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Shehbazia (*Shehbazieae*, Cruciferae), a new monotypic genus and tribe of hybrid origin from Tibet

Shehbazia (*Shehbazieae*, Cruciferae) – новый монотипный род и триба гибридного происхождения из Тибета

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Summary. Generic placement of the Tibetan *Dontostemon tibeticus* known before 2000 as *Nasturtium tibeticum* is discussed in the light of available molecular and morphological data. The species is recognized as a representative of the newly described genus *Shehbazia*. In view of the purported intertribal (*Chorisporae* × *Dontostemoneae*) hybrid origin of the genus, the tribe *Shehbazieae* is established in order to accommodate *Shehbazia* correctly into the currently accepted system of Cruciferae. Morphological and molecular evidences of the hybrid origin of the taxon are briefly discussed.

Аннотация. В работе рассмотрено положение в системе крестоцветных тибетского эндемика *Dontostemon tibeticus*, до 2000 года известного под названием *Nasturtium tibeticum*. Вид отнесен к новому монотипному роду *Shehbazia*. Кратко обсуждены морфологические и молекулярные данные, которые позволяют предполагать возникновение данного таксона в результате древней межтрибовой гибридизации (*Chorisporae* × *Dontostemoneae*), в связи с чем также установлена новая триба *Shehbazieae*.

Nasturtium tibeticum Maxim. described by Maximowicz (1889) based on N.M. Przewalski's collections from Tibet, represents rather rare case in Cruciferae B. Juss. and, in particular, in *Nasturtium* W.T. Aiton, when a species had not experienced a single change of generic placement for more than one hundred years. As late as only in 2000, Al-Shehbaz using morphological, geographical, and ecological evidences, clearly demonstrated that the species is unrelated to the predominantly North American/European *Nasturtium* and placed it in the Central/North Asian *Dontostemon* Andrzej. ex C.A. Mey. (for details, see Al-Shehbaz, 2000). Simultaneously, the first author of the present communication paid attention to the superficial similarity in habit and leaf morphology of *D. tibeticus* to the species of *Chorispora* R. Br. ex DC. but, due to serious differences in the fruit structure, no nomenclatural adjustment has been proposed. Both suggestions received support when molecular phylogenetic data became

available. In the ITS-based study, German et al. (2009) found *D. tibeticus* associated with both tribes *Chorisporeae* C.A. Mey. and *Dontostemoneae* Al-Shehbaz et Warwick and tentatively assigned it to *Chorisporeae* based on the closer affinity of *D. tibeticus* to this tribe. German et al. (2009) also noticed that inclusion of *D. tibeticus* into the analysis strongly affected the position of both tribes with respect to each other. This item was further targeted in the combined cp/nr DNA-based phylogenetic study of *Chorisporeae* (German et al., 2011) and a remarkable incongruence between the positions of the discussed taxon on *trnL-F*- and ITS-based trees was found. While cp data indicated inclusion of *D. tibeticus* into 100% supported *Chorisporeae* clade, it fell out from the latter in most analyses of the ITS data set. Furthermore, its presence in the ITS matrix disturbed monophyly of the *Dontostemoneae*, otherwise supported as monophyletic. Based on the above findings, it was concluded that *D. tibeticus* has to be placed into a genus of its own (German et al., 2009, 2011) but its tribal affiliation was not resolved (German et al., 2011). Addressing these items taxonomically is the principal goal of the present communication.

Material and Methods

For PCR amplification we used DNA from two different herbarium vouchers (T.N. Ho & al. 1643 (MO) and D.E. Boufford et al. 33576 (GH)), which was used in the previous publications (German et al., 2009, 2011). Amplification and sequencing the primers for ITS are given in German et al. (2009, 2011). Forward and reverse sequences from each individual were manually edited in CHROMAS Lite 2.1 (Technesylum Pty Ltd) and combined in single consensus sequences. The sequences of all samples were aligned with CLUSTAL X (Thompson et al., 1997) and subsequently corrected manually in MEGA 6 (Tamura et al., 2013). To test for multiple ITS copies within individuals of *D. tibeticus*, we also cloned PCR amplicons using the TOPO TA Cloning kit (Invitrogen) according to the instructions of the manufacturer. The DNA of 15 clones was isolated with NucleoSpin plasmid kit (Macherey-Nagel, Düren, Germany) according to the instructions of the manufacturer and prepared for sequencing. Sequencing was performed on ABI 377XL automatic sequencer with universal M13 forward and reverse primers.



Fig. 1. Fragments of alignments of variable sites of ITS1 (above) and ITS2 (below) of *Dontostemon tibeticus* and representatives of *Chorisporeae* (*Chorispore* spp., *Parrya* R. Br. spp.), *Dontostemoneae* (*Clausia aprica* (Steph. ex Willd.) Korn.-Tr., *Dontostemon* spp.) and related tribes *Anchonieae* DC. (*Eremoblastus caspicus* Botsch.) and *Hesperideae* Prantl (*Hesperis sibirica* L.).

Results and Discussion

Sequences of all 15 clones are mostly identical to the sequences obtained before from the same individuals (AY558942, AY558970; FN821592). No multiple ITS copies were found. Newly obtained sequences were submitted to the GenBank (accession numbers: LN713849–LN713863).

Among the phenomena causing conflicting topologies of cp and nr DNA-based trees, are for instance, incomplete lineage sorting and long branch attraction (Soltis, Kuzoff, 1995; Wang et al., 2014), but one of the most common and serious reasons is hybridization. This obviously is the case in *D. tibeticus*. Long branch attraction can be ruled out because various phylogenetic data evaluation methods were applied including the Bayesian approach, and this should overcome the problem (Wang et al., 2014). Morphological and molecular data (ITS) pattern of *D. tibeticus*, as shown below, argues in favor of a hybrid origin rather than of incomplete lineage sorting. Especially noteworthy is the result obtained when ITS matrix from German et al. (2011) was split and separate ITS1 and ITS2 trees were constructed. *Dontostemon tibeticus* grouped with the *Dontostemoneae* in the first case and with *Chorispora* in the latter (trees not shown). Such topologies were

congruent with the results of both BLAST search (<http://www.ebi.ac.uk/>) and comparison of variable sites of the sequences (Fig. 1). However, resemblance in the alignment (Fig. 1) was only moderately pronounced. *Dontostemon tibeticus* is apparently not a recent hybrid and the time elapsed since the hybridization event was enough for homogenization of parental ITS copies and subsequent evolution of the formed sequence. This might explain why a clear additive pattern as in the recent hybrids is not observed.

Thus, among three modes of ITS evolution after hybridization – (1) a loss of one copy and fixation of the second driven by unidirectional concerted evolution; (2) formation of a new and hybrid ITS type via concerted evolution representing a mixture of the two original parental ITS sequences; and (3) presence of both ITS copies (Koch et al., 2003, 2010 and examples therein) – the second is apparently peculiar for *D. tibeticus*. This suggestion is supported by the absence of any other copies of ITS checked by cloning which excludes the above options 1 and 3. Interesting is the way of homogenization resulted not in a commonly observed mosaic structure of the derived sequence but instead in ITS1 and ITS2 remained closer to the first and the second parent, respectively.

Table 1

Comparison of some morphological characters of *Dontostemon tibeticus* with members of tribes *Chorispora* and *Dontostemoneae*.

Taxon/Character	<i>CHORISPOREAE</i>	<i>DONTOSTEMONEAE</i>	<i>D. tibeticus</i>
Leaves	Entire to <i>deeply pinnate</i> with few or <i>numerous</i> , remote or <i>connivent lobes</i>	Entire, rarely pinnate with few remote lobes	<i>Deeply pinnate</i> with <i>numerous connivent lobes</i>
Petioles	<i>Strongly expanded</i>	Not or somewhat expanded	<i>Strongly expanded</i>
Stigmas	<i>Strongly 2-lobed</i> with <i>connivent (sub)decurrent lobes</i>	Capitate-2-lobed with free, non or slightly decurrent lobes	<i>Strongly 2-lobed</i> with <i>connivent subdecurrent lobes</i>
Median filaments	Free, toothless, not (e. g., <i>Chorispora</i>) or dilated (e. g., some <i>Parrya</i> s. l.) at base	United or, if free, either <i>toothed</i> and dilated along the whole length, or toothless and dilated in lower half	Free, expanded and sometimes minutely <i>denticulate</i> at base
Anthers	Obtuse, rarely apiculate	Apiculate, rarely obtuse	Obtuse
Cotyledon	Accumbent	Incumbent, more rarely accumbent or oblique	Accumbent
Style	<i>Conical</i>	Cylindrical	<i>Conical</i>
Petals	Yellow, white, lavender, purple, brownish, blackish, sometimes bicolor with darker claws	White (entirely or rarely with purplish claws), lavender, purple	White to lavender with purplish claws

Characters shared by *D. tibeticus* with *Chorispora* are *italicized* and those with *Dontostemoneae* are in addition given in ***bold***.

The supposed hybrid nature of *D. tibeticus* is also reflected in morphological characters (table 1). Although more character states are shared with species of *Chorispora*, first of all *Chorispora*, the intermediate position is more or less obvious (especially if only *Chorispora* is taken into consideration among the genera of relevant tribe) although high role of parallelism in the evolution of morphological structures of Cruciferae should be kept in mind. We hypothesize that *D. tibeticus* originated by ancient hybridization between representatives of the two tribes *Chorispora* and *Dontostemoneae*, close to or most likely congeneric with *Chorispora* and *Dontostemon*, the prior being the maternal parent. The paternal one could be the ancestor of the two closely related and the only *Dontostemon* species occurring in Tibet, *D. pinnatifidus* (Willd.) Al-Shehbaz et H. Ohba and *D. glandulosus* (Kar. et Kir.) O.E. Schulz.

Despite detection of old hybrids is in principle a rather difficult task (Marhold, Lihová, 2006), testing the validity of the hypothesis seems to be a worthy goal. For this, comparative chromosome painting might be a very powerful method as was shown for various taxa across the Cruciferae (e. g., Mandáková, Lysak, 2008; Mandáková et al., 2010a, 2012). In particular, it helped to reveal the intertribal hybrid origin of indigenous Australian Cruciferae taxa (Mandáková et al., 2010b) of the tribe *Microlepidieae* Al-Shehbaz, Warwick, Mummenhoff et M.A. Koch. Another case of successful natural intertribal hybridization in the family was detected in the New Zealandian *Pachycladon* O.E. Schulz (Joly et al., 2009; Zhao et al., 2010) recently also assigned to *Microlepidieae* (Heenan et al., 2012). In both mentioned cases, hybridization was preceded by a long-distant (intercontinental) dispersal of ancestral species, and exclusively taxa of the major evolutionary lineage I of the family (as first outlined by Beilstein et al., 2006) have been involved. Thus, *D. tibeticus* represents the third example of an origin via very distant hybridization in the family with some remarkable differences from the other two cases. Like in the above mentioned taxa, its putative ancestors are also confined to just one evolutionary lineage of the Cruciferae. However, in this case, lineage III is involved in which no intertribal hybridization have been revealed yet. Besides, unlike in the above taxa, no long-distant dispersal of the ancestral taxa apparently took place before the formation of *D. tibeticus*.

Taxonomically, *D. tibeticus* is accommodated here to a new genus named after the leading expert

in the field of systematics of Cruciferae worldwide, Ihsan Ali Al-Shehbaz, in recognition of his enormous contribution to the study of the global diversity of this family and, in particular, his first and considerable correction of taxonomic position of *Nasturtium tibeticum*, hereafter *Shehbazia tibetica*. Taking into consideration the assumed origin of the genus and currently accepted system of the family (Al-Shehbaz, 2012), it is assigned to a new tribe also described herein.

Shehbazia D.A. German, **gen. nov.** – From habitually similar high mountain species of *Chorispora*, *Shehbazia* immediately differs in dehiscent vs. indehiscent fruits, white to pink petals with distinctly darker, deep purple claws (vs. more or less monochromatic), flattened at base and sometimes slightly toothed (vs. neither dilated nor toothed) filaments of median stamens, minute stigma, etc. From representatives of *Dontostemon*, the new genus differs in pinnatifid to pinnatisect leaves with numerous (to 11) and at least distally connivent lobes (vs. entire, dentate or, if pinnatifid, with up to four distant lobes), conical (vs. cylindrical) style and sub-decurrent (vs. diverged) stigma lobes. Unlike both genera, *Shehbazia* is lacking capitate glands (only gland-like warts are present on fruits).

Typus: *Sh. tibetica* (Maxim.) D.A. German.

Shehbazia tibetica (Maxim.) D.A. German, **comb. nov.** – *Nasturtium tibeticum* Maxim., 1889, Fl. Tangut. 1: 54, tab. 26, fig. 12–30. – *Dontostemon tibeticus* (Maxim.) Al-Shehbaz, 2000, Novon 10 (4): 334.

Described from NE Tibet: «Сев.-вост. Тибет: водораздельный хребет между Хуан-хэ и Голубой р., в ущелье у реки, в небольшом количестве, 10 июня в нач. цв.; в горах по р. Тала-чу, 18 июня с перв. цв.; в альпийской области по р. Бэ-чу, 7 июля [18]’84 в цв. и почти спел. плод. / [NE Tibet: watershed between Huang He and Blue River [Yangtze], in the gorge near the river, not common, 10 June with first flowers; in the mountains along the river Tala-Chu, 18 June with first flowers; in the alpine zone along the river Be-Chu, 7 July 1884 with flowers and almost ripen fruits]» (Maximowicz, 1889).

Lectotypus (Grubov in Buzunova, 2000: 71): [China, Xizang] «Tibet borealis. Regio alpina ad fl. Bo-tschu. 25 VI / 7 VII 1884, [fl., fr. submit.], N.M. Przewalski» (LE!; syn – B!, LE!, P!, PE!).

Note. The same specimen (Fig. 2) the same year was independently designated as lectotype by Al-

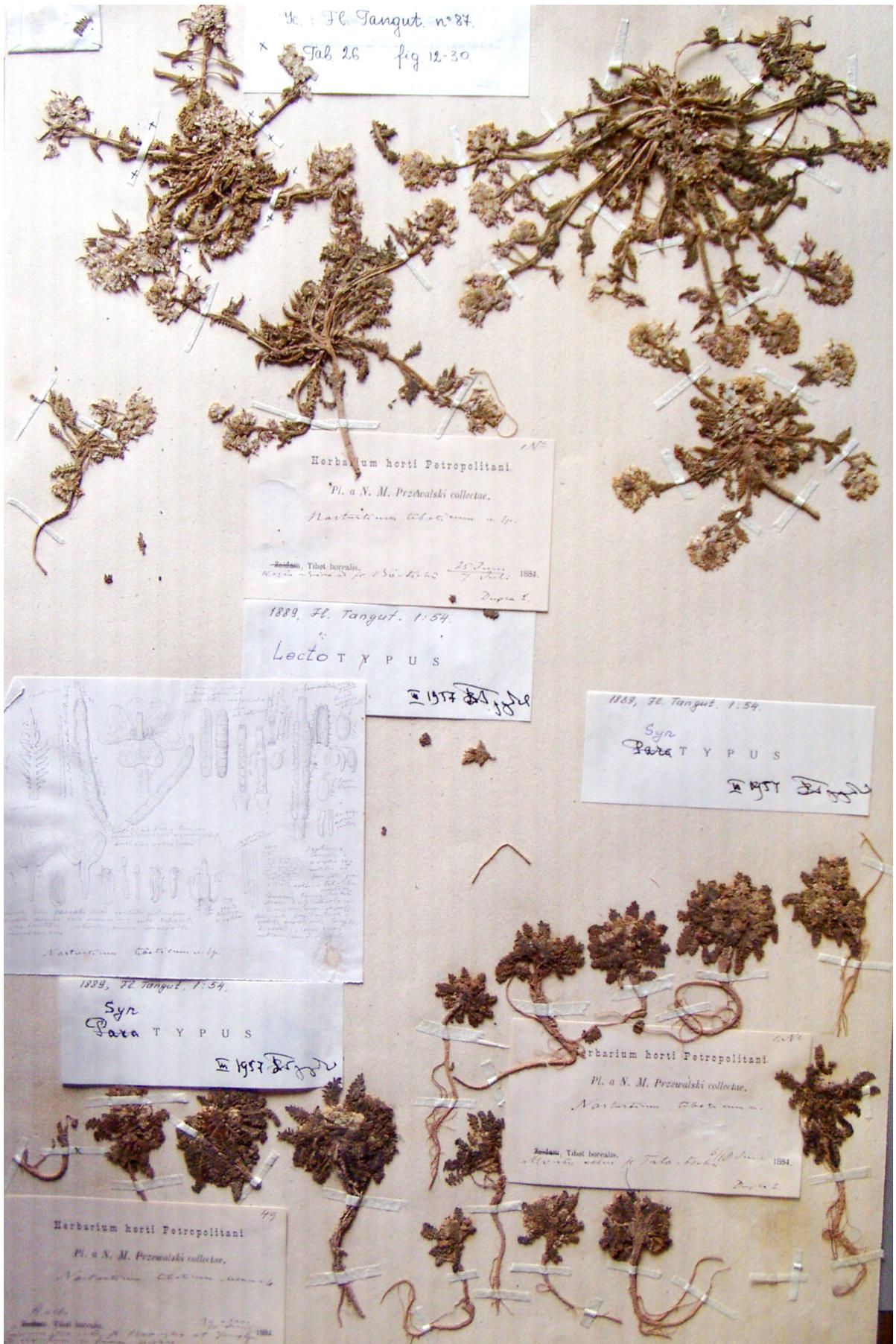


Fig. 2. Lectotype of *Shehbazia tibetica* (specimen composed of five plants above; mounted on one sheet with two syntypes below; LE).

Shehbaz (2000: 34) and Grubov (l. c.). Relevant issue of *Novon* was published 14 December while «Catalogue ...» was signed for printing on 16 October 2000 and thus the choice of Grubov has priority.

Shehbazieae D.A. German, **trib. nov.** – Plants biennial. Trichomes simple; gland-like warts present on fruits. Root somewhat fleshy, stems procumbent, few, simple. Basal leaves pinnatifid to pinnatisect, petiolate; cauline leaves similar, few, shortly petiolate to sessile, not auriculate at base. Racemes ebracteate. Lateral sepals slightly saccate at base. Petals bicolor, well differentiated to the white to pink obcordate blade and deep purple claw. Lateral filaments flattened and sometimes slightly toothed at base. Ovules 12–20 per ovary. Fruits terete, dehiscent siliques. Style conical; stigma deeply bilobed,

lobes subdecurrent. Seeds plump, neither margined nor winged; cotyledon accumbent. The tribe belongs to the evolutionary lineage III of the Cruciferae.

Typus: *Shehbazia* D.A. German

A more detailed description of *Sh. tibetica* can be found in Al-Shehbaz (2000) and Zhou et al. (2001).

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