Monsanima tinguaensis (Apocynaceae), an enigmatic new species from Atlantic rainforest

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Abstract

A new species of Asclepiadoideae (Apocynaceae) with enigmatic taxonomic position was found in the Atlantic rainforest of Rio de Janeiro State, southeastern Brazil. To ascertain its affinities within the Asclepiadeae, we performed phylogenetic analyses using the plastid regions \textit{trnT-L} and \textit{rps16}. Our results strongly support the new species close to the Orthosiinae, but do not support any generic relationship for this species. Based on the floral morphology, especially the corona lobes, which are highly fused, and curved inward at the top, covering the gynostegium, the new species is assigned to \textit{Monsanima}. This genus is sister to the other Orthosiinae and included so far only one species narrowly endemic to the \textit{campos rupestres} of Bahia, in northeastern Brazil. The new species, \textit{Monsanima tinguaensis}, occurs in a protected area, Tinguá Biological Reserve. However, it is known only by the type specimen and is probably vulnerable to extinction.

Introduction

The Asclepiadoideae (Apocynaceae) are widely known because of their complex floral morphology, characterized by a remarkable synorganization (e.g., Endress 1996). The traditional taxonomy of the subfamily until the 1990s, based on few floral characters, was pragmatic but essentially artificial (e.g., Rapini 2002). Morphological convergences are common in the subfamily and relationships are often difficult to derive based exclusively on morphology. Therefore, the use of phylogenetic analyses—especially based on molecular data (e.g., Liede & Täuber 2000, 2002, Rapini et al. 2003, 2006, Liede-Schumann et al. 2005, 2014, Silva et al. 2012, Liede-Schumann & Meve 2013)—has profoundly changed the systematics of neotropical asclepiads.

The use of molecular phylogenetics, mainly based on plastid regions, clarified several relationships among neotropical Asclepiadoideae which had been missed based on morphology (Rapini 2012). This is clear when current subtribal classification of the subfamily (Endress et al. 2014) is taken into account. For instance, the American genera \textit{Funastrum} Fournier (1882: 388), \textit{Pentacyphus} Schlechter (1906: 605) and \textit{Philibertia} Kunth in Humboldt et al. (1819: 195), once considered synonyms of \textit{Sarcostemma} Brown (1810) (Holm 1950), were shown to belong to different subtribes: \textit{Funastrum} and \textit{Philibertia} to Oxypetalinae, and \textit{Pentacyphus} to Pentacyphinae; while the palaeotropical \textit{Sarcostemma} belongs to Cynanchinae (Liede & Täuber 2000, Endress et al. 2014). Another example is the segregation of the genera of Malme’s (1927) Orthosiaceae: \textit{Peplonia} Descaine (1844: 545), \textit{Gonioanthela} Malme (1927: 6) and \textit{Macroditassa} Malme (1927: 9)—three genera currently treated under \textit{Peplonia}—were shown to belong to Metastelmatinae (Rapini et al. 2004, Silva et al. 2012), while the other two genera of the former ‘Orthosiaceae’, \textit{Orthosia} Decaisne (1844: 526) and \textit{Jobinia} Fournier (1885: 327), are closer to \textit{Scyphostelma} Baillon (1890: 252), recently reinstated by Liede-Schumann & Meve (2013), and the recently proposed monotypic \textit{Monsanima} Liede-Schumann & Meve (2013: 66) in Orthosiinae (Liede-Schumann et al. 2005, Liede-Schumann & Meve 2013). Furthermore, most of the neotropical species of \textit{Cynanchum} Linnaeus (1753: 212) were shown to belong to Orthosiinae rather than to Cynanchinae (Liede-Schumann et al. 2005) and were recently transferred to \textit{Jobinia}, \textit{Scyphostelma} or \textit{Monsanima} (Liede-Schumann & Meve 2013).
The position of species in a classification intended to reflect phylogenetic relationships is sometimes difficult to establish and some species of Asclepiadoideae have been placed in unrelated genera, often in different subtribes. For instance, *Minaria volubilis* Rapini & U.C.S.Silva in Silva et al. (2012: 803) was originally described in *Melinia Decaisne* (1844: 588) (= *Philibertia*), a genus of Oxypetalinae, whereas *Minaria* T.U.P.Konno & Rapini in Konno et al. (2006: 424), a genus segregated from *Ditassa* Brown (1810: 38), belongs to Metastelmatinae (Silva et al. 2012). *Dipolepis australis* (Malme 1937: 2) Hechem & C.Ezcurra in Hechem et al. (2011: 643) was originally described in *Metastelma* Brown (1810: 41), a genus of Metastelmatinae, and later transferred to *Tweedia* Hooker & Arnott (1834: 291), a genus of Oxypetalinae, whereas *Dipolepis* Brown (1810: 30) belongs to its own subtribe Dipolepinae (Endress et al. 2014), not closely related to Metastelmatinae nor Oxypetalinae (Liede-Schumann et al. 2005, Rapini et al. 2007; Hechem et al. 2011). More recently, some species of *Metastelma* (Metastelmatinae) were shown to belong to Tassadinaceae and Gonolobinaceae, while several others remained unresolved in Metastelmatinae, with still uncertain generic relationships (Liede-Schumann et al. 2014).

Establishing phylogenetic relationships is particularly difficult for monospecific genera, such as *Kerbera* Fournier (1885: 290) (probably synonym of *Jobinia* in Orthosiinae; Liede-Schumann, personal observation) and *Schistonema Schlechter* (1906: 604), both currently treated under Oxypetalinae (Rapini et al. 2011), and *Hypolobus* Fournier (1885: 311), whose position in Oxypetalinae (Liede 1997) or Metastelmatinae (Fontella-Pereira & Konno 1999) was under debate until recently (but see Endress et al. 2014). In such cases, the singular morphology makes ordinary classification uncertain. Likewise, new species whose relationships cannot be confidently established based on morphological characters—either because they have no clear affinity with any known species or because they could be assigned to unrelated taxa—can benefit from phylogenetic analysis, which may guide authors in generic or subtribal placement.

During the preparation of Rio de Janeiro’s Flora, a new species of Asclepiadoideae (Apocynaceae) with enigmatic taxonomic position was discovered in Serra do Mar, a mountain range parallel to the Atlantic coast, which extends from southern to southeastern Brazil and across the entire Rio de Janeiro State. The region is occupied by the Atlantic Forest domain, one of the most important biodiversity hotspots in the world (Myers et al. 2000), sheltering almost 20,000 species of plants in Brazil, approximately 40% of which are endemic (Forzza et al. 2012). Currently, there is only about 10% of the original Atlantic Forest remaining, which is distributed in small (< 50 ha) isolated patches, and Serra do Mar is the best preserved region, with approximately 30% of its rainforest still preserved (Ribeiro et al. 2009). The plant was collected in a protected remnant of preserved Atlantic Forest in the Rio de Janeiro metropolitan area.

The new species is a vine with a corona composed of sigmoid lobes fused almost to the top, covering the gynostegium, and pollinaria with pendent pollinia, which is a characteristic of the tribe Asclepiadeae. Because it is neither an Asclepias nor a Cynanchum, it almost certainly belongs to the MOOG clade, which is the oldest and largest Asclepiadoideae lineage in the New World (Rapini et al. 2007), encompassing about 780 species. It includes the subtribes Metastelmatinae, Oxypetalinae, Orthosiinae and Gonolobinae, besides the genera *Pentacyphus*, *Dipolepis* and *Tassadia Decaisne* (1844: 579), all of them currently treated as monogeneric subtribes (Endress et al. 2014). Coronas similar to the one found in the new species appear in unrelated species with the slender habit of the MOOG clade, such as *Peplonia asteria* (Vellozo 1829: t. 75) Fontella-Pereira & Schwartz (1983: 410) (for illustration, see Rapini et al. 2004) and *Ditassa thymifolia* (Schlechter 1916: 2) Liede & Meve (2013) (for illustration, see Liede-Schumann & Meve 2013), in Metastelmatinae, and *Monsanima morrenioides* (Goyder 1993: 21) Liede & Meve (2013: 66) (for illustration, see Goyder 1993) and *Jobinia grandis* (Handel-Mazzetti 1910: 4) Goes & Fontella-Pereira (2007: 12) (for illustration, see Fontella-Pereira & Schwarz 1982, as “Jobinia lutzii” Fontella-Pereira & Schwarz 1982), in Orthosiinae. However, corona morphology can vary greatly in Asclepiadoideae and should not be used alone to define generic relationships (e.g., Rapini 2002, Silva et al. 2012). Since we could not conclusively identify any species or group of species to which the new one is closely related, a phylogenetic analysis was conducted to reveal its relationships and facilitate its description in an appropriate genus.

**Material and Methods**

Trn*T-F (trn*T-L and trn*L-F intergenic spacers and trn*L intron) and rps16 (intron) sequences of the new Asclepiadoideae species were obtained (GenBank Accession numbers: *trn*T-L = KJ566592; *trn*L-F-trn*L: KJ566593; rps16 = KJ566594) as described in Silva et al. (2012) and aligned in a matrix with sequences of 58 species from previous studies (Rapini et al. 2003, Liede-Schumann et al. 2005, Hechem et al. 2011, Liede-Schumann & Meve 2013), representing all subtribes of neotropical Asclepiadeae. These regions were used to establish relationships within MOOG clade (Liede-
Schumann et al. 2005), and provided the main reference for subtribal circumscription of neotropical Asclepiadoideae (Endress et al. 2014). Two species of Asclepias Linnaeus (1753: 214) (Asclepiadaceae) and two of Cynanchum (Cynanchinae) were used as outgroup. The ingroup consisted of 10 species of Orthosiinae, sampling the four genera of the subtribe (4/4), 18 species of Metastelmatinae (9 sampled /12 accepted genera), 11 of Oxypetalinae (5/7 or 8), four of Gonolobinae (4/15), besides five species of Diplolepis (Diplolepinae), two of Tassadia (Tassadiinae), and two of Pentacyphus (Pentacyphinae).

We performed a maximum parsimony analysis in Paup v. 4 (Swofford 2002): a heuristic search from 1,000 replicates, starting by random stepwise addition, followed by TBR branch-swapping, saving no more than 10 trees per replicate. Trees in memory were then subjected to another round of TBR branch-swapping saving no more than 180,000 trees. Characters were treated as unordered and with equal weight. Branch bootstrap support (Bst) was calculated from 1,000 pseudo-replicates with random stepwise addition and TBR branch-swapping, retaining up to 20 trees per replicate. The Bayesian inference (BI) analysis was conducted in MrBayes (Ronquist & Huelsenbeck, 2003), with the four partitions (trnT-L, trnL, trnL-F and rps6) unlinked and under GTR+G substitution model, which was assessed as the best-fitting model for each individual partition in MrModeltest 2.3 (Nylander, 2008). Two simultaneous independent runs with four chains each (one cold and three heated) were used for the BI analysis. Chains were run for 3 million generations, starting with a random tree and saving a tree every 1,000 generations. Trees generated prior to convergence, at which point average standard deviation of split frequencies (ASDSF) reached ≤ 0.01, were excluded (burnin); the remaining trees were used to build a majority rule consensus in PAUP to provide branches posterior probabilities (PP).

Results

The matrix included 2834 characters (ca. 68% trnT-F; 32% rps16), 327 variable and 252 parsimony-informative. The parsimony analyses reached the limit of 180,000 trees with 833 steps, consistency index = 0.7971 (0.6472, when uninformative characters are excluded) and retention index = 0.8351. The Bayesian inference analysis converged at 864 generations; the remaining 2136 trees of each run were then used (4272 trees) to calculate the Bayesian consensus tree. As expected, our phylogenetic results are similar to those presented in Liede-Schumann et al. (2005). The seven subtribes (sensu Endress et al. 2014) of MOOG clade emerged in the strict consensus, most of them well supported: Pentacyphinae (PP = 100%; Bst = 100%), Diplolepinae (92%; 60%), Orthosiinae, including the new species (99%; 60%), Tassadiinae (100%; 100%), Oxypetalinae (100%; 81%) and Metastelmatinae (100%; 100%). In the strict consensus, the new species is unresolved in Orthosinae (Fig. 1), whereas in the Bayesian tree, it appears as sister to the rest of Orthosiinae, but this relationship is not well supported (Fig. 2).

Discussion

The new species should be treated in Orthosiinae. However, its relationship within the subtribe allows two generic classifications. If the new species forms a grade with Monsanima, either as sister group of the rest of Orthosiinae or as sister to the Orthosiinae core group (including Orthosia, Jobinia and Scyphostelma), it must be treated as a new genus. In this case, the corona folded inward over the gynostegium is probably a synapomorphy of Orthosiinae. Alternatively, the new species can also be sister to the monospecific Monsanima and the corona lobes fused in a tube inward atop the gynostegium can be a synapomorphy of the genus. In this case, it must be treated as the second species of Monsanima and points out a disjunction between the campos rupestres (rocky fields) of Chapada Diamantina in Bahia State, northeastern Brazil, to where M. morrenioides is endemic, and the Atlantic rainforest of Serra do Mar in Rio de Janeiro State, where the new species was discovered.

The new species bears the main diagnostic characters of Monsanima: it is a slender twining plant, with extra-axillary inflorescences shorter than leaves, flowers with corona lobes highly fused, folded inward over the gynostegium, anthers bearing wings shorter than dorsum, and pollinaria with short, broad caudicles. Although our results do not resolve the new species as sister to M. morrenioides, they also do not support an alternative relationship. Based on the present data, therefore, it seems plausible to describe the new species under Monsanima, rather than create a second monospecific genus in Orthosiinae.
FIGURE 1. MOOG tree generated from the analysis of *trnT-F* and *rps16* combined dataset (outgroup omitted) showing clades with ≥ 50% maximum parsimony bootstrap support (Bst). Numbers above branches indicate posterior probabilities (− = absent in Bayesian tree); numbers below branches indicate Bst. Dashed clade is present in the strict consensus of 180,000 most parsimonious trees but without Bst; arrows indicate clades absent in the strict consensus. The subtribal classification (*sensu* Endress et al. 2014) is mapped onto this topology: PEN = Pentacyphinae; TAS = Tassadiinae.
FIGURE 2. MOOG phylogram (ACCTRAN optimization) based on the majority rule consensus tree derived from the Bayesian analysis of trnT-F and rps16 combined dataset (outgroup omitted). The subtribal classification (sensu Endress et al. 2014) is mapped onto this topology: DIP = Diplolepinae; GON = Gonolobinae; MET = Metastelmatinae; ORT = Orthosiinae; OXY = Oxypetalinae; PEN = Pentacyphinae; TAS = Tassadiinae. Asterisks indicate branches with posterior probability < 90%.
**Taxonomic treatment**

*Monsanima tinguaensis* R.Santos & Fontella, *sp. nov.* (Fig. 3).

*Monsanima tinguaensis* is similar to *M. morrenioides* (Goyder) Liede & Meve because of the slender habit, extra-axillary inflorescences shorter than leaves, corolla with flattened lobes, almost entirely connate, free only at the apex, where they arch over the gynostegium, and pollinarium with short, flattened, broader than long, hyaline caudicles. However, it differs by the longer petiole (> 1 cm vs. < 0.3 cm long), larger (> 7.5 × 3 cm vs. < 3.5 × 0.5 cm), elliptic to oblong (vs. linear to narrowly lanceolate) leaf blade, corymbiform (vs. umbelliform) inflorescences, larger (> 10 mm vs. < 7 mm long), rotate (vs. campanulate) corolla, anthers with (vs. without) anther appendages, and longer pollinia (> 0.65 mm vs. ca. 0.5 mm long).


Slender vine; branches hirsute. Leaves with 3 or 4 collers at midrib base; petiole 1.3–2.9 cm long, hirsute; leaf blade 7.6–8.3 × 3.4–4 cm, elliptic to oblong, apex acuminate to mucronate, base truncate to slightly cordate, hirsute on both surfaces, especially on midrib. Inflorescences extra-axillary, corymbiform, 3–5 flowered, shorter than the leaves; peduncle ca. 7 mm long, densely hirsute. Flowers with pedicel 1.2–1.4 cm long, densely hirsute; sepals 6–7 × 0.8–1.5 cm, surpassing the corolla tube, narrowly triangular, hirsute on the abaxial surface, 1 collector in sinuses; corolla rotate, white with vinose stripes, tube 2–3 mm long, glabrous, lobes 8–12 × 4.5–6.5 mm, ovate, acuminate at the apex, glabrous; corona lobes 5–6 × 3–3.5 mm, slightly sigmoid, fluted, almost entirely connate, free only at the apex, longer than anthers, strongly incurvate over gynostegium. Gynostegium ca. 2 mm long, sessile; anthers 1.8–2 × ca. 2.2 mm, subtriangular, wings extended in a protuberant appendix over the dorsum, anther appendages ca. 0.5 mm long, ovate to slightly rhombical; retinaculum 0.47–0.49 × 0.09–0.15 mm, oblong, caudicles 0.13–0.14 mm long, horizontal, broader than long, flattened, hyaline, pollinia 0.66–0.68 × 0.23–0.26 mm, suboblong; style-head slightly mammillate. Fruits unknown.

**Distribution and habitat:**—*Monsanima tinguaensis* is known only from the type specimen, collected at the Pico do Tinguá (Tinguá Peak), in the Tinguá Biological Reserve. The Reserve was created in 1989 and is a UNESCO Biosphere Reserve since 1991 (IBAMA 2006). It is located between 22º28′–22º40′S and 43º10′–43º35′W, encompassing four municipalities (Nova Iguaçu, Duque de Caxias, Petrópolis and Miguel Pereira), and about 26,000 ha (IBAMA 2006). Most of the reserve (ca. 90%) is located in Serra do Mar and is dominated by mountain rainforest. The Maciço do Tinguá (Tinguá Massif) is the highest spot in the region, reaching over 1,600 m at the “Topo da Pedra do Tinguá” (Top of Tinguá Rock) (IBAMA 2006). At these high altitudes, beyond forest limits and at rock scrapes, an open vegetation called *campos de altitude* usually appears (Instituto de Pesquisas Jardim Botânico do Rio de Janeiro, 2002). The narrow distribution of *M. tinguaensis*, restricted to high altitudes in Serra do Mar, reassembles the distribution of *M. morrenioides*, which is known only from Pico das Almas in Chapada Diamantina, Bahia State. This disjunction suggests that the first lineage(s) to diverge in the evolution of Orthosiinae are currently restricted to small relics of preserved vegetation in mountain refuges.

**Conservation status:**—Rio de Janeiro has been floristically explored since the first naturalists visited Brazil in the 18’s century and collecting a new species in this State, especially close to metropolitan areas, seems to be unlikely. However, it is a megadiverse State and shelters an exceptionally high number of species with narrow distribution (< 10,000 km²), mainly in the Atlantic rainforest of Serra do Mar (Rapini et al. 2009). This circumstance suggests that *Monsanima tinguaensis* is a rare species, whose distribution is naturally narrow, currently likely restricted to only one of the few preserved areas of Atlantic rainforest in Rio de Janeiro. Known from a single location only, the new species is probably threatened like its congeneric *M. morrenioides*, which is Critically Endangered (Rapini et al. 2013). Nevertheless, it occurs in a reserve, with more than 20,000 km² of protected vegetation and should be classified as vulnerable according to the IUCN (2001) criterion D2.

In spite of the widespread destruction of Atlantic Forest, only 1% of its original area is under protection (Ribeiro et al. 2009). The Tinguá Biological Reserve is an exception in this long history of environmental degradation: it is in a metropolitan area in Rio de Janeiro and still shelters some of the most preserved forests in southeastern Brazil. Such a high level of conservation is due to one of the first acts of nature protection in Brazil, dating back to 1833. At that time, Dom Pedro II, the country’s Emperor, declared an area comprising three farms in the slope of Tinguá Massif as “Floresta Protetora” (Protective Forest; Instituto de Pesquisas Jardim Botânico do Rio de Janeiro 2002). Since the region presents a high number of rare species, other new relict species are expected to be found in the reserve.

**Etymology:**—The specific epithet “tinguaensis” refers to the locality where the plant was collected. In Tupi-Guarani, a Brazilian indigenous language, “Tinguá” means “the shape of a nose” in reference of the scarp appearance.
FIGURE 3. Monsanima tinguensis (from the holotype). A. branch with flowers; B. inflorescence; C. flower with a corolla lobe removed to show the corona; D. flower with a corolla lobe and part of corona removed showing the gynostegium; E. individual corona lobe dissected, lateral view; F. gynostegium; G. anther; H. pollinaria.
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References


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**APPENDIX.** GenBank accession numbers of sequences included in this study. *Species name: trnT-L, trnL/trnL-F, rps16.*