



Monsanima tinguagensis (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 *trnT-L* and *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 *Monsanima*. This genus is sister to the other Orthosiinae and included so far only one species narrowly endemic to the *campos rupestres* of Bahia, in northeastern Brazil. The new species, *Monsanima tinguagensis*, 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 *Funastrum* Fournier (1882: 388), *Pentacyphus* Schlechter (1906: 605) and *Philibertia* Kunth in Humboldt *et al.* (1819: 195), once considered synonyms of *Sarcostemma* Brown (1810) (Holm 1950), were shown to belong to different subtribes: *Funastrum* and *Philibertia* to Oxypetalinae, and *Pentacyphus* to Pentacyphinae; while the palaeotropical *Sarcostemma* belongs to Cynanchinae (Liede & Täuber 2000, Endress *et al.* 2014). Another example is the segregation of the genera of Malme's (1927) Orthosieae: *Peplonia* Descaine (1844: 545), *Gonioanthea* Malme (1927: 6) and *Macroditassa* Malme (1927: 9)—three genera currently treated under *Peplonia*—were shown to belong to Metastelmatinae (Rapini *et al.* 2004, Silva *et al.* 2012), while the other two genera of the former 'Orthosieae', *Orthosia* Decaisne (1844: 526) and *Jobinia* Fournier (1885: 327), are closer to *Scyphostelma* Baillon (1890: 252), recently reinstated by Liede-Schumann & Meve (2013), and the recently proposed monotypic *Monsanima* Liede-Schumann & Meve (2013: 66) in Orthosiinae (Liede-Schumann *et al.* 2005, Liede-Schumann & Meve 2013). Furthermore, most of the neotropical species of *Cynanchum* Linnaeus (1753: 212) were shown to belong to Orthosiinae rather than to Cynanchinae (Liede-Schumann *et al.* 2005) and were recently transferred to *Jobinia*, *Scyphostelma* or *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). *Diplolepis 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 *Diplolepis* Brown (1810: 30) belongs to its own subtribe Diplolepiniae (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 Tassadinae and Gonolobinae, 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 Gonolobinae (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*, *Diplolepis* 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

TrnT-F (*trnT-L* and *trnL-F* intergenic spacers and *trnL* intron) and *rps16* (intron) sequences of the new Asclepiadoideae species were obtained (GenBank Accession numbers: *trnT-L* = KJ566592; *trnL-F-trnL*: 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) (Asclepiadinaceae) 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%), Gonolobinae (100%; 100%), Oxypetalinae (100%; 81%) and Metastelmatinae (100%; 100%). In the strict consensus, the new species is unresolved in Orthosiinae (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.

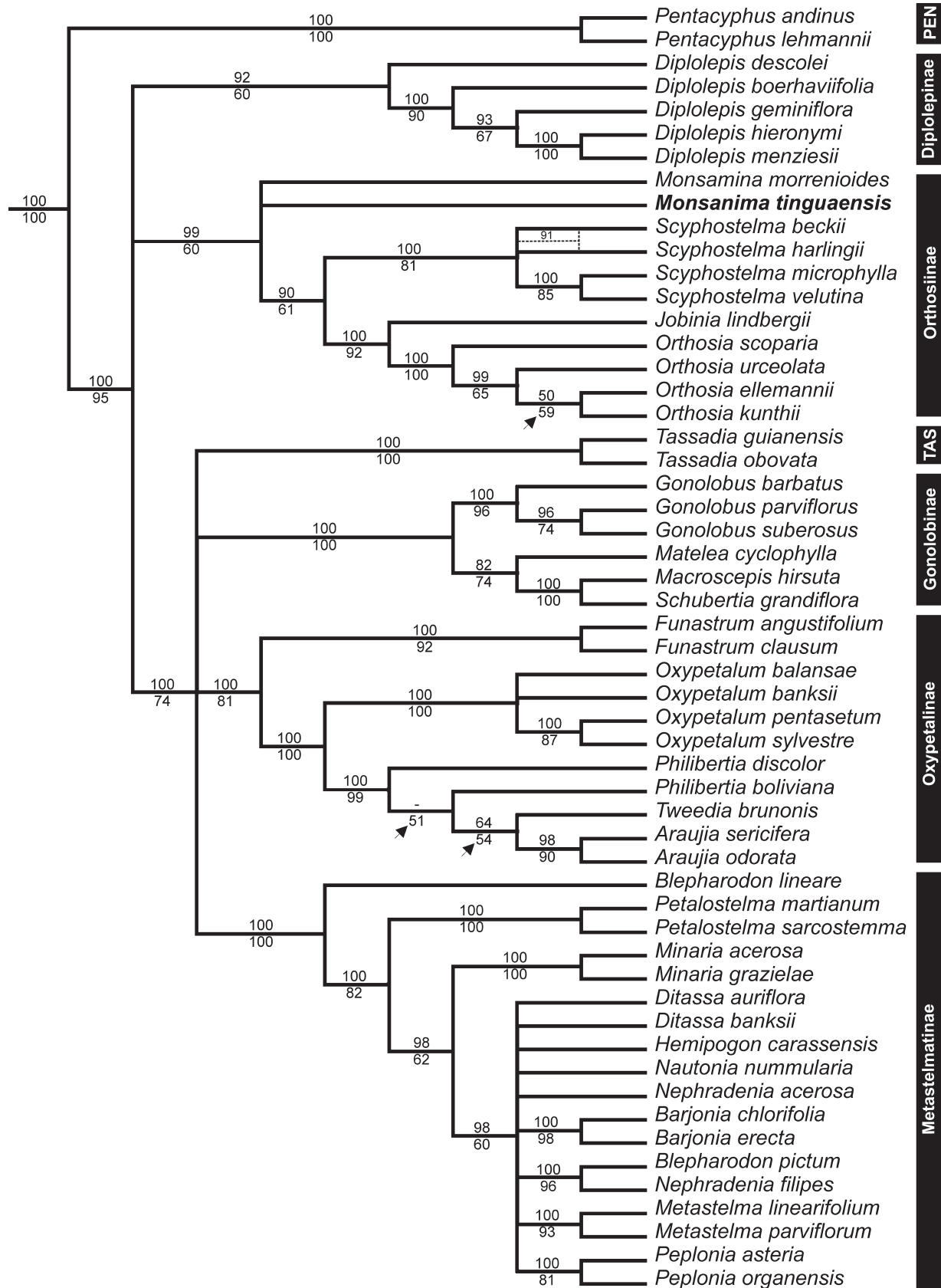


FIGURE 1. MOOG tree generated from the analysis of *trnT-F* and *rps16* combined dataset (outgroup omitted) showing clades with $\geq 50\%$ maximum parsimony bootstrap support (Bst). Numbers above branches indicate posterior probabilities (- = absent in Bayesian tree); numbers below branches indicate 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.

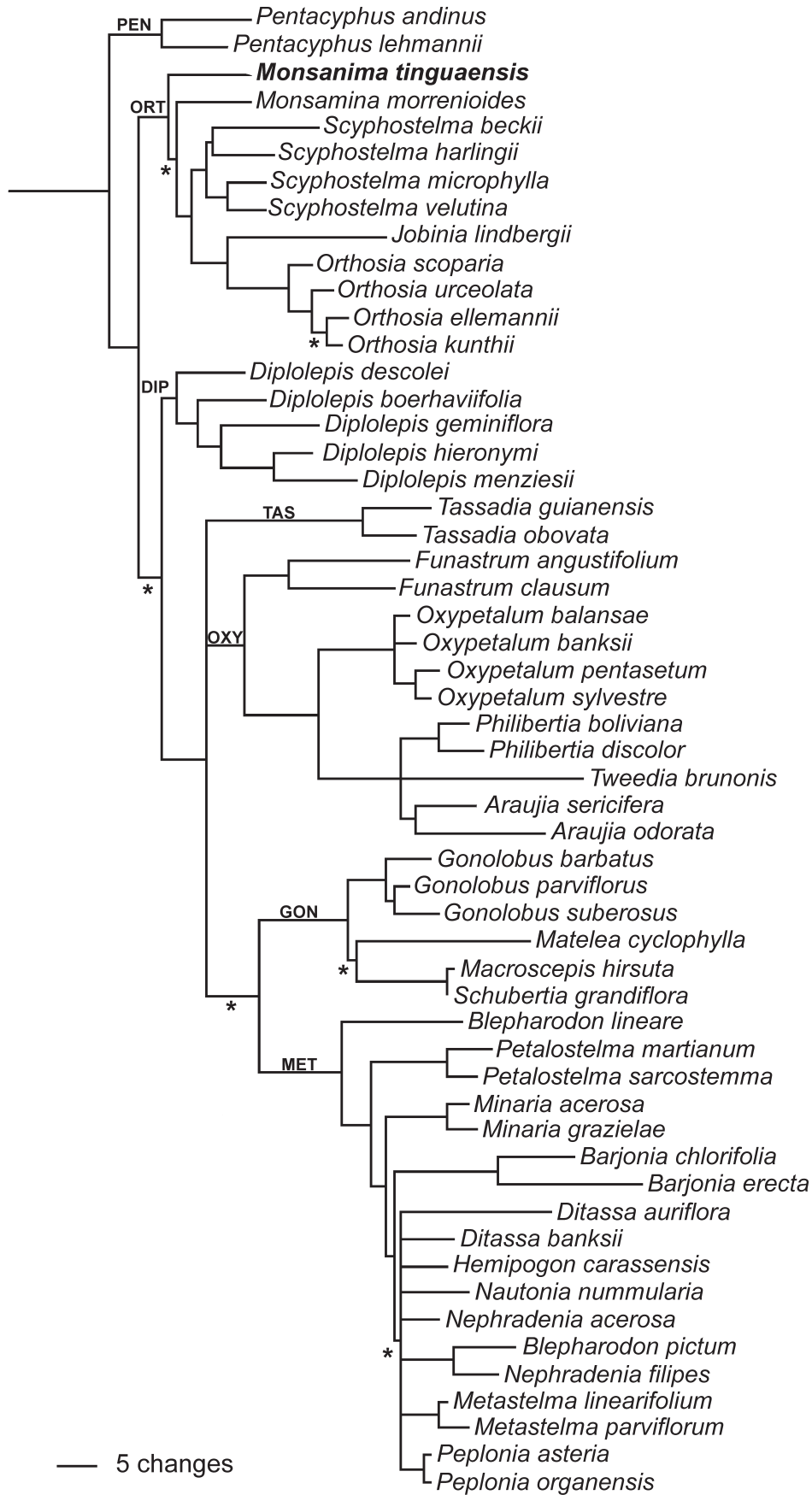


FIGURE 2. MOOG phylogram (ACCTRAN optimization) based on the majority rule consensus tree derived from the Bayesian analysis of *trnT-F* and *rps16* combined dataset (outgroup omitted). The subtribal classification (*sensu* Endress *et al.* 2014) is mapped onto this topology: DIP = Diplolepineae; GON = Gonolobinae; MET = Metastelmatinae; ORT = Orthosiinae; OXY = Oxypetalinae; PEN = Pentacyphinae; TAS = Tassadiinae. Asterisks indicate branches with posterior probability < 90%.

Taxonomic treatment

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

Monsanima tinguagensis is similar to *M. morrenioides* (Goyder) Liede & Meve because of the slender habit, extra-axillary inflorescences shorter than leaves, corona with fluted lobes, almost entirely connate, free only at the apex, where they arch over the gynostegium, and pollinaria with short, flattened, broader than long, hyaline caudicles. However, it differs by the longer petiole (> 1 cm vs. < 0.3 cm long), larger ($> 7.5 \times 3$ cm vs. $< 3.5 \times 0.5$ 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).

Type:—BRAZIL. Rio de Janeiro, Nova Iguaçu, “REBIO [Reserva Biológica] do Tinguá, trilha para o Pico, entre a primeira pedra e o pico”, 30 January 2002 (fl.), *J. Silva Neto et al. 1654* (holotype RB!).

Slender vine; branches hirsute. Leaves with 3 or 4 colleters at midrib base; petiole 1.3–2.9 cm long, hirsute; leaf blade 7.6–8.3 \times 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 \times 0.8–1.5 mm, surpassing the corolla tube, narrowly triangular, hirsute on the abaxial surface, 1 colleter in sinuses; corolla rotate, white with vinose stripes, tube 2–3 mm long, glabrous, lobes 8–12 \times 4.5–6.5 mm, ovate, acuminate at the apex, glabrous; corona lobes 5–6 \times 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 \times 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 \times 0.09–0.15 mm, oblong, caudicles 0.13–0.14 mm long, horizontal, broader than long, flattened, hyaline, pollinia 0.66–0.68 \times 0.23–0.26 mm, suboblong; style-head slightly mammillate. Fruits unknown.

Distribution and habitat:—*Monsanima tinguagensis* 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 22°28'–22°40'S and 43°10'–43°35'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. tinguagensis*, 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 relicts of preserved vegetation in mountain refuges.

Conservation status:—Rio de Janeiro has being 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 ($\leq 10,000$ km²), mainly in the Atlantic rainforest of Serra do Mar (Rapini *et al.* 2009). This circumstance suggests that *Monsanima tinguagensis* 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 ‘*tinguagensis*’ 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.

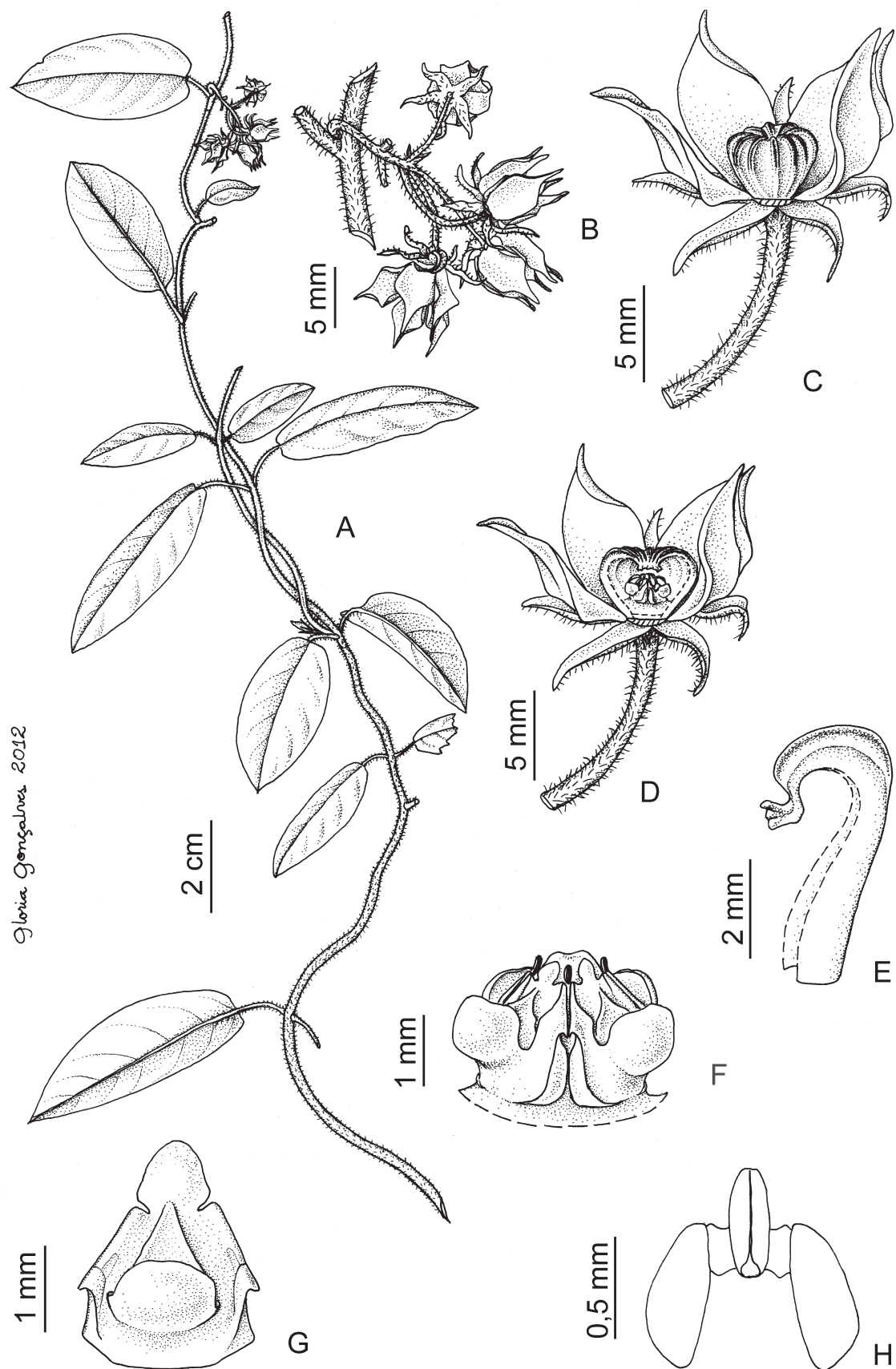


FIGURE 3. *Monsanima tinguensis* (from the holotype). A. branch with flowers; B. inflorescence; C. flower with a corolla lobe removed to show the corona; D. flower with a corolla lobe and part of corona removed showing the gynostegium; E. individual corona lobe dissected, lateral view; F. gynostegium; G. anther; H. pollinaria.

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References

- Baillon, H.E. (1890) *Histoire des plantes, vol. 19*. Librairie Hachette & Co., Paris, 476 pp.
- Brown, R. (1810) On the Asclepiadeae, a natural order of plants separated from the Apocineae of Jussieu. London. Preprinted from *Memoirs of the Wernerian Natural History Society* 1: 12–78 (1811).
- Decaisne, J. (1844) Asclepiadaceae. In: Candolle, A.L.P.P. de (Ed.), *Prodromus systematis naturalis regni vegetabilis, vol. 8*. Treuttel & Würtz, Paris, pp. 490–665.
- Endress, M.E., Liede-Schumann, S. & Meve, U. (2014) An updated classification for Apocynaceae. *Phytotaxa* 159: 175–194. <http://dx.doi.org/10.11646/phytotaxa.159.3.2>
- Endress, P.K. (1996) *Diversity and evolutionary biology of tropical flowers (Cambridge Tropical Biology Series)*. Cambridge University Press, Cambridge, UK, 311 pp.
- Fontella-Pereira, J. & Konno, T.U.P. (1999) Contribuição ao estudo das Asclepiadaceae brasileiras, XXXI. *Hypolobus* E. Fourn., um gênero extinto? *Bradea* 8(25): 139–143.
- Fontella-Pereira, J. & Schwarz, E.A. (1982) Estudo em Asclepiadaceae, XV. Chave para as espécies do gênero *Jobinia* Fournier ocorrentes no Brasil. *Boletim do Museu Botânico Municipal* 51: 1–18.
- Fontella-Pereira, J. & Schwarz, E.A. (1983) Estudos em Asclepiadaceae, XXVII. Novas combinações e novos sinônimos. *Bradea* 3(46): 410–412.
- Forzza, R.C., Baumgratz, J.F.A., Bicudo, C.E.M., Canhos, D.A.L., Carvalho Jr., A.A., Coelho, M.A.N., Costa, A.F., Costa, D.P., Hopkins, M.G., Leitman, P.M., Lohmann, L.G., Lughadha, E.N., Maia, L.C., Martinelli, G., Menezes, M., Morim, M.P., Peixoto, A.L., Pirani, J.R., Prado, J., Queiroz, L.P., Souza, S., Souza, V.C., Stehmann, J.R., Sylvestre, L.S., Walter, B.M.T. & Zappi, D.C. (2012) New Brazilian floristic list highlights conservation challenges. *BioScience* 62: 39–45. <http://dx.doi.org/10.1525/bio.2012.62.1.8>
- Fournier, E.P.N. (1882) Sur les Asclépiadées Américaines. *Annales des Sciences Naturelles; Botanique, série 6*, 14: 364–389.
- Fournier, E.P.N. (1885) Asclepiadaceae. In: Martius, C.F.P. & Eichler, A.W. (Eds.), *Flora brasiliensis, vol. 6, part 4*. Lindauer, München, pp. 189–332, t. 50–98.
- Goes, M.B. & Fontella-Pereira, J. (2007) Uma nova combinação e uma nova espécie de *Jobinia* E. Fourn. (Asclepiadoideae-Apocynaceae) no Sudeste do Brasil. *Bradea* 7(1): 11–15
- Goyder, D.J. (1993) Two new species of Asclepiadaceae from NE Brazil. *Kew Bulletin* 48: 21–24. <http://dx.doi.org/10.2307/4115743>
- Handel-Mazzetti, H.R.E. (1910, advance separate 1931) Asclepiadaceae und Apocynaceae. Denkschriften der Kaiserlichen Akademie der Wissenschaften, Wien. *Mathematisch-naturwissenschaftliche Klasse* 79(2): 377–388.
- Hechem, V., Calviño, C.I. & Ezcurra, C. (2011) Molecular phylogeny of *Diplolepis* (Apocynaceae-Asclepiadoideae) and allied genera, and taxonomic implications. *Taxon* 60: 638–648
- Holm, R.W. (1950) The American species of *Sarcostemma* R. Br. (Asclepiadaceae). *Annals of the Missouri Botanical Garden* 37: 477–560. <http://dx.doi.org/10.2307/2394402>
- Hooker, W.J. & Arnott, G.A.W. (1834) Contributions towards a flora of South America and the islands of the Pacific. 1. Extra-tropical South America. *Journal of Botany, Being a Second Series of the Botanical Miscellany* 1: 276–296
- Humboldt, F.W.H.A., Bonpland, A.J.A. & Kunth, C.S. (1819) *Nova genera et species plantarum (quarto editio)*, vol. 3, part 11. Librairie Grecque-Latine-Allemande, Paris, pp. 193–288.
- IBAMA – Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (2006) *Plano de Manejo – Reserva Biológica do Tingá*. Ministério do Meio Ambiente, Brasília. Available from: http://www.icmbio.gov.br/portal/images/stories/imgs-unidades-coservacao/rebio_tingua.pdf (accessed: 5 March 2014).

- Instituto de Pesquisas Jardim Botânico do Rio de Janeiro (2002) *Paisagem e Flora da Reserva Biológica do Tinguá: subsídios ao monitoramento da vegetação*. Relatório Técnico-Científico, Rio de Janeiro.
- IUCN (2001) *IUCN Red List Categories and Criteria*. Version 3.1. IUCN Species Survival Commission, Gland & Cambridge, UK.
- Konno, T.U.P., Rapini, A., Goyder, D.J. & Chase, M.W. (2006) The new genus *Minaria* (Apocynaceae). *Taxon* 55: 421–430.
<http://dx.doi.org/10.2307/25065588>
- Liede, S. (1997) Subtribes and genera of the tribe Asclepiadeae (Apocynaceae, Asclepiadoideae) – a synopsis. *Taxon* 46: 233–247.
<http://dx.doi.org/10.2307/1224093>
- Liede, S. & Täuber, A. (2000) *Sarcostemma* R. Br. (Apocynaceae–Asclepiadoideae). A controversial generic circumscription reconsidered: evidence from *trnL-trnF* spacers. *Plant Systematics and Evolution* 225: 133–140.
<http://dx.doi.org/10.1007/BF00985463>
- Liede, S. & Täuber, A. (2002) Circumscription of the genus *Cynanchum* (Apocynaceae–Asclepiadoideae). *Systematic Botany* 27: 789–801.
- Liede-Schumann, S. & Meve, U. (2013) The Orthosiinae revisited (Apocynaceae, Asclepiadoideae, Asclepiadeae). *Annals of the Missouri Botanical Garden* 99: 44–81.
<http://dx.doi.org/10.3417/2010130>
- Liede-Schumann, S., Nikolaus, M., Silva, U.C.S., Rapini, A., Mangelsdorff, R.D. & Meve, U. (2014) Phylogenetics and biogeography of the genus *Metastelma* (Apocynaceae–Asclepiadoideae–Asclepiadeae: Metastelmatinae). *Systematic Botany* 39: 594–612.
<http://dx.doi.org/10.1600/036364414X680708>
- Liede-Schumann, S., Rapini, A., Goyder, D.J. & Chase, M.W. (2005) Phylogenetics of the New World subtribes of Asclepiadeae (Apocynaceae—Asclepiadoideae): Metastelmatinae, Oxypetalinae, and Gonolobinae. *Systematic Botany* 30: 184–195.
<http://dx.doi.org/10.1600/0363644053661832>
- Linnaeus, C. (1753) *Species plantarum, vol. I*. Impensis Laurentii Salvii, Holmiae, 560 pp.
- Malme, G.O.A. (1927) Asclepiadaceae dusenianae in Paraná collectae. *Arkiv för Botanik utgivet av Kongliga Svenska Vetenskapsakademien* 21A(3): 1–48.
- Malme, G.O.A. (1937) Einige Beiträge zur Kenntnis südamerikanischer Asklepiadazeen. *Arkiv för Botanik utgivet av Kongliga Svenska Vetenskapsakademien* 29A(4): 1–9.
- Myers, N., Mittermeier, R.A., Mittermeier, C.G. Fonseca, G.A.B. & Kent, J. (2000) Biodiversity hotspots for conservation priorities. *Nature* 403: 853–858.
<http://dx.doi.org/10.1038/35002501>
- Nylander, J.A. (2008) MrModeltest v. 2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University, Upsala.
- Rapini, A. (2002) Six new species of *Ditassa* R. Br. from the Espinhaço Range, Brazil, with notes on generic delimitation in Metastelmatinae (Apocynaceae – Asclepiadoideae). *Kew Bulletin* 57: 533–546.
<http://dx.doi.org/10.2307/4110986>
- Rapini, A. (2012) Taxonomy “under construction”: advances in the systematics of Apocynaceae, with emphasis on the Brazilian Asclepiadoideae. *Rodriguésia* 63: 75–88.
<http://dx.doi.org/10.1590/S2175-78602012000100007>
- Rapini, A., Andrade, M.J.G., Giulietti, A.M., Queiroz, L.P. & Silva, J.M.C. (2009). Introdução. In Giulietti, A.M., Rapini, A., Andrade, M.J.G., Queiroz, L.P. & Silva, J.M.C. (orgs.) *Plantas raras do Brasil*. Conservação Internacional, Belo Horizonte, 23–35.
- Rapini, A., Berg, C. & Liede-Schumann, S. (2007) Diversification of Asclepiadoideae (Apocynaceae) in the New World. *Annals of the Missouri Botanical Garden* 94: 407–422.
[http://dx.doi.org/10.3417/0026-6493\(2007\)94\[407:DOAAIT\]2.0.CO;2](http://dx.doi.org/10.3417/0026-6493(2007)94[407:DOAAIT]2.0.CO;2)
- Rapini, A., Chase, M.W., Goyder, D.J. & Griffiths, J. (2003) Asclepiadeae classification: evaluating the phylogenetic relationships of New World Asclepiadoideae (Apocynaceae). *Taxon* 52: 33–50.
<http://dx.doi.org/10.2307/3647300>
- Rapini, A., Fontella-Pereira, J. & Goyder, D.J. (2011) Towards a stable generic circumscription in Oxypetalinae (Apocynaceae). *Phytotaxa* 26: 9–16.
- Rapini, A., Fontella-Pereira, J., Lamare, E. & Liede-Schumann, S. (2004) Taxonomy of *Peplonia* (including *Gonioanthele*) and a reinterpretation of Orthosieae (Asclepiadoideae, Apocynaceae). *Kew Bulletin* 59: 531–539.
<http://dx.doi.org/10.2307/4110907>
- Rapini, A., Koch, I., Valente, A.S.M., Fernandez, E.P., Monteiro, N.P. & Messina, T. (2013) Apocynaceae. In: Martinelli, G. & Moraes, M.A. (orgs.) *Livro Vermelho da Flora do Brasil*. Andrea Jakobsson e Instituto de Pesquisas Jardim Botânico do Rio de Janeiro, Rio de Janeiro, pp. 157–174.
- Rapini, A., Konno T.U.P. & Chase, M.W. (2006) Phylogenetics of South American Asclepiadoideae (Apocynaceae). *Taxon* 55: 119–124.
<http://dx.doi.org/10.2307/25065533>

- Ribeiro, M.C., Metzger, J.P., Martensen, A.C., Ponzoni, F.J. & Hirota, M.M. (2009) The Brazilian Atlantic Forest: How much is left, and how is the remaining forest distributed? Implications for conservation. *Biological Conservation* 142: 1141–1153.
<http://dx.doi.org/10.1016/j.biocon.2009.02.021>
- Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes 3. Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.
<http://dx.doi.org/10.1093/bioinformatics/btg180>
- Schlechter, R. (1906) Asclepiadaceae andinae. In: *Plantae novae andinae imprimis Weberbauerianae–II. Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* 37: 601–627.
- Schlechter, R. (1916) Asclepiadaceae andinae. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* 54 (Beibl. 119): 1–2.
- Silva, U.C.S., Rapini, A., Liede-Schumann, S., Ribeiro, P.L. & Berg, C. (2012) Taxonomic considerations on Metastelmatinae (Apocynaceae) based on plastid and nuclear DNA. *Systematic Botany* 37: 795–806.
<http://dx.doi.org/10.1600/036364412X648733>
- Swofford, D.L. (2002) PAUP*: Phylogenetic analysis using parsimony (and other methods), v. 4 beta 10. Sinauer Associates, Sunderland.
- Vellozo, J.M.C. (1829) *Florae Fluminensis, seu, Descriptionum plantarum parectura Fluminensi sponte mascentium liber primus ad systema sexuale concinnatus*. Typographia Naturali, Rio de Janeiro, 352 pp.
<http://dx.doi.org/10.5962/bhl.title.745>

Araujia sericifera Brot.: AJ428792, AJ428793/AJ428794, AJ699352. *Araujia odorata* (Hook. & Arn.) Fontella & Goyder: AJ704343, AJ704345/AJ704344, AJ704342. *Asclepias curassavica* L.: AJ704460, AY163664, AJ704459. *Asclepias syriaca* L.: AJ410178, AF214311/AJ410180, DQ660564. *Barjonia chloriifolia* Decne.: JN701896, AJ704467, AJ704463. *Barjonia erecta* (Vell.) K.Schum.: JN574691, JN574617, JN574478. *Blepharodon lineare* (Decne.) Decne.: AJ704465, AJ704468, AJ704466. *Blepharodon pictum* (Vahl) D.W.Stevens: AJ704468, AY163669, AJ704467. *Cynanchum montevidense* Spreng.: AJ290848, AJ290849/AJ290850, AJ699328. *Cynanchum roulinioides* (E.Fourn.) Rapini: AJ428732, AJ428733/ AJ428734, AJ704461. *Diplolepis boerhaviifolia* (Hook. & Arn.) Liede & Rapini: AJ428606, AJ428607/AJ428608, AJ699331. *Diplolepis descolei* (T.Mey.) Liede & Rapini: AJ699301, AJ699304/AJ699302, AJ699303. *Diplolepis geminiflora* (Decne.) Liede & Rapini: AJ410181, AJ410182/AJ410183, AJ699332. *Diplolepis hieronymi* (Lorentz) Liede & Rapini: AJ410211, AJ410212/AJ410213, AJ699333. *Diplolepis menziesii* Schult.: AJ699274, AJ699273/AJ699275, AJ699276. *Ditassa auriflora* Rapini: AJ704469, AJ704470/ AJ704471, AJ704472. *Ditassa banksii* R.Br. ex Schult.: AJ704474, AY163674, AJ704473. *Funastrum angustifolium* (Pers.) Liede & Meve: AJ428759, AJ428760/AJ428761, AJ699353. *Funastrum clausum* (Jacq.) Schult.: AJ290860, AJ290861/AJ290862, AJ699355. *Gonolobus barbatus* Kunth: AJ704262, AJ704261/AJ704263, AJ704264. *Gonolobus suberosus* (L) R.Br.: AJ704275, AJ704277/AJ704276, AJ704274. *Gonolobus parviflorus* Decne.: AJ704528, AY163689, AJ704529. *Hemipogon carassensis* (Malme) Rapini: JN574706, JN574635, JN574496. *Jobinia lindbergii* E.Fourn.: AJ704491, AY163694, AJ704490. *Macroscopus hirsuta* (Vahl) Schltr.: AJ704266, AJ704268/AJ704267, AJ704265. *Matelea cyclophylla* (Standl.) Woodson: AJ704271, AJ704269/AJ704272, AJ704273. *Metastelma linearifolium* A.Rich.: AJ428807, AJ428808/AJ428809, AJ699341. *Metastelma parviflorum* R.Br.: AJ428777, AJ428778/AJ428779, AJ699342. *Minaria acerosa* (Mart.) T.U.P.Konno & Rapini: AJ699286, AJ699287/AJ699288, AJ699285. *Minaria grazielae* (Fontella & Marquete) T.U.P.Konno & Rapini: AJ410202, AJ410203/AJ410204, AJ699339. *Monsanima morrenioides* (Goyder) Liede & Meve: AJ428684, AJ428685/AJ428686, AJ699348. *Monsanima tinguensis* R.Santos & Fontella: KJ566592, KJ566593, KJ566594. *Nautonia nummularia* Decne.: AJ410226, AJ410227/AJ410228, AJ699344. *Nephradenia acerosa* Decne.: AJ704497, AY163704/AY163705, AJ704496. *Nephradenia filipes* Malme: JN574718, JN574647, JN574508. *Orthosia ellemannii* (Morillo) Liede & Meve: AJ428780, AJ428781/AJ428782, AJ699350. *Orthosia kunthii* Decne.: AJ428783, AJ428784/AJ428785, AJ699351. *Orthosia scoparia* (Nutt.) Liede & Meve: HE611726, HE611780/HE611819, HE611862. *Orthosia urceolata* E.Fourn.: AJ704324, AJ704323/AJ704325, AJ704501. *Oxypetalum balansae* Malme: AJ704347, AJ704346/AJ704348, AJ704349. *Oxypetalum banksii* R.Br. ex Schult.: AJ704503, AY163710, AJ704502. *Oxypetalum pentasetum* (Rusby) Goyder & Rapini: AJ704253, AJ704252/ AJ704254, AJ704255. *Oxypetalum sylvestre* (Hook. & Arn.) Goyder & Rapini: AJ410244, AJ410245/AJ410246, AJ699363. *Peplonia asteria* (Vell.) Fontella & E.A.Schwarz: AJ704300, AJ704301/AJ704302, AJ704303. *Peplonia organensis* (Malme) Fontella & Rapini: AJ704487, AY163688, AJ704488. *Petalostelma martianum* E.Fourn.: JN574719, JN574648, JN574509. *Petalostelma sarcostemma* (Lillo) Liede & Meve: AJ428786, AJ428787/AJ428788, AJ699345. *Pentacyphus andinus* (Ball) Liede: AJ492149, AJ492150/AJ492151, AJ699335. *Pentacyphus lehmannii* (Schltr.) Liede: AJ290890, AJ290889/AJ290888, AJ704928. *Philibertia boliviana* (Baill.) Goyder: AJ704231, AJ704233/AJ704232, AJ704230. *Philibertia discolor* (Schltr.) Goyder: AJ704527, AY163700, AJ704526. *Schubertia grandiflora* Mart.: AJ428825, AJ428826/AJ428827, AJ699364. *Scyphostelma beckii* (Morillo) Liede & Meve: AJ704305, AJ704307/AJ704306, AJ704304. *Scyphostelma harlingii* (Morillo) Liede & Meve: AJ704309, AJ704308/ AJ704310, AJ704311. *Scyphostelma microphylla* (Kunth) Liede & Meve: AJ428681, AJ428682/AJ428683, AJ699347. *Scyphostelma velutina* (Morillo) Liede & Meve: HE611747, HE611796/HE611835, HE611879. *Tassadia guianensis* Decne.: AJ699278, AJ699280/AJ699279, AJ699277. *Tassadia obovata* Decne.: AJ699282, AJ699281/ AJ699283, AJ699284. *Tweedia brunonis* Hook. & Arn.: AJ704257, AJ704260/AJ704258, AJ704256.