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Biosystematic studies on the genus *Polygonatum* (Convallariaceae) IV. Molecular phylogenetic analysis based on restriction site mapping of the chloroplast gene *trnK*

With 3 Figures and 5 Tables

Summary

Phylogenetic relationships of 14 species and one variety of the genus *Polygonatum* as well as three species of the genus *Disporopsis* and *Heteropolygonatum roseolum* were analyzed based on mapped restriction site variation in a PCR-amplified chloroplast genome region, *trnK*. In agreement with earlier taxonomic treatments it was found that *Disporopsis* and *Heteropolygonatum* should be distinguished from *Polygonatum* at generic level, and that *P. sect. Polygonatum* can be recognized as a monophyletic group. Earlier series concepts in *P. sect. Polygonatum* based on chromosome number and characteristics of staminal filaments are not supported by the molecular data. The molecular data suggest that satellite chromosome morphology might prove valuable as a diagnostic character in *Polygonatum sect. Polygonatum*.

Introduction

The genus *Polygonatum* MILLER is currently classified in Convallariaceae (DAHLGREN et al. 1985) and comprises 57 species distributed in the warm-temperate to boreal zones of the Northern Hemisphere. TAMURA (1993a) subdivided the genus into two sections, i.e., sect. *Polygonatum* and sect. *Verticillata* BAKER. Section *Polygonatum* possesses alternate leaves, stout staminal filaments, perforated pollen exines, $x = 9, 10$ or 11

Zusammenfassung

Die phylogenetischen Beziehungen von 14 Arten und einer Varietät der Gattung *Polygonatum* sowie von drei Arten der Gattung *Disporopsis* und von *Heteropolygonatum roseolum* wurden mit Hilfe einer Kartierung der Restriktionsschnittstellen in einem PCR-amplifizierten Bereich des Chloroplasten-Genoms *trnK* untersucht. In Übereinstimmung mit früheren taxonomischen Bearbeitungen wurde gefunden, daß *Disporopsis* und *Heteropolygonatum* generisch von *Polygonatum* verschieden sind, und daß *P. sect. Polygonatum* eine monophyletische Gruppe ist. Frühere Gliederungen von *P. sect. Polygonatum* in Serien, die auf Chromosomenzahlen und Charakteristika der Filamente gegründet waren, wurden durch die molekularen Daten nicht bestätigt. Diese Daten deuten aber darauf hin, daß die Morphologie der Satelliten-Chromosomen ein brauchbares Merkmal innerhalb von *P. sect. Polygonatum* abgeben könnte.

as chromosome base number, and karyotypes consisting mainly of metacentric and submetacentric chromosomes. Section *Verticillata* has mostly opposite or verticillate leaves, slender filaments, mostly reticulate pollen exines, $x = 14$ or 15 as base chromosome number, and karyotypes consisting mainly of long subtelocentric and short metacentric chromosomes (TAMURA 1993a, 1993b; Table 1).

TAMURA (1993a) divided sect. *Polygonatum* into three series, i.e., ser. *Bracteata* L. ABRAMO-

Table 1
Morphological and karyological characters of the genus *Polygonatum*

Taxon	Phyllotaxis	Filaments	Pollen exine	Chromosomes
<i>Polygonatum</i> sect. <i>Polygonatum</i> ser. <i>Bracteata</i>	alternate	stout mostly thickening in upper part	perforate	$x = 9$ metacentric and submetacentric
<i>Polygonatum</i> sect. <i>Polygonatum</i> ser. <i>Polygonatum</i>	alternate	stout thickening in middle or without thickening	perforate	$x = 10$ metacentric and submetacentric
<i>Polygonatum</i> sect. <i>Polygonatum</i> ser. <i>Inflata</i>	alternate	stout thickening in lower part	perforate	$x = 11$ metacentric and submetacentric
<i>Polygonatum</i> sect. <i>Verticillata</i>	mostly opposite or verticillate	slender -	mostly reticulate	$x = 14$ $x = 15$ mostly long subtelocentric and shortmetacentric

VA, ser. *Polygonatum*, and ser. *Inflata* (SATAKE) M. N. TAMURA. Series *Bracteata* has filaments thickening mostly in their upper part and a chromosome base number of $x = 9$; ser. *Polygonatum* has filaments thickening in the middle or without thickening and $x = 10$, and ser. *Inflata* has filaments thickening in the lower part and $x = 11$ (TAMURA 1991, 1993a, 1993b; Table 1).

In our current understanding, *Polygonatum* is included in tribe Polygonateae together with *Disporopsis* HANCE, *Smilacina* DESF., and *Maianthemum* WIGG. *Disporopsis* comprises six species which are distributed in the warm-temperate zone of Southeast Asia, *Smilacina* comprises 25 species in the warm-temperate to boreal zones of Asia and North America and the tropical mountains of Central America, and *Maianthemum* comprises three species in the cool-temperate to boreal zones of the Northern Hemisphere.

The tribe is characterized by a horizontally creeping fleshy sympodial rhizome, an unbranched elongated aerial stem with dispersed foliage leaves, and pedicellate hypogynous syncarpous flowers. It may be divided into two groups: one group consisting of *Polygonatum* and *Disporopsis*, and the other group of *Smilacina* and *Maianthemum*. The former group shares a pseudo-terminal cauline foliage leaf, axillary inflorescences, connate tepals to which filaments are more or less adnate, and linear to oblong anthers, and *Disporopsis* differs from *Polygona-*

tum in having a corona. The latter group shares a terminal inflorescence, mostly free tepals and filaments, and oblong to orbicular anthers, and *Maianthemum* deviates from *Smilacina* in having dimerous flowers and cordate leaves (Table 2).

Recently, an undescribed convallariaceous plant was collected in Guangxi Prov., China, by Prof. DEZHI FU, Prof. HAI-NING QIN, and Mr. MIKINORI OGISU. The plant has a horizontal fleshy moniliform sympodial rhizome, a deciduous unbranched elongated aerial stem with alternately dispersed coriaceous foliage leaves, mostly two-flowered axillary and terminal drooping inflorescences, and pinkish pedicellate hypogynous flowers with connate tepals to which filaments are well adnate, lanceolate anthers, and syncarpous pistils. Some of these characters suggest this taxon as a member of the *Polygonatum-Disporopsis* group of the Polygonateae. The taxon lacks a corona and is more similar to *Polygonatum* than to *Disporopsis*. It differs from *Polygonatum* in having dimorphic stamens, imbricate tepals, and a terminal inflorescence in addition to axillary ones (Table 2). Accordingly, it can be considered as the type species of a new genus and is being newly described as *Heteropolygonatum roseolum* (TAMURA et al. submitted).

In the present study, the phylogeny of *Polygonatum*, *Disporopsis*, and *Heteropolygonatum* was analyzed by means of restriction site mapping of the *trnK* gene. This gene has pre-

Table 2
Morphological characters of the genera of tribe Polygonateae

Genus	Phyllotaxis	Leaf bases	Inflorescence		Flowers
<i>Polygonatum</i>	alternate, opposite or verticillate	attenuate ~ rotundate	axillary	mostly drooping	trimerous
<i>Heteropolygonatum</i>	alternate	obtuse	axillary and terminal	drooping	trimerous
<i>Disporopsis</i>	alternate	obtuse ~ rotundate	axillary	drooping ~ erect	trimerous
<i>Smilacina</i>	alternate	obtuse ~ rotundate	terminal	mostly erect	trimerous
<i>Maianthemum</i>	alternate	cordate	terminal	erect	dimerous

Genus	Tepals		Corona	Filaments	Anthers	
<i>Polygonatum</i>	connate	valvate	absent	adnate to tepals	monomorphic	linear ~ lanceolate
<i>Heteropolygonatum</i>	connate	imbricate	absent	adnate to tepals	dimorphic	lanceolate
<i>Disporopsis</i>	connate	valvate in lower part	present	adnate to tepals	monomorphic	lanceolate ~ oblong
<i>Smilacina</i>	mostly free	valvate ~ imbricate	absent	mostly free	monomorphic	oblong ~ orbicular
<i>Maianthemum</i>	free	valvate	absent	free	monomorphic	oblong ~ orbicular

viously been used by WOLFE et al. (1993 for Scrophulariaceae), MESSINGER et al. (1994 for *Ribes* L.), LISTON & KADEREIT (1995 for *Senecio* L.), SCHWARZBACH & KADEREIT (1995 for Papaveraceae) and TSUMURA et al. (1995 for conifers). It has been shown to be sufficiently variable for phylogenetic studies at generic and sometimes specific level.

The aims of the present study are to investigate (1) whether restriction site mapping of the *trnK* gene can contribute towards a consolidation of the classification of *Polygonatum*, (2) whether *Disporopsis* and *Heteropolygonatum* can be separated from *Polygonatum* or whether they should be included in this genus, and (3) to re-examine the characters which have been used for the classification of *Polygonatum* and allied genera.

Material and methods

The source of the material analyzed in the present study is given in Table 3. Two species and one variety of *Smilacina* and *Maianthemum* as the remaining genera of the Polygonateae sensu DAHLGREN et al. (1985) were used as outgroups. Voucher specimens are deposited mostly in HBG and KYO.

Total DNA isolation and PCR amplification followed the procedure of SCHWARZBACH & KADEREIT (1995). For PCR amplification, two oligonucleotide primers were synthesized: 5'-AACTAGTCGGATGGAGTAG-3' (*trnK*-2621) and 5'-CTCAACGGTAGAGTACTCG-3' (*trnK*-11). The primers *trnK*-2621 and *trnK*-11 are identical to positions 1835–1853 on strand B and 4397–4379 on strand A of the tobacco chloroplast genome, respectively (SHINOZAKI et al. 1986). The amplified region corresponds to the transfer RNA gene for lysine (*trnK*)

Table 3
Source of experimental material

Taxon	Locality	Collector	Chromosome no.
<i>Polygonatum</i> sect. <i>Polygonatum</i>			
ser. <i>Bracteata</i>			
<i>P. involucratum</i> (FRANCH. et SAV.) MAXIM.	Japan: Takine, Fukushima	M. N. TAMURA	2n = 18*
<i>P. cryptanthum</i> H. LEV. et VAN.	Japan: Tsushima Isls., Nagasaki	M. N. TAMURA	2n = 18*
<i>P. falcatum</i> A. GRAY	Japan: Mt. Onigajyo, Yamaguchi	M. N. TAMURA	2n = 18*
<i>P. trichosantherum</i> KOIDZ.	Japan: Nomosaki Cape, Nagasaki	M. N. TAMURA	2n = 18*
<i>P. multiflorum</i> (L.) ALL.	Germany: Jenisch Park, Hamburg	M. N. TAMURA	2n = 18
ser. <i>Polygonatum</i>			
<i>P. lasianthum</i> MAXIM.	Japan: Mt. Iohzen, Ishikawa	M. N. TAMURA	2n = 20
<i>P. humile</i> FISCHER	Japan: Mt. Ibuki, Shiga	M. N. TAMURA	2n = 20*
<i>P. canalicuratum</i> (MUHL.) PURSH	U.S.A.: Seven Springs, Pennsylvania	S. KAWANO	2n = 40
<i>P. odoratum</i> (MILLER) DRUCE	China: Jiuzhaigou, Sichuan	M. N. TAMURA	2n = 20*
var. <i>maximowiczii</i> (F. SCHMIDT) KOIDZ.	Korea: Ullung Isl.	M. N. TAMURA	2n = 20
ser. <i>Inflata</i>			
<i>P. macranthum</i> (MAXIM.) KOIDZ.	Japan: Mt. Tsurugi, Tokushima	M. N. TAMURA	2n = 22
<i>P. cyrtoneura</i> HUA	Taiwan: Mt. Chising, Taipei	M. N. TAMURA	2n = 22
<i>Polygonatum</i> sect. <i>Verticillata</i>			
<i>P. sibiricum</i> DELAR.	Korea: Mt. Torak, Ch'ungch'onbuk-do	M. N. TAMURA	
<i>P. verticillatum</i> (L.) ALL.	Austria: Obergurgl	K. KUBITZKI	2n = 28
<i>P. prattii</i> BAKER	China: Kunming Inst., Yunnan	Unknown	2n = 56*
<i>P. stenophyllum</i> MAXIM.	Korea: Mt. Samyong, Kangwon-do	M. N. TAMURA	
<i>P. cirrhifolium</i> (WALLICH) ROYLE	China: Kunming, Yunnan	M. N. TAMURA	2n = 30*
<i>P. oppositifolium</i> (WALLICH) ROYLE	Nepal: Dhankuta	H. HARA	2n = 30*
<i>Heteropolygonatum</i>			
<i>H. roseolum</i> M. N. TAMURA et OGISU	China: Mt. Dayao, Guangxi	D. Z. FU et al.	2n = 32
<i>Disporopsis</i>			
<i>D. longifolia</i> CRAIB	Thailand: Phu Kradung, Loei	M. N. TAMURA	
<i>D. aspera</i> (HUA) KRAUSE	China: Mt. Omei, Sichuan	M. N. TAMURA	
<i>D. arisanensis</i> HAYATA	Taiwan: Mt. Ari	M. N. TAMURA	
<i>Smilacina</i>			
<i>S. japonica</i> A. GRAY	Japan: Mt. Tsurugi, Tokushima	M. N. TAMURA	2n = 36
var. <i>robusta</i> (MAKINO et HONDA) OHWI	Japan: Mt. Arashima, Fukui	M. N. TAMURA	2n = 36
<i>Maianthemum</i>			
<i>M. dilatatum</i> (A. WOOD) NELSON et MACBR.	Germany: Botanic Garden, Hamburg	Unknown	2n = 36

Sections and series of *Polygonatum* are circumscribed according to TAMURA (1993a).

* chromosome number and karyotype published in TAMURA (1990, 1993a). All other chromosomal data are unpublished.

including the maturase-encoding gene *matK* (SUGITA et al. 1985; NEUHAUS & LINK 1987; Fig. 1).

Molecular characters were obtained from restriction fragment length polymorphisms (RFLPs) of the PCR amplification products. These were initially

digested with the following 18 restriction enzymes: *Bam*HI, *Bfal*, *Bgl*II, *Bsa*BI, *Bsa*II, *Dde*I, *Dra*I, *Eco*RI, *Eco*RV, *Hinc*II, *Msp*I, *Nde*I, *Rca*I, *Rsa*I, *Scr*FI, *Ssp*I, *Taq*I, and *Xmn*I. The enzymes *Apa*I, *Ava*I, *Bcl*II, *Bst*UI, *Eco*147I, *Hha*I, *Pst*I, and *Sph*I did not digest the DNA.