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Infrageneric reclassification and phylogenetic inference in the genus *Masdevallia* Ruiz & Pav. (Orchidaceae, Pleurothallidinae) - preliminary results of nrDNA based analysis

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Abstract: The genus *Masdevallia* is one of the largest genera within the subtribe Pleurothallidinae. lts present infrageneric classification, based on easily recognized morphological features, does not reflect natura! relationships and evolutionary trends within the genus. To test its relative utility in infrageneric classification and in resolving genus phylogeny, sequences of the Interna! Transcribed Spacer region (ITS1-5,8S-ITS2) were identified for 37 taxa and combined with additional 35 sequences taken from the GenBank. Results of maximum parsimony based on phylogenetic analysis indicate that the level of sequence divergence is sufficient to resolve infrageneric relationships. The implications of current results for the classification of *Masdevallia* are also briefly discussed. Many of the infrageneric changes made by C.A. Luer since his first classification of the genus in 1986 are not supported by results of this study.

Key words: Orchidaceae, Pleurothallidinae, *Masdevallia,* ITS, infrageneric classification, maximum parsimony (MP)

1. Introduction

The orchid genus *Masdevallia* Ruiz & Pav comprises mare than 400 taxa and is one of most species rich genera in the subtribe Pleurothallidinae Lindl. (tribe Epidendreae; subfamily Orchidaceae) (Luer 1986a, Luer 2000a, Pridgeon *et al.* 2005). Representatives of the genus are distributed from southem Mexico to southem Brazil, occurring mainly in montane forests, where they grow in cool and humid conditions (Luer 1986a, 1986b, 2000b). Plants are perennial, very small to large, weak to robust, epiphytic, lithophytic to terrestrial. They can be distinguished from other members of Pleurothallidinae by the calliferous petals and the lip hinged to the free, curved extension of the apex of the column-foot. Flowers are often large and showy, with variously connate and commonly tailed sepals. The aerial leaf-bearing secondary stem (ramicaul) is produced by the stout rhizome (primary stem) and is usually shorter than the coriaceous, erect or suberect leaf. The most remarkable orchid feature is the usually small lip, thick or thin, simple or divided by callus into epichile and hypochile.

Masdevallia was described by Spanish botanists Ruiz and Pavón in 1794 and named in honour of Jose Masdevall, physician in the court of Charles III of Spain. Reichenbach (1861) made the first attempt to indicate subdivisions of the genus by introducing the 'section' rank. The sections were then retained by Woolward (1896) in her monograph of *Masdevallia,* with additional subsectional divisions. However, the first detailed infrageneric classification of *Masdevallia* was published by Kranzlin (1925). This classification was rejected by Luer as 'error-ridden' and misleading in 1986. Instead, he proposed his own classification, dividing the genus into 5 subgenera, 17 sections and 12 subsections. His earlier revision of *Masdevallia* also resulted in recognition of new genera, e.g. *Dracula* Luer, *Dryadella* Luer, and *Trisetella* Luer. During the next decade, Luer made numerous changes in his classification by adding new sections or subsections, merging others, or finally raising same sections to subgeneric rank. Tuus, the most recent infrageneric classification of *Masdevallia* includes 10 subgenera, 13 sections and 13 subsections (Luer 2000a, 2000b, 2001). Such significant differences in division

of the genus are due not only to the large and still growing number of taxa, but also the relative lack of comprehensive morphological characters available to define groups of species. Luer (2000a, 2000b, 2001) based his delimitations of species within the genus on most easily recognized morphological features, to make the classification easy to use. Additionally, he noted that because of strong homoplasy rife within vegetative morphology, only the floral features, mainly the sculpture of the ovary, peduncle (the inflorescence-bearing stem), floral bracts, lip morphology, and number of flowers, might be used as primary diagnostic characters. This is true since only very few species of *Masdevallia* can be easily recognized by their habit alone (e.g. the unique, pendent, bluish leaves of *M. caesia* Roezl). Vegetatively, many *Masdevallia* species are too similar to each other, and without an inflorescence even the subgeneric rank is not recognizable. Nevertheless, Luer also stated that some of the floral features, like pedicel or shape and connation of sepals, are of lower value as diagnostic characters above the specific level, because of superficial similarities occurring often in many infrageneric taxa.

In 2001 Pridgeon *et al.* conducted an extensive phylogenetic analysis of Pleurothallidinae. The study, based on an analysis of nuclear and plastid DNA sequences, included 35 species of *Masdevallia*. The results confirmed the monophyletic character of the genus (with the exclusion of *M. erinacea* Rchb.f.) as well as its close relation to the genera previously recognized by Luer as *Dracula*, *Porroglossum*, and *Trisetella* (Pridgeon *et al.* 2001). However, mainly because of the relatively low level of molecular divergence among species of *Masdevallia*, those authors did not propose any further changes in infrageneric classification. Also, they stated that such a complicated infrageneric scheme appears to be unnecessary and unlikely to reflect any natural relationships and evolutionary patterns. Nevertheless, those authors suggested that such incongruence found between molecular-based phylogeny and morphological classification proves the need of further and more detailed studies upon the genus.

The aims of our study were: (*i*) to test whether increasing numbers of taxa analysed will increase the strength of the phylogenetic signal (by increasing the overall sequence divergence); and (*ii*) on the basis of results derived from phylogenetic analysis, to determine whether the current morphological classification of *Masdevallia* is phylogenetically informative.

To accomplish this goal, we sequenced nuclear ribosomal DNA internal transcribed spacer (ITS) regions. Obtained sequences where then combined with additional sequences, taken from GenBank resources, into a single matrix. Because of the preliminary character of this study and insufficient availability of DNA data (ITS sequences) from previous studies, no additional cpDNA markers were sequenced.

The ITS region has been used extensively in determining phylogenetic relationships within the Orchidaceae and proved to be a valuable molecular marker at the tribal (Douzery *et al.* 1999), subtribal Madiinae (Baldwin 1992), Laeliinae (Berg *et al.* 2000), Disinae (Douzery *et al.* 1999), Pogoniinae (Cameron & Chase 1999), Pleurothallidinae (Pridgeon *at el.* 2001)] and generic level [*Lycaste* and *Anguloa* (Ryan *et al.* 2000), *Cypripedium*, *Selenipedium* and *Paphiopedilum* (Cox *et al.* 1997), *Coelogyne* (Gravendeel *et al.* 2001)].

2. Material and methods

2.1. Plant sampling

To determine relationships within *Masdevallia*, we collected samples for 37 species. With the use of additional 35 GenBank sequences, we compared 72 taxa in total. The sampling covered 9 of 10 currently recognized subgenera with 12 of 13 sections and 11 of 13 subsections (Luer 2000a, 2000b, 2002). Due to the lack of plant material, our study did not include any representatives of subgen. *Cucullatia* Luer, sect. *Racemosae* Woolw. (subgen. *Masdevallia* Ruiz & Pav.) and of subsect. *Successiviflorae* Luer (subgen. *Polyantha* Luer, sect. *Polyanthae* Luer) and subsect. *Zahlbrucknerae* (Luer) Luer (subgen. *Pygmaeia* Luer, sect. *Amaluzae* Luer). Remaining subgenera, sections and subsections were sampled according to the number of species attributed (the most numerous taxa were sampled most extensively). However, in few cases we were unable to provide more than 1 species, thus some subgenera and sections might be sampled insufficiently. Outgroup taxa were selected among other members of the subtribe Pleurothallidinae. Plant samples were obtained from a private collection (Kusibab, Kraków) and the Botanical Garden of the University of Heidelberg.

2.2. DNA extraction, PCR and sequencing

DNA was extracted from 100 mg of fresh leaves or 20 mg of silica-dried leaves with the Genomic Mini AX Plant kit (A&A Biotech, Poland), following manufacturer's instructions. The ITS region (ITS1-5,8S-ITS2) was amplified via polymerase chain reaction (PCR) with primers 101F and 102R (Douzery *et al.* 1999) and in few cases universal primers ITS5 and ITS4 (White *et al.* 1990). The PCR protocol involved 30 cycles, starting with 5 min of initial premelt (94°C), then each cycle with 45 s of denaturation (94°C), 45 s of annealing (52°C), 1 min of extension (72°C), with final extension for 7 min (72°C). Because of the high GC content of the ITS region, DMSO (dimethyl sulfoxide) was used to reduce secondary structure formation of melted DNA strands and thus improve primer binding.

PCR products were purified with the High Pure PCR Product Purification Kit (Roche Diagnostics, Germany),

following manufacturer's protocol. Purified PCR products were sequenced by using the BigDye™ Terminator Cycle Sequencing Kit (Applied Biosystems, UK). Cycle sequencing conditions were as follows: 20 sec of initial denaturation followed by 25 cycles, each with 15 sec of denaturation (94°C), 20 sec of annealing (52°C) and 1 min of elongation (60°C). Sequencing reactions were purified by an ExTerminator (A&A Biotech, Poland), following manufacturer's protocol. Pelleted samples were sequenced on the Applied Biosystems 377 automated sequencer. Both strands (upstream and downstream) were sequenced to assure accuracy in base calling. Sequences were edited in FinchTV (Geospiza, USA), sequencing artifacts at the 5' and 3' ends were removed. All sequences were deposited in the GenBank (accession numbers in Table 1).

2.3. Phylogenetic analysis

Initial alignment was accomplished with ClustalX 1.8b and then corrected manually. Indels of 3_bp or longer were encoded as additional binary characters with the 'simple method', as described by Simmons and Ochoterena (2000). The final data set was analysed with PAUP version 4.0b4a (Swofford 2000), with *Lepanthospis* sp. (Cogn.) Ames, *Trichosalpinx blaisdellii* (S. Watson) Luer and *Scaphosepalum microdactylum* Rolfe designated as an outgroup. Full heuristic search was performed with optimality criterion set to maximum parsimony (MP). The initial MAXTREES option was set to 10_000 trees because of computer memory limitations. Tree-bisection-reconnection (TBR) was employed as a branch swapping algorithm with the MULTREES option in effect, 100 replicates of random sequence addition and ACCTRAN optimisation (Fitch parsimony). All characters were unordered and equally weighted. Individual gaps were treated as missing values. Internal support of clades was evaluated by the bootstrap (Felsenstein 1985) with 1000 bootstrap replicates. We define bootstrap support as weak for bootstrap values of 50-70%, moderate for 70-90%, and strong for 90-100%, respectively. The mean sequence divergence was calculated from uncorrected p-distance matrix under Pairwise Distance options in PAUP. For the resultant trees, tree length, Consistency (Ci), and Retention (Ri) Indices were recorded.

3. Results

The ITS region (ITS1-5,8S-ITS2) included a total of 776 characters yielding 248 variable positions (~32%), of which 156 were parsimony-informative (115 within the ingroup). The MP analysis yielded 6301

Table 1. GenBank accession numbers for all *Masdevallia* species used in this study, including the source publication

Masdevallia species	GenBank accession number	Sources
M. amabilis	GBAN-DQ923793	this study
M. amaluzae	GBAN-AF262799	Pridgeon *et al.* 2001
M. amanda	GBAN-DQ923763	this study
M. ampullacea	GBAN-AF262772	Pridgeon *et al.* 2001
M. aphanes	GBAN-AF262802	Pridgeon *et al.* 2001
M. barlaeana	GBAN-DQ923787	this study
M. bicornis	GBAN-DQ923764	this study
M. bicornis	GBAN-AF262792	Pridgeon *et al.* 2001
M. buccinator	GBAN-DQ923771	this study
M. caloptera	GBAN-DQ923766	this study
M. caloptera	GBAN-AF262773	Pridgeon *et al.* 2001
M. calura	GBAN-DQ923773	this study
M. campyloglossa	GBAN-DQ923781	this study
M. caudivolvula	GBAN-AF262770	Pridgeon *et al.* 2001
M. chaparensis	GBAN-AF262797	Pridgeon *et al.* 2001
M. chontalensis	GBAN-DQ923767	this study
M. citrinella	GBAN-AF262774	Pridgeon *et al.* 2001
M. civilis	GBAN-DQ923770	this study
M. coccinea	GBAN-AF262789	Pridgeon *et al.* 2001
M. collina	GBAN-AF262784	Pridgeon *et al.* 2001
M. colossus	GBAN-DQ923768	this study
M. coriacea	GBAN-AF262781	Pridgeon *et al.* 2001
M. cyclotega	GBAN-DQ923789	this study
M. datura	GBAN-DQ923761	this study
M. decumana	GBAN-AF262795	Pridgeon *et al.* 2001
M. discoidea	GBAN-DQ923759	this study
M. echo	GBAN-DQ923760	this study
M. erinacea	GBAN-AF262788	Pridgeon *et al.* 2001
M. floribunda	GBAN-AF260146	Van der Berg *et al.* 2000
M. harlequina	GBAN-DQ923790	this study
M. herradurae	GBAN-DQ923786	this study
M. heteroptera	GBAN-AF262800	Pridgeon *et al.* 2001
M. hieroglyphica	GBAN-AF262798	Pridgeon *et al.* 2001
M. ignea	GBAN-DQ923785	this study
M. impostor	GBAN-DQ923772	this study
M. infracta	GBAN-AF262785	Pridgeon *et al.* 2001
M. kuhniorum	GBAN-DQ923784	this study
M. kyphonantha	GBAN-AF262780	Pridgeon *et al.* 2001
M. lehmannii	GBAN-DQ923783	this study
M. limax	GBAN-AF262796	Pridgeon *et al.* 2001
M. macropus	GBAN-DQ923791	this study
M. melanoxantha	GBAN-DQ923782	this study
M. mentosa	GBAN-AF262777	Pridgeon *et al.* 2001
M. mooreana	GBAN-DQ923769	this study
M. murex	GBAN-DQ923765	this study
M. nidifica	GBAN-AF262787	Pridgeon *et al.* 2001
M. ophioglossa	GBAN-AF262790	Pridgeon *et al.* 2001
M. oreas	GBAN-AF262779	Pridgeon *et al.* 2001
M. ova-avis	GBAN-DQ923780	this study
M. pachyura	GBAN-DQ923792	this study
M. picturata	GBAN-AF262775	Pridgeon *et al.* 2001
M. pinocchio	GBAN-AF262778	Pridgeon *et al.* 2001
M. platyglossa	GBAN-DQ923779	this study
M. pyxis	GBAN-DQ923778	this study
M. racemosa	GBAN-AF262771	Pridgeon *et al.* 2001
M. reichenbachiana	GBAN-AF262783	Pridgeon *et al.* 2001
M. rolfeana	GBAN-DQ923788	this study
M. rubeola	GBAN-AF262791	Pridgeon *et al.* 2001
M. saltatrix	GBAN-AF262793	Pridgeon *et al.* 2001
M. sanctae-fidei	GBAN-DQ923795	this study
M. schroederiana	GBAN-DQ923777	this study
M. strobelii	GBAN-DQ923776	this study
M. teaguei	GBAN-AF262801	Pridgeon *et al.* 2001
M. titan	GBAN-AF262803	Pridgeon *et al.* 2001
M. torta	GBAN-DQ923775	this study
M. tovarensis	GBAN-DQ923774	this study
M. uniflora	GBAN-AF262769	Pridgeon *et al.* 2001
M. venezuelana	GBAN-AF262782	Pridgeon *et al.* 2001
M. ximenae	GBAN-AF262794	Pridgeon *et al.* 2001
M. sp. 124633	GBAN-DQ923762	this study
M. sp. 124981	GBAN-DQ923794	this study

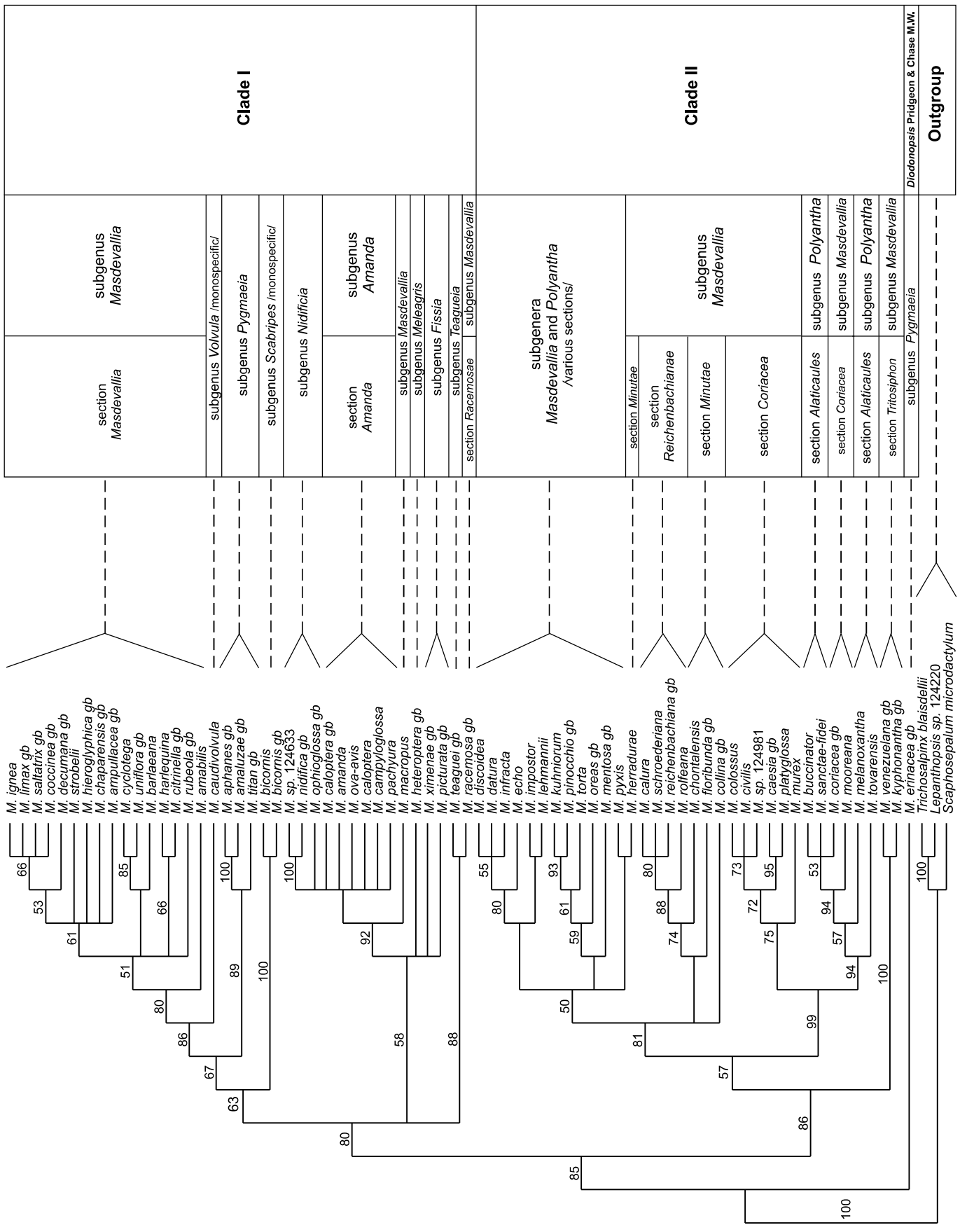

Fig. 1. Strict consensus of 6301 most parsimonious trees found in maximum parsimony (MP) analysis (bootstrap support above branches) combined with the most recent infrageneric classification of *Masdevallia* (subsections not shown). Sequences for species denoted with *gb* were obtained from the GenBank resources

most parsimonious trees with a length of 538 steps, with Ci=0.602 and Ri=0.797. The average number of changes per variable site is 2.1. The estimated transition/transversion (ts/tv) ratio is 2.08, which is congruent with the value of 2.0 expected for recently diverged sequences (Holmquist 1983; Matsumoto *et al.* 1998). The average mean sequence divergence is 3.3% for the ingroup, 8.6% for the outgroup, and 11.8% between ingroup and outgroup taxa. Strict consensus of all most parsimonious trees (bootstrap values above branches) combined with a scheme of recent infrageneric classification (Luer 2000b, 2001, 2002, 2003) is depicted in Figure 1.

Monophyly of *Masdevallia* is moderately supported (BP 85%) except for *M. erinacea*, with the other taxa falling into 2 major clades, also moderately supported (BP 80% and 86%, respectively). The first major clade is composed of species currently attributed to sect. *Masdevallia* Ruiz & Pav. of subgen. *Masdevallia* Ruiz and Pav. Subgenera *Volvula* Luer, *Pygmaeia* Luer, *Scabripes* Luer, *Nidificia* Luer, *Amanda* Luer, *Meleagris* Luer, *Fissia* Luer and *Teagueia* Luer, are grouped within 3 weakly to moderately supported subclades. The second major clade is composed of species attributed to subgen. *Masdevallia* and *Polyantha* Luer, grouped in 2 moderately to highly supported subclades (BP 81% and 99%, respectively) with sect. *Tritosiphon* (Schltr.) Sweet. (subgen. *Masdevallia*) as a sister group. No subsectional divisions congruent with the current morphological classification could be recognized; thus they are omitted in further discussion.

4. Discussion

4.1. ITS sequence divergence

The averaged ITS sequence divergence within the *Masdevallia* ingroup is lower than reported for other genera, e.g. 4% for *Iliamna* (Malvaceae) (Bodo Slotta 2000), 12.76% for *Gentiana* (Gielly *et al.* 1996), 11% for *Rubus* (Lawrence & Campbell 1999). However, the higher ITS sequence divergence at the infrageneric level can correspond to a higher level of homoplasy, as an excess of autapomorphic characters (Gielly *et al.* 1996) and thus significantly diminish the strength of phylogenetic resolution (e.g. *Gentiana*, sect. *Chondrophyllae*, Yuan & Kupfer 1997). The quality of phylogenetic resolution can be reduced not only by the overall low sequence divergence but also by hybridization with introgression events (Yuan & Kupfer 1997; Wendel & Doyle 1999; Small & Wendel 2000; Bodo Slotta 2000) Hybridization is commonly reported in *Masdevallia* (Luer 1986b, 2000a, 2000b), since many species grow in sympatry as well as many exhibit a variety of intermediate forms between other closely related taxa (e.g. species in subgen. *Masdevallia*, sect. *Reichenbachianae* Woolw. and *Minutae* Rchb.f. *ex* Woolw.). The high excess of transition over transversion (ts/tv= 2.08) along with significant homoplasy within morphological features and commonly reported hybridization events (contributing weak interbreeding barriers) would suggest a very recent origin and rapid radiations within the genus.

4.2. Sectional and subgeneric relationships within *Masdevallia*

According to the obtained results, subgenera *Masdevallia* and *Polyantha* – as currently delimited – are paraphyletic. The section *Masdevallia* (subgen. *Masdevallia*) is retained within the first major clade with moderately supported monophyly (BP 80%). All species of this section have single-flowered peduncles and smooth, simple and thin lips. The close relation of *Masdevallia caudivolvula* Kraenzl (subgen. *Volvula* Luer) is also moderately supported (BP 86%). This subgenus was previously described as sect. *Caudivolvulae* Luer within subgen. *Masdevallia* (Luer 1986b). Subgenera *Pygmaeia* Luer and *Scabripes* Luer, have weakly supported relations (BP 67% and 63% respectively) with subgen. *Masdevallia*, sect. *Masdevallia*. Monospecific subgen. *Scabripes* Luer was proposed by Luer in 2003 to include the unique species *Masdevallia bicornis* Luer. As stated by Luer (2000a), the existence of some morphological synapomorphies allowed him to classify this species as an unusual representative of subgen. *Masdevallia*. Monophyly of subgen. *Pygmaeia* is moderately supported (BP 89%), with the exceptional occurrence of *Masdevallia titan* Luer (subgen. *Masdevallia*, sect. *Durae* Luer). This subgenus was established by merging two sections, *Amaluzae* Luer and *Aphanes* Luer of subgen. *Masdevallia* and section *Pygmaeae* Luer of subgen. *Amanda* Luer to adopt 'loosely related' but small-sized species (Luer 2000b).

The subgenus *Amanda*, sect. *Amanda* Luer is highly supported, with the inclusion of subgen. *Nidificia* Luer (BP 92%). In a previous classification the latter subgenus was described as sect. *Nidificae* Luer within *Amanda* (Luer 1986b), which seems right. The occurrence of *M. macropus* F. Lehm. & Kraenzl. (subgen. *Masdevallia*, sect. *Masdevallia*) and *M. campyloglossa* Rchb.f. (subgen. *Masdevallia*, sect. *Coriacea* Rchb.f.) is unexpected and requires further investigation. Subgen. *Fissia* Luer, previously described as sect. *Fissae* Rchb.f. within subgen. *Amanda* (Luer 1986b), is not supported by molecular data, although its inclusion within *Amanda* receives relatively weak support (BP 58%). All species of the subgenera *Amanda*, *Nidificia* and *Fissia*, as currently delimited, have crested ovaries and peduncle rounded in cross-section. The position of *M. heteroptera* (subgen. *Meleagris* Luer) within species of subgen. *Amanda*, as previously described by Luer (if subgen.

Fissia is retained as a section), requires further taxonomic investigation. As stated by Luer (2003), sepals in *Meleagris* are substantially free near their base and the lip is hinged beneath to the extension of the column-foot, similarly as in many species of subgen. *Amanda*. Inclusion of other species from subgen. *Meleagris* in further analysis should help to resolve the relationship between both subgenera. The relation between *Masdevallia teagueia* Luer (subgen. *Teagueia* Luer, monospecific) and *M. racemosa* Lindl (subgen. *Masdevallia*, sect. *Racemosae* Woolw, monospecific), although moderately supported (BP 88%), is rather problematic since both species are very distantly related as long as morphology is considered.

The second major clade contains the remaining species from subgenera *Masdevallia* and *Polyantha*. The latter subgenus was previously described as section within subgen. *Masdevallia* (Luer 1986b). Sect. *Tritosiphon* of subgen. *Masdevallia*, previously described as subsection within sect. *Masdevallia*, is highly supported as monophyletic (BP 100%). All species within this section have solitary, tubular flowers (due to deep connation of sepals). The section *Coriacea* Rchb.f. (subgen. *Masdevallia*) is paraphyletic and moderately supported (BP 75%), with the exclusion of *Masdevallia mooreana* Rchb.f. and *Masdevallia coriacea* Lindl. The latter taxa are nested within species from sect. *Alaticaules* (Kraenzl.) Luer (subgen. *Polyantha*). The subclade containing both sect. *Alaticaules* and species from sect. *Coriacea* is highly supported (BP 94%) as well as its close relation to sect. *Coriacea s.s.* (BP 99%). These arrangements also require further taxonomic investigations, since both sections seem to be distantly related. For instance, the peduncle is rounded in cross-section in sect. *Coriacea* and triangular in sect. *Alaticaules,* although some clones of *Masdevallia infracta* Lindl. or *Masdevallia tovarensis* Rchb.f. occasionally can also produce rounded peduncles (Luer 1986b, 2000a).

The remaining subclade (BP 81%) contains sections *Minutae* and *Reichenbachianae* (subgen. *Masdevallia*) and a very weakly supported group (BP 50%) of species from various sections of subgenera *Masdevallia* and *Polyantha*. For this subclade we were unable to recognize any significant correlations with morphological classification. The section *Reichenbachianae* is moderately supported (BP 88%) and nested within species of sect. *Minutae*, indicating their close relations. According to Luer (2001), the ranges of both sections merge in Central America and many species from sect. *Reichenbachianae* have intermediate forms between both sections. Also, sect. *Reichenbachianae* is a Central American counterpart of sect. *Coriacea* from the Andean region, which is correlated with their separation into 2 moderately to highly supported subclades.

The isolated position of *Masdevallia erinacea* (subgen. *Pygmaeia*, sect. *Pygmaeae*) is congruent with results of Pridgeon *et al.* (2001), who included it into their new genus *Diodonopsis* (Rchb.f.) Pridgeon & M. W. Chase with 4 additional taxa *a priori* transferred from sect. *Pygmaeae (*Pridgeon & Chase 2001). As stated by Luer (2002) the proposal of the new genus was unwarranted, since sect. *Pygmaeae* was raised only to include those species that did not fit into any other sections and subsections within subgen. *Pygmaeia*. In our opinion, inclusion of other species from this section in a further analysis is required to resolve whether the proposal of a new genus was justified or not.

5. General conclusions

The ITS sequence divergence within the genus *Masdevallia*, after extending taxon sampling, remains rather low if compared to results from other studies at the infrageneric level. Despite the low sequence divergence, the contribution of phylogenetically informative characters is high (115 variable characters within the ingroup), yielding a relatively well-resolved phylogenetic tree. According to the obtained results, many of the infrageneric changes made by Luer since his first classification of the genus in 1986 are not supported. Because of frequent hybridization and possible introgression events within the genus, phylogeny based only on nuclear sequence data can be fairly reliable. Thus, further phylogenetic analysis should include at least 2 additional cpDNA markers (Shaw *et al.* 2005)

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