo, RJ		X	BR571777
<i>Aureliana pogogena</i>	NA		X	BR571801
<i>Aureliana pogogena</i>	NA		X	BM001016003
<i>Aureliana pogogena</i>	Road to Mendanha, MG		X	MO2430833
<i>Aureliana pogogena</i>	MG		X	BR587612
<i>Aureliana pogogena</i>	BA		X	BR553107
<i>Aureliana sellowiana</i> (Sendtn.) Barboza & Stehmann	Parelheiros, São Paulo, SP	X		BHCB145006
<i>Aureliana sellowiana</i>	Parelheiros, São Paulo, SP		X	BHCCB145007
<i>Aureliana sellowiana</i>	Serra do MarState Park, SP		X	BHCB133519
<i>Aureliana sellowiana</i>	Serra do MarState Park, SP		X	PMSP4402
<i>Aureliana tomentosa</i> Sendtn.	Santa Teresa, ES	X		BHCB115995
<i>Aureliana tomentosa</i>	Juiz de Fora, MG		X	BHCB124208
<i>Aureliana tomentosa</i>	Marilândia, ES		X	BHCB129418
<i>Aureliana velutina</i> Sendtn.	Nova Lima, MG	X		BHCB103620
<i>Aureliana velutina</i>	Abaíra, BA		X	K000788436
<i>Aureliana velutina</i>	Mendanha, MG		X	G00189970
<i>Aureliana velutina</i>	São Bartolomeu River, Brasilia, DF		X	K000788435
<i>Aureliana velutina</i>	Roncador River, Brasilia, DF		X	MO3272787
<i>Aureliana velutina</i>	Viçosa, MG		X	S128476
<i>Aureliana wettsteiniana</i> (Witasek) A.T.Hunz. & Barboza	Porto União, SC	X		BHCB143430
<i>Aureliana wettsteiniana</i>	Tijucas do Sul, PR		X	MO04627874
<i>Aureliana wettsteiniana</i>	Volta Grande, PR		X	S128517
<i>Aureliana wettsteiniana</i>	Volta Grande, PR		X	S128478
<i>Aureliana wettsteiniana</i>	Morretes, PR		X	BHCB10519
<i>Aureliana wettsteiniana</i>	Campo Grande, São Paulo, SP		X	WU0037952
<i>Aureliana</i> sp. nov.	Moji das Cruzes, SP	X		BHCB115939
<i>Capsicum hunzikerianum</i> Barboza & Bianch.	Conservation Area Boracéia, SP	X		JRS 04830
* <i>Discopodium penninervium</i> Hochst.	Tanzania	X		R. E. S. Tanner 3288
* <i>Nothoestrum longifolium</i> A.Gray	NA	X		BISH 431975
* <i>Nothoestrum latifolium</i> A.Gray	NA	X		H. St. John 26469
<i>Solanum trachytrichium</i> Bitter	Guarapuava, PR	X		JRS4239
* <i>Tubocapsicum anomalum</i> (Frach & Sav.) Makino	NA	X		Chen 231
<i>Vassobia breviflora</i> (Sendtn.) Hunz.	Road São Pedro do Sul – Santa Maria, RS	X		BHCB102084
* <i>Withania coagulans</i> (Stocks) Dunal	NA	X		Lester S.0678/69
* <i>Withania riebeckii</i> Schweinf. ex Balf.f.	NA	X		D'Arcy 17750
* <i>Withania somnifera</i> (L.) Dunal	NA	X		Lester S.0960

APPENDIX 2

Equivalence table: the first column refers to the names displayed in the phylogenetic tree. The following columns refer to the voucher information in Appendix 1.

Name in the phylogenetic tree	Taxon	Phylogenetic analysis	Collection site	Voucher
<i>Athenaea micrantha</i>	<i>Aureliana pogogena</i>	sp1	Road São José – Una, BA	BHCB126140
<i>Athenaea pogogena</i>	<i>Aureliana pogogena</i>	sp2	Conservation Area Serra Bonita, Camacan, BA	BHCB126160
<i>Aureliana darcy</i>	<i>Aureliana fasciculata</i>		Trindade, Paraty, RJ	BHCB115994

APPENDIX 3

GenBank accession numbers corresponding to the DNA sequences employed in this study. The names refer to the terminals on the phylogenetic tree. The italicized numbers indicate the sequences previously published and available at GenBank. NA, sequences not available.

Taxon	GenBank Accession Number					
	<i>ndhF</i>	<i>trnL</i> intron	<i>trnL-trnF</i>	<i>psaI-accD</i>	<i>trnC-ycf6</i>	ITS
<i>Athenaea anonacea</i>	KC549601	KC549620	KC549639	KC509471	KC509447	KC832788
<i>Athenaea cuspidata</i>	KC549598	KC549616	KC549635	KC509467	KC509443	KC832784
<i>Athenaea martiana</i>	KC549600	KC549619	KC549638	KC509470	KC509446	KC832787
<i>Athenaea micrantha</i>	KC549595	KC549612	KC549631	KC509463	KC509439	KC832780
<i>Athenaea picta</i>	KC549602	KC549621	KC549640	KC509472	KC509448	KC832789
<i>Athenaea pogogena</i>	KC549603	KC549622	KC549641	KC509473	KC509449	KC832790
<i>Aureliana angustifolia</i>	KC549597	KC549614	KC549633	KC509465	KC509441	KC832782
<i>Aureliana brasiliiana</i>	NA	KC549615	KC549634	KC509466	KC509442	KC832783
<i>Aureliana darcy</i>	EU580858	KC549617	KC549636	KC509468	KC509444	KC832785
<i>Aureliana fasciculata</i>	KC549599	KC549618	KC549637	KC509469	KC509445	KC832786
<i>Aureliana</i> sp. nov.	KC549611	KC549630	KC549649	KC509483	KC509462	KC832798
<i>Aureliana sellowiana</i>	KC549596	KC549613	KC549632	KC509464	KC509440	KC832781
<i>Aureliana tomentosa</i>	KC549604	KC549623	KC549642	NA	KC509450	KC832791
<i>Aureliana velutina</i>	KC549605	KC549624	KC549643	KC509474	KC509451	KC832792
<i>Aureliana wettsteiniana</i>	KC549606	KC549625	KC549644	KC509475	KC509452	KC832793
<i>Capsicum hunzikerianum</i>	KC549610	KC549629	KC549648	KC509482	KC509461	NA
<i>Discopodium penninervium</i>	EU126012	EU580986	EU580986	KC509476	KC509453	KC832794
<i>Nothocestrum longifolium</i>	EU580922	EU581038	EU581038	KC509477	KC509454	KC832795
<i>Nothocestrum latifolium</i>	EU580921	EU581037	EU581037	KC509478	KC509455	KC832796
<i>Solanum trachytrichium</i>	KC549609	KC549628	KC549647	NA	KC509460	NA
<i>Tubocapsicum anomalum</i>	EU580950	EU581066	EU581066	NA	KC509456	DQ314163
<i>Vassobia breviflora</i>	KC549608	KC549627	KC549646	KC509481	KC509459	DQ314190
<i>Withania coagulans</i>	EU580951	EU581068	EU581068	NA	NA	NA
<i>Withania riebeckii</i>	KC549607	KC549626	KC549645	KC509480	KC509458	NA
<i>Withania somnifera</i>	EU580952	EU581069	EU581069	KC509479	KC509457	KC832797