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Biodiv. Res. Conserv. 1-2: 14-17, 2006

State of research on the genus *Disticholiparis* Marg. & Szlach. (Orchidales, Malaxidinae)

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Abstract: The article is an introduction to taxonomic problems within the recently described genus *Disticholiparis* Marg. & Szlach. Genetic variation in the ITS region was analysed, which proved the distinct character of the genus *Disticholiparis*, as compared with other taxa of the former genus *Liparis*. A determination key to *Disticholiparis* and closely related Asiatic genera of the Malaxidinae, as well as a description of the genus are presented. Goals and methods of further research are listed and briefly characterized.

Key words: *Disticholiparis*, *Liparis*, Malaxidinae, Orchidales, taxonomy, systematics

1. Introduction

The genus *Disticholiparis* Marg. & Szlach. was proposed in 2004 (Margońska & Szlachetko 2004). Within the genus we joined 38 species, previously included in the section *Distichon* Ridley (1888) of the genus *Liparis* L. C. Rich., but about a dozen other taxa need verification and establishing of their systematic affiliation and taxonomic status. The examination of materials for a taxonomic revision of the subtribe Malaxidinae (Margońska, in preparation) and *Gynostemia Orchidalium* (Szlachetko & Margońska 2002) provided the background for distinguishing a set of morphological and anatomical features typical for this orchid group but at the same time unusual and unique within the subtribe Malaxidinae or even the order Orchidales.

This article presents initial results of genetic tests aiming to prove the distinct character of the genus *Disticholiparis*, as compared with other taxa of the former genus *Liparis*. Moreover, a determination key to the *Disticholiparis* and closely related Asiatic genera of the Malaxidinae, as well as a description of the genus are presented. Finally, aims and methods of further research are outlined.

2. Material and methods

DNA isolation. Total genomic DNA was extracted from 100 mg of fresh-frozen or 20 mg of silica-dried leaves (Chase & Hills 1991) by using the DNA Mini Plant kit (A&A Biotechnology, Poland) according to the manufacturer's protocol.

Amplification and sequencing. The ITS region (ITS1-5.8S-ITS2) was amplified via a polymerase chain reaction (PCR) with the primers AB101 and AB102R (Douzery *et al*. 1999). Both strands were sequenced to assure accuracy in base calling. Sequence Navigator was used to edit the sequences and each individual base position was examined for agreement of the two strands by using AutoAssembler. Before alignment, the sequence of each taxon was checked by using blast on the NCBI website.

Phylogenetic analysis. DNA sequences were aligned by ClustalX™ (Thompson *et al*. 1997) and adjusted by eye. The ITS region was analysed by using the heuristic search method of PAUP version 4.0b10 (Swofford 1998). The optimality criterion was parsimony with tree-bisection-reconnection (TBR) branch swapping and the MULTREES option in effect, simple addition and ACCTRAN optimization. Gaps were treated as missing values. Internal support of clades was evaluated by the bootstrap (Felsenstein 1985) with 500 bootstrap replicates. All characters were unordered and equally weighted (Fitch 1971). The aligned ITS matrix consisted of 705 bp of which 368 were variable and 245 were potentially parsimony informative. The bootstrap consensus tree, with a length of 650 steps, CI of 0.760 and RI of 0.834, is shown in Fig. 3.

Plant material. Sources of plants and vouchers are available upon request from the author.

3. Results

3.1. Systematics and key to the genus *Disticholiparis* and closely related genera of Malaxidinae

Systematics:

Order	Orchidales Bromhead
Family	Orchidaceae Juss.
Subfamily	Epidendrideae Lindl.
Tribe	Malaxideae Lindl.
Subtribe	Malaxidinae Benth. & Hook., 22 genera (Margońska 2003), over 1500 species
Genera	*Alatiliparis* Marg. & Szlach., 2000 (3 species) Asia
	Crossoglossa Dressl. & Dodson, 1993 (ca. 24 species) Americas
	Liparis L.C. Rich., 1818 (ca. 400 species) Eurasia, Americas
	Disticholiparis Marg. & Szlach., 2004 (38 species) Asia, Madagascar

Key:

1 Plants 1-leaved; inflorescence forming compact and articulately laterally flattened spike; floral bracts lanceolate to ovate or cucullate, arranged distichously, imbricate basally each other
Disticholiparis

1* Plants 1-many-leaved; inflorescence racemose, floral bracts lanceolate to triangular, arranged spirally along inflorescence rachis, distinctly separate from each other 2

2 Lip usually folded down about half of lamina length or erect, with similar form of marginal and remaining part of lamina, without strongly elongate, attenuate apex
Liparis

2* Lip always erect and ovate to oblong ovate, with apical portion thin, sagittate, with gently wavy margins, and a characteristic elongate, attenuate apex 3

3 Leaves distichously distributed along elongated and ascending stem; inflorescence dense to subdense, rachis straight; lip with 2 small, thick calli present bilaterally near lamina base; gynostemium distinctly abbreviated, erect, staminodes reduced
Crossoglossa

3* Leaves spirally distributed along distinct pseudobulb; inflorescence lax to sublax, rachis slightly zigzag; lip nectary just below lamina base, relatively large, globose; gynostemium short, gently arcuate, staminodes narrow or wide but always with two characteristic triangular folds at apical part near clinandrium and wing-like near column base
Alatiliparis

3.2. Description of the genus *Disticholiparis*

Autotrophic and sympodial orchids, epiphytic or sometimes terrestrial; from about 10 cm up to 50 cm high, with a well-developed root system and creeping rhizomes. Their only sole leaf blade always distinctly separated from the petiole and leaf sheath by a horizontal scar (Fig. 1).

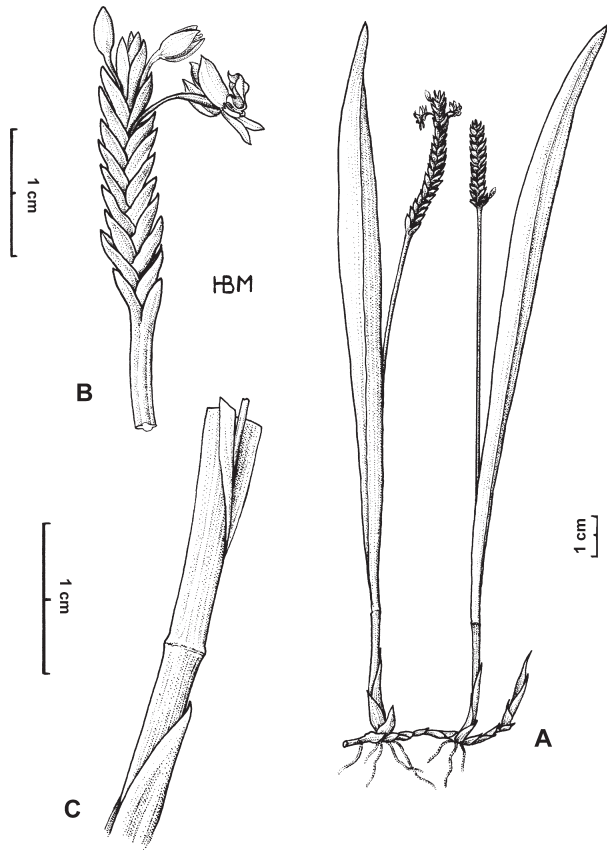

Fig. 1. *Disticholiparis gibbosa* (Finet) Marg. & Szlach: A – Habit; B – Inflorescence; C – Scar between leaf petiole and sheath (based on the type-specimen)

The most characteristic and unique features of the genus are: (*i*) spike form – always characteristically compact and articulately laterally flattened; and (*ii*) floral bracts lanceolate to ovate or cucullate, specifically arranged distichously and imbricate basally each other. Flowers short-lived, opening successively (almost 2-3 at the same time), distichously arranged, sometimes scented, entomophilous but probably sometimes autogamous. Adult flowers small, rarely over 1.5 cm in diameter, tepals usually recurved, particularly sepals, while young petals often erect. Lip outline can be erect (e.g. *D. compressa*) or often geniculate (e.g. *D. disticha*). Lip with pad-like callus (which holds off the column of gynostemium) at basal part, usually with a characteristic nectary positioned just near or below basal callus. Gynostemium always erect, of two kinds: usually short, robust, with a second, additional pair of staminodes in the form of two folds arranged symmetrically, one on

either side of the column (e.g. *D. gibbosa*) (Fig. 2); or sometimes long, narrow and slightly arcuate (e.g. *D. pandurata*).

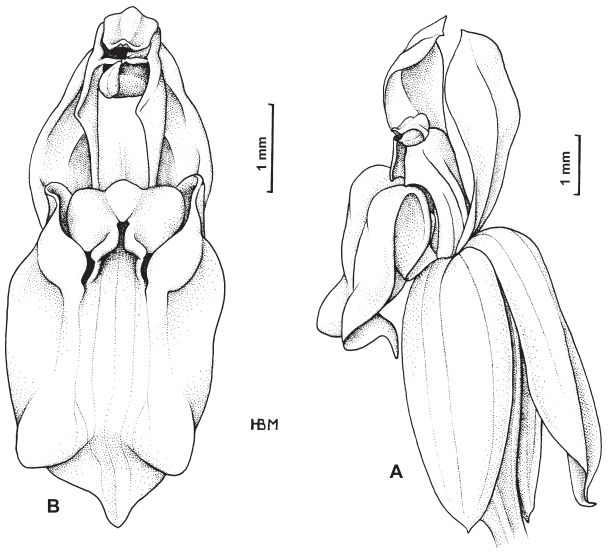

Fig. 2. *Disticholiparis gibbosa* (Finet) Marg. & Szlach. A – Flower – side view; B – Spread lip and gynostemium (based on the type-specimen)

4. Discussion and conclusions

Our initial results of genetic tests have already clearly proved the separate character of this orchid group from the other members of the former genus *Liparis* (Fig. 3). Recently Cameron (2005) also evidently emphasised the distinctness of this orchid group in relation to the other *Liparis* species on the basis of molecular data.

Representatives of the genus *Disticholiparis* seem to be a very interesting group of orchids from the taxonomic point of view, but rather difficult for research. Herbarium specimens and liquid-preserved collections are dispersed, mostly in foreign institutions, often wrongly determined. Many specimens are in poor condition or destroyed. As mentioned above, flowers of the orchids are short-lived, opening successively. For this reason many of the preserved specimens are without flowers and cannot be determined with the use of the classic taxonomic methods. Often herbarium materials are too old for biochemical and genetic techniques. Some specimens important for taxonomic studies are preserved in chemical liquids, e.g. formaldehyde and

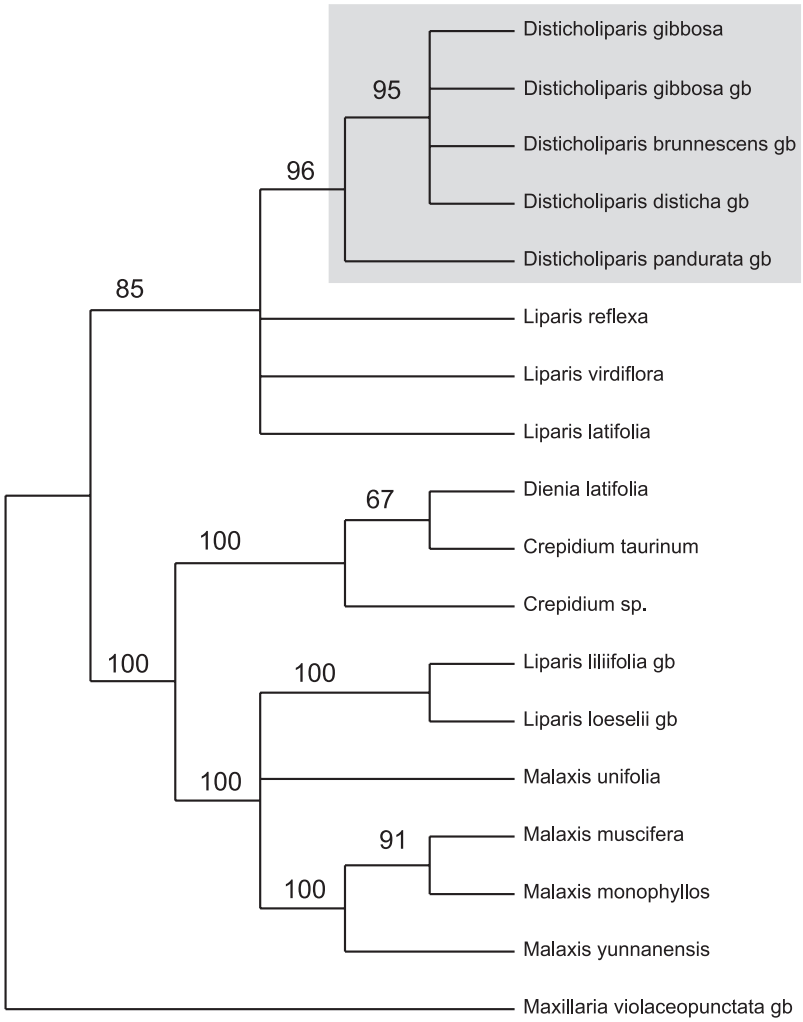

Fig. 3. Results of an analysis of the ITS region in the Malaxidinae (Orchidaceae): 50% majority rule consensus tree (MP); gb – sequences downloaded from the GenBank

glycerine. These chemical substances also make the use of biochemical and genetic methods impossible. Therefore the selection of high quality and representative taxonomic materials for research is very difficult.

Another basic taxonomic problem within the genus is the lack of clear and univocal criteria distinguishing every species. Many of the species are polymorphic. Members of this genus, except only few species (e.g. *D. gibbosa*), are rare or endemic, some of them known only from type-collections. Most of them are highly endangered, so it is not easy to collect alive plants for cultivation or tests, either.

The main aims of further studies will be:

- to review the species content of the genus – taxonomic verification of species, listing of synonyms according to the International Code of Botanical Nomenclature;
- to complete the taxonomic study of the genus both at species and section level: localisation of all existing type-collections, clear and univocal description of all taxa, creation of keys to determine infrageneric taxa;
- to establish the degree of similarity and relationship of *Disticholiparis* taxa and to recognize the real phylogenetic relations between the genus and other genera of the subtribe Malaxidinae – on the basis of data and samples selected during previous, basic research;
- to collect all accessible data about the ecology and biology of examined taxa;
- to map the distribution of *Disticholiparis* species;
- to assess the threats and to elaborate methods for protecting the researched taxa in natural habitats and in conservation culture.

These aims will be realised at the same time with the use of various methods:

- the basic database will be compiled with the use of classic methods (biometric, morphological, anatomical, and nomenclatoric studies) always in relation to type-specimens and protologues; this part of researche will be based on herbarium and liquid-preserved specimens, as well as on living plants, bibliographic and iconographic materials;
- later, the data will be digitalized and processed with the use of numerical techniques (phenetic analysis);
- correctly identified and well preserved younger herbarium specimens and fresh taxonomic samples will be studied by methods biochemical and genetic.

Modern systematics and taxonomic elaborations should be based not only on possibly the most numerous, available taxonomic materials, different kinds of samples (dry, conserved in liquid and alive specimens), the highest numbers of taxonomically important features, but by using all accessible methods, too. Only systematic elaborations like these can be complex sources of knowledge about researched organisms. Realisation of the project will conduce to elaborating the taxonomic revision of *Disticholiparis* at an infrageneric and species level, but also will help to work out a complete monograph of the genus. The obtained results will be an important part of a taxonomic revision of the whole subtribe Malaxidinae (Margońska, in preparation), as well.

Acknowledgments. I am grateful to MSc. Marcin Górniak for genetic analyses of taxonomic samples, and to the Curators of the herbaria AMES, AAU, BM, BP, C, C-GS, FI, G, K, MO, P, SING, US, WU, W-R, Z, for the loan of taxonomic materials and/or their hospitality during my visits. I am obliged to keepers of all visited scientific libraries, as well. Scientific work financed from the resources earmarked for science in years 2003-2005 as the Research Project no. 2 P04C 082 24.

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