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Molecular and Morphological Evidence for an Origin of the Aberrant Genus *Milula* within Himalayan Species of *Allium* (Alliacae)

Nikolai Friesen,[1] Reinhard M. Fritsch, Sven Pollner, and Frank R. Blattner [2]

Dept. of Taxonomy, Institute of Plant Genetics and Crop Plant Research (IPK), D-06466 Gatersleben, Germany

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Phylogenetic relationships between *Allium* and the monotypic Himalayan genus *Milula* were analyzed using sequences of the nuclear ribosomal DNA internal transcribed spacer (ITS) region and of the intergenic spacers from the chloroplast *trn*D(GUC)-*trn*T(GGU) region. Both marker systems unambig-uously placed *Milula spicata* within *Allium* subgenus *Rhizirideum*, close to *A. cyathophorum*. Morphologically the main difference between *Allium* and *Milula* is the conspicuous spicate inflorescence of *Milula* vs. the mostly capitate or umbellate inflorescences in *Allium*. Anatomical investigations of leaf characters support a close relationship of *Milula* with *A. cyathophorum* and *A. mairei*, whereas root characters are distinctive from other species of sect. *Cyathophora*. To maintain *Allium* as monophyletic, *Milula* has been included as *A. spicatum* in *Allium* subg. *Rhizirideum*.

***Key Words:* *Allium*; *Milula*; internal transcribed spacer (ITS); noncoding chloroplast DNA; phylogenetic analysis; taxonomy.**

INTRODUCTION

Milula spicata Prain, the only representative of the monotypic genus *Milula*, is a rare plant, growing on dry alpine pastures and steppe in the Central Himalayas at altitudes between 3000 and 4500 m. Its gross morphology (Prain, 1895) and onion-like smell (Stearn, 1960) strongly suggest that it is closely related to *Allium*. One conspicuous character differentiating *Milula* and *Allium* is the elongated, spicate inflorescence of *M. spicata* (Fig. 1), whereas in *Allium*, apart from *A. regelii* Trautv. with two to three superposed tiers, only capitate or umbellate inflorescences occur. When Prain described this plant in 1895 creating a new genus he noted that its appearance is "so completely that of an *Allium* that at first sight one feels inclined, in spite of its spicate inflorescence and its solitary bract, to treat it as the type of a somewhat aberrant section in that comprehensive genus" (Prain, 1895, p. 25). Despite this statement, to prevent further extension of the borders of the genus *Allium*, he described a new genus which he named *Milula*, an anagram of *Allium* to indicate the close relationship of the two taxa (Stearn, 1960). He also established a new subtribe Milulae of Alliae to accommodate his new genus, and this was subsequently treated as a tribe Milulae, next to Alliae by Krause (1930). Hutchinson (1959), reclassifying the Liliaceae and Amaryllidaceae on the basis of inflorescence characters, retained Miluleae in Liliaceae while transferring Allieae to Amaryllidaceae. Traub (1972) even established a monotypic family Milulaceae, whereas Takhtajan (1987) included *Milula* within the family Alliaceae in the subfamily Allioideae. Apart from the obvious overall similarity of the two genera this close classification is supported by karyological and seed structure data.

[1] Present address: Botanical Garden, University of Osnabrück, D-49076 Osnabrück, Germany.
[2] Towhom correspondence should be addressed.

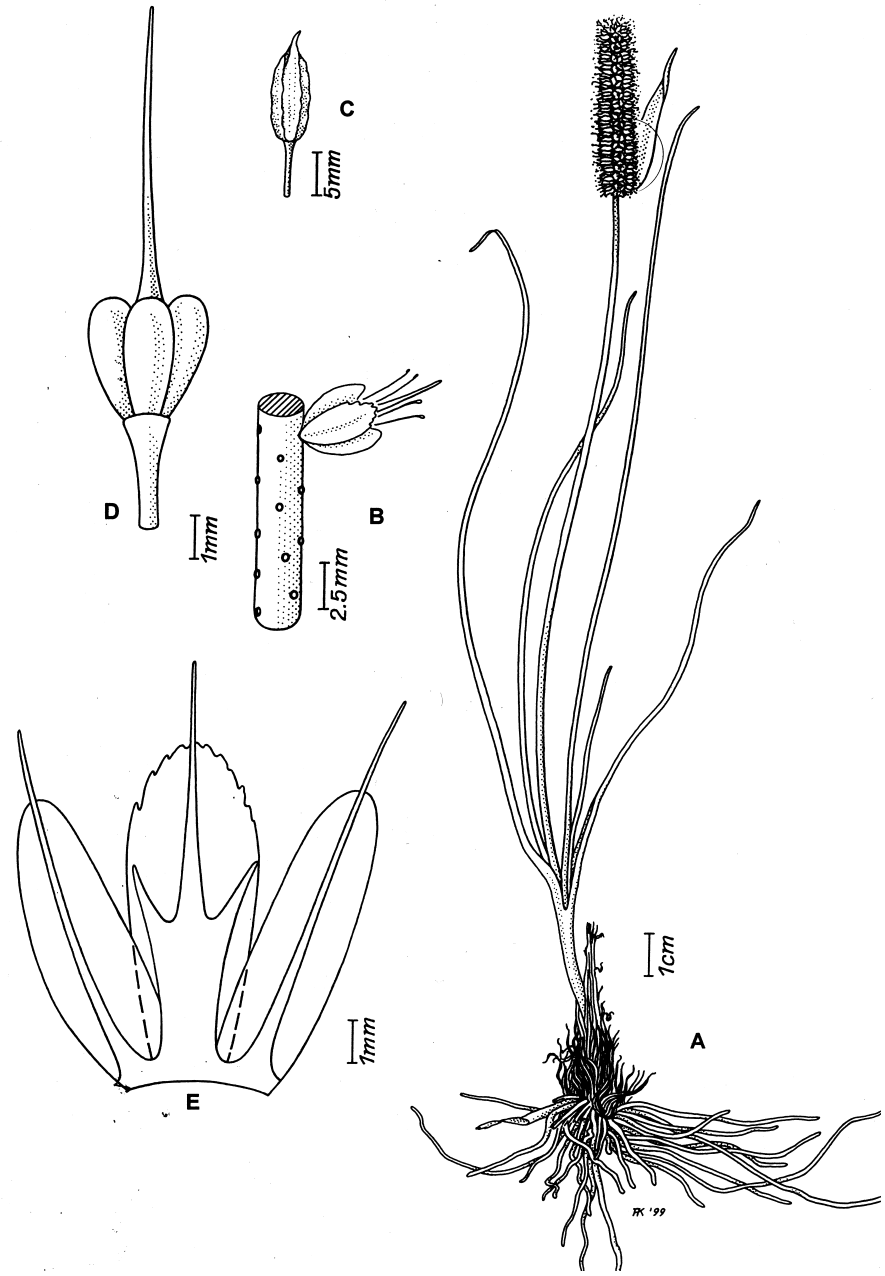

FIG. 1. *Milula spicata*. A. Plant habit; B. Single flower, neighboring flowers removed to show the attachment of the flower to the inflorescence axis; C. Young bud of the infloresecence; D. Gynoecium; E. Stamens with perianth segments.

Milula spicata has 2n=16 chromosomes (Öhzatay, 1978) with a similar karyotype to that of *Allium mairei* Levl. (unpublished result of H. Ohle). This karyotype is commonly found in species of *Allium* subgenus *Rhizirideum* (Friesen, 1988). The testa epidermis cells of *M. spicata* are characterized by undulated anticlinal cell walls and granulate sculptures (Kruse, 1994). Related though not identical testa types are also found among other species of *Allium*, for example in section *Butomissa*: *A. ramosum* and *A. tuberosum* (Kruse, 1984; Friesen, unpubl.) or in *A. haneltii* (Fritsch *et al.*, 1998). In addition to morphological data a phylogenetic analysis of chloroplast *rbc*L-sequences, which placed *Milula* as sister taxon to *Allium* in the Alliaceae (Fay and Chase, 1996), supports the close relationship of the two taxa.

In an ongoing investigation of the phylogeny of *Allium* using molecular markers we searched for a good outgroup taxon to root the ingroup in cladistic analyses. According to the results of the study of Fay and Chase (1996) and the general morphological similarity, *Milula* should be the appropriate candidate for this purpose. However, problems arose because no living plants of *Milula* were available and only badly fragmented DNA could be extracted from old herbarium specimens. This circumvented sequencing

of the entire *rbc*L-gene by Fay and Chase (1996) and they used a partial sequence of 670 basepair (bp) length for their analysis. A second problem is the relatively small sample size (three species) of *Allium* (incl. *Nectaroscordum*) in their study. Taking into account the broad similarity of *Milula* and *Allium*, these results do not really clarify the interrelationship of the two taxa. In February 1999 one of the author (NF) received recently collected herbarium material of Tibetan species of *Allium* and *Milula* for investigation and determination, collected by S. and G. Miehe (University of Marburg, Germany) and B. Dickoré (University of Göttingen, Germany). The quality of DNA isolated from these herbarium specim-ens was very good and allowed PCR amplification of fragments larger than 1000 bp length.

To investigate the position of *Milula* in comparison to *Allium* and other taxa of the Alliaceae we used DNA sequences of the internal transcribed spacer region (ITS) of the nuclear ribosomal DNA (nrDNA) and the *trn*D(GUC)-*trn*T(GGU) intergenic spacers of the chloroplast DNA (cpDNA). The ITS region, including the 5.8S rDNA and the two spacers ITS-1 and ITS-2, has been shown to be a valuable source of evidence particularly at the generic and intrageneric levels (e.g., Baldwin, 1992; Baldwin *et al*., 1995; Sang *et al*., 1994, 1995; Wolfe and Elisens, 1995; Wendel *et al*., 1995; Liston *et al*., 1996; Roelofs *et al*., 1997; Noyes and Rieseberg, 1998; Dubouzet and Shinoda, 1999). The relatively fast evolving chloroplast spacers and introns are widely used in systematic studies at lower taxonomic ranks (e.g., Morton and Clegg, 1993; Hodges and Arnold, 1994; Kelchner and Clark, 1997). They are easily amplifiable with universal primers, annealing in the highly conserved genes flanking the variable regions (Taberlet *et al*., 1991; Savolainen *et al*., 1994; Demesure *et al*., 1995). The comparison of phylogenetic hypotheses derived from nuclear and (mostly maternally inherited) chloroplast markers, allows the detection of concealed hybridization in the group under study (Rieseberg *et al*., 1996; Friesen *et al*., 1999) and gives additional support for groups found by both independent marker systems.

The first ITS sequences obtained in this study clearly showed that *Milula* sequences closely resembled the *Allium* ITS region. Due to this finding we were able to concentrate on finding the precise position of *Milula* near or within *Allium*. To further corroborate these initial findings we conducted a study on morphological features of *M. spicata*. If *Milula* is part of *Allium* it should possess some of the distinctive characters which can be used to differentiate subgenera and sections of the genus. Many of these characters can be found in leaf and root anatomy, which therefore were closer examined.

MATERIALS AND METHODS

Plant materials – For the analysis of the position of *Milula* in the Alliaceae we used two outgroup species [*Tulbaghia fragrans* Verdoorn and *Nothoscordum bivalve* (L.) Britton] which are clearly outside of *Allium* (Fay and Chase, 1996; Mes *et al*., 1997) together with 14 *Allium* species. The sources of plant material and vouchers of the accessions used in this study are listed in Table 1.

Anatomical investigation – Leaves and roots of the herbarium specimens of *M. spicata* were soaked overnight in glycerol/water 1:9. They were sectioned by hand with a razor blade. After differential staining with Astra blue (Merck) and Safranine (Fluka) the sections were transferred into glycerol gelatin, microscopically analyzed and photographed (Fritsch, 1988).

DNA extraction – DNA was isolated with the NucleoSpin Plant kit (Macherey-Nagel) according to the instructions of the manufacturer. The concentration and qualitiy of the extracted DNA was checked on an agarose gel.

Molecular methods – The ITS region (ITS-1, 5.8S rDNA, and ITS-2) was PCR-amplified using two primers ITS-A together with ITS-B (Blattner, 1999). PCR was carried out in a Grant Autogene II thermocycler (Grant Instruments, Cambridge, England) programmed for 95° C - 2 min [55° C - 30 sec, 70° C - 1 min, 95° C - 20 sec]$_{30}$ 70° C - 7 min. Amplification was carried out with 1 U Taq DNA polymerase (Boehringer Mannheim) in the supplied reaction buffer, 0.2 mM of each dNTP, 50 pmol of each primer and 10-25 ng of total DNA in 50 µL reaction volume. The PCR products were electrophoresed on an agarose gel, cut out, and purified with the NucleoSpin Extract kit (Macherey-Nagel). After checking DNA concentration on a 1.8% agarose gel, about 40 ng of the PCR product was used in a 10µL-cycle sequencing reaction with the ABI BigDye Terminator Kit on an ABI 377 DNA seqeuncer (ABI, Foster City, CA) according to the instructions of the manufacturer. ITS-A and ITS-B were used as sequencing primers.

The cpDNA region between *trn*D-*trn*T was amplified with universal primers developed by Demesure *et al*. (1995). Touchdown PCR was carried out in a volume of 50 µl with the following program: 94° C - 2 min [94° C - 20 sec, 65° C (-1° C/cycle) - 1 min, 72° C - 1 min]$_{10}$ [94° C - 20 sec, 55° C - 45 sec, 72° C - 1 min]$_{40}$ 72° C - 7 min. The PCR products were purified using Nucleospin Extract Kit following the protocol of the manufacturer and dissolved in 30µl TE. About 40 ng DNA were sequenced with the ABI BigDye Terminator Kit with the following temperature profile [94° C - 15 sec, 45° C - 1 min, 60° C - 3 min]$_{50}$ and analyzed on an ABI 377 DNA sequencer. As sequencing primers both PCR primers

TABLE 1

Origin of the investigated accessions of *Allium, Milula, Nothoscordum*, and *Tulbaghia*. TAX: accession numbers of the Department of Taxonomy, IPK Gatersleben, EMBL: sequence database accession numbers of the nrDNA ITS sequences and the cpDNA *trn*D-*trn*T sequences.

Genus	Species	TAX	Origin	EMBL	
Subgenus				ITS	*trn*D-*trn*T
Allium					
Amerallium Traub	*cernuum* Roth	3154	Germany, Garden origin	AJ250289	AJ270281
	insubricum Bois. & Reut.	0230	Germany, B. G. Marburg	AJ250291	AJ270284
	wallichii Kunth	2441	Germany, Garden origin	AJ250294	AJ270292
	hookeri Thw.	2013	China, Kunming	AJ250297	AJ270283
Caloscordum (Herbert) R.M.Fritsch	*neriniflorum* (Herb.) Baker	2379	Mongolia, Sumber	AJ250292	AJ270287
Nectaroscordum (Lindl.) Traub	*siculum* Ucria	2192	Germany, Garden origin	AJ250299	AJ270290
Rhizirideum (G.Don ex Koch) Wendelbo	*angulosum* L.	2778	Kazakhstan, village Kievskoe	AJ250287	AJ270279
	carolinianum DC	2570	Tajikistan, Anzob pass	AJ250290	AJ270280
	polyrrhizum Regel	2359	Mongolia, Talbulagu, Baruun urt	AJ250296	AJ270288
	mairei Levl.	6087	China, Yunnan, Eastern Lijiang Range	AJ250298	AJ270286
	kingdonii Stearn	6086	China, Yunnan, Eastern Lijiang Range	AJ250286	AJ270285
	cyathophorum Bur. & Franch.	2825	Norway, B. G. Oslo	AJ250288	AJ270282
	ramosum L.	2755	Russia, Buryatia, Lake Gusinoe	AJ250295	AJ270289
	tuberosum Rottl. ex Spr.	2454	India, Ladakh	AJ250293	AJ270291
Milula	*spicata* Prain (1)	6080	Tibet, Tsangpo Valley	AJ250284	AJ270293
	spicata Prain (2)	6081	Tibet, Upper Cuamda Chu	AJ250285	AJ270294
Nothoscordum	*bivalve* (L.) Britton	2621	UK, Wellesbourne	AJ250301	AJ270295
Tulbaghia	*fragrans* Verdoorn	2165	UK, London, Chelsea Physic Garden	AJ250300	AJ270296

and two newly developed internal primers (SP34: 5'-GAC GGA CTG TAA ATT CGT TGA C-3' and SP35=SP34 reverse complement) in the tRNA-Thyr gene [*trn*Y(GUA)] were used.

Data analysis – EMBL sequence accession numbers are given in Table 1. Initial alignments for both data sets were conducted with ClustalX (Thompson *et al*., 1997) using the 'Slow-Accurate' alignment algorithm with a gap opening penalty of 20 and a gap elongation penalty of 0.1 in the pairwise and the multiple alignment procedures, using the IUB DNA weight matrix. These initial alignments were improved manually according to the outlines discussed below to get reliable positional information (EMBL alignment number DS40337 for the ITS alignment and DS40206 for the cpDNA alignment). For the ITS data Fitch parsimony and generalized parsimony analyses (Swofford *et al*., 1996) were performed with PAUP* 4d65 (a test version kindly provided by D. Swofford, Smithsonian Institution, Washington) with the branch and bound search option, MULPARS, ACCTRAN, TBR branch-swapping, and gaps treated as missing. For generalized parsimony a stepmatrix weighting transitions vs. transversions 1:2 was used. *Tulbaghia fragrans* was specified as the outgroup taxon (Fay and Chase, 1996). For bootstrap (Felsenstein, 1985) and decay analyses (Bremer, 1988) the same settings were used as in the initial tree searches. A maximum likelihood analysis (Kishino and Hasegawa, 1989) with the 'Dnaml' module of the PHYLIP 3.573 program package (Felsenstein, 1993), procedure and settings as given in Blattner & Kadereit (1999), resulted in the same tree topology as generalized parsimony and is therefore not separately shown. The cpDNA spacer sequences, where genetic distances are smaller than in the ITS data and many characters rely on insertions/deletions (indels) rather than base mutations, were analyzed with Fitch parsimony (settings as above). Twelve shared indels were coded in a binary matrix, which was appended to the alignment for cladistic analysis. A combined data set including ITS and cpDNA data was analyzed with Fitch parsimony (settings as above) and generalized parsimony with a weighting scheme as above for all characters with the exception of the shared indels, which were treated as unordered characters.

RESULTS

ITS sequences – Within the 14 *Allium* species and *Milula* the lengths of ITS regions range from 612 bp in *A. cyathophorum* to 655 bp in *M. spicata* and *A.*

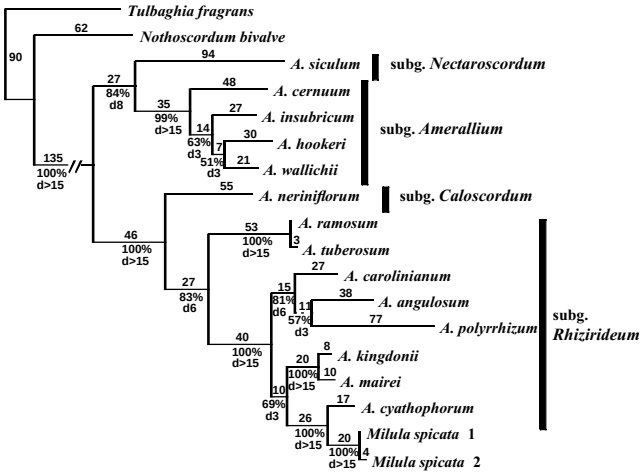

FIG. 2. Most parsimonious tree of a generalized parsimony analysis of the nrDNA ITS region weighting transitions vs. transversions 1:2. Fitch parsimony analysis resulted in two most parsimonious trees which differ from the tree shown only with respect to the position of *A. polyrrhizum*, which is not resolved in the consensus of the two trees (branch dashed). Above the branches the numbers of character transformations are given, below the bootstrap (%) and decay values are presented. The branch connecting the outgroup species and *Allium* is not drawn to scale.

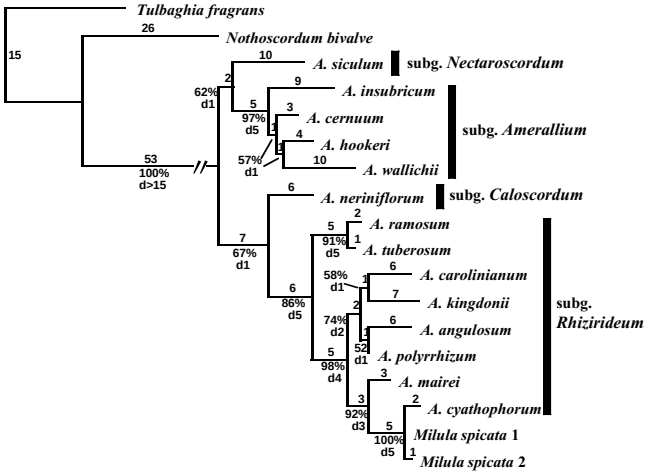

FIG. 3. One of three most parsimonious trees of a Fitch parsimony analysis of the cpDNA *trn*D-*trn*T region. The tree topology is identical to the strict consensus tree of this analysis. The branch connecting the outgroup species and *Allium* is not drawn to scale. See Fig. 1 for explanation of numbers at the branches.

siculum. *Nothoscordum bivalve* and *Tulbaghia fragrans* have ITS regions of 671 and 642 bp length, respectively. GC contents of the ITS region are between 42-46% in *Allium*, and 47% and 51% in *Tulbaghia* and *Nothoscordum*. Aligning the individual sequences resulted in an alignment (DS40337) of 695 bp length. In ITS-1, 213 (81%) sequence positions are variable, in ITS-2, 201 (75%) positions and even in the 5.8S rDNA 39 (23%) variable positions could be detected. Genetic distances (Kimura, 1980) range from 6% Kimura-distance (between *Milula* and *A. cyatophorum*) to 47% (*Milula* to *A. siculum*). This intrageneric variation is high in comparison with others plant groups (e.g., Baldwin, 1992; Kim and Jansen, 1994; Baldwin *et al.*, 1995; Sang *et al.*, 1995; Kornkven *et al.*, 1998). The inclusion of the outgroups *Nothoscordum* and *Tulbaghia* increases the amount of variable sites only by about 5%. Genetic distances between the outgroup taxa and *Allium*/*Milula* are between 51% and 70% Kimura-distance.

Noncoding cpDNA – The length of the *trn*D-*trn*T region in *Allium* and *Milula* ranges from 745 bp in *A. siculum* to 890 bp in *A. mairei* and 927 bp in *M. spicata* (Tax6081). In *Tulbaghia* it is 1176 bp long, in *Nothoscordum* 1169 bp. The length of the complete alignment (DS40206) is 1190 bp and it contains 12 shared indels, 90 unique and 69 parsimony informative base changes. Within the *trn*Y(GUA) gene, which is within the sequenced region, the species of *Allium* and *Milula* lack a single T compared to the outgroup species, reducing the length from 84 to 83 bp. The second gene, *trn*E(UUC) with 78 bp length shows no sequence variation. Genetic distances in the cpDNA sequences are up to 10% Kimura-distance between *Allium* and the outgroup taxa and between 0.5% and 4.7% within *Allium* and *Milula*.

Phylogenetic analysis – Fitch parsimony analysis of the ITS data produced two equally parsimonious trees (1097 steps length, CI 0.687, RI 0.720). Only one of the two trees (Fig. 2) was found by generalized parsimony. The only difference between the trees concerns the position of *A. polyrrhizum* which groups with either *A. angulosum* or *A. carolinianum*. *Allium* is clearly separated from the outgroup species (100% bootstrap support, d>15) and *Milula* is part of *Allium* and did not occur in a sistergroup position to *Allium*. Within *Allium* it is sistergroup to *A. cyathophorum* (sect. *Cyathophora* from S China).

Two major clades were found in *Allium*: subgenera *Nectaroscordum* (x=9) and *Amerallium* (x=7) on one side, with subgenera *Caloscordum* and *Rhizirideum*, and *Milula* (all x=8) on the other side. The species of former subgenus *Bromatorrhiza* occur at two different positions in the tree: section *Bromatorrhiza* (*A. wallichii* and *A. hookeri*, x=7) are clearly placed in subgenus *Amerallium* and species of sections *Coleoblastus* (*A. kingdonii* and *A. mairei*) and *Cyathophora* (*A. cyathophorum*) group in subg. *Rhizirideum*.

Fitch parsimony analysis of the *trn*D-*trn*T region resulted in three equally parsimonious trees of 208 steps length (CI 0.899, RI 0.889) which differ only with respect to the position of *A. cyathophorum* in

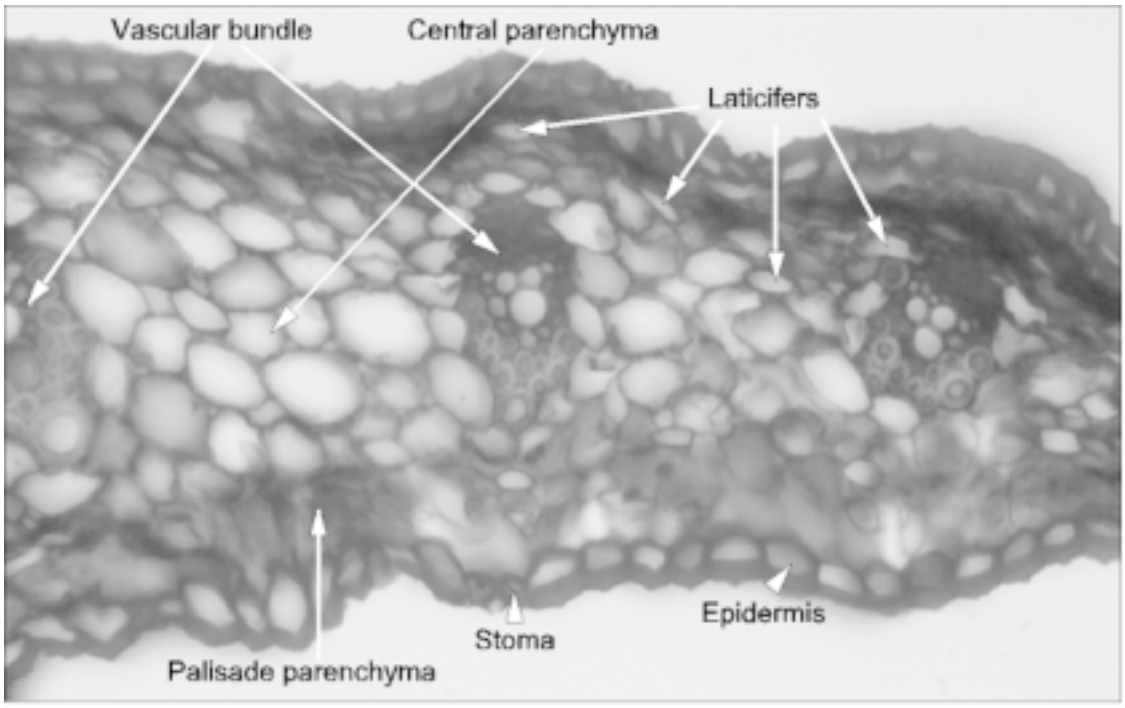

FIG. 4. Detail of a cross-section of a *Milula spicata* leaf blade (x150).

relation to the two accessions of *M. spicata* (Fig. 3). The only difference between the ITS tree and the cpDNA based tree concerns *A. kingdonii* which here is sister taxon of *A. carolinianum* instead of *A. mairei* in the ITS tree. Bootstrap support and decay values are generally lower in the cpDNA analysis which might be due to lower over-all character numbers supporting individual branches.

The analyses of the combined data resulted in trees with topologies identical to the corresponding trees from the sole ITS data. Fitch parsimony gave two most parsimonious trees (1312 steps, CI 0.717, RI 0.737) with bootstrap values comparable to the values of the ITS analysis except for the two different groups *A. kingdonii* is joining in the cpDNA vs. the ITS analysis. For these clades the bootstrap values slightly decrease (from 81% to 76% and from 69% to 66%, respectively), due to the contradicting signals in both data sets. Generalized parsimony resulted in one tree (1294 steps long when characters treated as unordered, CI 0.718, RI 0.736) with bootstrap values generally slightly higher than in the tree from the ITS analysis. The higher number of characters in the ITS data set here seems to overwhelm the signal introduced by the cpDNA data. Due to identity of tree topologies to the ITS results the trees found in the analyses of combined data sets are not shown separately.

Anatomy of leaves and roots – The leaves of *M. spicata* showed only one row of identically orientated vascular bundles (Fig. 4). The outer walls of the epidermal cells were strongly thickened with a longitudinal rib in its median part. One layer of true palisade parenchyma was present, and many laticifers were located in the transitional area between palisade cells and the other chlorenchyma. The chlorenchyma consisted of longitudinally elongated arm cells with short protuberances ("peg-cells") gradually passing into the chloroplast-free central parenchyma with large intercellular spaces. *Milula spicata* shares a rare combination of leaf anatomical characters with *A. cyathophorum* and *A. mairei*/*A. amabile* (Fritsch, 1988), that are absent in any other *Allium* group: one row of vascular bundles, many intraparenchymatic laticifers, and the presence of arm cell parenchyma. However, the parenchyma cells did not develop long arms like in *A. cyathophorum* (Fritsch, 1988), and the anatomical structure of *Milula* can be regarded as less specialised than those of the above cited *Allium* species. One row of vascular bundles combined with intraparenchymatic laticifers and an unspecialised parenchyma is present in many species of *Nothoscordum, Ipheion, Tulbaghia,* and *Agapanthus,* all distantly related to *Allium* (Fritsch, unpubl.) underlining that this is an apomorphic character state in Alliaceae *s.l.*

The roots of *M. spicata* had a thick cortex consisting of 10-15 cell layers with moderately but equally thickened walls. The rhizodermis, the large-celled exodermis, and the endodermis consisted of one cell

layer only; they did not show any special shape or wall thickenings. The central bundle had five to eight xylem and phloem spurs in an alternating arranged. This is widespread anatomical root structure present in many sections of subg. *Rhizirideum*, whereas species of the sections *Cyathophora* and *Coleoblastus* have knot-like thickened cortex cell walls (Fritsch, 1992).

DISCUSSION

Molecular data unambiguously align the monotypic genus *Milula* with species of *Allium* subg. *Rhizirideum* from Central Asia and the Himalayan region. Two major clades were found in *Allium* based on DNA analysis: subgenera *Nectaroscordum* (x=9) and *Amerallium* (x=7) on one side, with subgenera *Caloscordum* and *Rhizirideum*, and *Milula* (all x=8) on the other side. This result support the division of *Allium* into two large groups as found in earlier chloroplast DNA analyses (Linne von Berg *et al*., 1995; Mes *et al*., 1999) and the breaking up of subg. *Bromatorrhiza* which appears to be an artificial taxon (Samoylov *et al*., 1995, 1999).

One conspicuous feature of the ITS data are the unusually large intrageneric genetic distances within *Allium*. Kimura distances above 40%, as found in this study and by Dubouzet and Shinoda (1999), often characterize the most distant genera within subfamilies or even families (e.g., Baldwin *et al*., 1995; Hsiao *et al*., 1999; Noyes and Rieseberg, 1999; Blattner and Kadereit, 1999). Intrageneric distances in other plant families are mostly less than 10% (Baldwin *et al*., 1995). These findings make *Allium* either an extraordinarily fast evolving taxon or it is of ancient origin, and molecular evolution is not accompanied by the rise of comparable numbers of taxonomic categories. The distribution of *Allium* in the Northern Hemisphere, which fits well with the pattern of Tertiary geofloral elements (Hanelt *et al*., 1992; Friesen *et al*., in prep.), together with the restricted ability of long distance dispersal due to the lack of specialized dispersal mechanisms, make the second hypothesis more likely than postulating fast molecular evolution in this genus. Thus in age and genetic variation *Allium* easily resembles plant families in other groups of angiosperms. Large genetic distances often result in severe problems finding reliable sequence alignments. In our study the ITS sequences of members of different subgenera are rather distinct and hard to align. Therefor the initial ClustalX alignment had to be improved manually. An ITS data set representing the entire genus *Allium* (Friesen *et al*., in prep.) was the basis for these alterations because homologous parts of the ITS region are easier to align when additional sequences are available, connecting otherwise very different members of *Allium*.

The comparison of ITS data with the independent cpDNA data set clearly shows that the results obtained are stable despite possible ambiguities in the ITS alignment. The analyses differ only with respect to the position of *A. kingdonii* which is either sistergroup to *A. mairei* (ITS) or to *A. carolinianum* (cpDNA) and the position of *A. insubricum* within subg. *Amerallium*. To resolve these conflicting hypotheses a much larger sample of species would be necessary to avoid errors eventually introduced by taxon selection. Thus, it is not possible to decide whether hybridization or sample errors are the reason for the different positions of these taxa in nuclear and chloroplast based analyses. However, the inconsistencies in the position of *A. kingdonii* do not influence the interpretation of the systematic position of *Milula*. In both data sets *Milula* was the sistergroup of *A. cyathophorum* (ITS) or appeared in an unresolved polytomy with this taxon (cpDNA) with high bootstrap and decay support (100%/d>15 and 100%/d5). These findings clearly include *Milula* in subg. *Rhizirideum*.

The comparison of molecular data with morphological characters resulted in very similar systematical conclusions. According to leaf anatomy, *M. spicata* shows characters identical to *Allium* sections *Cyathophora* and *Coleoblastus*, which support the ITS- and cpDNA-based results. Root characters again support a position of *Milula* in subg. *Rhizirideum* but differ from sects. *Cyathophora* and *Coleoblastus*. Treating *Milula* as own genus thus would render *Allium* paraphyletic. Whereas paraphyletic taxa at a supraspecific level are inevitable, at least as long as evolution is at work (Sosef, 1997; Maddison, 1997; Bachmann, 1998), maintaining paraphyletic entities at higher taxonomic level is hotly debated (e.g., Queiroz and Gauthier, 1994; Brummitt, 1997). Because only minor differences, apart from the spicate inflorescence, distinguish *Milula* from *Allium*, we see no necessity to leave *Allium* as paraphyletic, but include *Milula* in *Allium* subgenus *Rhizirideum*. The obvious differences in root anatomy compared to species of sects. *Cyathophora* and *Coleoblastus*, together with the unique inflorescence suggests that *Milula* should be placed in its own section within subg. *Rhizirideum* (see taxonomical remarks below). Although no formal cladistic analysis of morphology was conducted, one can predict that the high level of corresponding morphological characters would also merge *Milula* and *Allium*. The highly aberrant spicate inflorescence of *Milula* is the major difference, which is not paralleled by additional characters distinguishing the two genera. Some other examples, where unusual

morphological characters than led taxonomists to make erronously groupings, are well known. Most of them could be uncovered by the use of molecular markers. An excellent example is *Heterogaura* Rothr. in the Onagraceae. *Heterogaura*, a monotypic genus, differs from its closest relatives in *Clarkia* Pursh by having of only four fertile stamens instead of eight and by one- or two-seeded nutlets instead of a dehiscent capsule (Sytsma and Gottlieb, 1986). Soltis *et al*. (1990) demonstrated that the monotypic *Conimitella* Rydb. is part of *Mitella* L. (Saxifragaceae). Capsule and petal characters differentiate these genera. Again capsule morphology led to the genus *Roemeria* Medik. which cpDNA data (Kadereit and Sytsma, 1992) and a cladistic analysis of morphological characters (Kadereit *et al*., 1994) revealed to be part of *Papaver* L. *Wilkesia* A.Gray from the Hawaiian archipelago, however, exhibits a complete different habit (yucca-like rosette tree), compared with *Dubautia* Gaudich, from within it arose (Baldwin *et al*., 1990). Whereas this last example might illustrate functional constraints of herbs gaining woodiness (Givnish *et al*., 1995; Blattner and Kadereit, 1999) the other characters listed could have arisen by single macromutations (Kadereit, 1994) resulting in prominent morphological differences. To bring such mutations to fixation they should have a selective advantage (e.g., Harrison, 1920) or occur in small populations, where founder events and lineage sorting results in random genetic drift (Hagedoorn and Hagedoorn, 1921; Dubinin and Romaschoff, 1932). For the spicate inflorescence of *Milula* no conclusions about a possible selective advantage of this distinct feature can be drawn because no data about reproductive biology and pollinators exist up to now. However, the rich geological and ecological diversity of the Himalayan mountain ranges together with habitat isolation due to changing climatic conditions during the formation of the Himalayas, might well promote rapid speciation in small, isolated populations (Jork, 1996) thus allowing the fixation of peculiar or rare morphological characters.

TAXONOMICAL REMARKS

Allium L.

Subgenus *Rhizirideum* (G. Don ex Koch) Wendelbo

Section *Milula* (Prain) Friesen comb. nova – *Milula* Prain in Sci. Mem. Med. Offic. Army India, Part IX, tab. 1 (1895).

Allium spicatum (Prain) Friesen comb. nova - *Milula spicata* Prain in Sci. Mem. Med. Offic. Army India, Part IX: 57, tab. 1 (1895); in Ann. R. Bot. Gard. Calcutta V: 165, tab. 200 (1896).

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