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Phylogenetics of the neotropical liana genus *Pedersenia* (Amaranthaceae: Gomphrenoideae) and discovery of a new species from Bolivia based on molecules and morphology

Abstract

Borsch T., Ortuño Limarino T. & Nee M. H.: Phylogenetics of the neotropical liana genus *Pedersenia* (Amaranthaceae: Gomphrenoideae) and discovery of a new species from Bolivia based on molecules and morphology. – Willdenowia 41: 5–14. – Online ISSN 1868-6397; © 2011 BGBM Berlin-Dahlem.
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The monophyletic genus *Pedersenia* comprises eleven species in the Neotropics, one of which, *P. volubilis*, is here described as new to science, being an endemic of mixed semi-deciduous Bolivian-Tucumán Interandean lower sub-humid forest at mid elevations of the Department of Santa Cruz, Bolivia. Among the liana species of *Pedersenia*, it differs by twining stems, synflorescences constituted by terminal stems of several metres and small, lanceolate leaves. It is also characterised by two synapomorphic substitutions in the *matK* CDS. Sequence data of *trnK/matK* show considerable divergence between morphologically very similar liana species of tropical evergreen broad-leaved forests from different geographical regions. The phylogeny suggests the only self-sustaining tree-like species from the dry inter-Andean valleys to be derived from lianas. Allopatric speciation fostered by the ecological and geographical differentiation of the forest communities may have led to morphologically cryptic species, underscoring the need for an integrated morpho-molecular revision of the genus *Pedersenia*.

Resumen

El género monofilético de *Pedersenia* actualmente comprende once especies aceptadas del neotrópico, una de las cuales, *P. volubilis*, se describe como nueva especie. Esta es endémica de una mezcla del bosque semi-decíduo interandino y el bosque subhúmedo subandino Tucumano-Boliviano, del departamento de Santa Cruz, Bolivia. Entre las especies de lianas, *P. volubilis* se diferencia por los tallos volubles y la ramificación de las sinflorescencias terminales de varios metros y por sus hojas lanceoladas. Además la especie se caracteriza por dos sustituciones en las secuencias del gen *matK*. Los datos moleculares (*trnK/matK*) muestran una considerable divergencia entre las especies de lianas morfológicamente muy similares de bosques húmedos de diferentes regiones geográficas. Las únicas especies autosostenibles arborescentes de los valles secos interandinos parecen haber derivado de lianas. La especiación alopatrica provocada por la diferenciación ecológica y geográfica de las comunidades forestales puede haber desembocado morfológicamente en especies crípticas, lo que subraya la necesidad de una revisión integral morfo-molecular del género *Pedersenia*.

Additional key words: *Pedersenia volubilis*, molecular systematics, *matK/trnK*, cryptic species, endemics, Boliviano-Tucumano biogeographical province

Introduction

The genus *Pedersenia* Holub represents a neotropical lineage of subfamily Gomphrenoideae of the Amaranthaceae. Most species of *Pedersenia* are woody lianas growing up to 10 m high on trees in various tropical forest types, whereas a small group of shrubs are endemics to

the dry valleys of the Andes in Bolivia and Peru. *Pedersenia* has suffered a complicated taxonomic history. The first species were described by Martius (1825) under *Trommsdorffia* Mart. (*T. argentata* Mart., *T. aurata* Mart., *T. pulverulenta* Mart.; published again 1826). Morphologically, these species are characterised by hermaphro-

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ditic flowers, bilobed stigmas and an androeceum that is basally fused into a cup with broadly rounded pseudostaminodia. Dietrich (1839) merged *Trommsdorffia* with *Iresine*, without providing any arguments. However, his decision seems plausible as the species of both genera are similar in general appearance. They possess complex thyrsoid synflorescences with hundreds to even thousands of flowers. Both genera are also similar in their life form spectra (lianas, shrubs, small trees) and all species of both genera develop conspicuous white trichomes at the abaxial side of the tepals at maturity, serving for dispersal of the deciduous mature flowers.

Several additional species with the above mentioned *Trommsdorffia* features were described under the generic name *Iresine* by Willdenow (1806; *I. canescens* Humb. & Bonpl. ex Willd.), Uline & Bray (1896; *I. completa* Uline & W. L. Bray), Urban (1907; *I. domingensis* Urb.), Standley (1917; *I. cardenasii* Standl., *I. costaricensis* Standl., *I. wrightii* Standl.), Fries (1920; *I. macrophylla* R. E. Fr.), Suessenguth (1934; *I. flavopilosa* Suess., *I. laurifolia* Suess., *I. tenuis* Suess., *I. weberbaueri* Suess.), Chodat & Hassler (1903; *I. hassleriana* Chodat) and Chodat & Rehfoos (1927; *I. guaranitica* Chodat).

Moquin-Tandon's (1849) transfer of *Trommsdorffia* to the large tropical genus *Alternanthera* was not accepted by other authors. Species of *Alternanthera* can easily be recognised by globose or subglobose stigmas, providing a synapomorphy for the monophyletic genus *Alternanthera* within *Gomphrenoideae* (Sánchez del-Pino & al. 2009). The synflorescences consist of much bigger sessile or stalked heads of densely arranged flowers, very different in appearance from those in *Iresine* or *Trommsdorffia*.

Borsch (1995) analysed the pollen morphology of Central American species of *Trommsdorffia* and found similarities with *Pfaffia* Mart. rather than with *Iresine*. Pollen in *Trommsdorffia*, *Pfaffia* and other gomphrenoid genera was later described as metareticulate (Borsch & Barthlott 1998), whereas *Iresine* type pollen has broadly vaulted mesoporia and evenly spread tectal microspines (Borsch 1998). Ongoing molecular and palynological work on *Iresine* (Borsch, Flores, Zumaya & Müller, pers. comm.) provides evidence that species with *Iresine* type pollen form a clade distinct from those with metareticulate pollen. Phylogenetic analyses of *Pfaffia* and its allies, based on chloroplast *trnK/matK*, *atpB-rbcL* spacer and nuclear ITS sequences (Borsch, Ortuño & Müller, pers. comm.) and of the *Gomphrenoideae* as a whole using *trnL-F* and *rpl16* intron sequence data (Sánchez del-Pino & al. 2009) further reveal a rather isolated position of *Trommsdorffia*. Therefore, the restoration of the genus *Trommsdorffia* by Pedersen (1997), following the concept of Martius (1825), is strongly substantiated by molecular phylogenetic hypotheses. However, while working on *Asteraceae*, Holub (1998) found that the earlier name *Trommsdorffia* Bernh. is validly published (Bernhardi 1800). Holub therefore replaced *Trommsdorffia* Mart.

by the new name *Pedersenia* and provided new combinations for a number of species as they had been accepted by Pedersen (1997).

The genus *Pedersenia* has never been revised and species limits, particularly of the lianas, are unclear. Several characters such as leaf shape and venation patterns, colour and size of tepals were put forward as diagnostic characters for species but often can be found to vary gradually. Following the synopsis by Pedersen (1997, 2000) the genus comprises 8 to 12 species. Some of these species appear to be endemics of Central America (*P. costaricensis* (Standl.) Holub in Costa Rica, *P. completa* (Uline & Bray) Borsch in Nicaragua and Panama (Borsch 2001) or the few tree-like species of the inner Andean dry valleys of Bolivia and Peru (*P. weberbaueri* (Suess.) Holub, *P. cardenasii* (Standl.) Holub; Borsch 1993; Borsch & al. in press). However, distribution patterns of the species occurring in the Caribbean and in the South American lowlands cannot be assessed at this point because of the lack of a reliable taxonomic treatment.

The *Amaranthaceae* comprise about 80 species in Bolivia and several new endemic species have been described recently, for example in *Gomphrena* (Ortuño & Borsch 2005, 2006). In the course of a collaborative project on the diversity and systematics of the *Amaranthaceae* of Bolivia and of preparing the treatment of the family for the Catálogo de las plantas vasculares de Bolivia (P. Jørgensen, S. G. Beck & M. H. Nee, in prep.), herbarium specimens of *Pedersenia* were encountered which deviate by leafy synflorescences with an apparently winding main axis. Such plants were then also collected during fieldwork in the department of Santa Cruz, in Florida Province west of Santa Cruz de la Sierra.

To get insights into the phylogenetic position of these deviating plants within *Pedersenia* and to support a taxonomic treatment, we used sequences of the *trnK* group II intron including the *matK* gene. Sequences of *trnK/matK* have been shown to contain high levels of historic signal and thus constitute a highly efficient phylogenetic marker at various levels in land plants (Hilu & al. 2003; Müller & al. 2006; Borsch & Quandt 2009). This region was also successfully used for analyses of phylogeography and speciation in *Aristolochia* (Watanabe & al. 2006) and *Erica arborea* (Désamoris & al. 2001). Weising & Gardner (1999) located universal primers for amplifying cp microsatellites in the 5' part of the *trnK* group II intron.

Aims of this study were to clarify the taxonomic status of the deviating plants of *Pedersenia* using both molecular and morphological data and to describe them as a new species. Furthermore, the utility of molecular markers to assess species diversity in taxonomically difficult genera such as *Pedersenia* should be tested.

Material and methods

Taxon sampling and plant material — Fieldwork was carried out in the surroundings of Santa Cruz de la Sierra

and the Parque Nacional Amboró in Bolivia in autumn 2002. Material of *Pedersenian* was examined from the herbaria B, BOLV, LPB, MEXU, MO, NY and USZ (Thiers 2008+). Types of names of other *Pedersenian* species were examined morphologically in the wider context of this project and of work underway for a treatment in Flora Neotropica by the first author.

DNA isolation, amplification and sequencing — Genomic DNA was isolated from silica gel dried or herbarium material following a modified CTAB triple extraction protocol (Borsch & al. 2003). The *trnK* intron including *matK* was then amplified in two overlapping halves using primers *trnK2F* bryo (Wicke & Quandt 2009) + *ACmatK1400R* and *ACmatK490F* + *trnK2R*. Otherwise, primer sources and PCR conditions were as described in Müller & Borsch (2005). Gel purified products were directly sequenced via Macrogen Inc. (Seoul, South Korea). Pherograms were edited and assembled using PhyDE (Müller & al. 2005+).

Sequence alignment, coding of length mutational events and phylogenetic analysis — The *matK/trnK* alignment of the *Amaranthaceae* by Müller & Borsch (2005) was used to add further sequences following the rules outlined by Löhne & Borsch (2005) using PhyDE (Müller & al. 2005+). Several mutational hotspots (see Appendix II) were excluded from the matrix subjected to tree inference. Indels were coded using the Simple Indel Coding method (Simmons & Ochoterena 2000). Phylogeny was reconstructed using parsimony with PRAP (Müller 2004). Ratchet settings were 200 iterations with 25 % of the positions randomly upweighted (weight=2) during each replicate and 10 random addition cycles. The number of steps for each tree and the homoplasy indices (CI, RI, RC) were calculated with PAUP*v.4.0b10 (Swofford 1998). Node support was calculated using 10 000 jackknife replicates, TBR branch swapping, 36.788 % of characters deleted in each replicate, and one tree held. Other methods such as Bayesian or Maximum Likelihood were not employed in this study because the data set for *Pedersenian* still is rather small and only one node between different taxa within the crown group gains statistical support. The presence of many autapomorphies for species was better illustrated (potential species identifiers in case of terminal branches) by plotting character state transitions (ACCTRAN) directly on the tree.

Results

Pedersenian volubilis Borsch, Ortuño & M. Nee, **sp. nov.** Holotype: Bolivia, Depto. Santa Cruz, Prov. Florida, carretera antigua a Cochabamba, “Achira”, 18°10'S, 63°49'W, 1100 m, 22.9.2002, T. Borsch & J. Coimbra 3537 (LPB; isotypes: B, USZ).

A Pedersenian hassleriana caulium parte distali volubili 2–3 m longa e nodis cunctis copiose ramosa; foliorum

ramos synflorescentiae axillantium lamina multo minore 25–50(–60) mm longa 10–15 mm lata anguste ovata vel lanceolata; tepalis omnino scariosis pallide brunneis vel perpallide viridi-brunnescentibus prominenter trinerviis exterioribus 2 glabris; nec non basibus nucleotidicis in positone 317 sequentiae geneticae *matK* in A et in positione 1132 in T mutatis differt.

Woody liana, 5–10 m long; stems branched, the upper parts twining around branches of other woody plants; bark pale brown to cream, somewhat shiny, glabrous. *Leaves* opposite, petiolate, petiole 3–4 mm long, blade narrowly ovate to lanceolate, 25–50(–60) × 10–15 mm, acuminate at apex, cuneate and very narrowly decurrent on the petiole, glabrous or very sparsely pubescent on the lower surface with short, simple, appressed trichomes. *Synflorescences* constituted by the upper parts (2–3 m) of the main twining stems, with many, up to 15 cm high, narrowly pyramidal, mostly erect, 2–3 times branched thyrsoid structures, branching from opposite axils, often up to higher branching orders subtended by small cauline leaves. *Flowers* generally hermaphroditic, solitary on a 0.3 mm long peduncle, arranged in subglobose paracladia of 8–15 flowers, the axes very densely pubescent with white, 2–3-celled, uniseriate trichomes with a rough surface. *Bracts* triangular or rounded-triangular, 0.5–0.7 mm long, 1-veined, membranaceous, pale brown, dorsally sparsely pubescent with trichomes concentrated near the midvein; trichomes 1–2 celled, uniseriate, 0.2–0.3 mm long with a rough surface. *Bracteoles* distinctly shorter than the flower, suborbicular, boat-shaped (saccate), 0.6–0.7 mm long, 1-veined, membranaceous, pale brown, dorsally sparsely pubescent with trichomes concentrated near the apex; trichomes as in bracts. *Tepals* broadly ovate to ovate-oblong, the lateral margins mostly parallel, the apex mucronate, outer two tepals 1.5–1.7 mm long, inner 1.2–1.4 mm long, only slightly narrower than outer, all distinctly 3-veined with prominent veins, scarious, very pale greenish brown to pale brown, no thinner margin differentiated laterally, the outer two tepals glabrous, the inner densely pubescent with appressed, 0.5–0.8 mm long, uniseriate, 2–3-celled, white trichomes with rough surface; basal dispersal trichomes stiff, very slightly undulate, smooth, 1.7–2 mm at maturity, very pale brown. *Stamina* with linear, 0.8–1.1 mm long filaments united for 15 % into a staminal cup with pseudostaminodia; staminal cup broadly rounded to quadrangular-obtuse, 0.3 mm high, much shorter than the free part of the filament, brown; anthers unilocular, 0.3–0.35 mm long. *Ovary* uniovulate, style 0.1–0.15 mm long but rather distinct, stigma with two erect triangular-rounded, 0.15 mm long lobes, dark blackish brown. *Fruit* a subglobose, indehiscent utricle, with a minute corolla; seeds not known.

Molecular data — The multiple sequence alignment of *trnK/matK* comprised five mutational hot spots (see Ap-

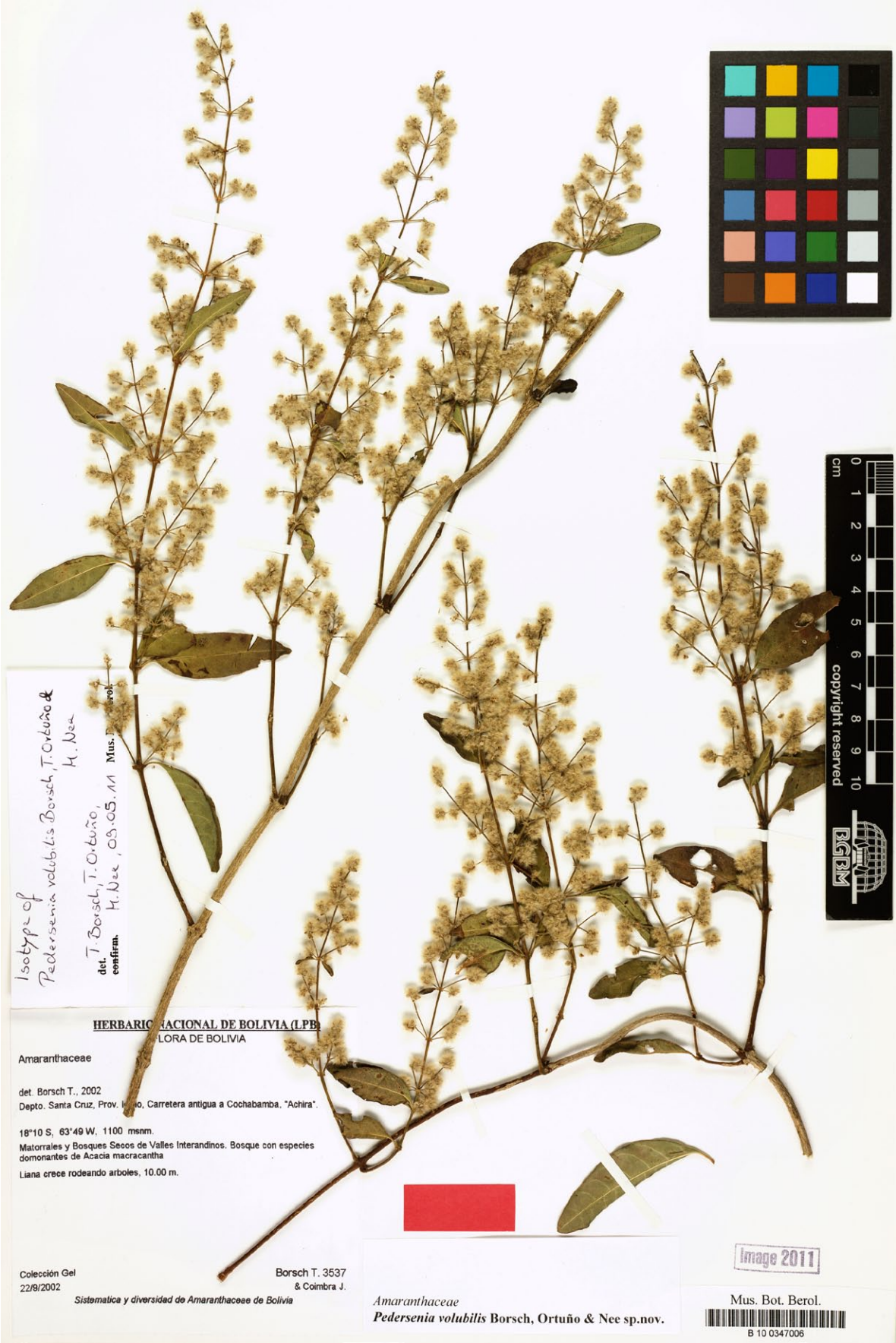


Fig. 1. *Pedersenia volubilis* – isotype at B.

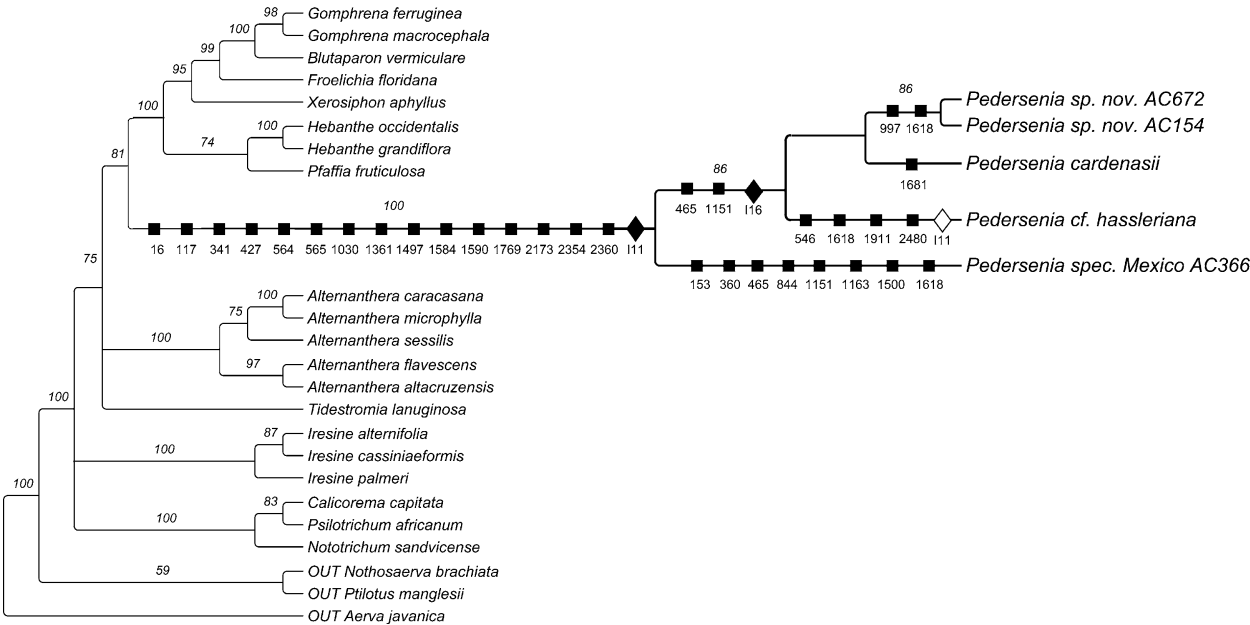


Fig. 2. Strict consensus of two shortest trees obtained from the *trnK/matK* data set. Numbers above branches are Jackknife support values. Black boxes indicate unambiguous character state transformations (substitutions) referring to the *Pedersenia* clade with an indication of the character number in the matrix. Two indels are mapped with the rhombus symbol, a reversal is indicated by an empty box.

pendix 2) that were excluded from the analysis. The *trnK/matK* matrix comprised 2512 characters of which 30 were from indels (in total 401 variable but uninformative, 250 variable and informative). Two shortest trees with a length of 933 steps (CI=0.808, RI=0.741, RC=0.599, HI=0.192) were recovered. The strict consensus tree (Fig. 2) depicts unambiguous character state transitions relevant for *Pedersenia*, most of which are apomorphic. The two samples of *P. volubilis* share substitutions in positions 317 (C substituted by an A) and 1132 (G substituted by a T; in parallel also in *Gomphrena*) of the *matK* coding region which, based on the current taxon sampling, are unique identifiers of this species within the genus.

Additional specimens seen — BOLIVIA: SANTA CRUZ: PROV. FLORIDA: 2 km NW of Samaipata, 18°10'S, 63°53' 30"N, 1675 m, 21.7.1994, *M. Nee* 45266 (C, LBP, MEXU, MO, NSW, NY, SI, USZ); 6.5 km (by road), 3 km (by air) NE of central square in Mairana on road to Yunga de Mairana, 18°06'S, 63°56'W, 1800 m, *M. Nee* 49304 (CTES, LPB, NSW, MO, NY, US, USZ); Camino Pampa Grande y Oleoducto, a 5.6 km de Pampa Grande, 18°04'S, 64°05'W, *P. Acevedo Rodriguez*, *L. Arroyo* & *B. Mostacedo* 4484 (LPB, USZ).

Habitat and ecology — *Pedersenia volubilis* is known from the Boliviano-Tucumano biogeographical province in Bolivia (Navarro & Maldonado 2002). Within this province, the collections *Nee* 45266 and 49304 and *Acevedo & al.* 4484 are from inter-Andean lower sub-humid forests (Bosque subhúmedo inferior interandino Boliviano-Tucumano; Navarro & Maldonado 2002) that

generally occurs at an altitudinal range between 1500 and 1900 m between 18°05'S and 63°55'W, on the southern margin of the Amboró National Park, Prov. Florida. Depending on differences in precipitation and temperature (ranging from 545 mm per year at Mairana to 930 mm per year at Monteagudo) these mesophytic forests are composed of a variable mixture of deciduous and evergreen plants. The canopy has a height of 15–20 m and the forests have a well developed understorey reaching 8 to 10 m. This vegetation series is transitional between the inter-Andean dry valley vegetation and the subhumid semi-open forests (bosque ralo) of the hill ranges (seranías). The type collection *Borsch & Coimbra* 3537 is from the same kind of vegetation but the collecting locality is drier and dominated by *Acacia aroma* Hook. & Arn., a species usually found in the Inter-Andean dry forest (Ibisch & Mérida 2004) and in the Prepuna (López 2000). The collection *Nee* 49304 was found at the upper limit of the dry forest of *Schinopsis haenkeana* Engl. with the transition to a more humid montane forest with *Clethra scabra* Pers., *Mimosa lepidota* Herzog and *Agarista boliviensis* (Sleumer) Judd.

Distribution — The new species is known from four collections; the actual range of the species may follow the distribution of the “Bolivian-Tucumano Interandes lower subhumid” forest. As suggested by Navarro & Maldonado (2002) some areas with climatic and geomorphological characteristics similar to the province of Florida also occur in the departments of Chuquisaca and further south to the department of Tarija. Based on the distribution of characteristic floristic elements of the vegetation

series (Navarro & Maldonado 2002) such as *Schinopsis haenkeana* (Kazuya & al. 2006; Meneses & al. 2009), a similar vegetation could occur furthermore in the department of Cochabamba. This means that *Pedersenia volubilis* could have a wider range of distribution in Bolivia than currently known. However, floristic studies and plant collecting in all these areas (e.g. Ortuño & Borsch 2006; Antezana & Navarro 2002; Meneses & al. 2009; López & Ortuño 2008) have not yielded any further specimens.

Conservation status — *Pedersenia volubilis* can hardly be evaluated using the criteria established by the International Union for Conservation of Nature (IUCN 2001), due to the limited numbers of plants known and the lack of any reliable estimate of the frequency of this species even in the area between Samaipata and Mairana, where it has been collected. Nevertheless, the conservation status of the respective forests was assessed (Dirección de ordenamiento territorial 2008) applying the IUCN criteria on the ecosystem scale and using the characterisation of the vegetation by Navarro & Maldonado (2002). Therein the “Bolivian-Tucuman Interandes lower subhumid forest” is categorised as vulnerable (VU) because the natural vegetation is increasingly becoming fragmented by agricultural activities and grazing and degraded through selective tree felling.

Discussion

Phylogenetic relationships in *Pedersenia* — The Alternantheroid clade (Sánchez-del Pino & al. 2009; *Alternanthera* + *Pedersenia* + *Tidestromia*) is not resolved by our *trnK/matK* data, whereas the Gomphrenoid and the Iresinoid clades (although the latter is not well represented) are found. *Pedersenia* is shown inconsistently (8% JK) as sister to the Gomphrenoid clade sensu Sánchez-del Pino & al. (2009), suggesting that especially the existence of the Alternantheroids needs to be further tested by additional characters. The genus *Pedersenia* is well supported as monophyletic based on *matK/trnK* sequences (Fig. 2), corroborating the combined analysis of *trnL-F* and *rpl16* sequence data (Sánchez del-Pino & al. 2009) but with a somewhat different taxon sampling. Synapomorphies for *Pedersenia* are the staminal cup with more or less broadly rounded pseudostaminodia and filaments that are linear in their free part and the distinctly bilobed stigma with broad to triangular lobes that are laterally not completely covered with stigmatic papillae (Borsch 1995, 2001). Five subequal, broadly lanceolate tepals with a rounded, obtuse to even somewhat hooded apex and three distinct veins are not found in other genera of *Gomphrenoideae*. Other morphological characters are less specific to *Pedersenia*. Pollen grains are metareticulate (Borsch 1995, 1998) as in all core *Gomphrenoideae* but resemble with their dodecaedric form and complete tecta the pollen found in most species of *Alternanthera*. *Pfaffia* and relatives have very similar pollen but with a higher aperture number and, as a

consequence, globose form (Borsch 1998). Also, the long trichomes that develop at the abaxial side of the tepals at maturity and obviously serve dispersal are found in a similar manner in some species of *Alternanthera* (e.g. *A. congesta* Suess. & Stützer, *A. laguroides* Standl.).

Within *Pedersenia*, relationships are resolved but only moderately supported. The specimen from Mexico (hitherto not assigned to any species) appears as sister to the South American taxa. It also has a high number of apomorphies. Within the South American core group (86% JK), the three sampled species are well differentiated but relationships lack statistical support. However, it is noteworthy that the erect tree-like *P. cardenasii* from the dry inner-Andean valleys of Bolivia is nested within terminals, all of which are lianas. The tree therefore suggests the erect habit to have evolved from lianas within the context of colonising the dry valley habitats. Further sampling of taxa and characters (adding to the *trnK/matK* data set) is needed to corroborate this hypothesis and to further illuminate phylogenetic relationships in *Pedersenia*.

*Species diversity in *Pedersenia* and delimitation of the new species*

— The genus *Pedersenia* currently contains eight species names of mostly still uncertain status according to The Plant List (2010). Pedersen (1997), who presented a synopsis based on a discussion of morphological similarities, accepted seven species (under *Trommsdorffia*). Holub (1998) then published new combinations for these taxa under *Pedersenia* without any further analysis. Pedersen (2000) argued that *P. hassleriana* also is a distinct species and added a further new combination. Both authors had overlooked that *Pedersenia completa* (Uline & Bray) Borsch, **comb. nov.** ($\equiv$ *Iresine completa* Uline & W. L. Bray in Bot. Gaz. 21: 349. 1896) is another morphologically distinct taxon from Central America that does not belong to the Iresinoid clade (Borsch 1995, 2001) but to *Pedersenia* based on floral and pollen morphology. The recently described *Pfaffia miraflorensis* Agudelo & Franco-Rosselli also belongs to *Pedersenia*. However, it remains to be seen if this species is an endemic of Colombia as stated by Agudelo-H. (2008) or whether it belongs to either Central American-Caribbean or South American species. There are other taxa of *Pedersenia* with only little vaulted and hairy pseudostaminodia (e.g. *P. completa*). Considering current morphology-based taxonomic hypotheses there are eleven species in *Pedersenia*. Nevertheless, about twice as many names exist for somewhat diverging specimens, several at infra-specific rank, which need further study.

Although several type specimens were examined by Pedersen (1997, 2000) to group them into morphospecies, it is important to note that especially the lianas within *Pedersenia* differ mostly by size and indumentum (density, length and colour of trichomes) of the tepals, the stiffness and colour of the long trichomes at the base of the tepals, which serve in dispersal, the complexity of the synflorescences and the size and venation of the

leaves. All of these characters may vary gradually and more specimens needed for the application of descriptive statistics are hardly available. All these characters may easily be influenced by abiotic conditions, so it is unclear in how far morphological variation is simply caused by environmental modifications.

Nee (2004) identified the other specimens of *Pedersenia* that occur in the Parque Nacional de Amboró as *P. argentata* (Mart.) Holub. However, the plants in the lowland areas of Bolivia (see *P. cf. hassleriana*, Fig. 2) may better be identified as *P. hassleriana*. The diagnosis of the latter species in Chodat & Hassler (1903 under *Iresine*) is very short and basically refers to a stronger fused staminal cup consisting of a darker brown and thicker tissue, with more emarginate pseudostaminodia than in *P. aurata*. Pedersen (1997) argued that *P. argentata* is conspecific with *P. aurata* and merged them under the former name although the tepals differ in size, texture and strength of the veins. Plants similar to the type of *P. aurata* (Martius 1825, 1826 under *Trommsdorffia*) occur widely in South America and appear to be distributed in the areas of evergreen tropical broad-leaved forests from the lowlands to mid elevations of the Andes. This taxon may be replaced by *P. hassleriana* in the more seasonal semideciduous forests (e.g. bosque semidecuido chiquitano) of southern Bolivia, Argentina, and Paraguay, and, according to Pedersen (2000), by *P. macrophylla* in still drier, seasonal forests of the Chaco. On the other hand, specimens with emarginate pseudostaminodia occur throughout South America, indicating that the diagnostic characters of *P. hassleriana* put forward by Chodat & Hassler (1903 under *Iresine*) are not overly reliable. This may just give some examples of the difficult taxonomy in the genus *Pedersenia* that can hardly be resolved using morphology alone.

Pedersenia volubilis deviates morphologically from all other lianas by the richly branched upper stems that wind around trunks of other woody plants with long (2–3 m) terminal parts of which thyrsoid structures (here termed synflorescences) branch off laterally from all nodes. Its leaves are much smaller (only half the length) than in all other liana species of *Pedersenia*, and contrary to other species often subtend also synflorescence branches of higher order. Floral morphological characters are more difficult to delimit but the pale brown or very light greenish brown, scarious tepals with three very prominent veins, the outer two of which are glabrous, appear to be characteristic. Sequences of *matK/trnK* of the two different individuals examined are identical and converge on two synapomorphies that provide molecular support for the identity of this species (Fig. 2).

Conclusion and future work

Pedersenia may contain cryptic species with only minor morphological differences but different autecology and long separated biogeographic histories. As primary forest lianas, their evolution may be closely connected to

the history of certain vegetation types. Consequently, geographically well-isolated species of *Pedersenia* have become distant genetically although morphological differences are scarce. In comparison to other lineages within the *Amaranthaceae*, the evolution of different habits in *Pedersenia* is restricted to the self-supporting tree-like relatives of *P. cardenasii* growing in the inner Andean dry valleys of Bolivia and Peru. Ecological differences between species probably do not relate to pollination and dispersal, explaining largely similar floral structures. However, there are so far no data on the kind of pollinators in species of *Pedersenia*. This study indicates considerable sequence differentiation despite of high morphological similarity in lianas from Mexico (*P. sp.* AC366; collected in evergreen broad-leaved primary forests of Chiapas) and from Bolivia, provisionally identified as *P. hassleriana* (AC108). There are a number of other recent examples that describe considerable speciation of clades occurring in geographically and ecologically patterned areas. Sotuyo & al. (2007) provide an example of “morphostatic radiation” as a consequence of isolation and local adaptation in *Caesalpinia* (*Fabaceae*) in dry forests of Mexico. Saerkinen & al. (2011) found a much higher diversity of endemics in *Mimosa* (*Fabaceae*) in inner-Andean dry valleys in Peru. Similar cases of morphologically cryptic species are also known from temperate regions such as *Veronica* in the Mediterranean (Martínez-Ortega & al. 2004). While all of these cases likely involve allopatric speciation of closely related taxa, Gurushidze & al. (2008) provided evidence for genetically well differentiated but morphologically similar and therefore cryptic species in *Allium* that nowadays occur sympatrically. Although there are so far no molecular data on population structure for *Pedersenia*, field observations suggest that deviating taxa of this genus are generally not found in the same habitat. Therefore, morphologically static, allopatric speciation appears to be the main pattern of diversification in *Pedersenia*. Future work has to complement detailed morphological examination of geographically distinct specimens with the sequencing of molecular markers. The *trnK/matK* region is certainly a good candidate although it has to be optimised for degraded DNA as sampling will have to rely largely on herbarium material to allow representativity. The lianas of *Pedersenia* are difficult to encounter in the field and due to the wide distribution of the genus throughout the woody vegetation of the Neotropics without major concentration of species in certain geographical regions, collecting is time and resource intensive. However, diversity in *Pedersenia* may in fact be considerably higher than the currently recognised eleven species.

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Appendix 1. Plant material used for the molecular phylogenetic part of this study.

Collections made in the field first list country and locality in the case of both herbarium specimens and silica gel dried samples. The DNA isolate number is generally given as a unique identifier for each sample; it follows the specimen data and precedes the EMBL/GenBank accession number. In case sequences were published prior to this study the respective reference is provided instead of the specimen data.

Outgroups: *Amaranthaceae*: *Amaranthoideae*: *Aerva javanica* Juss.: Müller & Borsch (2005), AY514793. *Nothosaerva brachiata* Wight: Müller & Borsch (2005), AY514806. *Ptilotus manglesii* (Lindl.) F. Muell.: Müller & Borsch (2005), AY514824.

Ingroup: *Amaranthaceae*: *Amaranthoideae*: *Calicorema capitata* (Moq.) Hook. f.: Müller & Borsch (2005), AY514807. *Nototrichium sandwicense* (Gray) Hillebr.: Müller & Borsch (2005), AY514817. *Psilotrichum africanum* Oliv.: Müller & Borsch (2005), AY514822. — *Amaranthaceae*: *Gomphrenoideae*: *Alternanthera altacruzensis* Suess.: Sage & al. (2007), AM887483. *A. caracasana* Kunth: Hilu & al. (2003), AF542595. *A. flavescens* Kunth: Sage & al. (2007), AM887484. *A. microphylla* R. E. Fr.: Sage & al. (2007), AM887485. *A. sessilis* (L.) DC.: Müller & Borsch (2005), AY514796. *Blutaparon vermiculare* (L.) Mears: Müller & Borsch (2005), AY514798. *Froelichia floridana* Moq.: Müller & Borsch (2005), AY514799. *Gomphrena ferruginea* Pedersen: Ortuño & Borsch unpubl. data [Ortuño & Borsch 3650 (B, LPB)], AM887525. *G. macrocephala* A.

St.-Hil.: Müller & Borsch (2005), AY514801. *Hebanthe occidentalis* (R. E. Fr.) Borsch & Pedersen var. *occidentalis*: Müller & Borsch (2005), AY514821. *H. grandiflora* (Hook) Borsch & Pedersen: this paper [T. Borsch, H. Flores & H. Ochoterena 3882, AC365 (B, MEXU)], FR870370. *Iresine alternifolia* (Uline & W. L. Bray) S. Watson: Sage & al. (2007), AM887490. *I. cassiniaeformis* Standl.: Sage & al. (2007), AM887489. *I. palmeri* S. Watson: Müller & Borsch (2005), AY514804. *Pedersen* *cardenasii* (Standl.) Holub.: Sage & al. (2007), AM887491. *P. cf. hassleriana* (Mart.) Holub.: Sage & al. (2007), AM887522. *P. volubilis* Borsch, Ortuño & M. Nee: this paper [T. Borsch & Coimbra 3537, AC154 (B, LPB)], FR870371; [M. Nee 45266, AC672 (NY)], FR870372. *P. sp. AC366*: this paper [T. Borsch 3887, AC366 (B, LPB)], FR870373. *Pfaffia fruticulosa* Suess.: Borsch, Flores, Zumaya & Müller unpubl. data [T. Borsch 3522 (B, LPB)], AM887492. *Xerosiphon aphyllus* (Pohl ex Moq.) Pedersen: Ortuño, Borsch & Müller, unpubl. data [S. Splett 701 (B)], AM887523.

Appendix 2. List of indels.

- Gap of 95 nt in *Ptilotus*.
- “CWAAT” SSR in *Hebanthe grandiflora*.
- “TAAAGAA” partial SSR in *Alternanthera caracasana* and *A. microphylla*.
- “T” in *A. microphylla*, overlapping with 3.
- “T” SSR in *Alternanthera altacruzensis*. HS1 (microsatellite) in *trnK* 5' part.
- “A” of unknown origin in *Ptilotus*.
- 1 nt gap in *Gomphrena* and *Blutaparon*, probably deletion.
- 5 nt gap in *Gomphrena macrocephala*, probably deletion.
- “A” SSR in *Tidestromia*.
- “TAAGG” SSR in *Tidestromia*. HS2 in *trnK* 5' part. HS3 in *trnK* 5' part (one substitution in *Pedersen* AC108 coded separately).
- “AGAGAATCC” SSR in four samples of *Pedersen* (AC103, AC154, AC366, AC372).
- “A” in *Alternanthera altacruzensis*, probably insertion.
- “AAAAATAA” SSR in *Pfaffia*.
- “AAA” SSR in *Tidestromia*. HS4 (microsatellite) in *trnK* 5' part just adjacent *matK* CDS, one nt shorter in AC366 (coded in indel matrix).
- 9 nt gap in *Calicorema*.
- 9 nt gap in *Tidestromia*.
- 6 nt gap in *Nototrichium*. HS5 inversions of one codon in *matK* CDS (but not variable in *Pedersen*).
- “TTAGTY”-SSR with one substitution at the end, autapomorphic to *Alternanthera sessilis*.
- “AATTGATC” partial SSR in *Aerva*; motif not exactly clear in sparsely sampled outgroup.
- “AMTGATC” SSR in *Alternanthera* and *Gomphrena ferruginea*.
- Second repeat of 21 in *Alternanthera sessilis*.
- “TTTATTTTGATA” SSR in *Pfaffia*.
- “CTTTTCTATA” SSR in *Alternanthera flavescens*.
- “CTGAAA” SSR in *Ptilotus*.
- “AAAT” in all *Alternanthera*.
- “ATGCTTATACAATAAAT” SSR in *Alternanthera sessilis*.
- “TGAGTATTCAAC” SSR in *Ptilotus*.
- “TTTGTT” SSR in *Alternanthera sessilis*.
- “TC” SSR in *Alternanthera caracasana* and *A. microphylla*.
- “T” SSR in *Iresine cassiniaeformis*.