Eric Yasuo Kataoka

Systematics of *Martinella* Baill. (Bignonieae, Bignoniaceae)

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Universidade de São Paulo
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Abstract

Martinella Baill. is a small genus of Neotropical lianas within tribe Bignonieae (Bignoniaceae). The genus is monophyletic, well supported by morphological and molecular characters. Members of *Martinella* have a continuous interpetiolar ridge surrounding the stem, bilobed or 4-5-parted calyces, and minute triangular prophylls of the axillary buds. The most recent taxonomic treatment of *Martinella* recognized three species: *Martinella insignis* A.H. Gentry ex Zuntini & L.G. Lohmann, endemic to the Atlantic Forest of eastern Brazil, *Martinella iquitoensis* A. Samp. [= *Martinella insculpta* Sprague & Sandwith], and *Martinella obovata* (Kunth) Bureau & K. Schum., the latter two are widely distributed species from southern Mexico to Bolivia. Generic circumscription remained unchanged since the description of *Martinella*, although species delimitation and phylogenetic relationships among species within the genus remained unclear or unknown. In this dissertation, I investigated phylogenetic relationships of *Martinella*, and conducted a taxonomic revision. The phylogenetic reconstruction was based on a hybrid approach that combined high throughput sequencing (HTS) with Sanger sequencing data to infer the phylogeny of *Martinella* based on broad sampling of characters and individuals. Three complete and three nearly-complete plastomes were sequenced, assembled, and annotated. In addition, sequences of the plastid markers *ndh*F and *rpl*32-*trn*L and the nuclear marker *pep*C were obtained for additional samples, covering the morphological diversity and geographic distribution of members of the genus. The tree that resulted from the analysis of the complete dataset (Sanger + HTS) is fully resolved, representing the most robust estimate of phylogenetic relationships of *Martinella* to date. This phylogeny identified five main clades that are recognized as five species in the taxonomic revision of the genus. These five species represent the three previously recognized species plus two new species, *Martinella lanuginosa* Kataoka & L.G. Lohmann and *Martinella tomentosa* Kataoka & L.G. Lohmann. The taxonomic revision of the genus presents detailed descriptions for all five taxa, a complete list of synonyms, distribution maps, illustrations, and indications of conservation status for all species recognized. This thesis highlights the importance of in-depth taxonomic studies of selected lineages, especially in megadiverse regions such as the Neotropics, where sampling lacunae still persist.

Keywords: Amazonia, genomics, neotropical region, phylogeny, taxonomy.

Resumo

Martinella Baill. é um gênero pequeno de lianas neotropicais pertencente à tribo Bignonieae (Bignoniaceae). O gênero é monofilético, bem sustentado por caracteres morfológicos e moleculares. Os membros de *Martinella* apresentam uma crista interpeciolar contínua em torno do caule, cálices bilobados ou 4-5-lobados, e profilos da gema axilar triangulares e reduzidos. O tratamento taxonômico mais recente de *Martinella* reconheceu três espécies: *Martinella insignis* A. H. Gentry ex Zuntini & L.G. Lohmann, endêmico da Mata Atlântica do leste do Brasil, *Martinella iquitoensis* A. Samp. [= *Martinella insculpta* Sprague & Sandwith], e *Martinella obovata* (Kunth) Bureau & K. Schum., as duas últimas são amplamente distribuídas do sul do México até a Bolívia. A circunscrição genérica permaneceu inalterada desde a descrição de *Martinella*, embora a delimitação das espécies e as relações filogenéticas dentro do gênero permanecessem pouco claros ou desconhecidos. Nesta dissertação, investiguei as relações filogenéticas de *Martinella* e realizei uma revisão taxonômica. A reconstrução filogenética foi baseada em uma abordagem mista que combinou dados de sequenciamento em larga escala (HTS) e dados de sequenciamento Sanger para inferir a filogenia de *Martinella* com base em uma ampla amostragem de caracteres e indivíduos. Três plastomas completos e três plastomas quase completos foram sequenciados, montados e anotados. Além disso, sequências dos marcadores plastidiais *ndh*F e *rpl*32-*trn*L e do marcador nuclear *pep*C foram obtidas para amostras adicionais, cobrindo a diversidade morfológica e a distribuição geográfica dos membros do gênero. A árvore resultante da análise da matriz de dados completa (Sanger + HTS) é totalmente resolvida, representando a estimativa mais robusta das relações filogenéticas de *Martinella* até o momento. Essa filogenia identificou cinco clados principais que são reconhecidos como cinco espécies na revisão taxonômica do gênero. Essas cinco espécies representam as três espécies previamente reconhecidas, e dois novos táxons, *Martinella lanuginosa* Kataoka & L.G. Lohmann e *Martinella tomentosa* Kataoka & L.G. Lohmann. A revisão taxonômica do gênero apresenta descrições detalhadas de todos os cinco táxons, uma lista completa de sinônimos, mapas de distribuição, ilustrações, e indicações do *status* de conservação de todas as espécies reconhecidas. Esta dissertação destaca a importância de estudos taxonômicos aprofundados de linhagens selecionadas, especialmente em regiões megadiversas como a região neotropical, onde lacunas de amostragem ainda persistem.

Palavras-chave: Amazônia, filogenia, genômica, região neotropical, taxonomia.

Contents

Thesis Outline 1

General Introduction 2

References 6

Chapter One – Integrating plastome and targeted loci data to reconstruct the phylogeny of *Martinella* Baill. (Bignonieae, Bignoniaceae) 8

Chapter Two – Taxonomic revision of *Martinella* Baill. (Bignonieae, Bignoniaceae) 33

Conclusions 83

Thesis Outline

This Master's thesis focuses on the systematics of *Martinella* Baill., a genus of Neotropical lianas from tribe Bignonieae (Bignoniaceae). My desire to study biodiversity comes from my fascination for the Neotropical biota, which motivated me to understand its diversity into more depth and to contribute important knowledge in alpha taxonomy. In this study, I investigated the phylogenetic relationships of *Martinella*, and conducted a taxonomic revision of the genus. The main findings of my research are presented in this thesis, which contains an introduction, two chapters, and conclusions. The two chapters correspond to manuscripts that were structured following the guidelines of the scientific journals in which we intend to publish those papers. Further details about these chapters are given below.

Chapter One focuses on the phylogeny of *Martinella*, which was reconstructed based on a combination of high throughput sequencing data (i.e., newly generated sequences of complete or nearly-complete chloroplast genomes), and Sanger sequences of two chloroplast markers (*ndh*F and *rpl*32-*trn*L), and one nuclear gene (*pep*C). Five different datasets were used to reconstruct the phylogeny of the genus under Maximum Likelihood and Bayesian criteria. These analyses led to the most robust phylogenetic tree of *Martinella* to date, which has direct implications for the taxonomy of the genus.

Chapter Two focuses on the taxonomic revision of *Martinella*, which was based on extensive fieldwork, a comprehensive analysis of herbarium specimens, and the phylogenetic information obtained in Chapter One. A general overview on the morphology, taxonomic history, geographic distribution, reproductive biology, economic and ethnobotanical uses is presented. This information is followed by a detailed evaluation of the individual species, including descriptions for all taxa, a full list of synonyms, distribution maps, illustrations, and general comments.

General Introduction

The Neotropical region stands out for its rich biodiversity, even when compared to tropical areas around the world (Antonelli & Sanmartín, 2011). This region includes most of Central and South America, extending from central Mexico to southern Brazil (Morrone, 2014). The Neotropics also comprise a diverse array of ecosystems, ranging from some of the most arid to those with the highest rainfall on the planet (Fiaschi et al., 2015). The striking Neotropical diversity has historically intrigued researchers, which makes studies involving organisms of this region very promising to address evolutionary and biogeographic questions (Hughes et al., 2013).

The Bignoniaceae includes 82 genera and approximately 860 species (Lohmann & Ulloa, 2006 onwards). The family is Pantropical but occurs predominantly in the Neotropics (Olmstead et al., 2009). Members of the Bignoniaceae are trees, shrubs, herbs or lianas that are easily recognized by the compound and opposite leaves, showy and tubular flowers with four didynamous stamens and one staminode, and capsules with winged seeds (Gentry, 1980). Eight major clades are currently recognized: *Tabebuia* alliance, Pantropical clade, and tribes Bignonieae, Catalpeae, Jacarandeae, Oroxyleae, Tecomeae, and Tourretieae (Olmstead et al., 2009). Of these, tribe Bignonieae Dumort. is the largest, encompassing 21 genera and ca. 393 species, nearly half of the known species in the family (Lohmann & Taylor, 2014).

Members of Bignonieae are lianas or shrubs that occur in wet forests of Central and South America, seasonally dry tropical forests and the South American *Cerrados* (Lohmann, 2006). This megadiverse clade is characterized by a stem anatomy with differential cambial activity producing discontinuous 4-32 phloem wedges (Gentry, 1980; Pace et al., 2011; Lohmann & Taylor, 2014). Moreover, members of Bignonieae are readily recognized by the compound and opposite leaves with the terminal leaflet generally modified into a tendril, and by a septicidal capsule (Gentry, 1980; Lohmann, 2006).

Historically, the generic classification of Bignonieae was very unstable (see Figure 1 in Lohmann & Taylor, 2014). This is attributed to the focus of previous classifications on highly variable and homoplastic reproductive characters, such as corolla shape and color, fruit shape and ornamentation (Gentry, 1973; Lohmann & Taylor, 2014). The indication that vegetative characters could be potentially more informative to the generic classification of Bignonieae is relatively recent (Gentry, 1980). This idea was corroborated by a broad phylogenetic study of the whole tribe that

identified certain vegetative characters as putative synapomorphies for many clades (Lohmann, 2006). Few Bignonieae genera remained with a constant circumscription over the last 200 years (see Lohmann & Taylor, 2014). *Martinella* Baill. is one of those few genera whose circumscription remained unchanged since its description by Baillon (1888) (see Table 1 in Lohmann & Taylor, 2014).

Members of *Martinella* are distributed throughout wet forests of Central America and north/northeastern South America (Zuntini & Lohmann, 2014). A continuous interpetiolar ridge on the stems and 2-4-parted calyces represent putative synapomorphies of the genus (Lohmann, 2006; Lohmann & Taylor, 2014). Additional characteristics include: (i) minute and triangular prophylls of the axillary buds, (ii) basal portion of the corolla tube narrower and slightly more elongated than the calyx, (iii) inflated and campanulate corollas, and (iv) hummingbird pollination (*Martinella*-type flower) (Gentry, 1974; Lohmann & Taylor, 2014).

The monophyly of *Martinella* was strongly supported by the molecular phylogeny of tribe Bignonieae based on sequences of two markers (*ndh*F and *pep*C) for the two species recognized at that time, i.e., *Martinella obovata* (Kunth) Bureau & K. Schum and *Martinella iquitoensis* A. Samp. [= *Martinella insculpta* Sprague & Sandwith] (Lohmann, 2006) (Figure 1). The most recent synopsis of the group recognized three lianescent species within the genus (Zuntini & Lohmann, 2014): *Martinella insignis* A.H. Gentry ex Zuntini & L.G. Lohmann, *M. iquitoensis* [= *M. insculpta*], and *M. obovata*. *Martinella insignis* is restricted to northern Atlantic Forest and is known from few records. This species inhabits sandy soils, has membranaceous leaflets, trifid tendrils, "pocket-like" domatia, 5-lobed calyces, and yellow corollas (Zuntini & Lohmann, 2014). *Martinella iquitoensis* [= *M. insculpta*] is widely distributed in the Amazon basin and has been reported to grow on sandy soils. This species has coriaceous leaflets, trifid tendrils, botryoid inflorescences and dark-purple corollas (MacBride, 1961; Zuntini & Lohmann, 2014). *Martinella obovata* is the most widely distributed species, occurring from Central America to southern Amazon, and growing on different soil types. This species is characterized by membranaceous to coriaceous leaflets, trifid tendrils, racemose inflorescence, and lilac/purple corollas (MacBride, 1961; Zuntini & Lohmann, 2014).

Despite the straightforward circumscription of the genus, the delimitation of the Amazonian *Martinella* spp., namely the morphologically diverse *M. iquitoensis* and *M. obovata*, has long been considered problematic. These taxa show overlapping character states such as corolla color, leaflet texture, and tendril type, complicating their correct identification (MacBride, 1961; Zuntini & Lohmann, 2014). Wide geographic distribution and phenotypic plasticity may contribute to such high morphological variation in both species, which led

previous researchers to suggest that these taxa may represent a species complex (Zuntini & Lohmann, 2014).

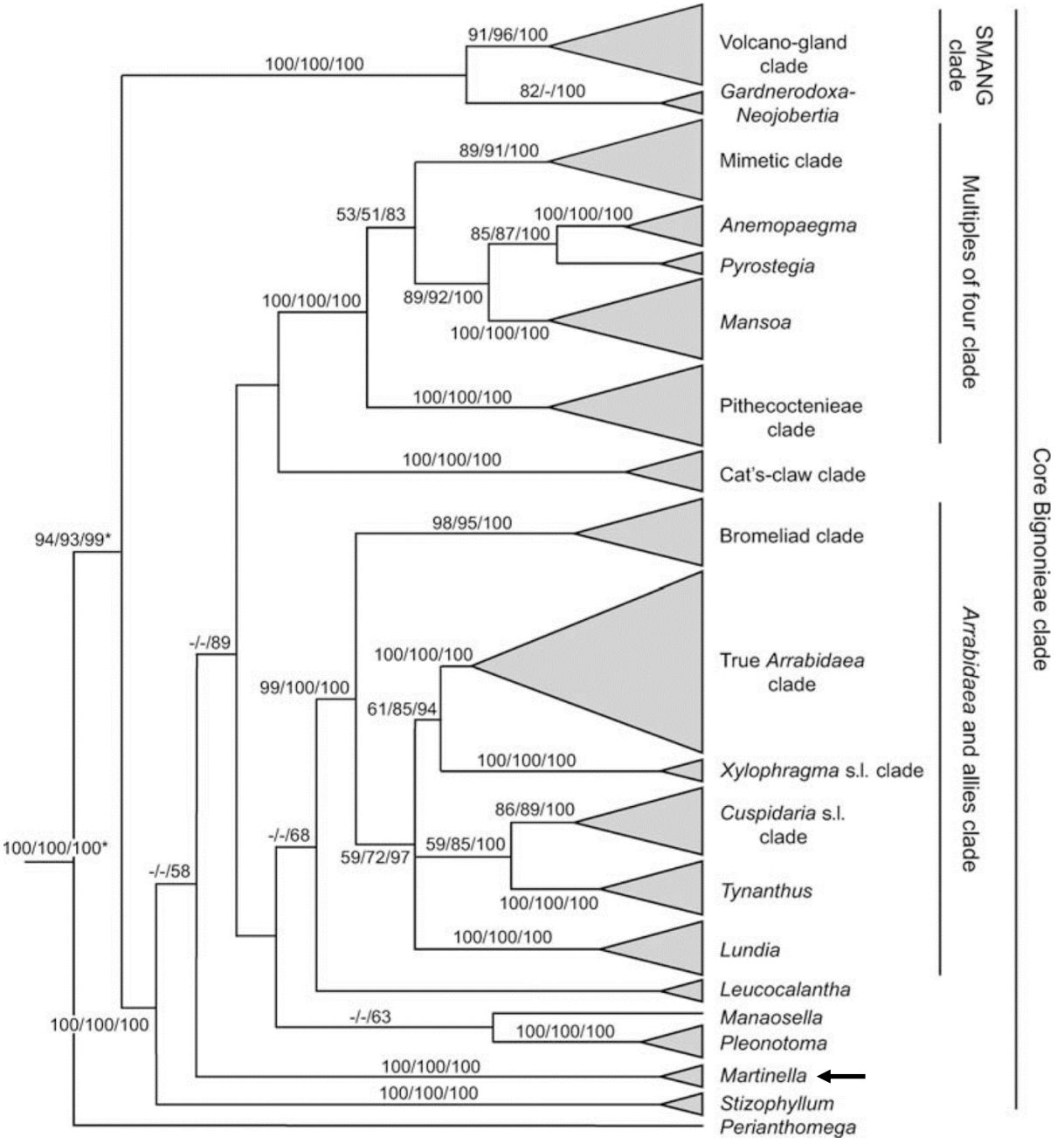

Figure 1. Phylogenetic relationships among the 19 species groups of tribe Bignonieae recovered by Lohmann (2006). The phylogenetic placement of *Martinella* is indicated by a black arrow. Values shown at nodes indicate, respectively, parsimony bootstrap, likelihood bootstrap and posterior probability.

The advent of high throughput sequencing (HTS) technologies is allowing us to rapidly obtain molecular data for a myriad of applications at relatively low costs. The nature of HTS is quantitative as it counts how many times each base has been sequenced (Straub et al., 2012), which then gives the empirical per-base coverage (or sequencing depth) of a given genome

(Sims et al., 2014). Another interesting feature of HTS is that many samples can be pooled together and sequenced in a single run (for a review of applications, see McCormack et al., 2013). Thus, in phylogenetic studies, one would no longer need to choose between sampling more taxa or more characters, as this technique allows us to maximize both. Consequently, the field of Plant Systematics is greatly benefiting from HTS technologies, especially using approaches such as genome skimming (e.g., Straub et al., 2012; Reginato et al., 2016), and target enrichment (e.g., Carlsen et al., 2018; Moore et al., 2018). These applications have recovered robust and highly supported phylogenetic hypotheses, allowing us to address fundamental questions in Plant Systematics.

Recent approaches successfully applied HTS-generated data in combination with data obtained using traditional Sanger sequencing for phylogeny reconstruction (e.g., Williams et al., 2016; Fonseca & Lohmann, 2018). This strategy is particularly interesting as it allows the integration of publicly available sequences of target genes with newly generated HTS data, maximizing character and taxon sampling, thus contributing to advance our understanding about the phylogeny of many plant groups.

This dissertation aimed to: (i) reconstruct phylogenetic relationships of *Martinella* using Sanger and HTS-generated data, and (ii) conduct a taxonomic revision of *Martinella*.

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Integrating plastome and targeted loci data to reconstruct the phylogeny of *Martinella* Baill. (Bignonieae, Bignoniaceae)

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Abstract

The rapid development of high throughput sequencing (HTS) technologies revolutionized the field of molecular systematics by allowing the generation of large amounts of DNA sequence data at relatively low costs. Large datasets often lead to robust phylogenetic hypotheses, with high branch support and resolution. In this study, we use a hybrid approach that combines HTS data with traditional targeted loci data to infer the phylogeny of *Martinella* Baill. (Bignonieae, Bignoniaceae), a group of Neotropical lianas. The genus traditionally includes three species, although two additional putative new species have been recently identified based on morphology. We sequenced, assembled, and annotated three complete and three nearly-complete plastomes representing the three species currently recognized plus one of the putative new taxa. In addition, we obtained sequences of the plastid *ndh*F and *rpl*32-*trn*L and the nuclear *pep*C markers via Sanger sequencing for 15 additional individuals of *Martinella*, covering the range of geographic distribution of all species in the genus. Two additional outgroups were sampled, leading to a final dataset with 23 individuals and multiple molecular markers. We used these data to assemble five different dataset combinations in order to evaluate tree topology and support values, as follows: (i) plastid dataset (23 individuals); (ii) nuclear dataset (19 individuals); (iii) plastid-nuclear dataset (23 individuals); (iv) plastome dataset (eight individuals); and (v) combined dataset (23 individuals). Targeted loci data recovered highly supported general relationships among species, but with low resolution, while HTS data recovered a fully resolved tree with maximum support in most branches. By combining HTS and Sanger sequencing data, we were able to maximize taxon and character sampling, which led to a fully resolved tree with moderate support. This tree is considered as the most robust and reliable estimate of the phylogeny of *Martinella* to date and is used as basis to evaluate its taxonomy. This phylogeny strongly supports the recognition of five taxa, including the previously recognized *M. obovata*, *M. iquitoensis* and *M. insignis*, plus two new Amazonian species. Our study highlights the importance of in-depth studies of individual lineages, especially in Amazonia, where important sampling lacunae still remain.

Keywords: Amazon, biodiversity, high throughput sequencing, lianas, Neotropical flora.

1. Introduction

In recent years, we have witnessed great advances in the acquisition and analysis of large molecular datasets due to the rapid development of high throughput sequencing (HTS) technologies (Lemmon and Lemmon, 2013; Wicke and Schneeweiss, 2015). These datasets have been used to reconstruct robust molecular phylogenies and to address key questions in plant systematics. The high number of chloroplasts per plant cell allowed sequencing of complete plastomes, which have rapidly become the molecule of choice for many phylogenomic studies (Straub et al., 2011; Straub et al., 2012). The widespread use of chloroplast genes for phylogeny reconstruction is also explained by its haploid nature and uniparental inheritance, allowing us to generate robust phylogenies at various taxonomic levels.

The HTS *status quo* contrasts with datasets traditionally amassed using Sanger sequencing. The latter method targets loci with 600 – 800 bp, meaning that datasets generated using Sanger technology are much smaller, apart from being more time consuming and proportionally more expensive than those generated with HTS (Wicke and Schneeweiss, 2015). Despite that, data generated via Sanger sequencing revolutionized our understanding of phylogenetic relationships in Angiosperms (Chase et al., 1993; Soltis et al., 2000). Roughly two decades of combined efforts of many research groups resulted in the accumulation of large amounts of molecular data that are publicly available. By integrating Sanger data with genome sequences obtained via HTS, we are able to maximize the utility of the data available in repositories and reconstruct robust phylogenies based on high character and taxon sampling (e.g., Uribe-Convers et al., 2017; Fonseca and Lohmann, 2018).

In-depth phylogenetic studies of selected plant clades have the potential to shed light on patterns and processes of evolution (Daly et al., 2012). When those studies are conducted in conjunction with morphological investigations, we are able to interpret novel morphological findings in light of new phylogenetic information (e.g., Pace et al., 2016; Frazão and Lohmann, 2018). These approaches allow for comparative and integrative analyses that enable us to address longstanding evolutionary questions about the origin and maintenance of biological diversity in mega diverse areas of the globe, such as the Neotropics (Antonelli et al., 2009).

Martinella Baill. is a small genus of Neotropical lianas in tribe Bignonieae. The monophyly of the genus is strongly supported by molecular data and morphological characters. Members of *Martinella* have showy tubular flowers (Fig. 1), and a continuous ridge at the interpetiolar region representing a putative synapomorphy (Fig. 1) (Lohmann, 2006; Lohmann and Taylor, 2014). Species of *Martinella* are widely distributed throughout the Neotropics, from

southern Mexico to the Amazon (Lohmann and Taylor, 2014). Three species are currently recognized in the genus, two of which are widely distributed in wet forests of Central and South America, while one species is restricted to the northern portion of the Brazilian Atlantic Forest (Zuntini and Lohmann, 2014). The widely distributed Amazonian species are known for their complicated taxonomy, owing to overlapping morphological characters (MacBride, 1961; Zuntini and Lohmann, 2014).

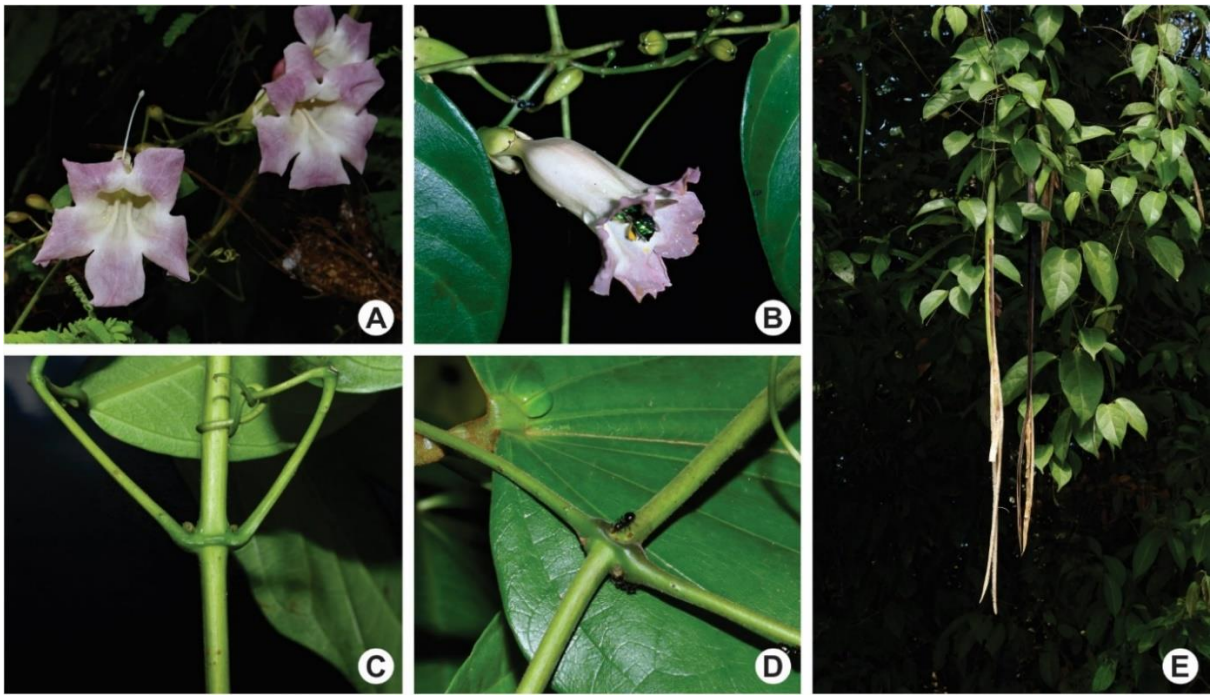

Figure 1. Morphology of *Martinella* Baill. **A-B** Flowers of *Martinella obovata* (Kunth) Bureau & K. Schum., in B, a flower being visited by an Euglosini bee. **C-D** Interpetiolar region with a continuous ring that surrounds the stem, a generic synapomorphy, in D, ants visiting patelliform glandular trichomes at the interpetiolar region. **E** Branches bearing fruits that can reach up to 1.5 m long. Photo **B** by G. Gerlach, all other photos by E. Kataoka.

In this study, we compiled a robust dataset with previously published and newly generated DNA sequence data using both Sanger sequencing and HTS approaches. We used this dataset to infer the phylogeny of *Martinella* based on a comprehensive sampling of molecular markers and taxa, covering the breadth of morphological variation and known geographic distribution of all species in the genus. The robust phylogenetic framework reconstructed was then used as basis to identify independently evolving lineages that are diagnosed by morphology, which served as basis to evaluate species limits within the genus.

2. Material and methods

2.1. Sampling

Our sampling scheme was designed to represent the breadth of morphological variation and geographic distribution of members of *Martinella*. Special attention was given to *Martinella obovata* (Kunth) Bureau & K. Schum., the most morphologically variable and broadly distributed taxon, long considered as a species complex (MacBride, 1961; Gentry, 2009; Zuntini and Lohmann, 2014). Overall, we sampled 23 specimens, representing 21 *Martinella* and two outgroups (Table 1). Individuals of *Martinella* sampled represent all three species currently recognized, i.e., *M. insignis* A.H. Gentry ex Zuntini & L.G. Lohmann, *M. iquitoensis* A. Samp., and *M. obovata* (Zuntini and Lohmann, 2014), plus two putative new species. For Sanger sequencing, we used the same two chloroplast markers (*ndh*F and *rpl32-trnL*) and one nuclear gene (*pepC*) that have been successfully used to infer phylogenetic relationships within Bignonieae (Lohmann, 2006; Kaehler et al., 2011; Fonseca and Lohmann, 2015; Medeiros and Lohmann, 2015). For the ingroup taxa, 15 accessions were sequenced using Sanger sequencing, while six specimens were sequenced using HTS. *Adenocalymma pedunculatum* (Vell.) L.G. Lohmann and *Anemopaegma arvense* (Vell.) Stellfeld ex J.F. de Souza were selected as outgroups based on the phylogenetic relationships known for the Bignonieae (Lohmann, 2006), and the availability of previously published plastomes (Firetti et al., 2017; Fonseca and Lohmann, 2018).

2.2. DNA extraction

Total genomic DNA was extracted from leaves, either from herbarium specimens or silica-dried samples collected in the field. We used the commercial genomic DNA purification kit Invisorb Spin Plant Mini Kit (Stratec Molecular, Berlin, Germany), following the manufacturer's instructions. We then performed gel electrophoresis by loading 2 μL in a 1% agarose gel, run at 80 V for approximately 60 min, and compared the resulting bands to a size standard 1 Kbp DNA ladder (Promega, Madison, USA). We quantified DNA concentration and purity in NanoDrop 2000 (ThermoFisher Scientific Inc., Wilmington, USA) or Qubit 2.0 (Life Technologies, California, USA).

2.3. Sanger sequencing: PCR conditions, cloning, sequencing, sequence editing, and alignment

PCR primers, annealing temperatures and elongation time follow Zuntini et al. (2013). A standard PCR program was implemented as follows: one initial step at 95°C for 5 min; 40 cycles

at 95°C for 30 s, 48–56°C for 30 s, 72°C for 30 s to 2 min, and a final step at 72°C for 5 min. The PCR program parameters varied depending on primers' annealing temperatures (Zuntini et al., 2013) and the Taq DNA polymerase used, in which case we followed the manufacturer's instructions for elongation time. Amplifications were conducted in 25 μL reactions. For the chloroplast markers, each reaction included 7.25 μL deionized water, 0.5 μL $MgCl_2$ (25mM), 1.25 μL dimethyl sulfoxide (DMSO) (Sigma-Aldrich, St. Louis, USA), 1.25 μL of each primer (10 μM), 12.5 μL KAPA2G Fast ReadyMix PCR Kit (KAPA Biosystems, Boston, USA), and 1 μL template DNA. Amplification of the nuclear marker used the same conditions, except for 12.5 μL GoTaq Green Master Mix (Promega, Madison, USA), and primers IV_119F and V_25R (Zuntini et al., 2013).

For the *pepC*, PCR products were purified with a glycogen precipitation protocol (Sambrook et al., 1989), and then ligated with pGEM-T Easy Vector System I (Promega, Madison, USA). We prepared competent *E. coli* cells (DH10B strain), which were transformed using the Ec3 electroporation program on a MicroPulser Electroporator (Biorad, California, USA). After overnight incubation, we selected positive colonies to perform colony PCR. For each sample, depending on the transformation success and colony growth, 10 to 20 transformant colonies were selected and transferred to a "back-up plate" using a sterile toothpick; cells were resuspended in 20 μL deionized water and boiled for 5 min in a thermocycler at 95 ºC. Subsequently, 1 μL of the boiled colonies were used as template for a PCR reaction using M13 universal primers in order to check for positive amplification of the expected 630 bp (Zuntini et al., 2013). These amplifications used an initial step of 95°C for 5 min; 30 cycles of 95°C for 45 s, 53°C for 1 min, 72°C for 90 s, and a final step of 72°C for 10 min. For these verification-only PCRs (25 μL final volume) we used the following reagents: 14.3 μL deionized water, 5 μL 5X Green GoTaq Flexi Buffer (Promega, Madison, USA), 2 μL $MgCl_2$ (25mM), 0.5 μL dNTPs PCR nucleotide Mix 10mM (Promega, Madison, USA), 1.0 μL of each primer (10 μM), 0.2 μL of "homemade" Taq DNA polymerase, and 1 μL template DNA. For each sample, up to four colonies with positive amplification of the expected fragment size were selected for sequencing. We performed gel electrophoresis after all PCRs. We loaded 2 μL of the PCR product on an 1% agarose gel, run at 80 V for approximately 60 min, and compared to a size standard 1 Kbp or 100 bp DNA ladder (Promega, Madison, USA). All PCR products were purified and sequenced at Macrogen Inc. (Seoul, South Korea).

Raw sequences of *ndh*F, *rpl*32-*trn*L and *pepC* were carefully analyzed and edited in Geneious 9.1.6 (Kearse et al., 2012). Only base calls with Phred quality score equal to or greater than 20 were accepted for contig assembly; bases that did not meet the threshold were coded as

'N'. Consensus sequences were then aligned using MAFFT 7 (Katoh and Standley, 2007) using default settings. The resulting alignments were visually inspected in Geneious 9.1.6 and poorly aligned regions were removed using GBlocks (Castresana, 2000) on the Castresana's Lab Server (http://molevol.cmima.csic.es/castresana/Gblocks_server.html) using all three options for a less stringent selection.

2.4. High throughput sequencing: Library preparation, plastome assembly, annotation and alignment

We performed genome skimming for six specimens of *Martinella* (Table 1). For library preparation, five micrograms (5 μg) of genomic DNA were fragmented using a Covaris S-series sonicator to obtain DNA fragments of approximately 300 bp. The sonicator parameters for 50 μL of sample were: 10% duty factor, 200 cycles per burst, 50 s treatment time and intensity five. Short-insert libraries were constructed using the NEBNext DNA Library Prep Master Mix Set and NEBNext multiplex oligos for Illumina (New England Biolabs Inc., Ipswich, USA). We verified expected size selection by agarose gel electrophoresis of library products against a size standard Promega 100 bp ladder (Promega Corporation, Madison, USA). We determined DNA library concentration on an Applied Biosystems 7500 Real-Time PCR System using the Kapa Library Quantification Kit (Kapa Biosystems Inc., Wilmington, USA) and/or using fluorometry on Qubit 2.0 (Life Technologies). The DNA library was pooled together with other 15 samples sequenced on an Illumina HiSeq 2000 system (paired-end) (Illumina Inc., San Diego, USA).

For plastome assembly we used the Fast-Plast pipeline (https://github.com/mrmckain/Fast-Plast; McKain and Wilson, unpublished). Illumina adaptors were removed, and low-quality reads were trimmed using Trimmomatic 0.35 (Bolger et al., 2014). Using Bowtie2 (Langmead and Salzberg, 2012), the trimmed reads were mapped against a custom database containing the plastomes published in Fonseca and Lohmann (2017) with default parameters. The mapped reads were then assembled *de novo* using SPAdes 3.1.0 (Bankevich et al., 2012) with k-mer sizes 55 and 87, with the only-assembler option. When longer contigs were not readily obtained, the pipeline used afin (https://github.com/mrmckain/Fast-Plast/tree/master/afin), an iterative approach to map more reads onto the contigs allowing their expansion. For all afin analyses, we used default parameters. Coverage analyses were performed using Jellyfish 2.1.3 (Marçais and Kingsford, 2011) using the estimate of 25-mer abundance. We established a coverage threshold of 20 X,

i.e., base pairs with coverage less than the threshold value were replaced by an N. The final contigs were visualized in Geneious 9.1.6.

Table 1. Taxa sampled for phylogenetic reconstruction of *Martinella* Baill. using high throughput sequencing (HTS) and Sanger sequencing data. Samples for HTS are in bold, the remaining samples were sequenced for targeted loci (*ndh*F, *rpl*32-*trn*L, *pep*C). ✓ and × indicate whether Sanger sequencing for each marker was successful or not, and GenBank accession codes are indicated in sequences published in previous studies.

Species	**Voucher**	**Locality**	**Markers**		
			*ndh*F	*rpl*32-*trn*L	*pep*C
M. insignis 1	**Zuntini, A. R. 151 (SPF)**	**Brazil, Espírito Santo, Linhares**	**HTS**	**HTS**	✓
M. insignis 2	Zuntini, A. R. 321 (SPF)	Brazil, Espírito Santo, Linhares	✓	✓	✓
M. iquitoensis 1	**Kataoka, E.Y. 339 (SPF)**	**Brasil, Amazonas, Manaus**	**HTS**	**HTS**	✓
M. iquitoensis 2	Kataoka, E.Y. 344 (SPF)	Brasil, Amazonas, Manaus	✓	✓	✓
M. iquitoensis 3	Kataoka, E.Y. 370 (SPF)	Brasil, Mato Grosso, Apiacás	✓	✓	×
M. iquitoensis 4	Kataoka, E.Y. 372 (SPF)	Brasil, Mato Grosso, Cotriguaçu	✓	✓	✓
M. iquitoensis 5	**Kataoka, E.Y. 404 (SPF)**	**Brasil, Acre, Bujari**	**HTS**	**HTS**	✓
M. iquitoensis 6	Kataoka, E.Y. 407 (SPF)	Brasil, Acre, Bujari	✓	✓	✓
M. obovata 1	Torke, B.M. 211 (MO)	French Guiana	✓	✓	✓
M. obovata 2	Giraldo, R. 181 (HUA)	Colombia, Antioquia, Mutatá	✓	✓	✓
M. obovata 3	Roldán, F.J. 2068 (HUA)	Colombia, Antioquia, Remedios	✓	✓	×
M. obovata 4	Kataoka, E.Y. 273 (SPF)	Brasil, Roraima, Caracaraí	✓	✓	✓
M. obovata 5	Vargas, L.D. 4556 (MO)	Costa Rica, Guanacaste, Liberia	✓	✓	✓
M. obovata 6	Kataoka, E.Y. 360 (SPF)	Brasil, Pará, Belém	✓	✓	✓
M. obovata 7	Kataoka, E.Y. 380 (SPF)	Brasil, Mato Grosso, Aripuanã	✓	✓	✓
M. obovata 8	**Kataoka, E.Y. 390 (SPF)**	**Brasil, Acre, Rio Branco**	**HTS**	**HTS**	✓
M. obovata 9	Kataoka, E.Y. 406 (SPF)	Brasil, Acre, Bujari	✓	✓	✓
M. obovata 10	**Gomes, B. M. 647 (SPF)**	**Brazil, Amazonas, Caracaraí**	**HTS**	**HTS**	✓
M. sp. nov 1	**Souza, M.A.D 39 (SPF)**	**Brazil, Amazonas, Manaus**	**HTS**	**HTS**	✓
M. sp. nov 2 – 1	Lohmann, L.G. 616 (MO)	Peru, Madre de Dios, Manu	✓	×	DQ222760
M. sp. nov 2 – 2	Ferreira, L. 109 (NY)	Brazil, Acre, Brasileia	✓	×	×
A. pedunculatum	Fonseca, L.H. 267 (SPF)	Brazil, Minas Gerais, Diamantina	MG008313	MG008313	MG831917
A. arvense	Firetti, F. 237 (SPF)	Brazil	MF460830	MF460830	×

Plastome annotation was conducted in Geneious 9.1.6 using plastomes of closely related species (Firetti et al., 2017; Fonseca and Lohmann, 2017) as references to transfer annotations with at least 75% similarity. The annotation of tRNAs was conducted in tRNA-Scan (Lowe and Eddy, 1997). Plastome gene maps were generated in OGDRAW (Lohse et al., 2007) using the online interface (https://chlorobox.mpimp-golm.mpg.de/OGDraw.html).

Plastome alignments were conducted in MAFFT 7 (Katoh and Standley, 2013) using default settings. Alignments were visually inspected in Geneious 9.1.6 and assessed on sequence variability. Poorly aligned regions of noncoding regions were removed using GBlocks (Castresana, 2000) on the Castresana's Lab Server

(http://molevol.cmima.csic.es/castresana/Gblocks_server.html) using all three options for a less stringent selection. We did not use GBlocks to remove poorly aligned regions of coding sequences because it altered reading frames, complicating subsequent partitioning analyses.

2.5. Phylogenetic analyses

For phylogeny reconstruction, we used the data generated with Sanger sequencing and HTS to construct five different datasets: (i) **plastid dataset** (23 individuals), including sequences of cpDNA markers obtained with Sanger (*ndh*F and *rpl*32-*trn*L) for 15 individuals plus sequences for these same markers obtained with HTS for six individuals; (ii) **nuclear dataset** (19 individuals), including sequences of the nuclear *pepC* obtained with Sanger sequencing; (iii) **plastid-nuclear dataset** (23 individuals), including the combined sequences of cpDNA markers (*ndh*F and *rpl*32-*trn*L) and the nuclear marker *pepC* obtained with Sanger sequencing (i.e., a combination of plastid and nuclear datasets); (iv) **plastome dataset** (eight individuals), including coding and noncoding sequences of the chloroplast genome obtained with HTS; and, (v) **combined dataset** (23 individuals), including the plastome plus plastid-nuclear datasets combined.

For the plastid dataset, we extracted the *ndh*F and *rpl*32-*trn*L sequences of plastomes and aligned those with all other sequences obtained via Sanger sequencing. For the genomic chloroplast datasets, we used the Large Single Copy (LSC), Small Single Copy (SSC) and only one of the Inverted Repeats (IR). From the LSC, SSC and IR, we separated coding sequences (CDS) and noncoding sequences (NCS) based on the annotated plastomes, using the rtracklayer and Biostrings packages of Bioconductor (https://www.bioconductor.org/) in R (R Development Core Team, 2018).

We selected models of molecular evolution in Partition Finder (Lanfear, 2012) using the corrected Akaike Information Criteria (AICc) and searching for models implemented in MrBayes. We concatenated alignments of each CDS and NCS. For the CDS we used 111 partitions as input, based on CDS/gene annotations, and one partition for all the NCS. PartitionFinder reduced the inputted 111 partitions to 32.

All phylogenetic analyses were performed with Maximum Likelihood (ML) using RaxML 8.2.9 (Stamatakis, 2014), and Bayesian Inference (BI) using MrBayes 3.2 (Ronquist et al., 2011) on CIPRES (Miller et al., 2010). For ML analyses, the GTRCAT model of evolution was used for all partitions. The ML node support was estimated using a rapid bootstrap analysis with 1,000 replicates. For BI, we used the evolutionary models selected in PartitionFinder. We

ran two independent runs, each with 10 million generations and four Markov chains, using default priors. Trees were sampled every 1,000 generations. Support values of BI were estimated using posterior probabilities. We considered that convergence and stationarity of the independent Markov chains was reached when the average standard deviation of split frequencies was < 0.01. Consensus trees were obtained after 25% of the trees had been discarded (burn-in).

We assessed the combinability of plastid and nuclear datasets using the Congruence Among Distance Matrices (CADM) (Campbell et al., 2011) implemented in R. To implement this test, we reduced the plastid dataset to the same number of terminals for which we had nuclear data (19 terminals).

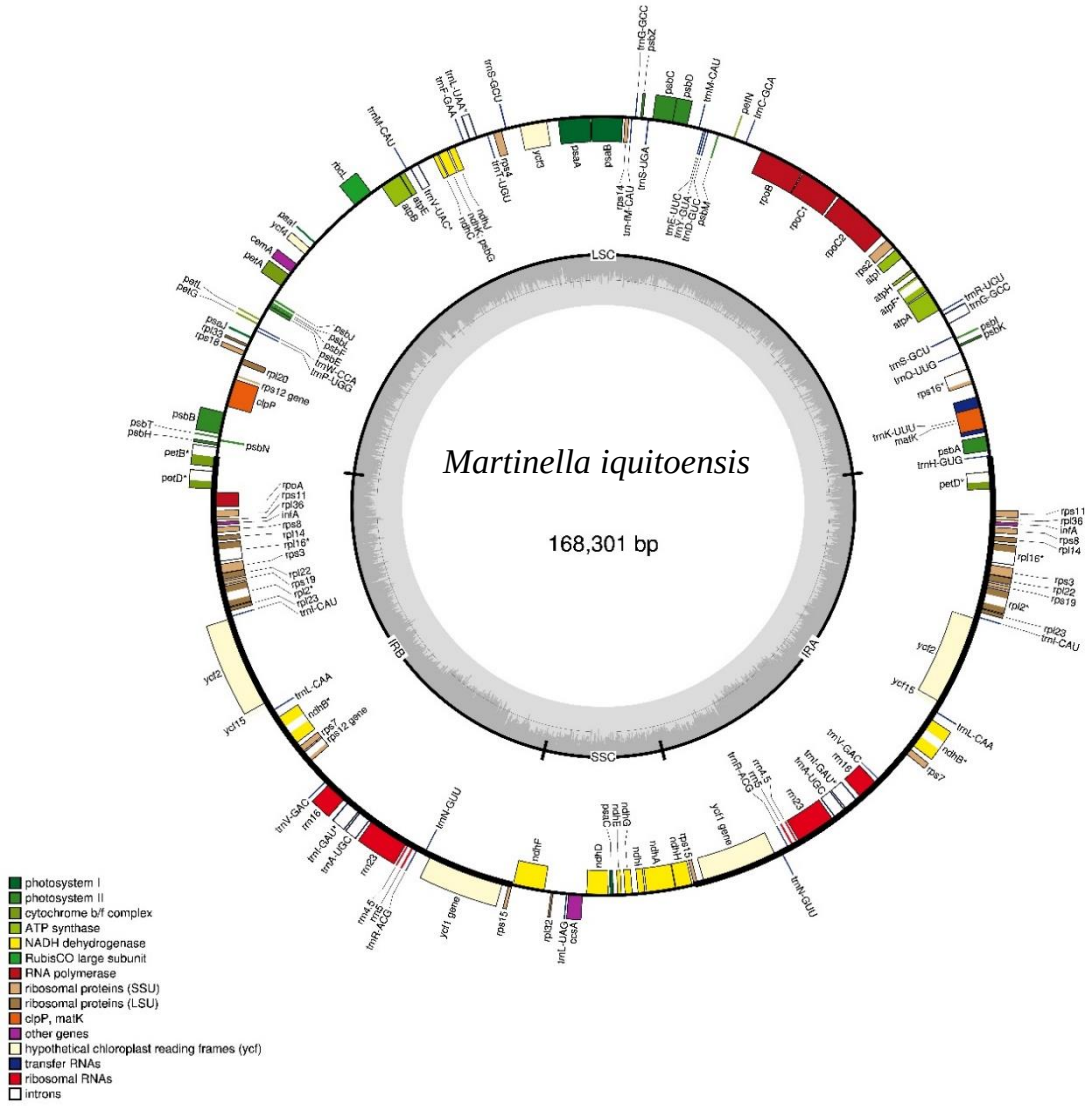

Figure 2. Gene map of the annotated plastome of *Martinella iquitoensis* A. Samp. (accession *M. iquitoensis* 5). Genes drawn inside the circle are transcribed clockwise, and those drawn outside are transcribed counterclockwise. Genes belonging to different functional groups are color-coded. The darker gray in the inner circle corresponds to GC content, and the lighter gray corresponds to AT content.

3. Results

3.1. Sanger sequencing

High quality sequences were obtained for almost all sampled specimens (Table 1). For the *ndh*F, we obtained sequences for 15 specimens, ranging from 2,051 to 2,101 bp. For the *rpl*32-*trn*L, we obtained sequences for 14 specimens, ranging from 997 to 1,051 bp. For the *pepC*, we obtained sequences for 19 specimens, ranging from 586 to 763 bp.

3.2. Plastome assembly, annotation and variation

We assembled three complete and three partial plastomes of *Martinella*. We obtained a minimum of 7,168,560 and a maximum of 15,587,593 reads with average length of 101 bp. Of these, a minimum of 240,111 and a maximum of 1,166,879 reads were mapped against reference plastomes. The assembled plastomes have the typical quadripartite structure with two single-copy regions (LSC and SSC) separated by two repeated and inverted regions (IRs) (Fig. 2).

We assembled complete plastomes with sizes ranging from 167,518 to 168,301 bp and partial plastomes with sizes ranging from 97,620 to 127,078 bp (considering the LSC, SSC and only one IR as a conservative measure due to low coverage regions that were coded as 'N'). Mean plastome coverage varied from 197.7x to 1636.7x. The annotated genes (CDS) varied from 60 (for the incomplete plastome) to 79 (for the complete plastome) (Table 2). The plastomes are relatively conserved, with approximately 98.3% pairwise sequence identity. Noncoding regions are more variable than coding regions, with 96.2% and 98.1% pairwise identity, respectively.

Table 2. *Martinella* plastome descriptions. LSC (Large Single Copy), IR (Inverted Repeat), SSC (Small Single Copy). Lengths are given in base pairs (bp).

Species	Number of raw reads	Number of mapped reads	Plastome coverage	Plastome length (LSC + IR+ SSC)	LSC length	IR length	SSC length	GC content	CDS
M. insignis 1	13,190,987	771,488	197.7 x	125,937	74,796	38,722	12,419	37.5 %	79
M. obovata 8	13,792,333	911,214	1091 x	128,239	75,938	39,638	12,663	37.3 %	79
M. obovata 10	7,168,560	502,748	273.1 x	127,078	75,237	39,223	12,618	37.5 %	79
M. iquitoensis 1	14,373,590	1,073,066	1272 x	128,134	76,069	39,384	12,681	37.3 %	78
M. iquitoensis 5	15,587,593	1,166,879	1636.7 x	129,151	77,304	39,150	12,697	37.4 %	78
M. sp. nov. 1	9,154,900	240,111	229.5 x	97,620	-	-	-	39.5 %	60

3.3. Phylogenetic analyses of the plastid and nuclear datasets

The aligned matrices of the *ndh*F contains 2,071 characters, of which 37 (1.79%) are parsimony informative; the *rpl*32-*trn*L matrix contains 909 characters, of which 30 (3.3%) are parsimony informative; and the *pepC* matrix contains 625 characters, of which 60 (9.6%) are parsimony informative. The best-fit models of DNA substitution were the GTR+G for the *ndh*F and *rpl*32-*trn*L matrices, and HKY+G for the *pepC*.

The analyses of the plastid and nuclear datasets recovered trees with different levels of resolution between datasets (Figs. S1 and S2). In both analyses, *Martinella* was recovered as monophyletic [bootstrap (bp) = 99%, posterior probability (pp) = 1.0)] with two main lineages, sister to each other: (i) the Amazonian clade, including *M. iquitoensis*, *M. obovata*, and two putative new species, and (ii) the Atlantic Forest clade, including *M. insignis* exclusively (Figs. S1 and S2).

In the Amazonian clade of the plastid tree, four main subclades were recovered: one subclade containing the two putative new species (bs = 99, pp = 1.0); two separate subclades containing *M. obovata* individuals (bs = 100 and 86, pp = 1.0 and 0.85, respectively); and, one subclade containing all individuals of *M. iquitoensis* (bs = 63, pp = 0.51) (Fig. S1). In the Amazonian clade of the nuclear tree, we recovered slightly different relationships and two main subclades: one subclade containing three specimens of *M. iquitoensis* (bs = 98, pp = 1.0), and the other containing *M. obovata*, the putative new species, and two individuals of *M. iquitoensis* (bs = 63, pp = 0.85) (Fig. S2). The relationships reconstructed using the nuclear dataset are not supported by morphology, instead, the relationships appear to reflect geographic proximity among samples (Table 1, Fig. S2).

Generally, the trees derived from the analyses of the plastid dataset recovered relationships among main clades and outgroups, but the ingroup remained poorly resolved (Fig. S1). In contrast, the trees derived from the analyses of the nuclear dataset showed fewer polytomies and higher resolution among closely related species (Fig. S2). Topologies recovered using the ML and BI criteria for each dataset were identical, only differing in support values, which were generally higher in the majority rule consensus tree that resulted from the BI analyses.

Results derived from the CADM test (W = 0.61) indicated high degree of similarity between topologies that were significantly congruent after 1000 permutations (p = 0.01). As such, the plastid and nuclear datasets were combined into a single data matrix. The analyses of the combined plastid-nuclear dataset also recovered a monophyletic *Martinella* (bs = 97, pp =

1.0) and *M. insignis* as sister to the Amazonian clade (Fig. 3). The resulting trees showed improved resolution and higher support within the Amazonian clade, within which two main subclades were recovered: one subclade containing four individuals of *M. iquitoensis* (bs = 100, pp = 1.0), and the other subclade containing individuals of *M. iquitoensis*, *M. obovata*, and the two putative new species (bs = 77 and pp = 0.99). Within the latter, four other small subclades were recovered: one containing the two putative new species (pp = 1.0), one containing individuals of *M. obovata* from Colombia and Costa Rica (bs = 70, pp = 0.98), one containing individuals of *M. obovata* from central, southern, and eastern Amazonia (pp = 1.0), and one subclade containing two individuals of *M. iquitoensis* from central Amazonia (bs = 97, pp = 1.0) (Fig. 3).

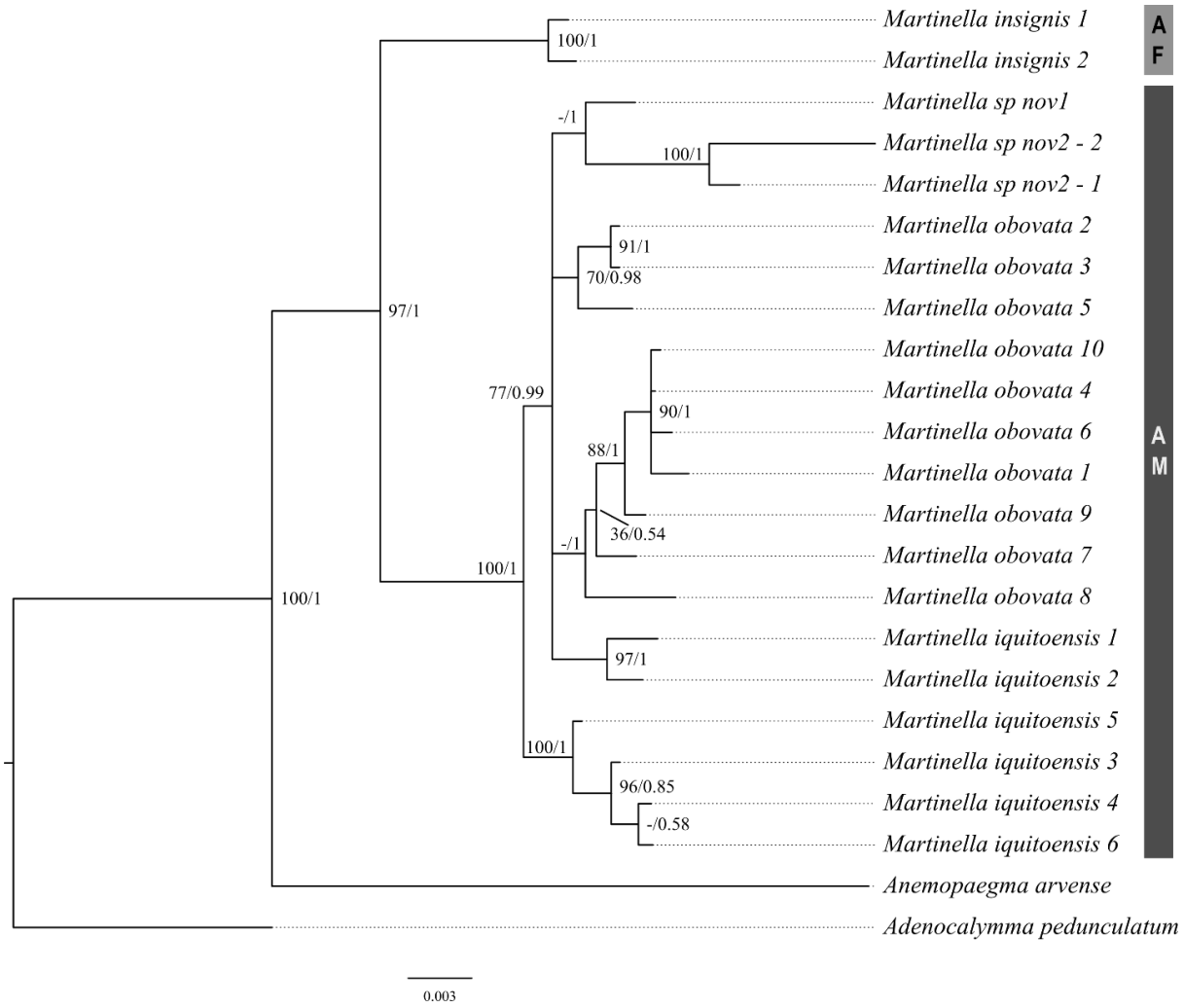

Figure 3. Bayesian majority rule consensus tree that resulted from the analysis of the plastid-nuclear dataset (concatenated alignments of the *ndh*F, *rpl*32-*trn*L and *pep*C markers). Bootstrap (from the Maximum Likelihood tree) and posterior probability values, respectively, are shown at nodes. Branch lengths indicate number of substitutions per site as measured on the scale bar. AF: Atlantic Forest clade, AM: Amazonian clade.

Some incongruences emerged between the topologies that resulted from the ML and BI analyses, namely: (i) a clade containing *Martinella sp. nov.* 1 and *Martinella sp. nov.* 2 was recovered in the BI topology, but not in the ML topology, (ii) a clade with *M. obovata* 8 as sister to other individuals of *M. obovata* from central and eastern Amazonia recovered on the BI topology, and (iii) the placement of three individuals of *M. iquitoensis* (accessions 3, 4 and 6), that varied between ML and BI topologies. However, all these incongruences were weakly supported in the ML tree (bp < 54).

3.4. Phylogenetic analyses of the plastome dataset

The aligned plastome dataset includes eight terminals (six *Martinella* specimens, and two outgroups). The CDS matrix contains 69,939 characters, of which 878 (1.26%) are parsimony informative, and the NCS matrix contains 49,167 characters, of which 467 (0.95%) are parsimony informative. The plastome data matrix (CDS + NCS) contains 119,106 characters, of which 1,345 (1.13%) are parsimony informative. Partition finder identified 33 partitions. The GTR was selected as the best-fit model of DNA substitution for eight partitions, the GTR+I was selected for five partitions, the GTR+G was selected for five partitions, the GTR+I+G was selected for one partition, the HKY was selected for eight partitions, the HKY+G was selected for two partitions, and the F81 was selected for four partitions.

The ML and BI trees recovered from the analyses of the plastome dataset were topologically identical and included maximum support values for all nodes except from one (Fig. 4). These trees showed the same general relationships among lineages obtained with the plastid and nuclear datasets, and also recovered a *M. insignis* sister to the Amazonian clade (bp = 100, pp = 1.0). However, a fully resolved Amazonian clade was recovered, with *Martinella sp. nov.* 1 appearing as sister (bp = 100, pp = 1.0) to a clade including *M. iquitoensis* and *M. obovata* (bp = 97, pp = 0.99).

3.5. Phylogenetic analyses of the combined plastome and Sanger dataset

Due to high congruence among topologies recovered in the analyses of the Sanger and plastome dataset – as indicated by visual inspection and the CADM congruence test –, we combined all our data into a combined dataset (plastome + plastid + nuclear datasets). The final alignment contained 119,432 characters, of which 1,433 (1.2%) are parsimony informative. We used the same partitioning scheme and evolutionary models implemented in the analyses of the individual data partitions.

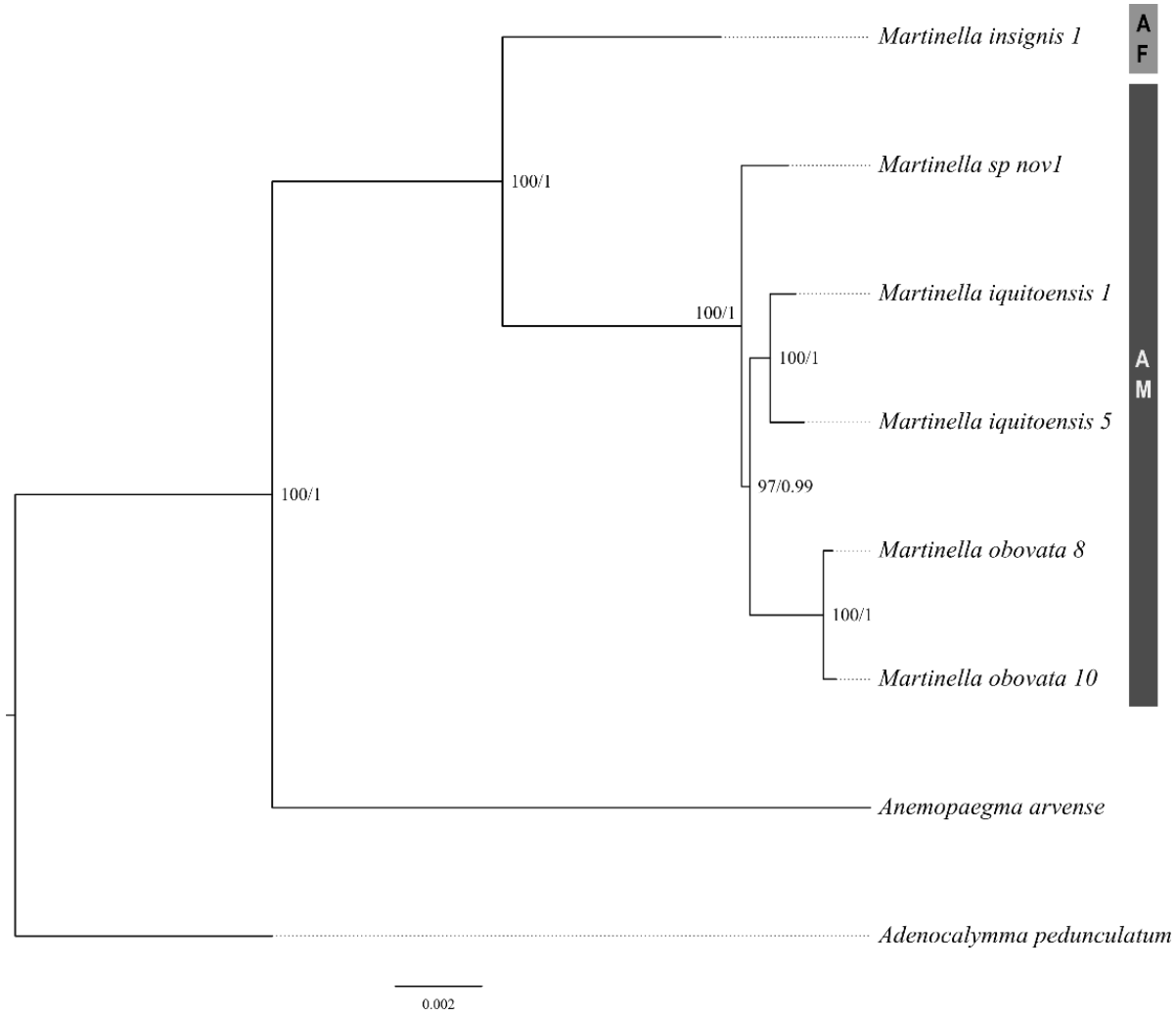

Figure 4. Bayesian majority rule consensus tree that resulted from the analysis of the plastome dataset. Bootstrap (from the Maximum Likelihood tree) and posterior probability values, respectively, are shown at nodes. Branch lengths indicate number of substitutions per site as measured on the scale bar. AF: Atlantic Forest clade, AM: Amazonian clade.

Similar to the relationships recovered in the analyses of the other datasets, *M. insignis* emerged as sister to the Amazonian clade (bs = pp = 1.0), within which a well-supported clade containing *Martinella sp. nov.* 1 and *Martinella sp. nov.* 2 (bs = 61, pp = 1.0) emerged as sister to a weakly supported clade with *M. iquitoensis* plus *M. obovata* (bs = 53, pp = 0.89) (Fig. 5). Despite weak support within the Amazonian clade, all species were recovered as monophyletic, and the topologies that resulted from the ML and BI analyses were identical. The topology that resulted from these analyses was generally congruent with topologies obtained through the analyses of all other datasets, with only minor differences in the placement of few accessions.

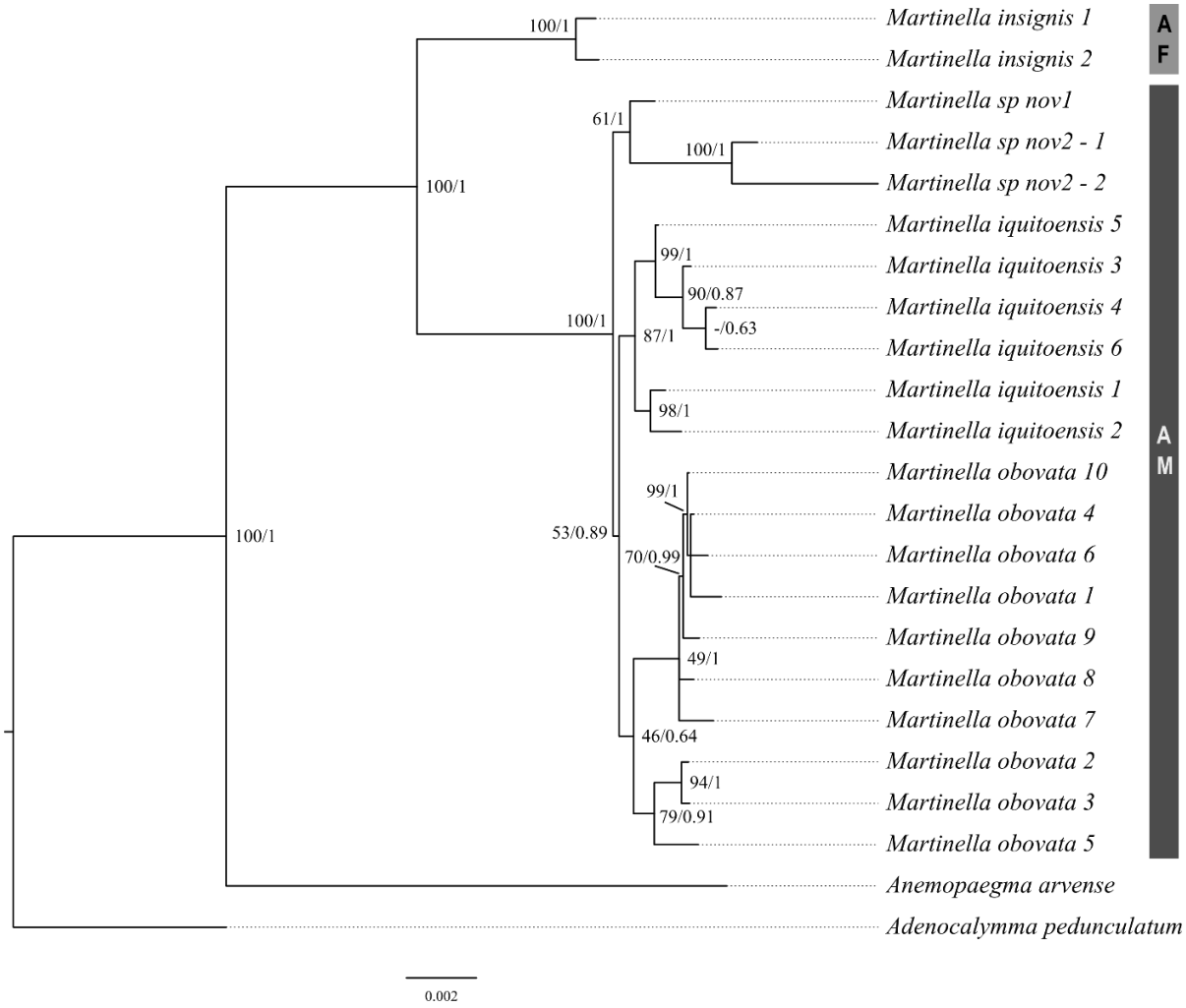

Figure 5. Bayesian majority rule consensus tree that resulted from the analysis of the combined dataset. Bootstrap (from the Maximum Likelihood tree) and posterior probability values, respectively, are shown at nodes. Branch lengths indicate number of substitutions per site as measured on the scale bar. AF: Atlantic Forest clade, AM: Amazonian clade.

4. Discussion

In this study, we inferred the phylogeny of *Martinella* using data generated by Sanger sequencing and HTS technology. We assembled and annotated six chloroplast genomes, and sequenced targeted chloroplast and nuclear loci for 15 specimens, representing all three species currently recognized plus two putative new species. This approach allowed us to compile a large DNA sequence dataset (119,432 characters) that is representative of the morphological diversity and distribution of *Martinella* as a whole. We evaluated changes in phylogenetic tree support and resolution with increased taxon and character sampling in five datasets. The most comprehensive dataset, i.e., HTS and Sanger data combined, led to a well-resolved and robust phylogeny for *Martinella*. This tree was used to identify monophyletic lineages supported by morphology and to evaluate the circumscription of individual species. In the following

subsections we discuss our main findings and implications for species recognition within the genus.

4.1. Plastome structure

Species of *Martinella* show a typical quadripartite plastome organization with a large and a small single copy region separated by two inverted repeat regions. Despite the typical organization, complete plastomes of *Martinella* ranged from 167,518 to 168,301 bp, being among the largest chloroplast genomes in the Angiosperms. For example, *Pelargonium* × *hortorum* has the largest land plant plastome sequenced to date, with 217 Kbp (Chumley et al., 2006), while the large plastomes of the Mimosoid legumes range from 163 to 175 Kbp (Dugas et al., 2015), and those of tribe Crescentieae (Bignoniaceae) are 154 Kbp in size (Moreira et al., 2016). Within tribe Bignonieae, plastome sizes range from 157,025 to 159,407 bp in *Adenocalymma* spp. (Fonseca and Lohmann, 2017), 167,413 to 168,987 bp in *Anemopaegma* spp. (Firetti et al., 2017), and 153,776 bp in *Tanaecium tetragonolobum* (Jacq.) L.G. Lohmann (Nazareno et al., 2015). Variation in plastome size can be caused by gene loss, expansion or contraction of the IRs and/or variation in intergenic regions (Xiao-Ming et al., 2017). In *Martinella*, we observed an expansion of the IRs, with the duplication of 11 genes (i.e., *pet*D, *rpo*A, *rps*11, *rpl*36, *inf*A, *rps*8, *rpl*14, *rpl*16, *rps*3, *rpl*22 and *rps*19) and a partial duplication of *pet*B, which shifted the boundaries of the LSC and IRb, from *rps*19 to the intermediate portion of the *pet*B. This same pattern of variation in plastome structure was documented in *Anemopaegma* spp. (Firetti et al., 2017).

4.2. Phylogeny of Martinella

By comparing topologies recovered using five distinct datasets amassed with Sanger sequencing and/or HTS, we were able to evaluate phylogenetic support and resolution with increasingly larger datasets. The analyses of targeted chloroplast and nuclear loci recovered highly congruent tree topologies, which allowed these datasets to be combined into a single data matrix. The tree recovered from the analyses of the combined Sanger dataset (plastid + nuclear) led to improved resolution and increased support compared to trees reconstructed using the plastid and nuclear datasets individually, likely due to the higher number of characters. This finding is consistent with earlier phylogenetic studies of other genera of Bignonieae, such as *Adenocalymma* (Fonseca and Lohmann, 2015), *Lundia* (Kaehler and Lohmann, 2012), and *Tynanthus* (Medeiros and Lohmann, 2015). However, within the Amazonian clade of

Martinella, relationships among and within main clades remained unresolved and weakly supported.

The phylogeny of *Martinella* inferred using a newly generated plastome dataset for a subset of taxa led to a fully resolved and supported topology (bp > 97 and pp > 0.98). This pattern corroborates the importance of plastome data for addressing phylogenetic relationships at different taxonomic levels, and the potential of these data for resolving interspecific relationships within Bignonieae (Firetti et al., 2017; Fonseca and Lohmann, 2018).

The integration of HTS and Sanger sequencing data maximizes taxon and character sampling, representing a very promising approach in plant systematics (Williams et al., 2016; Fonseca and Lohmann, 2018). In our combined analysis of the plastid, nuclear, and plastome datasets, we recovered a fully resolved tree for *Martinella* that is congruent with the topologies recovered from all other datasets analyzed. Therefore, the tree recovered using a super matrix approach and the combined dataset is considered our best estimate of phylogenetic relationships within *Martinella*. Although the combined tree showed the same relationships recovered in the analysis of the plastome dataset, support values within the Amazonian clade were generally low (min. bs = 46, min. pp = 0.63). These findings are expected when super matrix approaches are adopted given the high percentage of missing data for taxa for which only Sanger sequencing data is available (Williams et al., 2016; Uribe-Convers et al., 2017).

4.3. Taxonomic implications

Our phylogenetic reconstruction recovered five strongly supported clades, three of which correspond to currently recognized taxa, i.e., *Martinella insignis* (Atlantic Forest), *Martinella iquitoensis* (Amazon), and *Martinella obovata* (Amazon). In addition, our analyses support the recognition of two new Amazonian taxa, previously hypothesized to represent new species based on morphology and extensive fieldwork (E.Y. Kataoka pers. obs.). These new taxa are known from only a few specimens and will be formally published on the taxonomic revision of the whole genus (Kataoka and Lohmann, in prep. – Chapter Two). These findings highlight the importance of in-depth taxonomic and phylogenetic studies of selected lineages in the Amazon region (Fine and Lohmann, 2018), where despite historical efforts in biological surveys and research, important lacunae still remain (Hopkins, 2007).

5. Conclusions

We inferred the phylogeny of *Martinella* using a combined approach that integrated HTS and Sanger sequence data. This approach allowed us to amass a comprehensive DNA sequence dataset with over 119,000 characters for accessions representing the range of geographic distribution of the genus. We analyzed five different dataset combinations that reconstructed highly congruent topologies with minor variations in tree resolution and support. The Sanger and HTS dataset combined into a super matrix resulted in the best estimate of phylogenetic relationships within the genus and recovered five clades. Three of these clades correspond to currently recognized species, and two correspond to new Amazonian taxa, corroborating initial hypotheses based on morphology.

Although we recovered a well-resolved and robust phylogeny of *Martinella*, further studies integrating nuclear data, perhaps using a target-capture approach, could improve estimates of relationships and support within the Amazonian clade. In addition, the newly generated complete chloroplast sequences would allow for the development of molecular markers for intraspecific studies focusing on population level questions, especially in the widespread Amazonian species. These studies have the potential to address hypotheses about the origin and maintenance of Amazonian biodiversity as a whole.

Acknowledgements

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Supplementary material

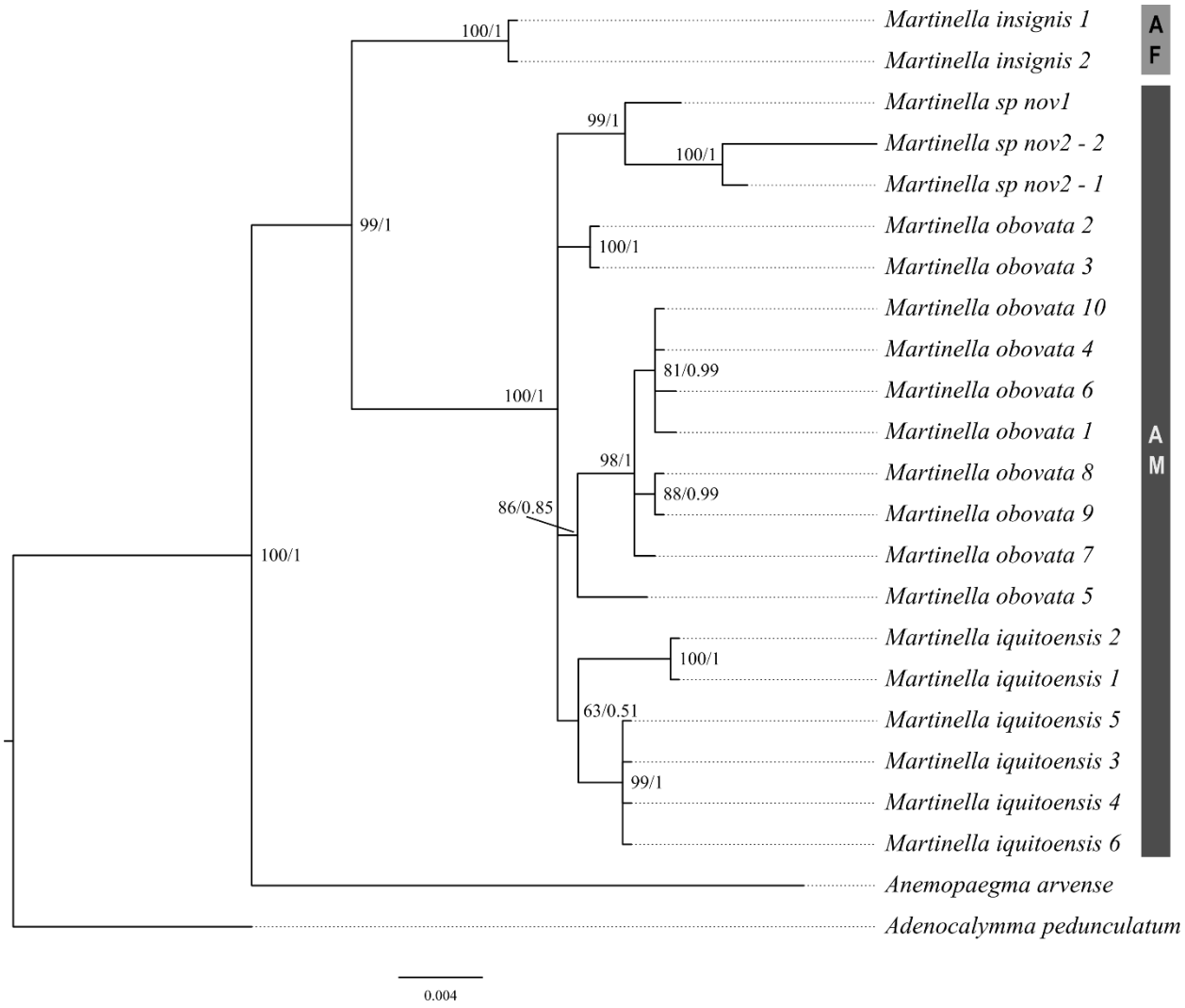

Figure S1. Bayesian majority rule consensus tree that resulted from the analysis of the plastid dataset (concatenated alignments of the *ndh*F and *rpl*32-*trn*L markers). Bootstrap (from the Maximum Likelihood tree) and posterior probability values, respectively, are shown at nodes. Branch lengths indicate number of substitutions per site as measured on the scale bar. AF: Atlantic Forest clade, AM: Amazonian clade.

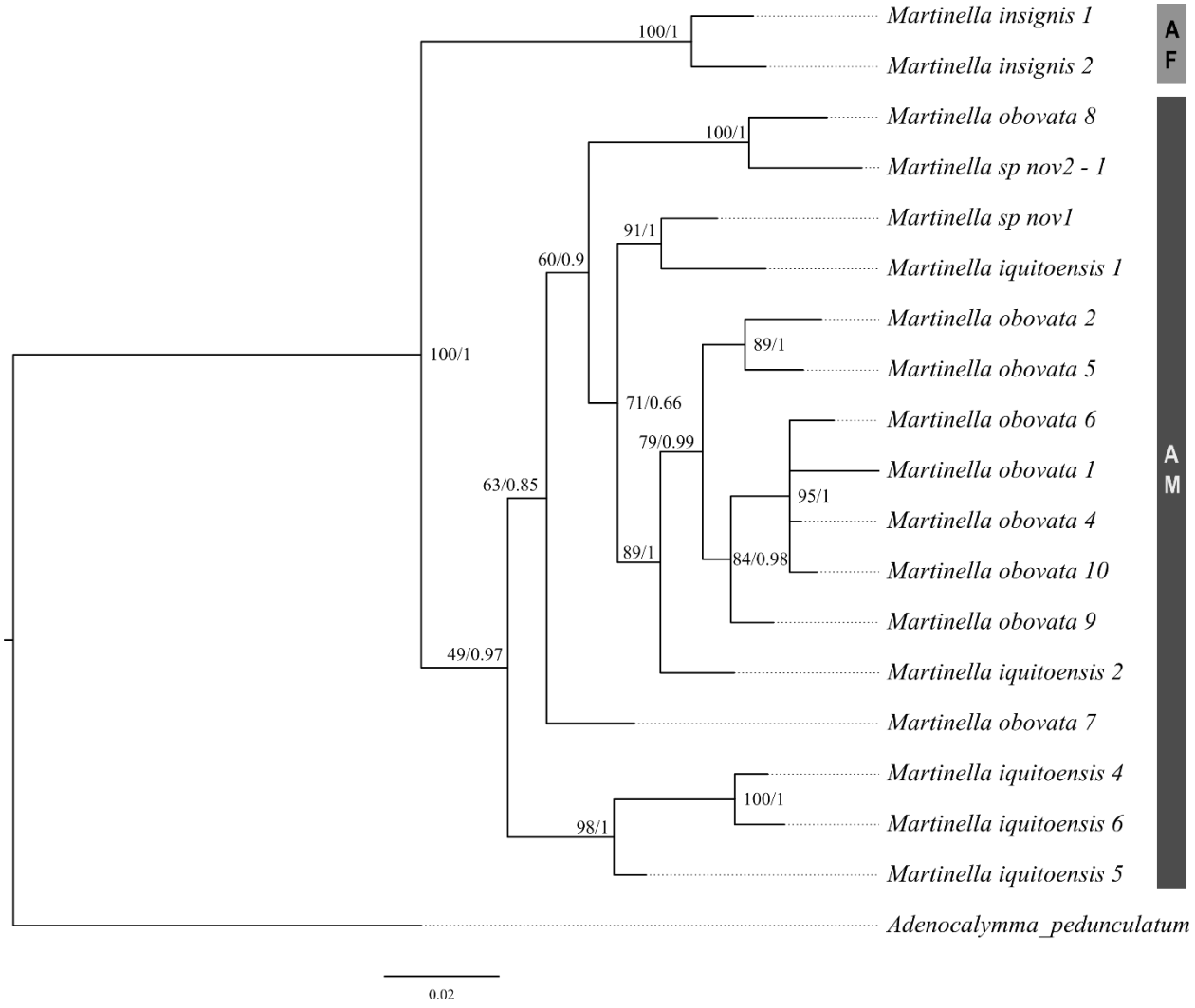

Figure S2. Bayesian majority rule consensus tree that resulted from the analysis of the nuclear dataset (alignment of the *pep*C marker). Bootstrap (from the Maximum Likelihood tree) and posterior probability values, respectively, are shown at nodes. Branch lengths indicate number of substitutions per site as measured on the scale bar. AF: Atlantic Forest clade, AM: Amazonian clade.

Taxonomic revision of *Martinella* Baill. (Bignonieae, Bignoniaceae)

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Abstract

Martinella Baill. is a genus of Neotropical lianas in tribe Bignonieae (Bignoniaceae). The genus is monophyletic and well supported by morphological and molecular characters. Members of *Martinella* are characterised by a continuous interpetiolar ridge surrounding the stem, bilobed or 4-5-parted calyces, and minute triangular prophylls of the axillary buds. Generic circumscription remained unchanged since the description of the genus, although unclear species limits remained. Based on extensive fieldwork, herbarium work, and a molecular phylogenetic hypothesis for the genus, we here recognise five species of *Martinella*. Of these, three were recognised in earlier treatments for the genus, while two represent new species described here, *Martinella lanuginosa* Kataoka & L.G. Lohmann **sp. nov.** and *Martinella tomentosa* Kataoka & L.G. Lohmann **sp. nov.** An older name was discovered for *Martinella iquitoensis* A. Samp., which is now treated as a synonym of *M. insculpta* Sprague & Sandwith. In addition, lectotypes are designated for five species names, namely *Spathodea obovata* Kunth, *Bignonia martinii* DC., *Bignonia fockena* Miq., *Tabebuia cordata* Benth., and *Doxantha longisiliqua* Bert. ex Spreng, and a neotype is designated for *Martinella gollmeri* K. Schum. This work provides a full taxonomic treatment for *Martinella*, including a complete list of synonyms, morphological descriptions, illustrations, photographs, distribution maps, conservation status, and comments for all five species recognised.

Keywords: Amazon, Lianas, Neotropical Flora, Taxonomy

Introduction

Martinella Baill. (Bignonieae, Bignoniaceae) is a genus of Neotropical lianas, whose monophyly is supported by molecular phylogenetic studies (Lohmann 2006), and by morphological features. Namely, minute triangular prophylls of the axillary buds and interpetiolar ridges surrounding the stems are thought to represent morphological synapomorphies of the genus (Lohmann and Taylor 2014). Additionally, silvery or whitish leaflets on the abaxial surface, bilobed or 4-5-parted calyces, and corollas with a constricted basal portion and an upper campanulate portion also characterise species in the genus.

The circumscription of *Martinella* remained very stable over the years (Lohmann and Taylor 2014). Despite generic stability, species delimitation remained poorly known, which led some authors to consider the Amazonian *Martinella* as part of a species complex due to overlapping morphological characters (MacBride 1961, Zuntini and Lohmann 2014). This taxonomic uncertainty motivated the present study.

Taxonomic history

Baillon (1888) described *Martinella* based on reproductive characters such as the irregularly 2-4-lobed calyces, bilabiate corollas with a wide tube (presumably referring to what was named *Martinella*-type flower nearly a century later, see Gentry 1974), stipitate ovary that sits on a large nectariferous disk, and glabrous, flattened and narrow fruits. Even though the genus was described based on *Bignonia martinii* DC. [= *Martinella obovata* (Kunth) Bureau & K. Schum.], the name *Martinella* was not directly associated with the specific epithet "martinii" in the protologue (Baillon 1888). Only in Schumann (1894) the name *Martinella* was formally associated with the epithet "martinii", when the new combination *Martinella martinii* (DC.) Baill. ex. K. Schum was officially proposed. Schumann (1894) also proposed a new species, *Martinella gollmeri* K. Schum [= *Martinella obovata* (Kunth) Bureau & K. Schum.], based on shallow and bowl-shaped nectariferous disk, and frizzy calyces. Two years later, Bureau and Schumann (1896) transferred *Spathodea obovata* Kunth into *Martinella*, proposing the new combination *Martinella obovata* (Kunth) Bureau & K. Schum., which became the accepted name for *Martinella martinii*.

A new species, *Martinella insculpta* Sprague & Sandwith, was described in 1934 by Sprague and Sandwith, with two other names being published shortly after, *M. iquitoensis* A. Samp. (Sampaio 1935) and *M. manaosiana* A. Samp. (Sampaio 1936); these names are synonyms of *M. insculpta*. More recently, Zuntini and Lohmann (2014) published a new species

endemic to the Atlantic Forest, *M. insignis* A. H. Gentry ex. Zuntini & L.G. Lohmann, expanding the distribution of this predominantly Amazonian genus.

Martinella's taxonomy and nomenclature were comprehensively treated by Alwyn Gentry in Flora of Ecuador (Gentry 1977) and *Flora de Venezuela* (Gentry 1982). In these treatments, Gentry provided a list of synonyms for *Martinella obovata* that included *M. insculpta*, which is here treated as a separate taxon. In the most recent synopsis of the genus (Zuntini and Lohmann 2014), three species were recognised: *M. insignis* A.H. Gentry ex. Zuntini & L.G. Lohmann, *M. iquitoensis* A. Samp. [= *M. insculpta*] and *M. obovata* (Kunth) Bureau & K. Schum.

Five main clades were recovered in a molecular phylogeny inferred for *Martinella* (Kataoka and Lohmann in prep.) and each clade is here recognised as a distinct species. Of these, three correspond to previously known species, i.e., *Martinella insculpta* Sprague & Sandwith, *Martinella insignis* A.H. Gentry ex Zuntini & L.G. Lohmann, and *Martinella obovata* (Kunth) Bureau &. K. Schum., while two correspond to new taxa, i.e., *Martinella lanuginosa* Kataoka & L.G. Lohmann and *Martinella tomentosa* Kataoka & L.G. Lohmann.

Geographic distribution and habitat

Members of *Martinella* are distributed in wet forests of Central America, northern South America, the Amazon and the Atlantic Forest of Brazil, between 0–1700 m above sea level (a.s.l.) (Zuntini & Lohmann 2014). Among the five *Martinella* species recognised in this study, *M. obovata* is the most frequent and broadly distributed, occurring from southern Mexico to southern Amazon, reaching as far south as Bolivia and the Brazilian state of Mato Grosso do Sul. *Martinella insculpta* is also widely distributed in Central America and Amazonia but is less common when compared to its sympatric *M. obovata*. The other three species in the genus are rare, with narrow distribution ranges. The only known records of *M. tomentosa* are from Central Brazilian Amazon (Amazonas state), while *M. lanuginosa* is only known from western Brazil (Acre state), Peru, and northern Colombia. Lastly, *M. insignis* is the only member of *Martinella* that is restricted to the Atlantic Forest, occurring in the states of Espírito Santo and Bahia, in eastern Brazil.

Habitats

Species of *Martinella* occur in wet Neotropical forests. *Martinella insculpta*, *M. lanuginosa*, and *M. tomentosa* predominantly occur in *terra firme* forests in the Amazon, with

some reports of *M. insculpta* growing in white-sand soils. *Martinella obovata* preferentially occurs along riverbanks of the Amazonian white-water rivers, where many individuals were found during field expeditions, and where many collections were made over the years. *Martinella insignis* is restricted to sandy soils of the Atlantic Forest, growing close to the shore (Zuntini and Lohmann 2014).

Reproductive biology

All species of *Martinella* share a flower morphology that was described by Gentry (1974) as *Martinella*-type flower. This floral morphology is characterised by spathaceous, tubular or urceolate calyces, and by tubular, infundibuliform, or urceolate corollas that are straight and coriaceous, and can be red, magenta or white (Gentry 1974, Alcantara and Lohmann 2010). These traits suggest hummingbird pollination. All Amazonian species of *Martinella* fit in the pollination syndrome suggested by their floral morphology, which is supported by field observations with additional evidence of Euglossini bee visitation and/or pollination (Gentry 1974). Conversely, *M. insignis* has a yellow corolla that is unique in the genus. This corolla colour suggests bee pollination, although field studies are needed to confirm this prediction.

Economic and ethnobotanical uses

Bignoniaceae species are traditionally used for timber, handicraft, and medication (Gentry, 1992). *Martinella* species are widely used by Amazonian indigenous people to treat eye inflammation and conjunctivitis (e.g. Gentry and Cook 1984; Alexiades 1999). Extracts are made using the root's outer bark, which is scraped, macerated with water and the resulting juice is filtered and used as eye drops (e.g. Gentry and Cook, 1984; similar reports are available in herbarium specimens' labels, e.g., G.T. Prance 15557 and Lewis 14026).

The widespread use of *Martinella* extracts for medicinal purposes throughout the Amazon motivated chemical analyses, which led to the discovery of the alkaloids Martinelline and Martinellic acid from organic extracts of *M. iquitoensis* [= *M. insculpta*] (Witherup et al. 1995). Further studies have been conducted attempting to synthesise these compounds (e.g. Davies et al. 2013).

Cytology

Chromosome count for *Martinella obovata* is 2*n* = 40 (Goldblatt and Gentry 1979), corresponding to the most common chromosome number in genera of tribe Bignonieae (Cordeiro et al. 2017).

Morphology

Habit. All species of *Martinella* are lianas, although seedlings until ca. 70 cm tall are self-supporting shrubs (see Figures 1A-C).

Stems. Mature stems of *Martinella* bear lenticels and solid pith with four phloem wedges in cross section. Four phloem wedges is the predominant condition within tribe Bignonieae and is also found in *Adenocalymma*, *Callichlamys*, *Cuspidaria*, *Fridericia*, *Lundia*, *Manaosella*, *Neojobertia*, *Tanaecium*, *Tynanthus*, *Pachyptera*, *Pleonotoma*, and *Stizophyllum* (Lohmann 2006, Lohmann and Taylor 2014). Young stems are cylindrical and generally remain cylindrical at maturity but become tetragonal in *Martinella insculpta*. A continuous ring surrounds the interpetiolar regions of stems (see Figure 1E). The stem surface is smooth, sparsely to densely covered with trichomes. Stems over ca. 4 cm in diameter often have sparse lenticels. Non-lenticelled parts of the stem are pale to dark green, often with dark blotches.

Leaves. In Bignonieae, leaves are commonly 2- or 3-foliolate with the terminal leaflet generally modified in a tendril (Gentry 1980, Lohmann and Taylor 2014). In mature individuals of *Martinella*, leaves are exclusively 2-foliolate, bearing a trifid tendril, with discolorous leaflets that bear glandular trichomes on the abaxial side (see Figures 1C, D, F, G). Young individuals of *M. insculpta* and *M. obovata* show a unifoliolate first leaf and lack tendrils (see Figures 1A-B). In some taxonomic treatments, *Martinella* was described as bearing simple or trifid tendrils (MacBride 1961, Gentry 1977, Zuntini and Lohmann 2014). However, careful examination of herbarium specimens revealed that most seemingly simple tendrils corresponded to trifid tendrils with missing parts. Trifid tendrils represent the ancestral character state within tribe Bignonieae and were maintained in *Martinella* (Sousa-Baena et al. 2014). Leaf venation is brochidodromous in all species of *Martinella*. Leaf domatia is only found in *M. insignis,* as pocket-like structures at the axil of the midvein with the secondary veins, on the abaxial side of leaflets, mainly at the basal portion.

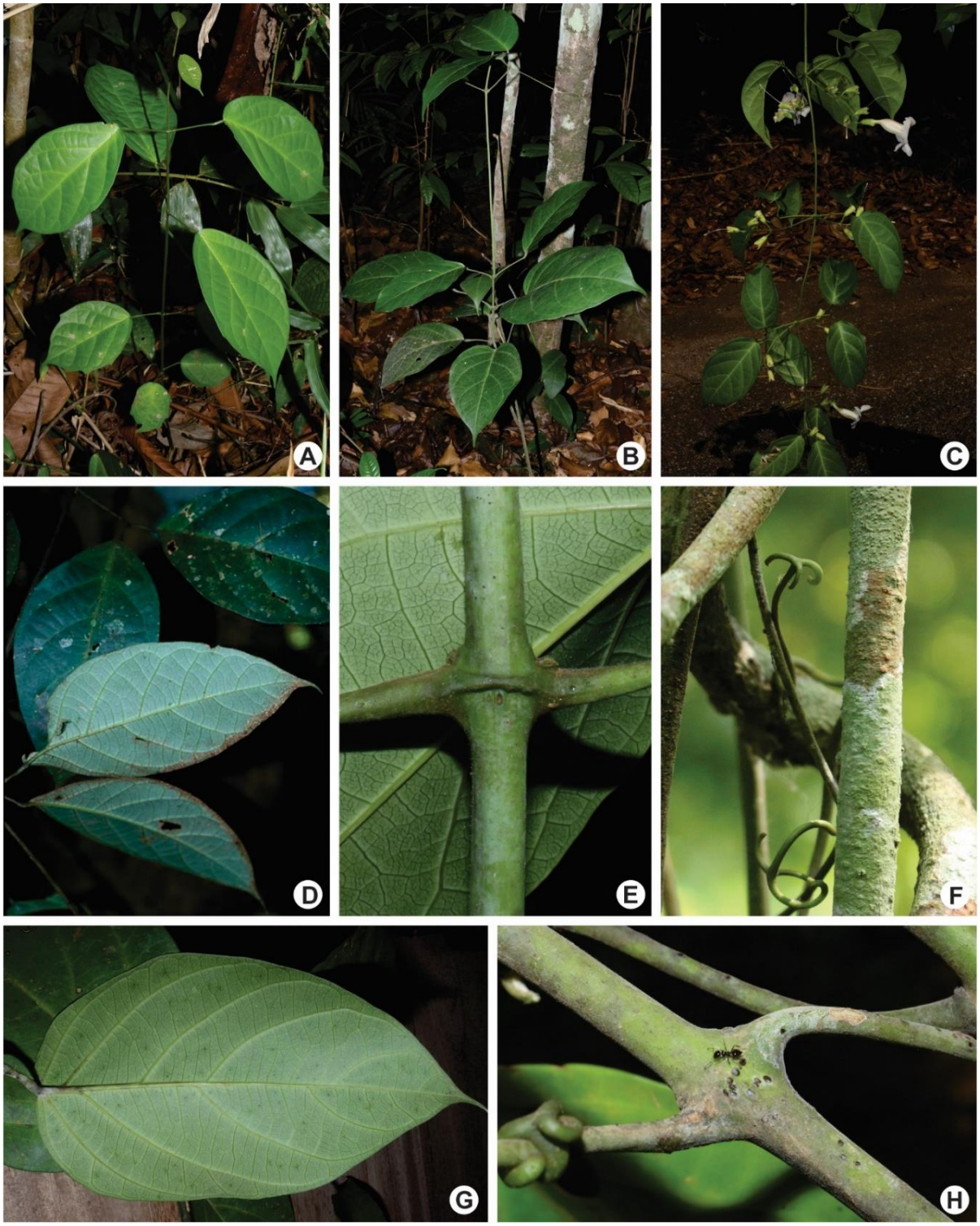

Figure 1. Vegetative characters of *Martinella* Baill. **A**-**B** Young individuals of *M. obovata* and *M. insculpta* bearing unifoliolate leaves; **C** Branch of *M. obovata*; **D** Discolorous leaflets of *M. insculpta*; **E** Interpetiolar ridge of *M. obovata*; **F** Trifid tendril of *M. insculpta*; **G** Abaxial side of leaflets of *M. insculpta* with patelliform glandular trichomes; **H** Interpetiolar region of *M. insculpta* with patelliform glandular trichomes being visited by ants. Photos taken by E.Y. Kataoka, except from photo D, taken by R. Foster.

Prophylls of the axillary buds. Structures traditionally referred to as pseudostipules (Gentry 1980) were subsequently shown to actually refer to well-developed prophylls of the axillary buds (Lohmann and Taylor 2014). Prophylls are very useful structures for the identification of genera and species in tribe Bignonieae, often representing morphological synapomorphies of generic-level clades (Lohmann and Taylor 2014). All species of *Martinella* have minute triangular prophylls, a putative morphological synapomorphy of the genus (Lohmann 2006) (see Figures 1E, H).

Trichomes. Four main types of trichomes are found in tribe Bignonieae: (i) non-glandular (eglandular) trichomes, (ii) peltate glandular trichomes, (iii) stipitate glandular trichomes, and (iv) patelliform/cupular glandular trichomes (Nogueira et al. 2013). Trichome distribution on the plant body is highly variable, although some genera of tribe Bignonieae are readily recognised by diagnostic patterns of trichome distribution, e.g., *Adenocalymma*, with patelliform glandular trichomes on the prophylls of the axillary buds, floral bracts, calyces, bracteoles, and fruits (Lohmann and Taylor 2014; Fonseca and Lohmann in prep.), and *Pachyptera*, with a field of patelliform glandular trichomes at the interpetiolar region and petiole apex (Francisco and Lohmann 2018). In species of *Martinella*, three trichome types are found: (i) non-glandular trichomes (referred to as simple eglandular trichome hereafter), (ii) stipitate glandular trichomes, and (iii) patelliform glandular trichomes (commonly referred to as "glands" in the literature). The simple eglandular trichomes are distributed throughout the plant, with variation in density depending on the plant organ; stipitate glandular trichomes are found in high density on the whole plant of *M. insignis* and *M. lanuginosa* and at different parts of the plant in other species with variable density; patelliform glandular trichomes are found on the whole plant of all members of *Martinella*, mainly at the interpetiolar region, and at the base of the abaxial surface of leaflets (see Figures 1E, H). The patelliform glandular trichomes can also be found on stems, inflorescences, and calyces of *M. insculpta* and *M. obovata*.

Inflorescences. Flowers of *Martinella* are organised in axillary and/or terminal inflorescences that bear six to 26 flowers, although only few of them open at a time. Botryoid inflorescences are found in *M. insculpta*, racemes are found in *M. obovata*, and thyrsi are found in *M. insignis*, *M. tomentosa*, and *M. lanuginosa*. Botryoids and racemes are very lax, contrasting with thyrsi, which shows higher levels of branching and a higher number of flowers per inflorescence.

Calyx. Calyx morphology is highly variable within Bignonieae (Lohmann and Taylor 2014). However, this trait is quite constant within *Martinella*, with the tubular-campanulate and

irregularly 2- to 4-lobed calyx (or regularly 5-lobed in *M. insignis*) representing a putative generic synapomorphy (Lohmann 2006, Lohmann and Taylor 2014).

Corolla. Corolla shape is an additional distinctive feature in *Martinella*. All species show tubular-campanulate corollas, with a tubular basal portion that is long and much narrower than the markedly campanulate upper portion, giving an inflated appearance compared to the basal portion. This distinctive corolla morphology was described by Gentry as the *Martinella*-type flower (Gentry 1974). Corolla colour varies from pale lilac (*M. obovata*) to dark magenta (*M. insculpta*) or yellow (*M. insignis*). Corolla colour in *Martinella lanuginosa* and *Martinella tomentosa* is unknown, but seems to vary from lilac to magenta, as in other Amazonian species.

Androecium. Members of *Martinella* have four didynamous stamens and one very reduced staminode (ca. 1 mm long). The stamens are inserted at the inferior portion of the corolla tube, at approximately 1/4 of the corolla length. Anthers are included with straight and divaricate thecae and glabrous filaments.

Pollen. Gentry and Tomb (1979) highlighted the usefulness of pollen morphology to generic-level identification of members of Bignonieae, although this trait shows convergent evolution. In *Martinella*, pollen is quite constant, tricolpate with reticulate exine (see Figure 2E) in all species. Similarly, reticulate pollen grains are also found in *Bignonia*, *Mansoa*, *Pachyptera*, and *Pyrostegia* (Gentry and Tomb 1979, Francisco and Lohmann 2018).

Gynoecium. As in most Bignonieae, *Martinella* has a bilocular ovary with two fused carpels with axillary placentation (Gentry 1980), a single style with lanceolate and bilamellate stigma. The ovary is terete, with a glabrous and smooth surface.

Fruits. Species of *Martinella* have linear, flattened, septicidal capsules with two valves. Capsules of *Martinella* are among the longest fruits in Bignonieae, reaching up to 1.5 m long and 3 cm wide, only comparable to *Dolichandra* fruits (Fonseca et al. 2017). In addition, the capsules are glabrous to puberulent.

Seeds. Seeds of *Martinella* are symmetrically winged, oblong and thin. The seed body is chartaceous, opaque and brown or green, even after dried (in *M. obovata*). The seed wings are membranous and translucent, likewise brown or green-coloured. Seeds are wind-dispersed as in many other clades of Bignoniaceae (Gentry 1979, Gentry 1980).

Figure 2. Reproductive characters of *Martinella* Baill. **A-C** Flowers of *M. obovata* in frontal (A) and lateral view (B and C); **B** Irregularly 3-parted calices, and corolla narrowly-tubular at base and campanulate at upper portion; **C** Euglossini bee visiting the flower of *M. obovata*; **D** Lateral view of *M. insignis* flower showing the typical corolla shape and 5-parted calyx; **E** Scanning election micrograph showing tricolpate and reticulate pollen grains of *M. obovata*; **F** Dried seeds of *M. obovata* with greenish wings; **G** Flat, narrow, and long (≤ 1.5 m) fruit of *M. obovata*. All photos taken by E.Y. Kataoka, except from photo C, taken by G. Gerlach and D, taken by A.R. Zuntini.

Material and methods

The taxonomic revision of *Martinella* was based on living specimens and observations made on fresh material during field expeditions, as well as on the analyses of herbarium specimens deposited in the following herbaria: IAN, INPA, MG, MO, NY, QCA, QCNE, R, RB, SPF, UFACPZ (acronyms follow Thiers, continuously updated). All specimens were examined either physically or digitally via high quality photographs of herbarium specimens. We accessed specimens' photographs via the JSTOR Global Plants (https://plants.jstor.org/),

the Reflora Virtual Herbarium (http://reflora.jbrj.gov.br/reflora/herbarioVirtual/), or websites of individual herbaria.

Field expeditions were conducted between June and December 2016 in the Brazilian states of Acre, Amazonas, Mato Grosso, Pará, and Roraima. All specimens were deposited at SPF. All accepted names are listed in alphabetical order. Nomenclatural discussions follow the International Code of Nomenclature for algae, fungi, and plants (Shenzen Code) (Turland et al. 2018). Citations of type specimens are followed by the herbarium acronym and barcode, unless otherwise stated between brackets.

Species delimitation

We used molecular phylogenomic data (Kataoka and Lohmann in prep.) combined with morphological data in an integrative manner to recognise species. We followed the criterion that separately evolving lineages represent different species (de Queiroz 2007). In addition, species must be diagnosable by unique combinations of morphological features (Cracraft 1983). Therefore, we recognised species as separately evolving lineages that share a unique combination of morphological characters.

Morphological descriptions

Morphological descriptions follow the general terminology adopted in Lohmann and Taylor (2014). Additional terms follow Radford (1974) for general morphology, Hickey (1973) for leaf shape and venation, Weberling (1992) for inflorescence type, Nogueira et al. (2013) for trichome type, and Gentry and Tomb (1979) and Halbritter et al. (2018) for pollen morphology. All measurements were carried out on dried specimens and/or rehydrated material. In addition, pollen from herbarium specimens were analysed using scanning electron microscopy (SEM) on a Zeiss DSM 970 scanning electron microscope. In the descriptions, characters shown in parentheses indicate rare conditions.

Distribution maps, conservation status and list of examined specimens

Distributions maps were produced in QGIS 2.18 (QGIS Development Team 2018). We used a final dataset that included a combination of two separate datasets: (i) a newly generated distribution dataset with approximately 150 records from collections made in the field and from digitised specimen labels, and (ii) a dataset with approximately 500 records compiled through the years (Lohmann unpublished data, described in detail in Meyer et al. 2017).

Conservation status was assessed based on the complete distribution dataset using the Geospatial Conservation Assessment Tool (GeoCAT; http://geocat.kew.org/) (Bachman et al. 2011). This tool considers the metrics Extent of Occurrence (EOO) and Area of Occupancy for 2 km² grids (AOO) to objectively assign conservation status based on the IUCN criteria A, B and D (IUCN, 2012; IUCN Standards and Petitions Subcommittee 2017).

The list of examined specimens was produced using the R package monographaR (Reginato 2016).

Taxonomic treatment

Martinella Baill., Hist. Pl. 10:30. 1888.

Type. *Martinella martinii* (DC.) Baill. ex K. Schum [=*Martinella obovata* (Kunth) Bureau & K. Schum.].

Description. *Lianas. Roots* with swollen portions. *Branches* terete, glabrous, puberulous or pubescent, eglandular trichomes simple, glandular trichomes stipitate or patelliform, with a continuous ridge at the interpetiolar region, with few interpetiolar patelliform trichomes; prophylls of the axillary buds minute, glabrous, puberulous or pubescent. *Leaves* 2-foliolate with the terminal leaflet generally modified in a trifid tendril; leaflets membranous, chartaceous or coriaceous, glabrous to pubescent, margins entire, revolute, more conspicuously when dried, with or without mite-domatia, with patelliform glands on the adaxial surface. *Inflorescences* axillary, botryoid, racemose, or a thyrse. *Flowers* with calyces tubular-campanulate, irregularly 2-4-lobed or (5-) lobed, lobe apices mucronate or aristate, chartaceous, with scattered patelliform glands; corolla deep purple, lilac or dark magenta (yellow), narrowly tubular at basal portion and wide campanulate at upper portion, straight to slightly curved, membranous, outer surface glabrous, inner surface glabrous with eglandular trichomes concentrated at stamen insertion; stamens included, glabrous, pollen tricolpate and reticulate; ovary terete, smooth, glabrous, with a single series of ovules per placenta, style glabrous, stigma rhombic, glabrous. *Capsules* drying brown, linear, flattened, smooth, glabrous, with calyx normally persistent; seeds oblong, winged, with wings opaque, green or beige.

Discussion. The nomenclatural type of *Martinella* may be encountered as *Martinella martinii* (DC.) Baill. in certain sources. However, Baillon (1888) stated on the original description: “Generis typus est *Bignonia Martini* DC.,” thus not directly associating the genus

name with the specific epithet and not officially proposing the new combination, as required for valid publication by Art. 33.1 (Turland et al. 2018). The name *Martinella martinii* (DC.) Baill. ex. K. Schum. was only validly published by Schumann (1894) based on the gathering of Martin s.n. from Cayenne, French Guiana (deposited at P), which represents the type specimen of *Bignonia martinii* DC. (de Candolle 1845).

As circumscribed here, *Martinella* comprises five species distributed from southern Mexico to eastern Brazil. A key to all species recognised is given below:

Key to species of *Martinella*

1 Calyx 5-lobed; corolla yellow; eastern Brazil (Atlantic Forest) ……..... ***M. insignis***

– Calyx irregularly 2-4-lobed; corolla lilac to deep purple; southern Mexico, the Antilles, Central America and South America (Amazon basin) ……..…..….. 2

2 Inflorescence racemose ….……...……..…… ***M. obovata***

– Inflorescence botryoid or a thyrse ……....…...…….. 3

3 Leaflet coriaceous, stem quadrangular in cross section …….....…. ***M. insculpta***

– Leaflet chartaceous, stem cylindrical in cross section ……..……...…… 4

4 Branches densely covered with simple eglandular trichomes; leaflets tomentose abaxially ……..……...…….…….. ***M. tomentosa***

– Branches densely covered with stipitate glandular trichomes; leaflets lanuginose abaxially ……..……...…….. ***M. lanuginosa***

1. *Martinella insculpta* Sprague & Sandwith, Bull. Misc. Inform. Kew 1934(3):101. 1934.

Figure 3

Martinella iquitoensis A. Samp., Ann. Acad. Bras. Sci. 7:123. 1935. [*Martinella iquitosensis*, orth. var.]. TYPE: PERU. Loreto: Iquitos, 23 February 1924, *J.G. Kuhlmann 1492* (holotype: RB-00536899!; isotypes: RB-00537289!, K-000449503 image!).

Martinella manaosiana A. Samp., Bol. Mus. Nac. RJ. 12(3-4):84. 1936. TYPE: BRAZIL. Amazonas: Manaus, Capuêra de terra firme, Villa Belizario, 25 July 1931, *A. Ducke* (holotype: RB-00536900!; isotype: K-000449502 image!, MO-074517 image!, R-000028732!).

Type. Guyana. Unknown locality, *Drake* s.n. (holotype: K-000449501 image!).

Description. *Lianas*; branches with solid pith, tetragonal when mature, cylindrical when young, green with dark blotches, drying brown or black, smooth, glabrescent, with simple eglandular trichomes and scattered patelliform glandular trichomes in higher densities at interpetiolar region; prophylls of the axillary buds covered with simple eglandular trichomes, with few patelliform glands. *Leaves* 2-foliolate, with the terminal leaflet generally modified into a trifid tendril; petioles terete, pulvinated, 31–73 mm long, glabrous, with few patelliform glandular trichomes; petiolules terete, pulvinated, 23–65 mm long, glabrous, with few patelliform glandular trichomes; leaflets discolorous, with abaxial surface lighter than the adaxial surface, coriaceous, ovate, apex acuminate, base cuneate or truncate, margins entire and slightly revolute, 15–32 × 8.2–23.5 cm, adaxial surface glabrous with simple eglandular trichomes at canaliculi of veins, abaxial surface glabrescent, with patelliform glandular trichomes concentrated near base and scattered along the midvein. *Inflorescences* botryoid, 8.7–22.3 cm long, puberulent with simple eglandular trichomes, and stipitate and patelliform glandular trichomes; bracts linear, 1.1–1.2 mm long, puberulent, with simple eglandular trichomes and stipitate glandular trichomes; pedicels terete, 11.1–15.2 mm, puberulent, with simple eglandular trichomes and stipitate glandular trichomes. *Flowers* with calyx green, chartaceous, campanulate, 11.8–16.8 × 5.5–8 mm, densely covered with simple eglandular trichomes and stipitate glandular trichomes, with few patelliform glandular trichomes, lobes 2-4, apex mucronate, puberulent; corolla dark magenta, membranous, 41.3–69.4 mm long, narrowly tubular basal portion 15–27.4 long × 2.9–4.3 mm wide, upper campanulate portion 26.3–41.9 long × 12–16.6 mm wide, slightly curved, lobes subcircular, ca. 8.7 × 12.7 mm; stamens in two lengths, longer ones 16–21.2 mm, shorter ones 9.8–14 mm, thecae 2.9–3.1 mm, glabrous; staminode ca. 1.8 mm, glabrous; gynoecium 34.8–45.2 mm long; ovary glabrous; style glabrous; stigma lanceolate, glabrous; nectariferous disk ca. 4.3 × 0.9 mm. *Capsules* linear, 70–88 × 1.1–1.6 cm, glabrous. *Seeds* ca. 4.7 × 0.9 cm.

Distribution and habitat. *Martinella insculpta* is widely distributed through the Amazon and in wet forests in Central America (Figure 4). This species occurs in *terra firme* forests, from 59–650 m a.s.l.

Etymology. The specific epithet means carved, engraved, referring to the aspect of leaflet venation on both sides, but especially on the adaxial surface.

Phenology. Flowering specimens were collected from February to November, while fruiting specimens were collected from August to December.

Figure 3. *Martinella insculpta* Sprague & Sandwith. **A** Flowering branch; **B** Interpetiolar region with patelliform glandular trichomes and minute and triangular prophylls; **C** Trifid tendril; **D** Abaxial side of leaflet with patelliform glandular trichomes; **E** Calyx external view; **F** Calyx indumentum; **G** Open flower showing anthers, trichome distribution, and reduced (ca. 1 mm) staminode; **H** Gynoecium; **I** Fruit, flattened with a smooth surface; **J** Winged seed. Illustrated by Klei Sousa, based on B.L. Stannard 423, SPF; E.Y. Kataoka 372 and 407, SPF; G.T. Prance 14914, INPA; L.O.A. Teixeira 958, INPA.

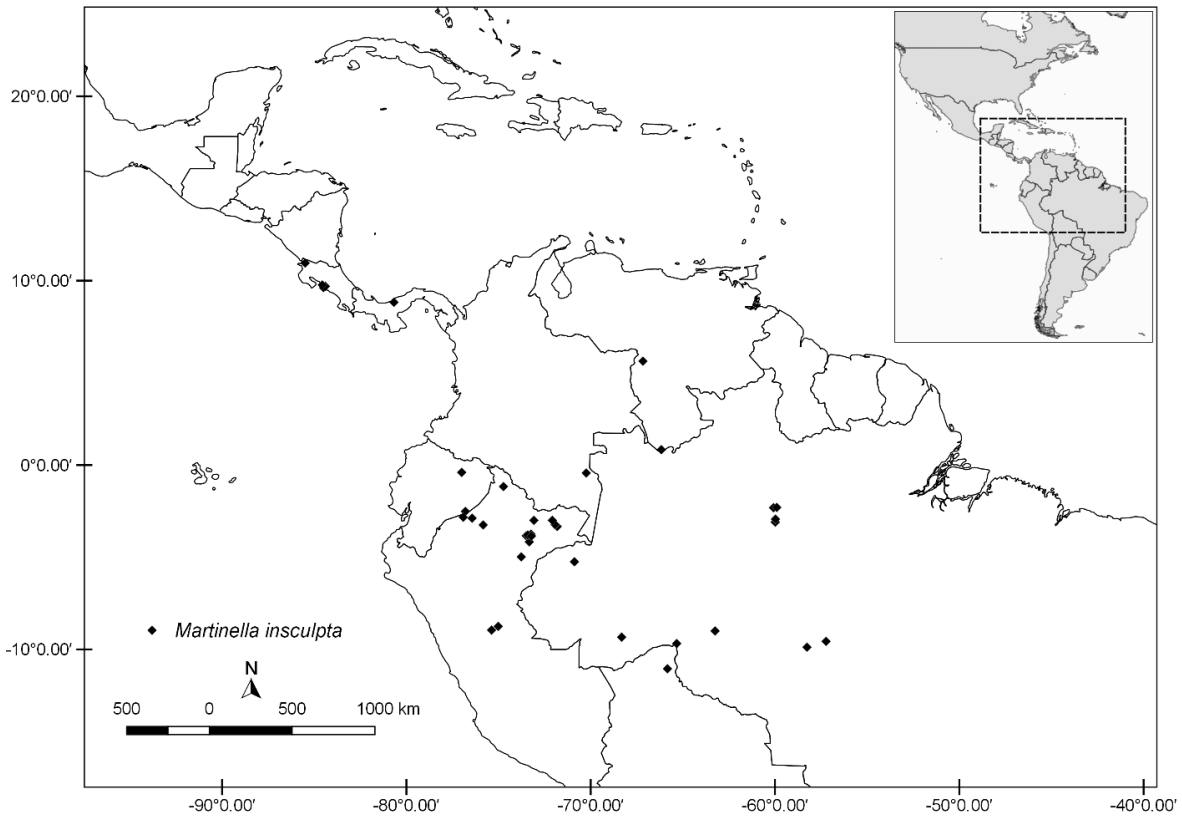

Figure 4. Distribution map of *Martinella insculpta*.

Conservation status. Least Concern (LC) based on the EOO of 4,002,910 km², and as Endangered (EN) based on the AOO of 164 km².

Discussion. We noticed an older name, *Martinella insculpta* Sprague & Sandwith (published in 1934), for *Martinella iquitoensis* A. Samp., which was only effectively published in 1935. Therefore, we synonymise *M. iquitoensis* under *M. insculpta*. In previous taxonomic studies (Floras), *Martinella insculpta* and *M. obovata* were considered part of a species complex due to overlapping morphological characters. However, new collections made in the field and a comprehensive analysis of herbarium specimens collected throughout the known geographic range of this species indicated that these two taxa indeed represent distinct units. Our decision was based on the following characters: (i) shape of mature stems in cross section (quadrangular in *M. insculpta* versus cylindrical in *M. obovata*), (ii) apex of petioles and petiolules (pulvinated in *M. insculpta* versus non-pulvinated in *M. obovata*), (iii) leaflet texture and size (coriaceous and consistently larger in *M. insculpta* versus chartaceous and smaller in *M. obovata*), and (iv) inflorescence structure (botryoid in *M. insculpta* and racemose in *M. obovata*). The combination of these distinctive morphological characters will aid in the correct identification of specimens that are often misidentified in herbarium sheets. In addition, a densely sampled phylogeny of *Martinella* revealed that *M. insculpta* and *M. obovata* indeed represent distinct evolutionary

lineages (Kataoka and Lohmann in prep.) providing further support for the recognition of these two taxa as separately evolving lineages with clear diagnostic traits.

Common uses. The bark of the roots of *M. insculpta* and *M. obovata* are used in traditional medicine.

Local names. *Martinella insculpta* and *M. obovata* are known by the local names *raiz dos olhos* (Brazil), *yuquilla toxju* (Peru), *raíz de ojo* and *uarakú-manaté* (Venezuela).

Specimens examined. BOLIVIA. Beni: Province of Vaca Diez, 17 km from the road between Riberalta and Guayaramerín on the old road to Cachuela Esperanza, ca. 18 km E of Riberalta. Primary forest, 230 m, 11°3'S, 65°50'W, 4 Sept 1981, *J.C. Solomon 6108* (MO). **BRAZIL. Acre**: Bujari, Estrada de acesso à área da Floresta do Antimary sem atividade de manejo florestal, 196 m, 9°19'46.1"S, 68°18'56.2"W, 10 Dec 2016, *E.Y. Kataoka 404* (SPF), Lado direito da entrada da trilha de acesso à torre de observação, 226 m, 9°20'5.3"S, 68°19'13.1"W, 12 Dec 2016, *E.Y. Kataoka 407* (SPF). **Amapá**: Rio Falsino, approx. 10 km upstreamf confluence with rio Araguari. West bank. Primary forest on terra firme, undulating terrain, 0°50'S, 51°45'W, 29 Sept 1983, *B.V. Rabelo 2386* (SPF). **Amazonas**: Rainforest, vicinity of Maturacá Mission, near rio Maturacá, on trail to helioport, 19 Oct 1970, *J.A. Steyermark 104035* (IAN), Rio Cuieiras just below mouth of rio Branquinho. Capoeira, 26 Sept 1971, *G.T. Prance 14914* (INPA, MG, R), Rio Purus, rio Ituxi. Serra near Namorado Novo between Rio Curuquetê and Rio Madeira at Abunã. Forest on Terra Firme, 5 Aug 1971, *G.T. Prance 14717* (INPA, MG, R); Barcelos, Rio Negro, próximo ao rio Arara, 1 May 1973, *A. Loureiro 37901* (INPA, SPF); Humaitá, Estrada Humaitá-Lábrea, km 59, a 6 km ao norte. Mata de terra firme, com muito Babassu, 6 June 1982, *L.O.A. Teixeira 958* (INPA, MG); Manaus, 22 June 1882, *Schwacke 3610* (RB), A partir da entrada da Reserva pela AM-010 (km 26), estrada sentido alojamento, lado esquerdo na bifurcação entre estrada de acesso ao alojamento e estação meteorológica, 139 m, 2°55'59"S, 59°58'31.9"W, 21 Sept 2016, *E.Y. Kataoka 344* (SPF), Bosque da Ciência do INPA, próximo ao Paiol da Cultura, subindo pela escadaria larga, 84 m, 3°5'56.5"S, 59°59'10.5"W, 20 Sept 2016, *E.Y. Kataoka 342* (SPF), Bosque da Ciência do INPA, próximo ao tanque dos peixes-boi, 69 m, 3°5'50.6"S, 59°59'15.1"W, 20 Sept 2016, *E.Y. Kataoka 339* (SPF), Estrada do Aleixo, km 7, 7 July 1977, *W.A. Rodrigues 9707* (INPA, SPF), Estrada do Aleixo, near Manaus; turnoff to Rio Negro at km 11 past INPA, 2 Dec 1974, *A.H. Gentry 13024* (INPA), Grounds of INPA at Manaus, 5 Apr 1974, *A.H. Gentry 11208* (INPA), Igapó, 14 June 1882, *C.A.W. Schwacke 425* (R), Igapó, 22 June 1882, *C.A.W. Schwacke 463* (R), Sede do INPA, Estrada do Aleixo, 30 July 1973, *P.L. Lisboa 6* (INPA); Presidente Figueiredo, Vila de Balbina, Represa da UHE de Balbina. Mata de beira de rio, 27 June 2007, *J.A.C. da Silva*

1294 (INPA); Rio Preto da Eva, Estrada Manaus-Itacoatiara, km 90, rio Preto. Terra firme, solo arenoso, 30 July 1961, *W. Rodrigues 2203* (INPA); Santa Isabel do Rio Negro, Rio Uneiuxi, Makú indian village, 300 km above mouth. Indian plantation, 23 Oct 1971, *G.T. Prance 15557* (INPA). **Mato Grosso**: Apiacás, Margem da estrada de acesso Paranaíta - Apiacás, 233 m, 9°33'53.3"S, 57°13'44.5"W, 12 Nov 2016, *E.Y. Kataoka 370* (SPF); Cotriguaçu, Margem da estrada de acesso Nova Bandeirante - Cotriguaçu, ca. 10 Km após travessia do rio Juruena, 251 m, 9°52'40.4"S, 58°15'47.1"W, 13 Nov 2016, *E.Y. Kataoka 372* (SPF). **Rondônia**: Rio Machado, curso inferior, igapó, Feb 1981, *M. Goulding 1324* (MG); Porto Velho, Represa Samuel southern end of E dike near quarry by road, ca 2 km S of end of main dike. Upland hillside forest, 9º00'S, 63º15'W, 7 June 1986, *W. Thomas 4974* (INPA). **COLOMBIA. Antioquia**: Primary/old secondary forest on west bank of river, 2 km N of Quebrada La Tirana. Tropical Wet/Very Wet Forest Transition zone. Rainfall approx. 4400 mm/year. Vic. Planta Providencia 28 km SW of Zaragoza. Valley of Río Anorí in areas surrounding the confluence of Quebrada La Tirana and Río Anorí, approx. 3 km upriver from Planta Providencia, 24 Mar 1977, *W.S. Alverson 266* (MO); San Luis, Autopiste Medellín - Bogotá. Sector Río Samaná - Río Claro, San Luis - Antioquia, 500 m, 3 Dec 1981, *J.J. Hernandez 99* (QCA). **COSTA RICA. Guanacaste**: Liberia, P.N. Guanacaste. Cuenca del Tempisque. Volcán Orosí, Estación Biológica Maritza. Trail to Cacao Station, ca. 1 km from Maritza comedor, ~ 100 km into first patch of primary forest. N side of trail. 0.1 ha Transect Maritza. Premontane moist forest, 650 m, 10°57'19.1748"N, 85°29'29.6432"W, 12 Mar 2003, *B. Boyle 7052* (MO). **Puntarenas**: Cantón de Garabito, R.B. Carara. Cuenca del Tárcoles. Sector Bijugual. Entrando a los bosques poco intervenidos, 600 m, 9°46'0"N, 84°34'0"W, 14 May 1998, *A. Rodríguez 3367* (MO). **San José**: Turrubares, San Juan De Mata. Area no protegida. Montelimar, 85 m, 9°37'24"N, 84°29'55"W, 15 Oct 2001, *A. Estrada 3072* (MO); Z.P. La Cangreja. Mastatal de Puriscal. Bosque primario en parches remanentes, 300 m, 9°41'45"N, 84°23'47"W, 21 Oct 1992, *J.F. Morales 910* (MO). **ECUADOR. Napo**: Coca (Puerto Francisco de Orellana), 8 km al N de Coca. Bosque humedo tropical. Suelo aluvial fertil. Bosque secundario, 250 m, 0°24'S, 77°0'W, 8 Apr 1985, *W. Palacios 272* (MO, QCA, QCNE); **Pastaza**: Kapawi, Río Pastaza. Village area, secondary and primary forests, and pastures, 235 m, 2°31'S, 76°48'W, 25-29 July 1989, *W.H. Lewis 14026* (QCNE). **PANAMA.** Colón, Teck Cominco Petaquilla mining concession. Forest along road, 220 m, 8°49'39"N, 80°40'28"W, 20 Feb 2008, *G. McPherson 20083* (MO). **PERU. Alto Amazonas**: Puranchim, río Sinchiyacu. Rainforest, terra firma and palm lowlands, 200 m, 2°50'S, 76°55'W, 3-7 Dec 1988, *W.H. Lewis 14389* (MO); Andoas, Capihuari, 5 km NE of Andoas on Rio Capihuari, near Ecuador border along oil pipeline, lateritic uplands alternating

with Mauritia swamps, 240 m, 17 Nov 1979, *A.H. Gentry 28200* (MO). **Huanuco**: Carretera marginal (in construction) km 4-12 south from Km. 86 of Pulcallpa-Tingo Maria road, 270 m, 8°45'S, 75°1'W, 1 June 1983, *A.H. Gentry 41384* (MO). **Loreto**: Pampa hermosa and vicinity, Río Corrientes, 1 km S of junction with Río Macusari. Low rainforest, mostly terra firma with scattered white sand, 160 m, 3°15'S, 75°50'W, 3-20 Dec 1985, *W.H. Lewis 9975* (MO). **Maynas**: Iquitos, Carretera de Peña Negra, ca. 7 km de Quisto Cocha, en terreno arenoso, monte despejado, 150 m, 12 July 1982, *M. Rimachi Y. 6180* (MO), Carretera Iquitos-Nauta, km 45. Bosque primario, 120 m, 4°10'S, 73°20'W, 12 June 1987, *R. Vásquez 9172* (MO), Iquitos, trail between extension of Yavari and Versailles, mostly highly disturbed upland scrub area, 11 Feb 1974, *M. Rimachi Y. 835* (MO), Puerto Almendras, Río Nanay. Bosque primario en suelo con arena blanca, 122 m, 3°48'S, 73°25'W, 18 July 1988, *R. Vásquez 10977* (MO), Río Nanay, Carretera de Picuruyacu, trocha de la granja de la marina, en terreno arenoso, 160 m, 26 July 1982, *M. Rimachi Y. 6270* (MO), Rio Momon; Momoncillo (Caserio), borde de pastizal de ganado, 17 Aug 1976, *J. Revilla 979* (MO), Santo Tomas (Iquitos), 100 m, 3°51'S, 73°13'W, 14 Apr 1979, *F. Ayala 1793* (MO); Napo, Environs of Río Santa María. Collected one hour upstream of the Secoya village of "Vencedor", 4 hours by outboard from the mouth of the Santa Maria river, 100 m, 1°10'S, 74°44'W, 15 May 1982, *S.R. King 492* (MO). **Pebas**: Primary forest behind Brillo Nuevo. Loreto, Rio Yaguasyacu, Brillo Nuevo, 11 Sept 1981, *R. Hahn 123* (MO). **SURINAME. Brokopondo**: Brownsweg, Near Brownsweg Nature Park. Little disturbed high mesophytic rain forest on slope, gravelly clay soil, 240 m, 4°35'41.28"N, 55°6'0"W, 1 Oct 2005, *K. Van Kerckhove MVK 114* (SPF). **VENEZUELA. Amazonas**: Atures, Transecto desde bosque alto denso con tatucos a orillas de río Cataniapo, hasta bosque medio ralo en parte alta de colina, a 1 km al oeste de San Pedro de Cataniapo, a unos 60 km al sur-este de Puerto Ayacucho, 100 m, 5°38'N, 67°10'W, 7 Mar 1981, *F. Guanchez 923* (MO); Rio Negro, 0 to 2 km west of Cerro de La Neblina base camp, which is on Río Mawarinuma, 140 m, 0°50'N, 66°10'W, 7 Feb 1984, *R. Liesner 15711* (MO), Near Cerro de La Neblina base camp, which is on Río Mawarinuma, 140 m, 0°50'N, 66°10'W, 25 Mar 1984, *R. Liesner 16956* (MO). **Rio Negro**: Territorio Federal Amazonas, Neblina Massif, bongo (dugout) trip down rio Mawarinuma for c. 2 km + NW from base camp at mouth of canyon, 140 m, 0°50'N, 66°10'W, 31 Mar 1984, *B.L. Stannard 423* (SPF).

2. ***Martinella insignis* A.H. Gentry ex Zuntini & L.G. Lohmann, Phytokeys 37:17-21. 2014.**

   Figure 5

Type. Brazil. Bahia: Itamaraju, Rodovia Itamarajú-Teixeira de Freitas, 3 km de Itamarajú (BR-101). Fazenda Chapadão, 3 November 1983, *R. Callejas, A. M. de Carvalho & L. M. Silva 1629* (holotype: MBM-94960 image!; isotypes: MO-074484 image!, NY-00483568 image!, RB-00058792!).

Description. *Lianas*; branches with solid pith, cylindrical, green, drying brown, striated, pubescent, densely covered with stipitate glandular trichomes; prophylls of the axillary buds densely covered with stipitate glandular trichomes. *Leaves* 2-foliolate, with the terminal leaflet generally modified into a trifid tendril; petioles terete, not pulvinated, 34.6–48.4 mm long, covered with stipitate glandular trichomes; petiolules terete, not pulvinated, 13.9–26.4 mm long, covered with stipitate glandular trichomes; leaflets discolorous, with abaxial surface lighter than the adaxial surface, membranous, ovate, apex acuminate to caudate, base cordate, margins entire and slightly revolute, 6.4–8.8 × 3.6–5.2 cm, adaxial surface glabrous, with stipitate glandular trichomes on the margins and at the canaliculi of main veins, abaxial surface pubescent, densely covered with stipitate glandular trichomes at main veins, pocket domatia on the axils of primary and secondary veins, few patelliform glandular trichomes concentrated near base and scattered along the midvein. *Inflorescences* in compound thyrsi, 7.5–11.5 cm long, sparsely to densely covered with stipitate glandular trichomes; bracts linear to narrowly elliptic, 5.2–20.2 × 0.6–2.5 mm, pubescent, densely covered with stipitate glandular trichomes; pedicels terete, 6.3–11.2 mm, sparsely to densely covered with stipitate glandular trichomes. *Flowers* with calyx pale green, chartaceous, campanulate, 13.1–17.2 × 6.1–11.9 mm, densely covered with stipitate glandular trichomes, lobes 5, apex aristate, aristae 1.8–4.3 mm long, pubescent, densely covered with stipitate glandular trichomes; corolla yellow, membranous, 41.5–47.3 mm long, narrowly tubular basal portion 15.8–18.5 mm long × 2.5–4.9 mm wide, upper campanulate portion 23.1–32 mm long × 12.5–15.2 mm wide, slightly curved, lobes subcircular, 7.5–9.1 × 8.6–9.5 mm; stamens in two lengths, longer ones 12.4–13.3 mm, shorter ones 12.0–12.5 mm, thecae 2.5–2.7 mm, glabrous; staminode 1.3–3.4 mm, glabrous; gynoecium 30.9–35 mm long; ovary glabrous; style glabrous; stigma lanceolate, glabrous; nectariferous disk 2.3–3.1 × 1.0–1.2 mm. *Capsules* linear, 40.2–90 × 1.1–1.5 cm, pubescent when immature, glabrous when developed. *Seeds* ca. 4.5 × 1.2 cm.

Distribution and habitat. *Martinella insignis* is endemic to the northern portion of the Brazilian Atlantic Forest (see Figure 6) and grows in sandy soils, in areas between 62–590 m a.s.l.

Etymology. The specific epithet means conspicuous, readily distinguishable, referring to the contrasting corolla colour when compared to other species.

Figure 5. *Martinella insignis* A.H. Gentry ex Zuntini & L.G. Lohmann. **A** Flowering branch; **B** Interpetiolar region; **C** Stipitate glandular trichomes; **D** Flower in lateral view; **E** Calyx (opened) and gynoecium; **F** Fruit; **G** Roots with swollen portions. Illustration reproduced from Zuntini and Lohmann (2014); illustrated by Klei Sousa, based on A.R. Zuntini 152 and 321, SPF; D. Sucre 5519, RB.

Phenology. Flowering specimens were collected between October and February, while fruiting specimens were collected in January and November.

Conservation status. Vulnerable (VU) based on the EOO of 12,721 km², and as Endangered (EN) based on the AOO of 32 km².

Discussion. *Martinella insignis* is the only representative of the genus that occurs in the Atlantic Forest, where it is likely to be very rare, given the few collections made to date. In addition, *M. insignis* is remarkably distinctive from its congeneric species due to the membranous leaflets with pocket-shaped domatia on the abaxial surface, 5-lobed and aristate calyces, and yellow corollas.

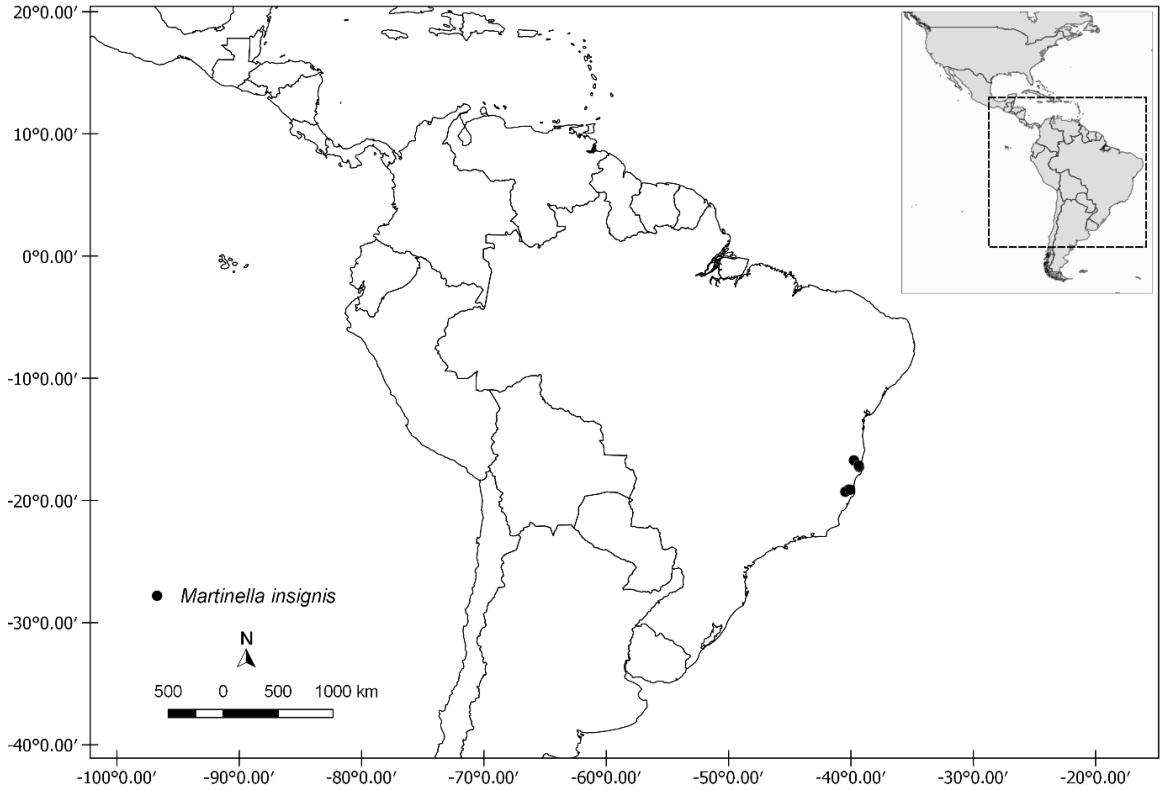

Figure 6. Distribution map of *Martinella insignis*.

Specimens examined. BRAZIL. Bahia: Itamaraju, Rodovia Itamaraju-Teixeira de Freitas (BR 101), 3 km de Itamaraju, Fazenda Chapadao. Mata higrófila sul Baiana, 3 Nov 1983, *R. Callejas 1629* (RB), RPPN Fazenda Riacho das Pedras, prop. Gersino Antônio Bronzon, Mata de Tabuleiro com pequenos distúrbios (extração seletiva), 78 m, 17°8'48"S, 39°21'53"W, 12 Feb 2007, *R.A.X. Borges 825* (RB, SPF). **Espírito Santo**: Governador Lindemberg, Pedra de Santa Luzia, prop. Firmino Sottele, 420-590 m, 19°17'17"S, 40°27'56"W, 7 Nov 2007, *V. Demuner 4481* (SPF); Linhares, Reserva Natural Vale. MME, 5 Oct 2011, *A.R. Zuntini 321* (SPF); Sooretama, Reserva Natural da Companhia Vale do Rio Doce ("Reserva de Linhares"), 62 m, 19°6'59.7"S, 40°4'21.7"W, 14 Dec 2007, *A.R. Zuntini 151* (SPF).

3. *Martinella lanuginosa* Kataoka & L.G. Lohmann sp. nov.

Figure 7

Type. PERU. Madre de Dios: Tambopata, Dist. Puerto Maldonado, Fundo Concepción, bosque ribereño, 200 m, 12º32'S 69º03'W, 22 August 2003, *I. Huamantupa, J. Vargas & J. Quispe, 3698* (holotype: CUZ not seen; isotype: MO-2981780 not seen, SPF-240817!).

Diagnosis. *Martinella lanuginosa* differs from other Amazonian species of *Martinella* by the lanuginose leaflets on the abaxial surface and inflorescences arranged in lax thyrsi, contrasting with the glabrous or tomentose leaflets on the abaxial surface and inflorescences botryoid or racemose found in all other Amazonian species.

Description. *Lianas*; branches with solid pith, cylindrical, green, drying light brown, smooth, pubescent, densely covered with stipitate glandular trichomes, with scattered patelliform glandular trichomes more frequently at interpetiolar region; prophylls of the axillary buds densely covered with stipitate glandular trichomes. *Leaves* 2-foliolate with the terminal leaflet generally modified into a trifid tendril; petioles terete, not pulvinated, 40–83.7 mm long, densely covered with stipitate glandular trichomes with few scattered patelliform glandular trichomes; petiolules terete, not pulvinated, 19.4–70.1 mm long, densely covered with stipitate glandular trichomes and occasional patelliform glandular trichomes; leaflets discolorous, with abaxial surface lighter than the adaxial surface, chartaceous, ovate, apex acuminate, base cordate, margins entire and slightly revolute, 6.5–14.4 × 5.2–11.2 cm, adaxial surface glabrous, with simple eglandular trichomes and stipitate glandular trichomes at canaliculi of veins, abaxial surface lanuginose, densely covered with simple eglandular trichomes with few patelliform glandular trichomes distributed along the midvein. *Inflorescences* in thyrsi, 6.5–11 cm long, densely covered with simple eglandular trichomes and stipitate glandular trichomes, with few patelliform glandular trichomes; bracts linear, 1.5–3 mm long, puberulent, densely covered with stipitate glandular trichomes; pedicels terete, 6.5–15.5 mm, densely covered with stipitate glandular trichomes. *Flowers* with calyx pale, chartaceous, campanulate, 18–21.6 × 8.9–12.3 mm, puberulent, covered with simple eglandular trichomes and stipitate glandular trichomes, lobes 2-4, apex mucronate, puberulent; corolla lilac, membranous, 62.6–69.6 mm long, narrowly tubular basal portion 20.6–21.9 mm long × 5.3–6.2 mm wide, upper campanulate portion 42–47.7 mm long × 16.9–20.7 mm wide, lobes subcircular, 7.3–8.3 × 12.4–13 mm; stamens in two lengths, longer ones 17.9–18.6 mm, shorter ones 12.3–13.3 mm, thecae 3–3.2 mm, glabrous; staminode ca. 1.2 mm, glabrous; gynoecium ca. 42 mm long; ovary

glabrous; style glabrous; stigma lanceolate, glabrous; nectariferous disk 3.3–3.4 × 1.1–1.15 mm. *Fruits* and *seeds* not seen.

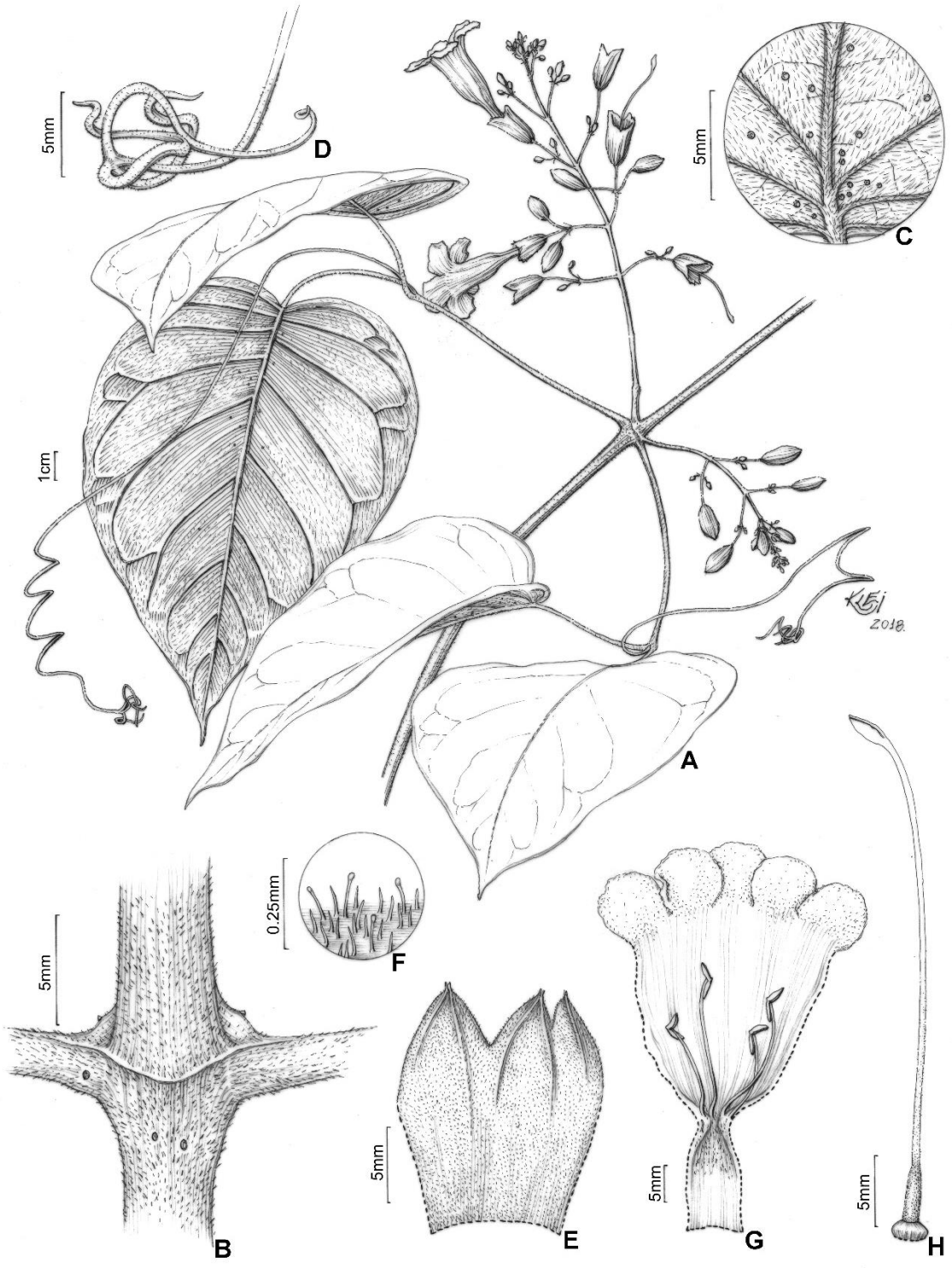

Figure 7. *Martinella lanuginosa* Kataoka & L.G. Lohmann. **A** Flowering branch; **B** Interpetiolar region with patelliform glandular trichomes; **C** Abaxial side of leaflet with lanuginose indumentum and patelliform glandular trichomes; **D** Trifid tendril; **E** Calyx external view; **F** Detail of calyx indumentum; **G** Open flower showing anthers, trichome distribution, and reduced (ca. 1.5 mm) staminode; **H** Gynoecium. Illustrated by Klei Sousa, based on A.H. Gentry 27233, MO; I. Huamantupa 3698, R. Rueda 414, SPF.

Distribution and habitat. *Martinella lanuginosa* is restricted to the western portion of the Amazon (see Figure 8), with known occurrences in *terra firme* in Brazil (Acre state) and eastern Peru.

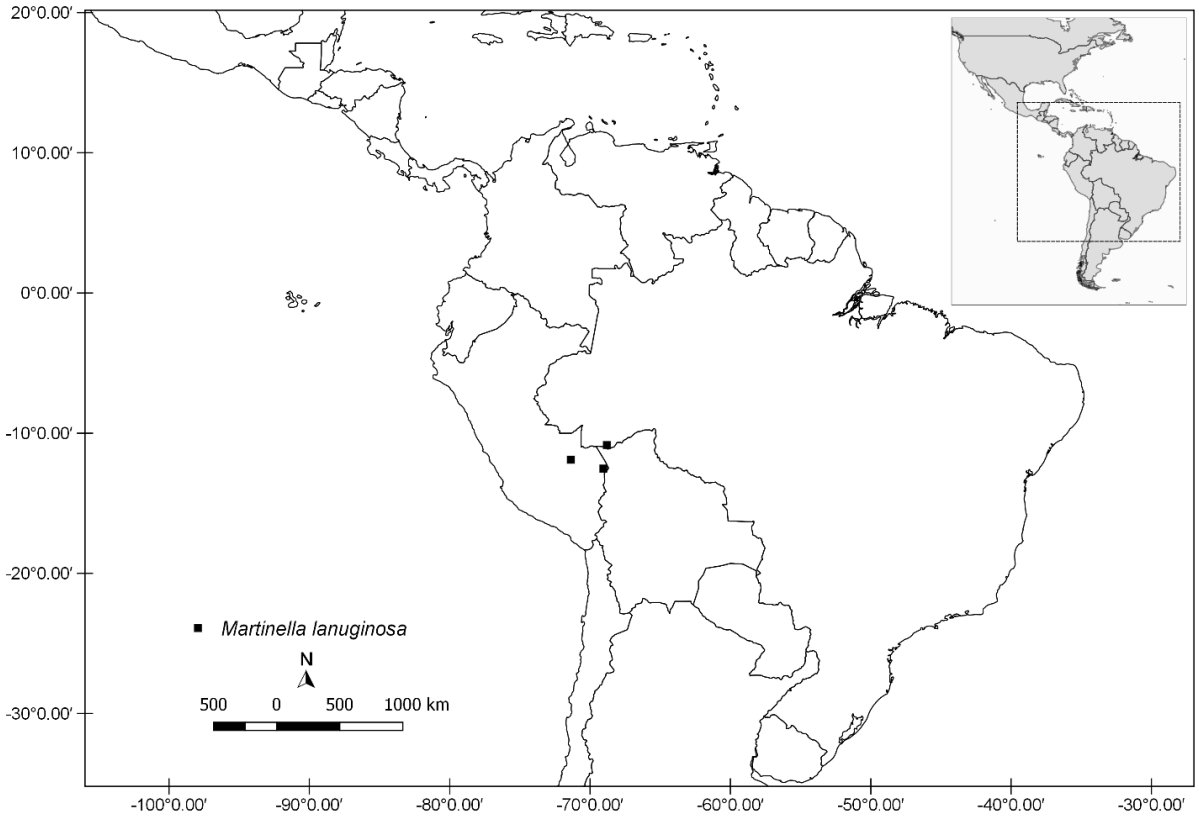

Figure 8. Distribution map of *Martinella lanuginosa*.

Etymology. The specific epithet relates to the lanuginose indumentum on the abaxial surface of leaflets, which confers a wool-like feel when touched.

Phenology. Flowering specimens were collected in late October.

Conservation status. Near Threatened (NT) based on the EOO of 24,543 km², and as Endangered (EN) based on the AOO of 12 km².

Discussion. *Martinella lanuginosa* is a new taxon discovered based on morphology and confirmed to represent an independent lineage based on molecular phylogenetic data (Kataoka and Lohmann in prep.). This new taxon is sister to *M. insculpta* and *M. obovata*. *Martinella lanuginosa* is readily distinguished from its Amazonian sister-taxa by the leaflets lanuginose on the abaxial side, a trait unique to this species. Very few specimens of *M. lanuginosa* have been collected, none of them bearing fruits and/or seeds.

Specimens examined. BRAZIL. Acre: Brasileia, Reserva Extrativista Chico Mendes. Seringal Porongaba, Colocação Dois Irmãos. Terra Firme, 10°51'N, 68°48'W, 28 Oct 1991, *L.*

Ferreira 109 (NY). **COLOMBIA**. **Bolivar**: Turbaco, Fundación Jardín Botánico "Guillermo Piñeres" y alrededore, 130 m, 23 May 1992, *R. Rueda 414* (MO). **PERU**. **Alto Amazonas**: Loreto, Yurimaguas Estación Experimental de North Carolina State, 10 years old second growth dominated by *Cecropia*, on sandy and yellow lateritic soil, 180 m, 9 Oct 1985, *A.H. Gentry 52144* (MO). **Madre de Dios**: Cocha Cashu camp, Manu National Park, Río Manu, mature forest on alluvial soil, 380 m, 24 Oct 1979, *A.H. Gentry 27233* (MO); Manu, Parque Nacional Manu, Estacion Biologica de Cocha Cashu. Mature floodplain, 150 m, 11°54'S, 71°22'W, 6 June 2001, *L.G. Lohmann 616* (MO).

4. *Martinella obovata* (Kunth) Bureau & K. Schum., in Mart., Fl. Bras. 8(2):161, tab. 84. 1896.

Figure 9

Spathodea obovata Kunth, Nov. Gen. Sp. (quarto ed.) 3:147. 1818 [1819]. *Bignonia obovata* (Kunth) Spreng., Syst. Veg. 2:830. 1825. *Macfadyena obovata* (Kunth) Miers, Proc. Roy. Hort. Soc. London 3:200. 1863. TYPE. Colombia. Magdalena: Turbaco, s.d., *F.W.H.A. von Humboldt & A.J.A. Bonpland 1391* (lectotype, designated here: P-00670823 image!).

Bignonia fockeana Miq., Linnaea 18:609. 1844. *Macfadyena fockeana* (Miq.) Miers, Proc. Roy. Hort. Soc. 3:200. 1863. TYPE. Suriname. Paramaribo, *Focke 924* (lectotype, designated here: U-0000750 image!).

Tabebuia cordata Benth., Bot. Voy. Sulphur 129. 1844. TYPE. Panama. Isthmus of Darién, *Barclay s.n.* (lectotype, designated here: K-000449504 image!).

Bignonia martinii DC., Prodr. 9:152. 1845. [as Martini]. *Martinella martinii* (DC.) Baill. ex K. Schum. In Engler & Prantl, Nat. Pflanzenf. 4(3b):216. 1894. TYPE. French Guiana. Cayenne, *Martin s.n.* (lectotype, designated here: P-00481520 image!; isolectotypes: P-00481521 image!, P-00481522 image!, U-0000749 image!, US-00125833 image!).

Doxantha longisiliqua Miers, Proc. Roy. Hort. Soc. 3:190. 1863. Non *Bignonia longisiliqua* Bert. ex Spreng., Syst. Veg. 2:830. 1825. nom. illeg. TYPE: Colombia. Santa Marta: *Bertero s.n.* (lectotype, designated here: G-DC-00133287 image!; isolectotype: MO-074584 image!).

Martinella gollmeri K. Schum. In Engler & Prantl, Nat. Pflanzenf. 4(3b):216. 1894. TYPE. Venezuela. *Gentry 10864* (neotype, designated here: MO-2241772!).

Anemopaegma leptosiphon Rusby, Mem. New York Bot. Gard. 7:354. 1927. TYPE. Bolivia. Ixiamos, 245 m, 15 December 1921, *M. Cardenas 1926* (holotype: NY-00313067 image!).

Arrabidaea duckei A. Samp., Bol. Mus. Nac. Rio de Janeiro 12(3-4):81. 1936. *Periarrabidaea duckei* (A. Samp.) A. Samp., Ann. Acad. Bras. Sci. 12:91. 1936. TYPE. Brazil. Manaus, *Ducke s.n.* (holotype: RB-00536852!; isotype: R-28626 [accession number]!).

Type. Colombia. Magdalena: Turbaco, s.d., *F.W.H.A. von Humboldt & A.J.A. Bonpland 1391* (holotype: P-00481520 image!).

Description. *Lianas*; branches with solid pith, cylindrical, green, drying brown, smooth, puberulent, covered with few stipitate glandular trichomes, with scattered patelliform glandular trichomes more frequently at interpetiolar region; prophylls of the axillary buds covered with simple eglandular trichomes. *Leaves* 2-foliolate, with the terminal leaflet generally modified into a trifid tendril; petioles terete, not pulvinated, 8.5–66 mm long, covered with stipitate glandular trichomes, with few scattered patelliform glandular trichomes; petiolules terete, not pulvinated, 5–40.4 mm long, covered with stipitate glandular trichomes, with occasional patelliform glandular trichomes; leaflets discolorous, with abaxial surface lighter than the adaxial surface (silver-like colour), chartaceous, ovate, apex acuminate, base cordate, margins entire and slightly revolute, 7.9–13.2 × 3.0–9.4 cm, adaxial surface glabrous, with simple eglandular trichomes and stipitate glandular trichomes at canaliculi of veins, abaxial surface glabrescent, with patelliform glandular trichomes concentrated near the base and scattered along the midvein. *Inflorescences* racemose, 11.9–15.5 cm long, sparsely covered with simple eglandular trichomes and stipitate and patelliform glandular trichomes; bracts linear, 1.5–2 mm long, puberulent, densely covered with simple eglandular trichomes and stipitate glandular trichomes; pedicels terete, 4–12.7 mm, puberulent, with simple eglandular trichomes and stipitate glandular trichomes. *Flowers* with calyx green, chartaceous, campanulate, 11.9–17.6 × 4.8–9.6 mm, puberulent, sparsely covered with simple eglandular trichomes and stipitate glandular trichomes, with few patelliform glandular trichomes, lobes 2-4, apex mucronate, puberulent; corolla light lilac to dark magenta, membranous, 55.2–58.5 mm long, narrowly tubular basal portion 15.6–17.2 long × 4.2–5.3 mm wide, upper campanulate portion 16.1–18.7 mm long × 14–15 mm wide, lobes subcircular, 10.5–11.7 × 12.7–15.7 mm; stamens in two lengths, longer ones 16–20 mm, shorter ones 12–12.6 mm, thecae 2.5–3.1 mm, glabrous; staminode 0.8–1 mm, glabrous; gynoecium 34–36.3 mm long; ovary glabrous; style glabrous; stigma lanceolate, glabrous; nectariferous disk 3.2–3.6 × 1.3 mm. *Capsules* linear, 20.5–116 × 1.1–2 cm, glabrous. *Seeds* ca. 4 × 1.1 cm.

Distribution and habitat. *Martinella obovata* is widely distributed through the Amazon and in wet forests of southern Mexico and Central America (Figure 10). This species

occurs between 0–1700 m a.s.l., in a wide range of habitat types within *terra firme* forests, preferentially along riverbanks, where the species is most common and abundant.

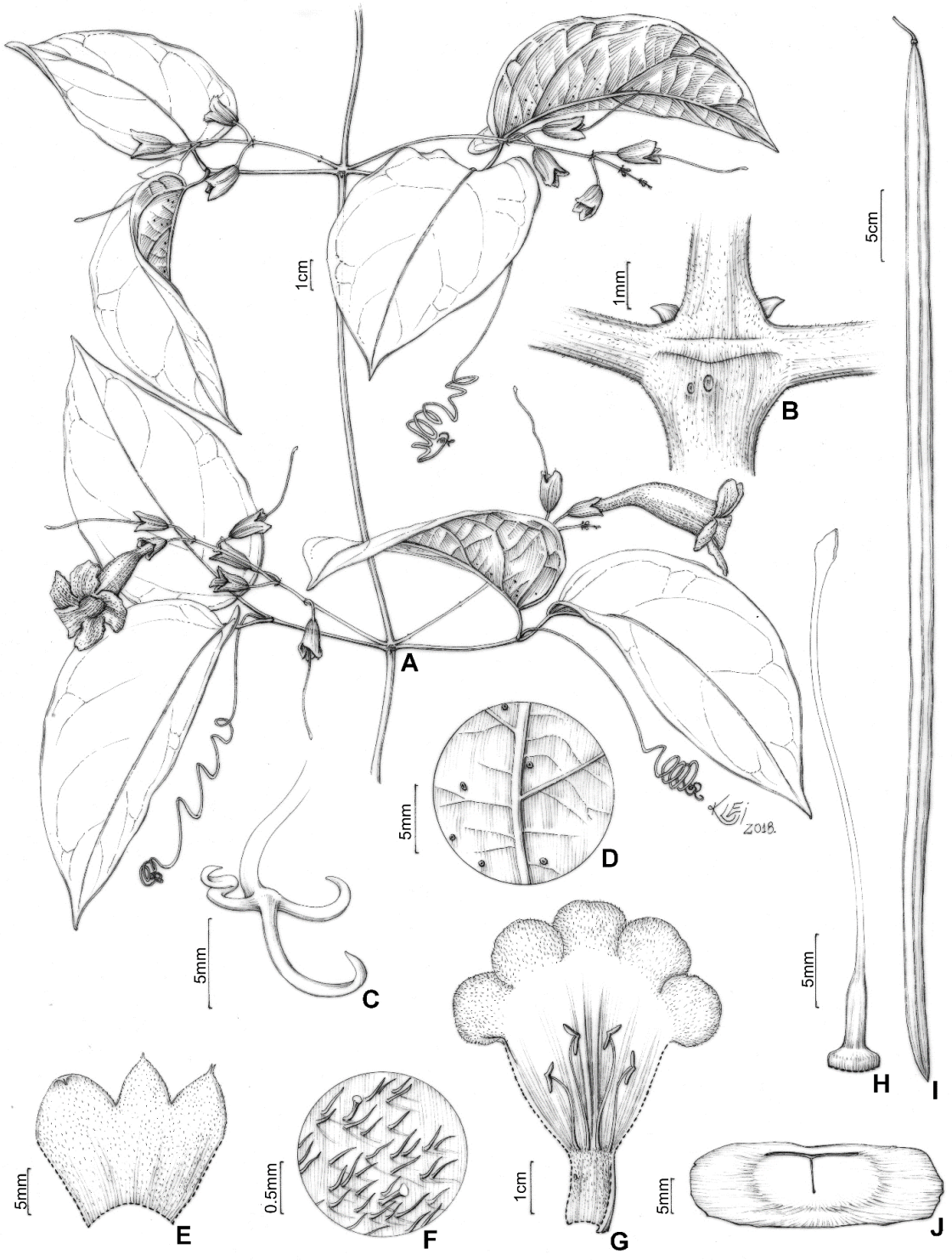

Figure 9. *Martinella obovata* (Kunth) Bureau & K. Schum. **A** Flowering branch; **B** Interpetiolar region with minute and triangular prophylls; **C** Trifid tendril; **D** Abaxial side of leaflet with patelliform glandular trichomes; **E** Calyx external view; **F** Detail of calyx indumentum; **G** Open flower showing anthers, trichome distribution, and reduced (ca. 1 mm) staminode; **H** Gynoecium; **I** Fruit flattened with a smooth surface; **J** Winged seed. Illustrated by Klei Sousa, based on E.Y. Kataoka 309, 329 and 360, SPF.

Phenology. Flowering and fruiting specimens were collected throughout the year.

Conservation status. Least Concern (LC) based on the EOO of 10,477,635 km², and as Vulnerable (VU) based on the AOO of 1,324 km².

Discussion. *Martinella obovata* is diagnosable by a combination of cylindrical stems in cross section, glabrous leaflets, racemose inflorescence, and calyx covered with simple eglandular and stipitate glandular trichomes. *Martinella obovata* is the most widely distributed species in the genus and occurs from southern Mexico to southern Amazon in a wide altitudinal range. Despite the wide geographic and elevational range, no clear morphological discontinuities were identified in our study. However, as expected, we did find some variation especially considering plasticity to local environmental conditions. Therefore, we recognise a widespread *M. obovata*, which is also supported by a robust phylogenetic inference for *Martinella* as a whole (Kataoka and Lohmann in prep.). The widespread *M. obovata* recognised here includes multiple synonyms, several of which required the designation of lectotypes or neotypes. Commentaries about the selection of those types are presented. *Spathodea obovata* Kunth was described based on a specimen from Turbaco (Colombia). We located a specimen annotated as holotype at P that matches the original description of this taxon and is here designate as lectotype. In the protologue of *Bignonia martinii,* De Candolle specifically cited a specimen deposited at P. Duplicates of the type collection are deposited at P, U and US. The sheet with flowers is here chosen as lectotype. *Bignonia fockeana* Miq. was described based on a specimen growing in a garden at Paramaribo, Suriname. We here designate a specimen collected by Focke at the same location and deposited at U as lectotype. *Tabebuia cordata* Benth. was described based on a specimen from Isthmus of Darien (Isthmus of Panama). A good quality specimen deposited at K and collected by Barclay is here designated as lectotype. *Bignonia longisiliqua* Bert. ex Spreng. is an illegitimate homonym. *Doxantha longiliqua* Miers was subsenquently published based on the same specimen of *B. longisiliqua*. The specimen cited in the protologue of *B. longisiliqua* and deposited at G-DC is here selected as a lectotype. The type specimen of *Martinella gollmeri* was destroyed at B; no isotypes or illustration have been found, and a neotype from Venezuela that matches the original description is here designated as a neotype.

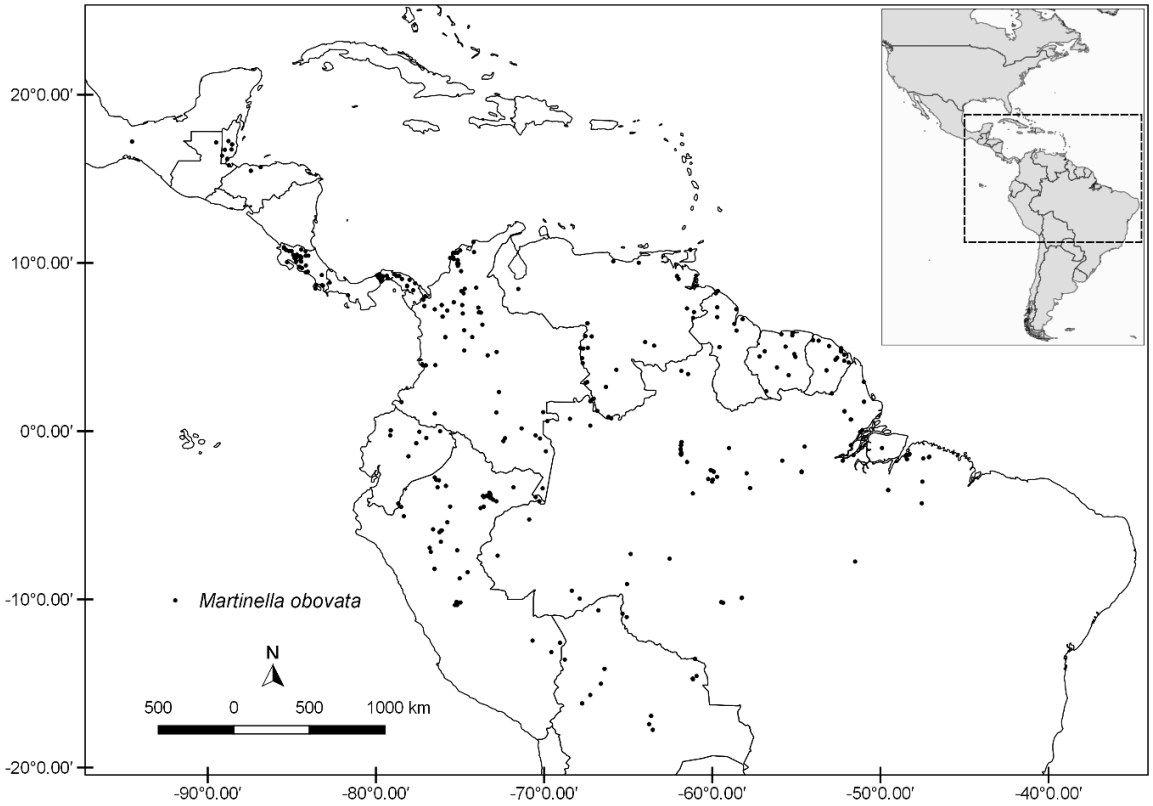

Figure 10. Distribution map of *Martinella obovata*.

Specimens examined. BELIZE. **Cayo**: Hummingbird highway, south of Belmopan, vicinity of mile 28, premontane wet forest, 70 m, 14 June 1973, *A.H. Gentry 8250* (MO), Vicinity of Grano de Oro lumber camp south of Millionario; disturbed forest and roadside, 500 m, 2 June 1973, *A.H. Gentry 7767* (MO). **BOLIVIA**. **Ballivian**: Comunidad Galilea, Canchon Tierra Negra, 200 m, 14°30'S, 66°37'W, 21 Oct 1994, *E. Rivero 183* (SPF). **Beni**: Isla de Espiritu, Prov. Ballivian, Espiritu en la zona de influencia del rio Yacuma. Sabana húmeda, 200 m, 13 Apr 1981, *St.G. Beck 5372* (MO). **Franz Tamayo**: Madidi, Chalalan, Sendero Silvador. Bosque amazonico preandino estacional, 350 m, 14°25'23"S, 67°55'26"W, 24 Nov 2004, *A. Araujo-M 1532* (SPF). **La Paz**: Prov. Nor Yungas, 4.1 km N of (below) Yolosa on road to Caranavi. Secondary growth, roadside, 1200 m, 16°11'S, 67°44'W, 6 Oct 1984, *J.C. Solomon 12474* (MO). **Santa Cruz**: Prov. Ichilo. Parque Amboró. Río Semayo, Nueva Palestina. A 35 km al E de la Ciudad de Santa Cruz, 480 m, 17°45.5'S, 63°32'W, 4 Mar 1990, *R.C. Quevedo S. 51* (MO). **Sara**: Camino de Santa Rosa del Sara a Buen Retiro, 274 m, 17°13'57"S, 63°39'1"W, 7 Jan 2009, *G.A. Parada 1394* (SPF). **BRAZIL**. Aramanahy, low and high land, 11 Jan 1932, *M.D. Cost 255* (IAN). **Acre**: Bujari, Margem direita do rio Antimary, sentido jusante a partir da ponte da BR-364, 154 m, 9°28'52.3"S, 68°20'55"W, 11 Dec 2016, *E.Y. Kataoka 406* (SPF);

Mâncio Lima, São Domingos, Campina, solo arenoso humoso, 7º23'57" S 72º45'41" W 23 Oct 1998, *C.A.C. Ferreira 11751* (INPA); Rio Branco, Próximo ao prédio do Herbário UFACPZ, ca. 60 m após fim da trilha de acesso do campus ao herbário, 182 m, 9°57'26.5"S, 67°52'27.8"W, 06 Dec 2016, *E.Y. Kataoka 390* (SPF). **Amapá**: Coastal Region survey. Right bank of rio Flechal, 1°45'N, 50°58'W, 13 Aug 1962, *J.M. Pires 52504* (IAN, MG), Rio Amapari, between Munguba and Serra do Navio, 25 Sept 1961, *N.Y.Bot. Garden 51176* (INPA), Rio Araguarí; terra firme, baixa, 22 July 1951, *R.L. Fróes 27594* (R), Rio Araguari, along river between Mongubas and Serra do Navio, 0°42'N, 51°45'W, 25 Sept 1961, *J.M. Pires 51176* (IAN, MG), Rio Araguari, porto Platon, 18 Sept 1961, *J.M. Pires 51057* (IAN, MG), Rio Araguari; terra firme, baixa, 22 July 1951, *R.L. Fróes 27592* (IAN), Rio Araguari; terra firme, baixa, 22 July 1951, *R.L. Fróes 27592a* (IAN); Matapi, Mata baixa, terra firme, solo argiloso e úmido, 28 Dec 1976, *B.G.S. Ribeiro 1645* (MG); Porto Grande, Fazenda Governador, Aporema, 10 Nov 1982, *M. Dantas 1428* (IAN). **Amazonas**: Estrada Manaus-Caracaraí km 39, Reserva Experimental de Silvicultura Tropical. Terra firme, solo arenoso, campina, 12 Sept 1977, *J. Ribamar 189* (INPA), Km 65, on road from Manaus to Boa Vista. Manioc plantation, sandy soil, 22 July 1974, *A. Lasseign P21164* (INPA), Mindu, em capoeira, 5 Sept 1947, *T. Guedes 14* (IAN, RB), Rio Negro, próximo ao rio Arara, 2º acampamento da SIDERAMA; mata de terra firme, solo arenoso, 2 May 1973, *A.A. Loureiro 37946* (RB, SPF); Barcelos, Parque Nacional do Jaú. Margem direita do Rio Negro, 35 m, 1°49'59.8"S, 61°29'38.5"W, 6 June 2016, *E.Y. Kataoka 247* (SPF), Rio Negro, between Ilha da Silva & Tapuruquara, 15 Oct 1971, *G.T. Prance 15281* (INPA), Rio Negro, próximo ao rio Arara, 2º acampamento da SIDERAMA; mata de terra firme, solo arenoso, 2 May 1973, *A. Loureiro s.n.* (INPA); Humaitá, Estrada Humaitá-Lábrea, km 77. Igarapé na beira da estrada, latossolo, 11 June 1982, *L.O.A. Teixeira 1076* (INPA); Itapiranga, Rio Uatumã, em frente ao igarapé Sta. Lizia, 1 km da margem direita do rio; mata de terra firme; solo argiloso, 16 Aug 1979, *C.A. Cid 448* (INPA); Lábrea, Rio Ituxi, vicinity of Boca do Curuquete, 8 July 1971, *G.T. Prance 14014* (INPA); Manaus, Banks of rio Tarumã and Praia Dourada, sandy substrate, 14 Dec 1974, *A.H. Gentry 13292* (INPA), BR 17, igarapé da Bolívia, terra firme, arenoso, capoeira, 3 June 1955, *J. Chagas s.n.* (INPA, MG), Cabeceira do igarapé da Cachoeira Alta, terreno arenoso, capoeira, 28 Nov 1960, *W. Rodrigues 1956* (IAN), Cabeceira do igarapé da Cachoeira Alta, terreno arenoso, capoeira, 28 Nov 1960, *W. Rodrigues 1956A* (INPA), Campos Sales, igarapé da Cachoeira Alta do Tarumã. Terra úmida, 22 Sept 1954, *J.C. de Almeida s.n.* (INPA), Campos Sales, km 10 da BR-17, terreno alagadiço, 30 Aug 1954, *J. Chagas s.n.* (INPA), Estrada da Forquilha. Solo arenoso, capoeira fechada, terra úmida, 11 Oct 1956, *J. Chagas s.n.* (INPA), Estrada do Aleixo, km 7 entrada a esquerda.

Terra firme, arenoso, capoeira aberta, 24 Aug 1956, *F. Mello s.n.* (INPA), Igarapé da Cachoeira Alta do Tarumã. Terra firme, solo arenoso, capoeira, 17 Sept 1962, *W. Rodrigues 4635* (INPA), Igarapé da Cachoeira Alta. Solo arenoso, úmido, capoeira, 11 Dec 1961, *W. Rodrigues 3846* (INPA), Km 10 da estrada do Aleixo. Terra firme, arenoso, capoeira aberta, 14 Sept 1955, *D. Coelho s.n.* (INPA), Margem do igarapé da Bolívia, estrada da BR - 17, terra firme, arenosa, capoeira, 3 June 1955, *J.C. de Almeida s.n.* (INPA), Margem do igarapé da Bolívia. Terra firme, arenoso, capoeira baixa, aberta, 27 July 1956, *D. Coelho s.n.* (INPA), Margem do igarapé do Binda. Terreno firme, arenoso, capoeira baixa, aberta, 27 July 1956, *D.F. Coelho 4001* (IAN), Margem do igarapé do Mariano. Terra firme, arenoso, capoeira, alta, 31 July 1956, *F. Mello s.n.* (INPA), Margem do igarapé do Mariano. Terra firme, arenoso, capoeira, alta, 31 July 1956, *F.C. Mello 4016* (IAN), Margem do igarapé do parque 10, terra firme, arenoso, capoeira grossa, 6 Sept 1955, *F.C. Mello 1830* (INPA, MG), Parque 10 de Novembro, terra firme, úmido, arenoso, capoeira fechada, alta., 29 Feb 1956, *J. Chagas s.n.* (INPA), Ponta Negra, 11 Feb 1977, *M. Silva 2089* (INPA), Ponta Negra, campina de solo arenoso, 22 June 1961, *W. Rodrigues 2059* (INPA), Praia de Lajes, opposite meeting of the waters, Manaus, 11 Feb 1977, *G.T. Prance 24374* (INPA), Ramal Pau Rosa. Na margem de um pequeno igarapé, 52 m, 02º50'42"S, 60º14'12"W, 11 June 2011, *R. Goldenberg 1554* (INPA), Reserva Florestal Adolpho Ducke, Rodovia Manaus-Itacoatiara, km 26, Lateral Oeste-Acará. Floresta de Baixio, 2°53'S, 59°58'W, 9 Aug 1995, *C.A. Sothers 558* (INPA, SPF), Reserva Florestal Adolpho Ducke, Rodovia Manaus-Itacoatiara, km 26, saída do Igarapé Acará. Área alterada. Solo argiloso, próximo ao baixio/igarapé, 02º53'S, 59º58'W, 6 June 1997, *C.A. Sothers 1013* (INPA), Trilha próxima à entrada da Reserva Ducke pela AM-010 (km 26). Ca. 300 m a partir da portaria da Reserva, trilha paralela à rodovia, 80 m, 2°54'49.72"S, 59°58'50.24"W, 22 Sept 2016, *E.Y. Kataoka 346* (SPF); Maués, Rio Maués-Assu, lado oposto a cidade de Maués. Capoeira de terra firme, solo argilo-arenoso, 3º23'S, 57º45'W, 21 July 1983, *C.A. Cid 4244* (INPA); Presidente Figueiredo, Balbina 193 km de Manaus, 12 Aug 1986, *C.A.A. Freitas 156* (INPA), Entorno. Entrada à direita na estrada indo para a vila, depois da entrada para o CPPMA, 1°00'S, 59°00'W, 29 Nov 2006, *J.G. de Carvalho-Sobrinho 1246* (INPA, SPF), Entorno. Picada da Suçuarana, 11 July 2007, *S. Sakagawa 435* (INPA, SPF); Rio Preto da Eva, 2-5 km N of Manaus-Itacoatiara road at Km 79 near Rio Preto da Eva, 100-200 m, 24 Nov 1974, *A.H. Gentry 12836* (INPA); São Gabriel da Cachoeira, Road Camanaús-Uaupés near Camanaús. Caatinga on white sand, terra firme, 1 Nov 1971, *G.T. Prance 15986* (INPA); Tabatinga, Próximo ao aeroporto. Sub-base do Projeto RADAM/BRASIL. Quadrícula SB-19xA. Ponto 02, 1 May 1976, *C.D.A. Mota 358* (INPA, MG). **Goiás**: Alto Horizonte, Estrada para o trevinho, na ponte do rio Formiga. Mata

de galeria, 324 m, 14°9'1"S, 49°17'9"W, 1 May 2012, *J.E.Q. Faria* 2653 (SPF). **Maranhão**: Viana, Jan 1960, *O. de Carvalho 13* (RB). **Mato Grosso**: Aripuanã, Margem direita do rio Aripuanã, acesso pelo 'Balneário Oásis', acima da cachoeira., 224 m, 10°9'53.1"S, 59°27'44.9"W, 14 Nov 2016, *E.Y. Kataoka 380* (SPF); Cotriguaçu, Margem esquerda do rio Juruena, sentido montante, a partir da área de embarque da balsa, 192 m, 9°55'13.6"S, 58°15'4.2"W, 15 Nov 2016, *E.Y. Kataoka 381* (SPF), Rio Juruena, ilha próximo à margem esquerda do rio, 199 m, 9°54'2.8"S, 58°13'38.8"W, 15 Nov 2016, *E.Y. Kataoka 383* (SPF); Juara, Margem direita do rio Apiacás, junto a primeira cachoeira; salto Apiacás. Solo arenoso úmido; floresta aluvial, 26 May 1988, *M. Macedo 1918* (INPA). **Oiapoque**: Amapá, Parque Nacional do Cabo Orange, igarapé Cova da Onça, 2°56'26.24"N, 50°59'3.147"W, 3 Aug 2006, *S.R.M. Silva 62* (MG). **Pará**: Approx. 18 km east of Tucurui and rio Tocantins, by BR 263. White-sand campina and campina forest (campinarana), 3º30'S, 49º32'W, 28 Oct 1981, *D.C. Daly 1016* (INPA, MG), Beira do rio Mapuá, várzea, entre Vila Emilia e Boca do Mapuá, 18 July 1950, *G.A. Black 50-9804* (IAN), Beira do rio Mojú. Fábrica e cereanías, 1 June 1954, *G.A. Black 54-16288* (IAN), Cacaual Grande; lado W. do Canal Novais Filho; capoeira de várzea, 4 July 1952, *G.A. Black 52-15399* (IAN, RB), Km 15, Belem Brasilia highway E of Belem, near check point, 9 Dec 1974, *A.H. Gentry 13159* (IAN, INPA, MG), Km 56 da BR-163, *O.H. Knowles 1476* (MG), Região do Anapú, rio Pracajaí, Portel, 16 Sept 1956, *R.L. Fróes 32723* (IAN); Afuá, Rio Cajuuna, mata de várzea, margem inundável, 12 a 02 Sept - Oct 1992, *U.N. Maciel 1899* (MG); Alemquer, Várzea. Amazonian Costa Rica, 28 July 1903, *A. Ducke s.n.* (MG); Anajás, June 1900, *A. Ducke s.n.* (MG); Aramanai, Belterra, Jan 1932, *R.C. Monteiro da Costa 255* (R); Aveiro, Orla da mata firme, rio Tapajós, 2 Apr 1924, *J.G. Kuhlmann 1887* (R, RB); Barcarena, Itupanema, quintal, 23 Oct 1985, *M.C. Amorozo 220* (MG); Belém, Baixa da Pedreira, 3 Nov 1960, *E. Oliveira 1146* (IAN), Capoeira do Black, 25 m, 1°26'11.1"S, 48°26'37"W, 04 Nov 2016, *E.Y. Kataoka 360* (SPF), Capoeira do Black, 25 m, 1°26'11.1"S, 48°26'37"W, 04 Nov 2016, *E.Y. Kataoka 361* (SPF), Embrapa Amazônia Oriental. Capoeira do Black, *A.C. da S. Andrade 141* (IAN), IPEAN grounds. Forest edge and roadside second growth, alt. near sea level, 7 Dec 1974, *A.H. Gentry 13123* (IAN, INPA, MG), On lands of Instituto Agronômico do Norte, 2 K so of Administration Building. Near Rio Guamá, 15 Feb 1944, *A. Silva 117* (IAN), Parque do Utinga, aproximadamente 1,5 km da entrada do parque à direita, três metros da beira da estrada, 8 July 2011, *F.F.P. Castro s.n.* (MG); Benevides, Rua na beira da BR316, no Balneário Olho d'água, 1°21'19"S, 48°15'47"W, 31 Oct 2012, *M.P. do Nascimento 521* (IAN); Breves, local onde foi feito um inventário florestal, Oct-Nov 1957, *J.M. Pires 6653* (IAN); Gurupa, Rio Moju, afluente do rio Amazonas, próximo ao porto de Gurupa.

Várzea alta, 8 Dec 1991, *G. dos Santos 314* (MG), Rio Moju, afluente do rio Amazonas, próximo ao porto de Gurupa. Várzea alta, 8 Dec 1991, *G. dos Santos 317* (MG); Monte Alegre, Rio Maicurú, Monte Alegre, várzea, 12 Sept 1953, *R.L. Fróes 30203* (IAN); Oriximiná, rio Paru do oeste. Mata de beira de rio, solo argiloso, 5 Sept 1980, *C.A. Cid 2155* (INPA, MG, RB); Ourém, 1°32'51"S, 47°6'36"W, Oct 2011, *F.C.A. Lucas 228* (IAN), Capoeira, 5 Dec 1903, *R.S. Rodr. s.n.* (MG); Paragominas, Rodovia Belém-Brasília. Rio Uraim, beira do rio, terreno alagado, 18 Jan 1966, *M. Silva 452* (MG); Porto Trombetas, Mineração rio do norte, 1991, *E. Soares 545* (INPA), Mineração rio do norte, 1991, *E. Soares 766* (INPA), Mineração rio do norte. Restinga do rio Trombetas, 1991, *E. Soares 345* (INPA), Mineração rio do norte. Restinga do rio Trombetas, 3 July 1991, *O.H. Knowles 1732* (INPA); Primavera, Subindo o rio Quatipuru, aproximadamente 5 km da ilha de Maçaranduba. Capoeira baixa, 24 Nov 1993, *R. Lisboa 2992* (MG); Santarém, Km 35 da estrada do Palhão, arredores do acampamento do igarapé Curupira. Capoeira, beira do igarapé, 30 Aug 1969, *M. Silva 2446* (MG), Rio Curuaúna, várzea inundável; região do planalto de Santarém onde foi feito o levantamento estatístico florestal pelo IAN, SPVEA e FAO, Oct 1954, *R.L. Fróes 31360* (IAN); Tapeirinha para Santarém, Terra firme, 25 Dec 1958, *F. Markgraf 3872* (RB); Vitória do Xingu, Usina Hidrelétrica de Belo Monte, 19 June 2012, *L.S. Lima 417* (MG). **Rondônia**: Porto Velho, BR 364, Rio Novo próximo ao acesso à UHE de Samuel. Mata secundária, solo argilo-arenoso, 16 Aug 1987, *A. Vasques 18* (INPA), Mata ciliar, UHE - Samuel, Rio Jamari, montante da UHE, margem direita, 10 May 1988, *M. Pereira 230* (RB). **Roraima**: Boa Vista, T.F. de Roraima, terra firme, beira do rio, 20 Feb 1964, *M. Silva 16* (IAN, MG); Caracaraí, Margem direita do Rio Branco, 26 m, 1°2'50.3"S, 61°52'6.7"W, 8 June 2016, *E.Y. Kataoka 273b* (SPF); Rorainópolis, Confluência Rio Negro/Rio Branco. Margem esquerda do Rio Branco, 33 m, 1°23'30.2"S, 61°50'20.7"W, 7 June 2016, *E.Y. Kataoka 250* (SPF), Foz do rio Branco no rio Negro. Mata de igapó com interferência da água branca do rio Branco, 1°23'27.1"S, 61°50'31.6"W, 13 May 2015, *B.M. Gomes 647* (SPF), Margem a jusante do rio Branco. Borda de Mata de várzea, 22 m, 1°4'41.92"S, 61°51'7.33"W, 8 June 2016, *A. Frazão 267* (SPF), Margem esquerda do Rio Branco, 27 m, 1°17'42.2"S, 61°51'1.7"W, 10 June 2016, *E.Y. Kataoka 330* (SPF), Margem esquerda do Rio Branco, 28 m, 0°38'47.7"S, 61°49'15.3"W, 9 June 2016, *E.Y. Kataoka 309* (SPF), Margem esquerda do Rio Branco, 28 m, 0°38'47.9"S, 61°49'15.5"W, 9 June 2016, *E.Y. Kataoka 310* (SPF), Margem esquerda do Rio Branco, 28 m, 0°38'54.1"S, 61°49'18.2"W, 9 June 2016, *E.Y. Kataoka 311* (SPF), Margem esquerda do Rio Branco, 28 m, 0°38'54.1"S, 61°49'18.2"W, 9 June 2016, *E.Y. Kataoka 312* (SPF), Margem esquerda do Rio Branco, 28 m, 0°50'30.3"S, 61°51'4.6"W, 8 June 2016, *E.Y. Kataoka 289* (SPF), Margem

esquerda do Rio Branco, 28 m, 0°50'30.3"S, 61°51'4.6"W, 8 June 2016, *E.Y. Kataoka 290* (SPF), Margem esquerda do Rio Branco, 28 m, 1°16'41.8"S, 61°50'13.9"W, 10 June 2016, *E.Y. Kataoka 329* (SPF), Margem esquerda do Rio Branco, 28 m, 1°5'46.9"S, 61°52'7.4"W, 10 June 2016, *E.Y. Kataoka 324* (SPF), Margem esquerda do Rio Branco, 29 m, 1°23'9.4"S, 61°50'54.4"W, 7 June 2016, *E.Y. Kataoka 251* (SPF), Margem esquerda do Rio Branco, 30 m, 0°39'20.1"S, 61°49'32.1"W, 9 June 2016, *E.Y. Kataoka 313* (SPF), Margem esquerda do Rio Branco, 31 m, 1°23'2.8"S, 61°51'3.7"W, 7 June 2016, *E.Y. Kataoka 252* (SPF), Margem esquerda do Rio Branco, 31 m, 1°23'22.9"S, 61°50'38"W, 10 June 2016, *E.Y. Kataoka 332* (SPF), Margem esquerda do Rio Branco, 33 m, 0°40'57.1"S, 61°50'37.8"W, 8 June 2016, *E.Y. Kataoka 302* (SPF), Margem esquerda do Rio Branco, 34 m, 1°20'39.7"S, 61°52'5.2"W, 7 June 2016, *E.Y. Kataoka 262* (SPF), Margem esquerda do Rio Branco, 37 m, 0°50'32.4"S, 61°51'5.7"W, 8 June 2016, *E.Y. Kataoka 288* (SPF). **COLOMBIA**. **Amazonas**: Leticia, Parque Nacional Natural Amacayacu. Cerca de la Cabaña en la boca del río Amacayacu. Bosque secundario, 14 June 1992, *R. Rueda 525* (MO). **Antioquia**: Cáceres, 10-15 km NE de Cáceres en la Troncal de la paz, 180 m, 7°40'N, 75°22'W, 6 Nov 1987, *R. Callejas 5380* (MO). **Bolivar**: Isla de Barú, entre Santa Ana y Playa Mojana, 20 m, 25 Aug 1986, *H. Cuadros 3070* (MO), Road to Pta. Barú, W of Cartagena across Canal de Dique, scrubby (dry) forest remnants, 20 m, 10°18'N, 75°35'W, 4 July 1984, *A.H. Gentry 47648* (MO); Cartagena, Along road ca. 7 km SW of Arroyo Grande, old secondary vegetation, 70 m, 10°36'N, 75°24'W, 31 July 1985, *J.L. Zarucchi 3901* (MO); Morales, Cgto Norosí, caminoa Tiquisionuevo, 200 m, Apr 1985, *H. Cuadros 2126* (MO); San Juan, Loma de los colorados, 2 km S of San Juan, disturbed moist forest, 300 m, 9°58'N, 75°10'W, 27 Oct 1989, *A.H. Gentry 68258* (MO); Turbaco, Camino al depositario, 100 m, 8 June 1982, *H.C. Villalobos 1373* (MO). **Córdoba**: Ayapel, Carreteable a Ayapel de la Carretera Caucasia - Nechi, Finca del diamante, 50 m, 8°12'N, 74°47'W, 5 May 1999, *F.J. Roldán 2807* (MO); Junction of río Tigre and río manso. Paramillo National Park. Transect no. 6, 200 m, 7°30'N, 76°5'W, 28 July 1988, *A.H. Gentry 63841* (MO). **Caquetá**: Solano, Sitio Araracuara, pista de aterrizaje. Colecciones sobre afloramiento rocoso, 200 m, 0°35'S, 72°25'W, 10-25 May 1998, *M.V. Arbeláez 1088* (MO). **Chocó**: Riosucio, Peye. Orillas del río Peye, 30 m, 5 June 1976, *E. Forero 1874* (INPA). **Sucre**: San Onofre, Corregimiento "Las Brisas", Alto de "Salas", Arroyo "Mambú", 11 Sept 1996, *A. Realpe 229* (SPF). **Valle**: Bajo Calima, pluvial forest, road to Juanchaco Palmeras, 100 m, 3°55'N, 77°2'W, 10 July 1984, *A.H. Gentry 47843* (MO). **COSTA RICA**. **Alajuela**: San Isidro de San Ramón, 1259 m, 10°4'46"N, 84°26'30"W, 25 Oct 1986, *G. Herrera 123* (MO); Cantón de Upala, Z.P. Tenorio. Cordillera de Guanacaste. Bijagua. Primary forest on a ridge at Volcán Tenorio. Premontane

rainforest, 1000-1500 m, 10°43'0"N, 85°1'0"W, 20 Apr 1995, *D. Penneys 485* (MO). **Guanacaste**: Liberia, P.N. Guanacaste, cuenca del Tempisque. Estación Cacao, alrededores de la estación, 1100 m, 10°55'36"N, 85°28'6"W, 7 Aug 2007, *A. Soto 1822* (MO), P.N. Guanacaste. Cordillera de Guanacaste. Estación Cacao, Sendero Maritza, 1100 m, 10°55'43"N, 85°28'10"W, 9 Feb 1995, *L. Angulo 42* (MO), P.N. Guanacaste. Cuenca del Tempisque. Sector Cacao, hacia la estacíon de 1.5 km, después del río Góngora, 650 m, 10°53'10.421"N, 85°28'19"W, 30 Apr 2000, *L. Acosta 1057* (MO), P.N. Rincón de la Vieja. Cuenca del Tempisque, sendero hacia el cráter, 1004 m, 10°47'19.3"N, 85°20'57"W, 31 May 2011, *L.D. Vargas 4556* (MO), Parque Nacional de Guanacaste. Estación Cacao, 1100 m, 10°55'45"N, 85°28'15"W, 31 Oct 1993, *C. Chávez 332* (MO), Parque Rincón de La Vieja. Del mirador siguiendo la fila al volcán Santa María, 1100 m, 10°46'N, 85°49'W, 22 Nov 1987, *G. Herrera 1364* (MO). **Heredia**: La Selva, Rio Sarapiqui near Puerto Viejo, tropical wet forest, junction SSO and LOC trails, 100 m, 10°26'N, 84°1'W, 8 Jan 1993, *A.H. Gentry 78642* (MO). **Puntarenas**: Monteverde, Pacific slope forest, 1450 m, 8 Aug 1985, *W.A. Haber 2204* (MO), Monteverde, Pacific slope forest, 1450 m, 8 Aug 1985, *W.A. Haber 2211* (MO); Golfito, P.N. Corcovado. Peninsula de Osa. Estacion Sirena. Sendero Espaveles, 0 m, 8°28'51"N, 83°35'42"W, 16 Jan 1997, *R. Aguilar 4986* (MO), Refugio de Vida Silvestre Golfito, camino a la Gamba, 94 m, 8°40'56"N, 83°11'50"W, 9 Oct 2008, R. Aguilar 11418 (MO, SPF); Puntarenas, San Luis. Monteverde. Camino a Veracruz, 1050 m, 10°16'35"N, 84°47'45"W, 16 Oct 1992, *A. Fernández 440* (MO). **San Jose**: Reserva Biológica Carara. Sector Bijagual. Sitio Sendero Bijagual-Quebrada Bonita, 550 m, 9°46'20"N, 84°33'50"W, 1 Nov 1990, *R. Zúñiga 327* (MO); Cantón de Dota, Faja costeña del Valle de Parrita. Faldas Cerro Narra, Quebrada Salitrillo, bosque primario cerca del cauce, 200 m, 9°28'55"N, 84°2'50"W, 19 July 1995, *J.F. Morales 4572* (MO). **ECUADOR. Cantón Archidona**: Napo, Faldas al sur del Volcán Sumaco. Carretera Hollin-Loreto, km 31. Comuna Challua Yacu. Bosque pluvial pre montano. Suelos de origen volcánico, 1200 m, 0°43'S, 77°36'W, 15-17 Nov 1988, *A. Alvarado 46* (MO). **Los Rios**: Hacienda Los Ocho, km 50 on road from Santo Domingo to Quevado. Wet Forest, 200 m, 4 Feb 1974, *A.H. Gentry 9640* (QCA). **Napo**: Archidona Cantón, Faldas al sur del Volcán Sumaco. Carretera Hollin-Loreto, km 31. Comuna Challua Yacu. Bosque pluvial pre montano. Suelos de origen volcánico, 1200 m, 0°43'S, 77°36'W, 15-17 Nov 1988, *A. Alvarado 46* (QCNE). **Orellana**: Along MAXUS (YPF) road at Km 50. Yasuni National Park, 250 m, 16 Mar 1997, *R.J. Burnham 1493* (QCNE). **Pastaza**: Shell, Vicinity of Shell, 1.2 km N of town; disturbed virgin forest in swampy area, 1092 m, 1°29'33"S, 78°3'57"W, 9 May 2003, *T.B. Croat 88873* (QCNE). **Pichincha**: Quito Cantón, Parroquia Puerto Quito. Reserva Florestal de ENDESA, 10 km al norte de Alvaro Pérez

Intriago. Bosque muy húmedo premontano. Bosque primario. Transectos, 650-800 m, 00°03'N, 79°07'W, 11 June 1990, *C.E. Cerón 10140* (MO, QCNE). **Sucumbios**: Lago Agrio Cantón, Reserva Cuyabeno. Laguna Grande. Bosque húmedo tropical. Bosque primario sobre suelo bien drenado alrededor de cabañas de Neotropic, 230 m, 0°0'S, 76°11'W, 15 Nov 1991, *W. Palacios 9153* (QCNE). **Zamora-chinchipe**: Nangaritza Cantón, Mizai, in rio Nangaritza valley, forest on slope above military post, 850 m, 4°18'S, 78°40'W, 31 July 1993, *A.H. Gentry 80967* (QCNE). **FRENCH GUIANA**. Montagne de Kaw. Borde de piste forestière, 40 m, 4°33'N, 52°9'W, 25 Mar 1988, *G. Cremers 9827* (MO); Cayenne, Ile de Cayenne. Bord de route. La rocade, entre les ronds points Baduel et la Madeleine à proximité du croisement avec la route Raban, 7 m, 4°56'N, 52°20'W, 9 Mar 2009, *C. Delnatte 1693* (MO). **Saül**: La Fumée Mountain, Antenne Nord. Non-flooded moist forest, 400 m, 3°37'N, 53°12'W, 14 May 1986, *S.A. Mori 17998* (MO). **GUYANA**. Bords de la Rivière du Maroni, 1861, *M. Melinon 13* (R); Kariako village, Barama river, North-West District Mora riparian forest around Kariako village, 145 m, 7°22'N, 59°42'W, 23 Dec 1995, *T. van Andel 680* (MO). **HONDURAS**. **Atlántida**: Campamento Quebrada Grande ca. 10 km south west of La Ceiba. At base of north slope of Pico Bonito, from camp to 2 km east of camp. Upland forest on slope, 80 - 180 m, 15°42'N, 86°51'W, 10 May 1993, *R.L. Liesner 26180* (MO). **Yoro**: Cordillera Nombre de Dios, hills S of San José de Texíguat. Evergreen rainforest on steep slopes, 350 m, 15°29'N, 87°26'W, 17 May 1991, *G. Davidse 34509* (MO). **MEXICO. Veracruz**: Hidalgotitlán, Afluente O del río Las Cuevas, +/- 7-9 horas a pie al S del de La Laguna, área arriba de las cascadas donde el arroyo corre en direccíon E-O, entre lomas con suelos prof.; selva alta perenifolia con mucha jimba, 350 m, 17°13'30"N, 94°30'30"W, 17 Apr 1982, *T. Wendt 3865* (MO). **PERU. Amazonas**: Bagua, Distrito Imaza, Comunidad de Yamakat. Bosque primario, 600 m, 5°3'24"S, 78°20'17"W, 6 June 1997, *R. Vásquez 23907* (MO). **San Martín**: Prov. Mariscal Caceres. Dtto Tocache Nuevo. Al sud oeste del Aeropuerto de Tocache Nuevo. En bosque secundario, 400 m, 12 Jan 1970, *J. Schunke V. 3691* (INPA). **Loreto**: Km 22 Yurimaguas-Tarapoto road, remnant patch of mature forest on white sand and adjacent scrub, 190 m, 6°'S, 76°13'W, 10 Oct 1985, *A.H. Gentry 52177* (MO); Iquitos, Laguna Quistococha, ca. 15 km SW of Iquitos, 8 July 1977, *J.C. Solomon 3448* (MO). **Madre de Dios**: Tobapata Province. Cuzco Amazónico Lodge. Lago Sandobal and río Madre de Dios. Lake edge; aguajal, 200 m, 12°35'S, 69°3'W, 14 Apr 1990, *P. Núñez 12071* (MO). **Maynas**: Alpahuayo (km 25, carretera Iquitos-Nat.), Estacíon IIAP. Bosque primario, 19 Oct 1984, *R. Vásquez 5769* (MO); Iquitos, Río Itaya, Sanangal, restinga over silty clay mostly disturbed, 110 m, 17 May 1980, *S. McDaniel 23739* (MO), Río Nanay, Carretera de Picuruyacu, en terreno arenoso, 160 m, 23 Sept 1981, *M. Rimachi Y. 5720*

(MO); Nauta, Carretera a Iquitos. Bosque inundable estacional, 150 m, 4°29'S, 75°35'W, 12 Dec 1986, *R. Vásquez 8604* (MO); Pebas, Río Ampiyacu, 19 July 1976, *J. Revilla 924* (MO). **Pasco**: Oxapampa, Dist. Palcazú. Comunidad nativa Alto Lagarto - Reserva Comunal Yanesha. Remanente de bosque primario, 584 m, 10°9'7"S, 75°23'32"W, 30 Oct 2009, *R. Rojas 7122* (SPF), Distrito Palcazú. Comunidad Nativa Centro Connás. Bosque primario remanente en borde de carretera, 373 m, 10°9'59"S, 75°16'5"W, 14 May 2010, *R. Vásquez 36497* (SPF). **Tocache Nuevo**: San Martín, Al sud oeste del Aeropuerto de Tocache Nuevo. En bosque secundario, 400 m, 12 Jan 1970, *J. Schunke V. 3691* (IAN). **SURINAME. Brokopondo**: NW of Brokopondo Stuwmeer Lake (E of Brownsberg Nature Reserve), Tonka island. Trail west from main compound. High forest on laterite soil, 15 m, 4°35'N, 55°7'W, 4 Feb 1999, *B. Hoffman 5299* (MO); Brownsweg, Near Brownsweg Nature Park. Little disturbed high mesophytic rain forest on slope, gravelly clay soil, 240 m, 4°35'41.28"N, 55°6'0"W, 7 Oct 2005, *S. Ruysschaert SRU 728* (SPF). **Paramaribo**: Bakboord farm and lake property, just within NE corner of Paramaribo city limits, ca. 1 km N of Kwattaweg, ca. 1/2 km E of Henri Fernandesweg. Secondary forest patch bordering fairly recently abandoned pastureland, 5°50'60"N, 55°12'59.97"W, 25 Nov 1996, *R.J. Evans 2591* (MO, RB). **Sipaliwini**: Kwamalasemutu village vicinity, 50 m, 2°22.5'N, 56°47.3'W, 23 Feb 1999, *M.J. Plotkin 1359* (MO). **VENEZUELA**. Delta Amacuro. Cienega de selva húmeda caliente y selva de galería, carretera Caño Guará - La Horqueta, 30-31 Jan 1982, *B. Stergios 3982* (MO). **Amazonas**: Road from San Fernando do Atapabo to Santa Barbara 12-40 km from San Fernando; thickets and forest, mostly on white sand, 110 m, 24 Mar 1974, *A.H. Gentry 10964* (MO); Atures, Bosque húmedo del río Cataniapo entre San Pedro de Cataniapo y comunidad El Milagro, 95 m, 6°25'N, 67°25'W, 12 Aug 1986, *A. Castillo 2179* (MO), Primary rainforest, along road between Paso el Diablo and Caño de Culebra, 25-30 km southeast of Puerto Ayacucho, 100 m, 12 May 1980, *J.A. Steyermark 122336* (MO). **Bolivar**: Reserva Florestal Itamaca. Selva pluvial del bajo río Botanamo, entre su desemboque al río Cuyunf hasta la boca del río Guarapín, 16-17 July 1983, *B. Stergios 6108* (MO).

5. *Martinella tomentosa* Kataoka & L.G. Lohmann sp. nov.

Figure 11

Type. BRAZIL. Amazonas: Manaus, Reserva Florestal Adolfo Ducke, Rodovia Manaus Itacoatiara, km 26, 02°53'S 59°58'W. 19 June 1995, *M.A.D de Souza & C.F. da Silva, 39* (holotype: SPF-102015!; isotypes: INPA!, MO!).

Diagnosis. *Martinella tomentosa* differs from other Amazonian species of *Martinella* by the tomentose leaflets and branches, and inflorescences arranged in thyrsi, as opposed to the glabrous to lanuginose leaflets and inflorescences arranged in racemes or lax thyrsi of all other Amazonian species.

Description. *Lianas*; branches with solid pith, cylindrical, green, drying brown, smooth, tomentose, densely covered with simple eglandular trichomes and stipitate glandular trichomes, with scattered patelliform glandular trichomes more frequently at interpetiolar region; prophylls of the axillary buds densely covered with simple eglandular trichomes and stipitate glandular trichomes. *Leaves* 2-foliolate, with the terminal leaflet generally modified into a trifid tendril; petioles terete, not pulvinated, 38.1–58.6 mm long, densely covered with simple eglandular trichomes and stipitate glandular trichomes with few scattered patelliform glandular trichomes; petiolules terete, pulvinated, 27–31.5 mm long, densely covered with simple eglandular trichomes and stipitate glandular trichomes with occasional patelliform glandular trichomes; leaflets concolorous, chartaceous, ovate, apex acuminate, base cordate, margins entire and slightly revolute, 16.5–19.0 × 12.0–14.0 cm, adaxial surface glabrescent with simple eglandular trichomes and stipitate glandular trichomes at the canaliculi of veins, abaxial surface tomentose, densely covered with simple eglandular trichomes and stipitate glandular trichomes, and patelliform glandular trichomes concentrated near the base and few scattered along the midvein. *Inflorescences* in thyrsi; 10–19.5 cm long, tomentose, densely covered with simple eglandular trichomes and stipitate and few patelliform glandular trichomes; bracts linear, ca. 2 mm long, pubescent, densely covered with simple eglandular trichomes and stipitate glandular trichomes; pedicels terete, 4.3–9.4 mm, pubescent, with simple eglandular trichomes and stipitate glandular trichomes. *Flowers* with calyx green, chartaceous, campanulate, 14–21.8 × 18.1–21.3 mm, densely covered with simple eglandular trichomes and stipitate glandular trichomes, with few patelliform glandular trichomes, lobes 2-4, apex acuminate, pubescent; corolla lilac, membranous, 44.9–48.7 mm long, narrowly tubular basal portion 14.5–14.9 mm long × 2.9–3.2 mm wide, upper campanulate portion 28.4–30.2 mm long × 12.1–14.9 mm wide, lobes subcircular, 6.5–7.8 × 11.8–12.1 mm; stamens in two lengths, longer ones 14.4–15.4 mm, shorter ones 10.3–10.5 mm, thecae 2.6–3.1 mm, glabrous; staminode not seen; gynoecium 28.4–36.3 mm long; ovary glabrous; style glabrous; stigma lanceolate, glabrous; nectariferous disk 2.3–2.5 × 1–1.1 mm. *Fruits* and *seeds* not seen.

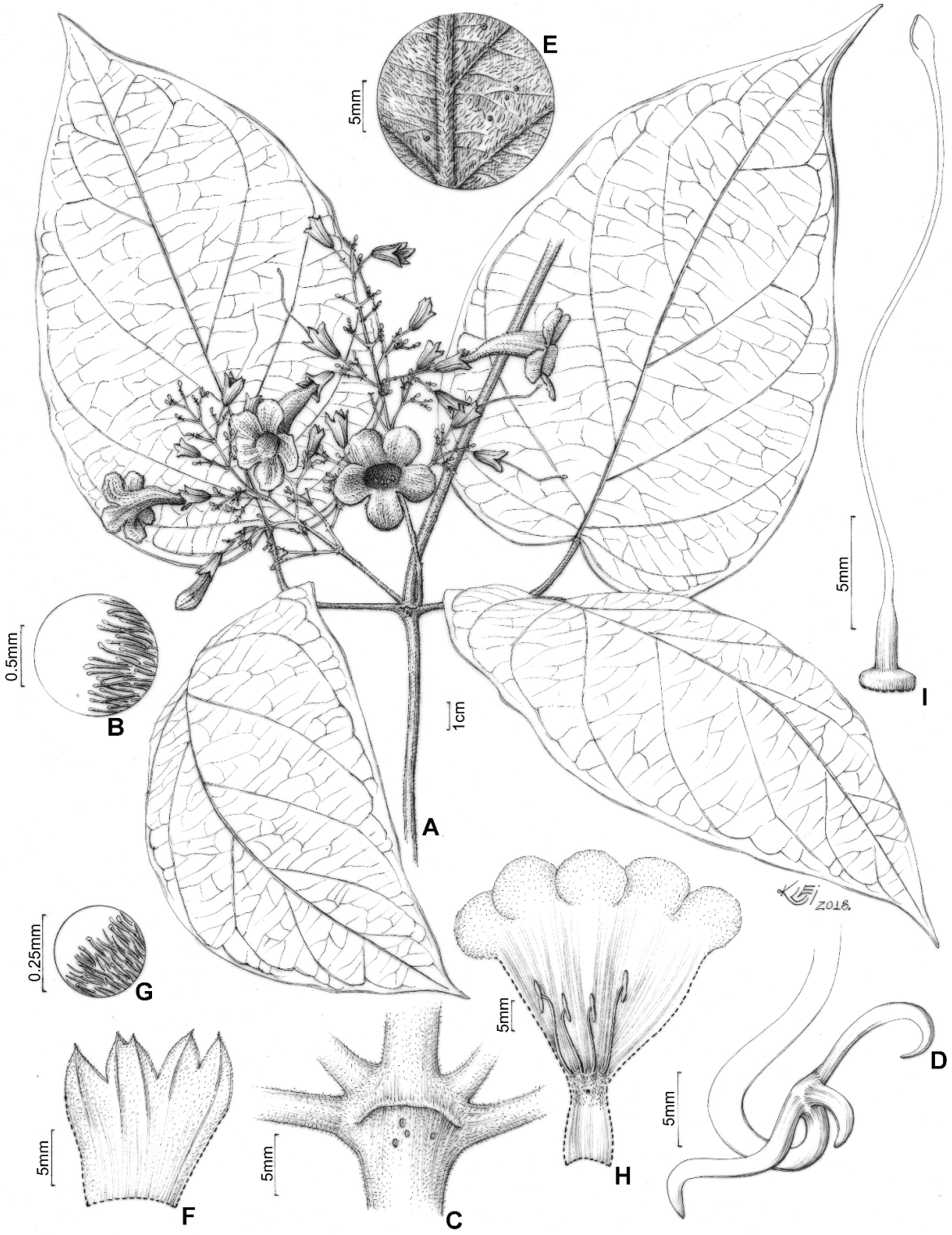

Figure 11. *Martinella tomentosa* Kataoka & L.G. Lohmann **A** Flowering branch; **B** Leaflet indumentum; **C** Ridge at the interpetiolar region and patelliform glandular trichomes; **D** Trifid tendril; **E** Abaxial side of leaflet with patelliform glandular trichomes; **F** Calyx external view; **G** Calyx indumentum; **H** Open flower showing anthers, trichome distribution, and reduced (ca. 1 mm) staminode; **I** Gynoecium. Illustrated by Klei Sousa, based on M.A.D. de Souza 39, SPF; W. Rodrigues 4444, INPA; M.F. Silva 855, INPA.

Distribution and habitat. *Martinella tomentosa* is restricted to the central portion of Amazonia (Figure 12), with known occurrences in *Terra Firme* forests from Brazil (Manaus state).

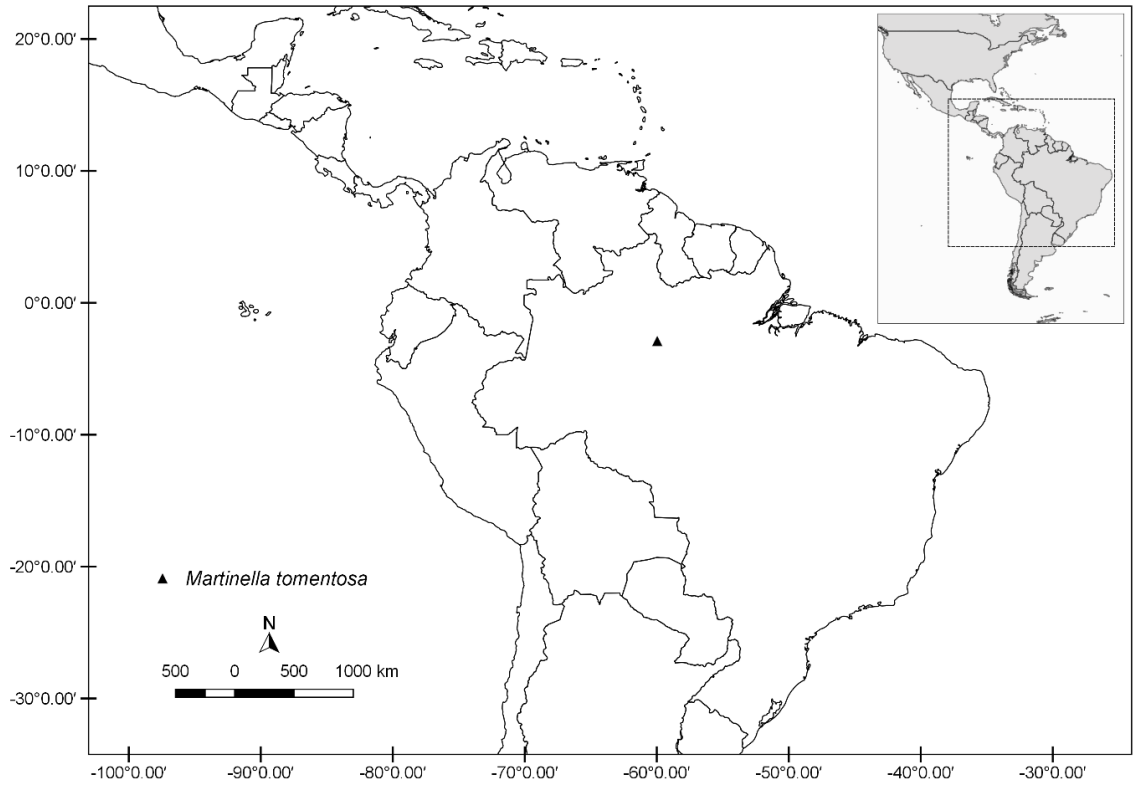

Figure 12. Distribution map of *Martinella tomentosa*.

Etymology. The species epithet relates to the distinguished tomentose indumentum in leaves and branches of *M. tomentosa*.

Phenology. Flowering specimens were collected in June.

Conservation status. Data deficient (DD); known from only three specimens of two localities.

Discussion. *Martinella tomentosa* is a new species whose description is strongly supported by morphological and molecular phylogenetic data (Kataoka and Lohmann in prep). The tomentose leaves and branches are the most striking characteristic that easily distinguish *M. tomentosa* from all other species of Amazonian *Martinella*. This new taxon is only known from very few collections from Central Amazonia, none of which was colleted during the fruiting season.

Specimens examined. BRAZIL. Amazonas: Estrada Castanho-Tupana, entre o km 50-40. Solo argiloso, margem da estrada sempre alagada, 18 July 1972, *M.F. Silva 855* (INPA);

Manaus, Igarapé do Passarinho, terreno; firme, argiloso, capoeira grossa, 15 May 1962, *W. Rodrigues 4444* (INPA), Reserva Florestal Adolfo Ducke, Manaus Itacoatiara, km 26, 2°53'S, 59°58'W, 28 Sept 1995, C.D. Leme 39 (INPA).

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Appendix I

Index to numbered collections

A. Alvarado 46 (obovata).

A. Araujo-M 1532 (obovata).

A. Castillo 2179 (obovata).

A. Ducke s.n. (obovata).

A. Estrada 3072 (insculpta).

A. Fernández 440 (obovata).

A. Frazão 267 (obovata).

A. Lasseign P21164 (obovata).

A. Loureiro 37901 (insculpta); s.n. (obovata).

A. Realpe 229 (obovata).

A. Rodríguez 3367 (insculpta).

A. Silva 117 (obovata).

A. Soto 1822 (obovata).

A. Vasques 18 (obovata).

A.A. Loureiro 37946 (obovata).

A.C. da S. Andrade 141 (obovata).

A.H. Gentry 11208 (insculpta); 28200 (insculpta); 41384 (insculpta); 27233 (lanuginosa); 52144 (lanuginosa); 7767 (obovata); 8250 (obovata); 9640 (obovata); 10964 (obovata); 12836 (obovata); 13024 (insculpta); 13123 (obovata); 13159 (obovata); 13292 (obovata); 47648 (obovata); 47843 (obovata); 52177 (obovata); 63841 (obovata); 68258 (obovata); 78642 (obovata); 80967 (obovata).

A.R. Zuntini 151 (insignis); 321 (insignis).

B. Boyle 7052 (insculpta).

B. Hoffman 5299 (obovata).

B. Stergios 3982 (obovata); 6108 (obovata).

B.G.S. Ribeiro 1645 (obovata).

B.L. Stannard 423 (insculpta).

B.M. Gomes 647 (obovata).

B.V. Rabelo 2386 (insculpta).

C. Chávez 332 (obovata).

C. Delnatte 1693 (obovata).

C.A. Cid 448 (obovata); 2155 (obovata); 4244 (obovata).

C.A. Sothers 558 (obovata); 1013 (obovata).

C.A.A. Freitas 156 (obovata).

C.A.C. Ferreira 11751 (obovata).

C.A.W. Schwacke 425 (insculpta); 463 (insculpta).

C.D. Leme 39 (tomentosa).

C.D.A. Mota 358 (obovata).

C.E. Cerón 10140 (obovata).

D. Coelho s.n. (obovata).

D. Penneys 485 (obovata).

D.C. Daly 1016 (obovata).

D.F. Coelho 4001 (obovata).

E. Forero 1874 (obovata).

E. Oliveira 1146 (obovata).

E. Rivero 183 (obovata).

E. Soares 345 (obovata); E. Soares 545 (obovata); E. Soares 766 (obovata).

E.Y. Kataoka 339 (insculpta); 342 (insculpta); 344 (insculpta); 370 (insculpta); 372 (insculpta); 404 (insculpta); 407 (insculpta); 247 (obovata); 250 (obovata); 251 (obovata); 252 (obovata); 262 (obovata); 273b (obovata); 288 (obovata); 289 (obovata); 290 (obovata); 302 (obovata); 309 (obovata); 310 (obovata); 311 (obovata); 312 (obovata); 313 (obovata); 324 (obovata); 329 (obovata); 330 (obovata); 332 (obovata); 346 (obovata); 360 (obovata); 361 (obovata); 380 (obovata); 381 (obovata); 383 (obovata); 390 (obovata); 406 (obovata).

F. Ayala 1793 (insculpta).

F. de C. Mello 1830 (obovata).

F. Guanchez 923 (insculpta).

F. Markgraf 3872 (obovata).

F. Mello s.n. (obovata).

F.C. Mello 4016 (obovata).

F.C.A. Lucas 228 (obovata).

F.F.P. Castro s.n. (obovata).

F.J. Roldán 2807 (obovata).

G. Cremers 9827 (obovata).

G. Davidse 34509 (obovata).

G. dos Santos 314 (obovata); 317 (obovata).

G. Herrera 123 (obovata); 1364 (obovata).

G. McPherson 20083 (insculpta).

G.A. Black 50-9804 (obovata); 52-15399 (obovata); 54-16288 (obovata).

G.A. Parada 1394 (obovata).

G.T. Prance 14717 (insculpta); 14914 (insculpta); 15557 (insculpta); 14014 (obovata); 15281 (obovata); 15986 (obovata); 24374 (obovata).

H. Cuadros 2126 (obovata); 3070 (obovata).

H.C. Villalobos 1373 (obovata).

I. Huamantupa 3698 (lanuginosa).

J. Chagas s.n. (obovata).

J. Revilla 924 (obovata); 979 (insculpta).

J. Ribamar 189 (obovata).

J. Schunke V. 3691 (obovata).

J.A. Steyermark 104035 (insculpta); 122336 (obovata).

J.A.C. da Silva 1294 (insculpta).

J.C. de Almeida s.n. (obovata).

J.C. Solomon 6108 (insculpta); 3448 (obovata); 12474 (obovata).

J.E.Q. Faria 2653 (obovata).

J.F. Morales 910 (insculpta); 4572 (obovata).

J.G. de Carvalho-Sobrinho 1246 (obovata).

J.G. Kuhlmann 1887 (obovata).

J.J. Hernandez 99 (insculpta).

J.L. Zarucchi 3901 (obovata).

J.M. Pires 6653 (obovata); 51057 (obovata); 51176 (obovata); 52504 (obovata).

K. Van Kerckhove MVK 114 (insculpta).

L. Acosta 1057 (obovata).

L. Angulo 42 (obovata).

L. Ferreira 109 (lanuginosa).

L.D. Vargas 4556 (obovata).

L.G. Lohmann 616 (lanuginosa).

L.O.A. Teixeira 958 (insculpta); 1076 (obovata).

L.S. Lima 417 (obovata).

M. Dantas 1428 (obovata).

M. Goulding 1324 (insculpta).

M. Macedo 1918 (obovata).

M. Melinon 13 (obovata).

M. Pereira 230 (obovata).

M. Rimachi Y. 835 (insculpta); 6180 (insculpta); 6270 (insculpta); 5720 (obovata).

M. Silva 16 (obovata); 452 (obovata); 2089 (obovata); 2446 (obovata).

M.A.D. de Souza 39 (tomentosa).

M.C. Amorozo 220 (obovata).

M.d. Cost 255 (obovata).

M.F. Silva 855 (tomentosa).

M.J. Plotkin 1359 (obovata).

M.P. do Nascimento 521 (obovata).

M.V. Arbeláez 1088 (obovata).

N.Y.Bot. Garden 51176 (obovata).

O. de Carvalho 13 (obovata).

O.H. Knowles 1476 (obovata); 1732 (obovata).

O.H. Knowles 1732 (obovata).

P. Núñez 12071 (obovata).

P.L. Lisboa 6 (insculpta).

R. Aguilar 4986 (obovata); 11418 (obovata).

R. Callejas 1629 (insignis); 5380 (obovata).

R. Goldenberg 1554 (obovata).

R. Hahn 123 (insculpta).

R. Liesner 15711 (insculpta); 16956 (insculpta).

R. Lisboa 2992 (obovata).

R. Rojas 7122 (obovata).

R. Rueda 414 (lanuginosa); 525 (obovata).

R. Vásquez 9172 (insculpta); 10977 (insculpta); 5769 (obovata); 8604 (obovata); 23907 (obovata); 36497 (obovata).

R. Zúñiga 327 (obovata).

R.A.X. Borges 825 (insignis).

R.C. Monteiro da Costa 255 (obovata).

R.C. Quevedo S. 51 (obovata).

R.J. Burnham 1493 (obovata).

R.J. Evans 2591 (obovata).

R.L. Fróes 27592 (obovata); 27594 (obovata); 30203 (obovata); 31360 (obovata); 32723 (obovata); 27592a (obovata).

R.L. Liesner 26180 (obovata).

R.S. Rodr. s.n. (obovata).

S. McDaniel 23739 (obovata).

S. Ruysschaert SRU 728 (obovata).

S. Sakagawa 435 (obovata).

S.A. Mori 17998 (obovata).

S.R. King 492 (insculpta).

S.R.M. Silva 62 (obovata).

Schwacke 3610 (insculpta).

St.G. Beck 5372 (obovata).

T. Guedes 14 (obovata).

T. van Andel 680 (obovata).

T. Wendt 3865 (obovata).

T.B. Croat 88873 (obovata).

U.N. Maciel 1899 (obovata).

V. Demuner 4481 (insignis).

W. Palacios 272 (insculpta); 9153 (obovata).

W. Rodrigues 2203 (insculpta); 1956 (obovata); 2059 (obovata); 3846 (obovata); 4635 (obovata); 1956A (obovata); 4444 (tomentosa).

W. Thomas 4974 (insculpta).

W.A. Haber 2204 (obovata); W.A. Haber 2211 (obovata).

W.A. Rodrigues 9707 (insculpta).

W.H. Lewis 9975 (insculpta); 14026 (insculpta); 14389 (insculpta).

W.S. Alverson 266 (insculpta).

Conclusions

This Master's thesis focuses on the phylogeny and taxonomic revision of *Martinella*. By combining traditional and novel approaches, we were able to infer the phylogeny of *Martinella* based on HTS and Sanger-generated DNA sequence data. This approach allowed us to assemble complete and nearly-complete chloroplast genomes of *Martinella*, and to construct a comprehensive dataset with over 119,000 characters for samples that represent the known range of geographic distribution. The best phylogenetic estimate of *Martinella* to date was reconstructed using Sanger and HTS datasets. The data obtained here was combined into a super matrix that recovered five main clades that correspond to the three previously recognized species (i.e., *M. insignis*, *M. iquitoensis* [= *M. insculpta*], and *M. obovata*), and two newly described Amazonian species (i.e., *M. lanuginosa*, and *M. tomentosa*). The newly generated complete chloroplast sequences would allow for the development of molecular markers for population genetics studies, especially within the widespread Amazonian species, in the future. Future investigations based on an even higher sampling of individuals (e.g., phylogeography) and a higher sampling of nuclear markers (e.g., target enrichment) can potentially improve support and clarify relationships among populations within the Amazonian clade. These studies are critical for a deep understanding about the origin and maintenance of the Amazonian biodiversity.

The phylogeny of *Martinella* reconstructed here supports the recognition of two new species that were previously identified solely based on morphology. These two new species, *M. lanuginosa* and *M. tomentosa*, were described and illustrated. In addition, the analysis of a comprehensive collection of specimens of *Martinella* allowed us to identify key morphological characters to distinguish *M. insculpta* from *M. obovata* such as the shape of mature stems in cross section (quadrangular in *M. insculpta* vs. cylindrical in *M. obovata*), leaflet texture (coriaceous in *M. insculpta* vs. chartaceous in *M. obovata*), and inflorescence structure (botryoid in *M. insculpta* vs. racemose in *M. obovata*).

This thesis highlights the importance of in-depth taxonomic studies, those of which represent the only way to uncover hidden diversity. These studies are particularly important in megadiverse regions, such as the Neotropics, where many sampling lacunae still remain.