# The analysis of some biological data on *Stachys kurdica* (*Lamiaceae*) in Turkey

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**Abstract.** *Stachys kurdica*, well adapted to live in sloping limestone crevices and steep screes, is distributed across particular routes in Southeast Turkey and North Iraq and also extends to Iran. This species belongs to the section *Fragilicaulis* and contains the following infraspecific taxa; var. *kurdica* and var. *brevidens*. These taxa share partly the same habitats and are distinguished by the calyx characters. Micromorphological studies of trichomes on the epidermal surface and nutlet surface by Tabletop Scanning Electron Microscopy and Light Microscopy have revealed non-glandular and glandular trichomes on the taxa. The nutlets were coloured from light to dark-brown, usually obovate, ventral sides flat, rooflike with rib, apex rounded, faintly granulate, wrinkly, glabrous and reticulate. Karyotypes of the taxa have been introduced for the first time to the scientific community and were obtained by the Image Analysis System. The karyotype analysis showed the taxa as diploid, with chromosome numbers of 2n = 34. In addition to these morphological characters, chromosomal and micromorphological differences further serve to distinguish the taxa. Therefore, this paper is the first study discussing together the morphological, micromorphological and karyotype structures of *S. kurdica*.

Key words: *fragilicaulis*, karyology, micromorphology, morphology

## Introduction

The genus *Stachys* is one of largest genera of *Lamiaceae* and includes *ca.* 370 species (435 taxa). It is distributed, above all, in the warm temperate regions of the Mediterranean and Southwest Asia, also in North America, South America and South Africa. The species are annual and perennial herbs and subshrubs (Bhattacharjee 1980; Govaerts 2015, Güner 2016). In Turkey, the genus *Stachys* comprises 88 species (114 taxa) and 59 taxa are endemic to Turkey. Species of the genus are mainly East Mediterranean elements (Bhattacharjee 1982; Scheen & al. 2010; Güner 2016).

Section *Fragilicaulis* R.Bhattacharje, which is distributed in Turkey, Iran and Iraq, is represented worldwide by 30 taxa (Bhattacharjee 1982; Güner & Akçiçek 2015; Güner 2016). Its species are suffrutescent saxatile perennials and their flowering stems are fragile below. They grow on sloping limestone rocks and in cliff crevices. *Stachys kurdica* Boiss. & Hohen, in subsection *Multibracteolatae* R.Bhattacharjee of section *Fragilicaulis*, is a perennial species which is distributed in southeastern Anatolia, West Iran and North Iraq. *S. kurdica* is an Irano-Turanian element and has two varieties: var. *kurdica* and var. *brevidens* Bornm. ex R.Bhattacharjee (Bhattacharjee 1982; Rechinger 1982). The infraspecific taxa of *S. kurdica*, which has been accepted as variety in some literatures, were evaluated as subspecies (*S.kurdica* subsp *kurdica*) by Salmaki & al. (2012). They studied the taxonomic revision of the genus *Stachys* species growing in Iran. *Stachys kurdica*, which was within the examined 32 *Stachys* taxa, was investigated morphologically. A morphological description and nutlet character of var. *kurdica* is given in this study.

Nutlet surface and trichome micromorphology provide some of the most useful taxonomic characters in some genera of *Lamiacea* (Salmaki & al. 2009; Satıl & al. 2012; Karaismailoğlu & Güner, 2019). Micromorphological studies of the genus *Stachys* have been on the increase in recent years. These studies have mostly concerned nutlet surface and trichome micromorphology (Falciani & al. 1995; Martin Mosquero & al. 2000; Salmaki & al. 2008, 2009; Rezakhanlo & Talebi 2010; Vundac & al. 2011; Erdoğan & al. 2012; Satıl & al. 2012; Giuliani & Bini 2012; Grujic & al. 2014).

The most comprehensive micromorphological studies of the genus Stachys were conducted by Salmaki & al. (2008, 2009) and Satıl & al. (2012). Salmaki & al. (2008) examined the seed micromorphology of 31 taxa belonging to the genus Stachys in Iran. Seven basic types were outlined according to surface properties: reticulate, colliculate, scalariform, verrucate, ruminate, foveate, and rugose. The reticulate type has been found especially useful in separating the species in the section. In another study, Salmaki & al. (2009) examined the seed micromorphology of 37 taxa belonging to the genus Stachys in Iran. They indicated that two basic types of trichomes can be distinguished: glandular and non-glandular. Satıl & al. (2012) have studied nutlet morphology of 32 taxa of Stachys sect. Eriostomum (Hoffmanns. & Link) Dumort. (Lamiaceae) by scanning electron microscopy (SEM). In their study, they have pointed out that nutlet micromorphological characters seem to be useful in the species-level taxonomy.

The chromosome numbers of 26 *Stachys* taxa were determined by Martin & al. (2011). The chromosome numbers reported as 2n = 30 belonged to subsection *Germanicae* R.Bhattacharjee. The chromosome numbers of *Stachys* species belonging to subsection *Creticae* R.Bhattacharjee were reported as 2n = 30. In subsection *Spectabiles* R.Bhattacharjee, all numbers were reported as 2n = 30 (Martin & al. 2011). Moreover,

some *Stachys* species were reported with chromosome numbers of 2n = 34 (Baltisberger & Lenherr 1984; Mulligan & Munro 1989; Baltisberger 1990, 2006). In our study, the chromosome number of the taxa was 2n = 4x = 34.

According to the available literature data, the morphology, karyologic, trichome micromorphology, and nutlet surface of *S. kurdica* have not been examined yet. In this study, we have carried out comparative 5 morphological, micromorphological and karyotype analyses of *S. kurdica* using a light microscope (LM) and scanning electron microscope (SEM).

## Material and methods

#### Morphological methods

Stachys specimens were collected from their natural habitats in Hakkari and Şırnak provinces, both in the flowering and fruit bearing periods of 2012-2015. The specimens were dried and stored at Gazi Herbarium (GAZI). Here are the localities of these taxa collected and analyzed in Turkey: S. kurdica var. kurdica – Şırnak: Köprülü – Uludere road, 26. km, after the Süvari gate, 10.06.2013, Ö.Güner 2353; Hakkari: near Çukurca, on limestone slopes, 1200 m, 10.06.2013, Ö.Güner 234; betwen Yüksekova and Dağlıca, Varegöz Valley, in rock crevices, 09.06.2013, Ö.Güner 2347a and S. kurdica var. brevidens - Hakkari, betwen Yüksekova and Dağlıca, Varegöz Valley, in rock crevices, 09.06.2013, Ö.Güner 2347b; 9 km from Kırıkdağ to Cehennem Dere, Kırıkdaği, in rock crevices, 1555 m, 11.06.2013, Ö. Güner 2348; 30 km from Yüksekova to Dağlıca, on cliffs and in crevices, 9.06.2013, Ö.Güner 2349.

#### Micromorphological methods

Trichomes on the epidermal (stem and leaves) and nutlet surface were studied by tabletop scanning electron microscopy (SEM). For SEM, small pieces of leaves and stem with nutlet grains were fixed on aluminum stubs with double-sided adhesive. The SEM micrographs were taken with a NeoScope JCM-5000 at an accelerating voltage of 10 kV. Furthermore, the trichomes on stem and leaves with nutlet surface were studied with Olympus BX53 light microscope (LM). Nutlets were examined for size, shape, colour, anticlinal and periclinal cell walls, hilum and ornamentation. These characters were determined according to various works (Bojňanský & Fargašová 2007; Stearn 1992; Satil & al. 2012; Salmaki & al. 2008; Moon & Hong 2006; Demissew & Harley 1992; Katarzyna & Katarzyna 2015). In trichome examination, emphasis was laid on trichome types and shape. Structure and classification of trichomes were determined according to various works (Metcalfe & Chalk 1950; Fahn 2000; Werker 2000; Giuliani & Bini 2008; Navarro & El Qualidi 2000; Salmaki & al. 2009).

#### Karyological methods

For the chromosome studies, root tip meristems were used as experimental material. When nutlets germinated (1-1.5 mm), the material was pretreated for 16 h in α-monobromonaphthalene at 4°C, fixed in 3:1 absolute alcohol-glacial acetic acid. Then the root tips were hydrolyzed with 1 N HCl for 11 minutes at 60 °C overnight, and stained with 2% aceto orcein for 2h at room temperature. The stained root tips were squashed in a drop of 45 % acetic acid and permanent slides were made according to the standard liquid nitrogen method; slides were dried for 24 h at room temperature and mounted in depex. The best metaphase photographs at 10×100 enlargement were taken by OLYMPUS BX51 microscope, with digital camera Pixera PVC 100C attachment. The chromosome counts in the mitosis metaphase were usually based on five different root tips from each individual.

Classification of chromosomes, length of long and short arm, haploid chromosome length, arm ratio, centromeric index, and relative chromosomal length were measured by Software Image Analyses (Bs200Pro) loaded on a personal computer. Chromosomes were classified according to the nomenclature of Levan & al. (1964). Classification of chromosomes as median (m), submedian (sm), subterminal (st), and terminal (T) was based on the analysis of metaphase chromosomes. Ideograms of these taxa were arranged in decreasing length.

For the analysis of karyotype asymmetry, the following methods were used. To describe karyotype asymmetry and to determine the karyotypic relationships between species, Huziwara (1962) had developed the total form percent (TF%); TF% = (Length of short arms in chromosome set / Total chromosome length in set) × 100. Then Arano (1963) had developed another karyotype asymmetry index: As K%. As K% = (Length of long arms in chromosome set / Total chromosome length in set) ×

100. Greilhuber and Speta (1976) had developed two indices to evaluate karyotype asymmetry called Syi index and Rec index. Syi = (Mean length of the short arms / Mean length of the long arms)  $\times$  100. Rec = ) /  $n \times 100$  (n = number of analyzed chromosome, CLi= length of each chromosome, LC= longest chromosome). Romero Zarco (1986) had provided a different method to measure karyotype asymmetry: the intrachromosomal asymmetry index (A1). A1= 1-) / n (bi = the average length of short arms in every homologous chromosome pair or group; Bi= the average length of long arms in every homologous chromosome pair or group; n= the number of homologous chromosome pairs or groups) and the interchromosomal asymmetry index (A2); A2= (s= standard deviation of the chromosome length; x= the mean of chromosome length). Watanabe & al. (1999) had defined the degree of asymmetry of karyotype (A). A = ) / n (where p and q are the lengths of long arm and short arm of chromosome i, and n is the haploid chromosome number of species.

Stebbins (1971) had distinguished twelve categories of karyotype asymmetry, but only ten of these were known to occur in higher plants. He had established them by recognizing three degrees of difference (A-C) between the largest and smallest chromosome of the complement, and four degrees (1–4) with respect to the proportion of chromosomes which are median pair with an arm ratio of less than 2:1 (e.g. Table 1).

Table 1. Stebbins' classification.

Ratio	Proport	ion of chromos	omes with arm	ratio <2:1
Largest/smallest	1.00(1)	0.99-0.51 (2)	0.50-0.01 (3)	0.00(4)
<2:1 (A)	1A	2A	3A	4A
2:1-4:1 (B)	1B	2B	3B	4B
>4:1 (C)	1C	2C	3C	4C

# Results

#### Morphological results

#### Stachys kurdica

Description: Suffrutescent perennial, with numerous stems, fragile, flowering stems 18–36 cm, erect, branched above, sparsely retrorse-pubescent or occasionally glabrous with sparsely sessile glands. Cauline leaves oblong-lanceolate to lanceolate, 1.4– $3 \times 0.4$ –1.2 cm, weakly serrate, dentate to subentire at margin, acute to obtuse at apex, subcordate to

cuneate at base; subsessile to 10 mm petiolate. Floral leaves similar but smaller,  $0.5-2.4 \times 0.2-$ 0.7 mm, flowers longer than verticillasters, gradually becoming shorter than calyx above, subentire to entire at margin, acute at apex, cuneate at base; shortly petiolate to subsessile. Verticillasters numerous, remote, 1–4.5 cm distant, 4–8-flowered. Bracteoles few, setaceous or seldom linear, 1–6 mm. Pedicels sessile to 6 mm. Calyx ± regular, infundibular, 5–9 mm, sparsely retrorse-pubescent with sparsely pubescent sessile glands; teeth subequal, broadly triangular-lanceolate with ± obtuse muticous at apex,  $1/5-1 \times$  tube, recurved in fruit. Corolla yellow, (9-)12-17 mm, tube exceeding the calyx, sparsely pubescent outside; limb bilabiate, upper lip 4-5 mm; the lower 3-lobed, middle lobe larger than 2 lateral lobes, 3-6 mm. Nutlets obovate, apex rounded, brown to dark-brown, wrinkly, glabrous,  $1.8-2.2 \times 1.3-1.5$  mm (e.g. Fig. 1).

- Calyx teeth broadly triangular, 1/5–1/4 × tube .....var. brevidens
- Calyx teeth broadly oblong-lanceolate, as long as or slightly shorter than tube, 3–4 mm ...... var. kurdica



Fig. 1. Habit (A, C), flowers (B, D) and drawings (E). *S. kurdica* var. *kurdica*, A. Habit, B. Verticillaster with flowered, E-I. Calyx, E-III. Corolla; *S. kurdica* var. *brevidens*, C. Habit, D. Verticillaster with flowered, E-II. Calyx, E-IV. Corolla.

#### Phenology, habitat and ecology

In the cities of Hakkari and Şırnak in Turkey, the infraspecific taxa of *S. kurdica* grow in similar types of habitats: steep sandstone, limestone slopes or in cliffs and crevices (e.g Fig. 2). They flower in June to July and fruit in mid-June and August. The varieties are Irano-Turanian elements and are distributed at *ca*. 1200–3800 m.



**Fig. 2.** Distribution map of *S. kurdica* (for Iranian data see Salmaki et al., 2012).

#### Micromorphological results

Trichomes can provide somewhat stable character sets, which are valuable for comparative systematic studies at every level of taxonomic categories (Metcalf & Chalk 1950; Navarro & El Oualidi 2000). Glandular trichomes of the members of Lamiaceae family mainly include peltate and capitate trichomes (Mannethody & Purayidathkandy 2018; Bokhari & Hedge 1971; Huang & Cheng 1971; Husain & al. 1990; Metcalf & Chalk 1950; Abu-Asab & Cantino 1987). In this study, the glandular trichomes of Stachys taxa were similar to this expression .Trichome structures of the examined taxa have shown similar characters (e.g. Fig. 3). In general, there were found eglandular (B type) and glandular trichomes (C and P type) on stem, leaves, corolla, and calyx ( Table 2). Eglandular trichomes were indicated as B1, B2 and B3; while glandular trichomes were indicated as C1, C2, C3, and P type (Table. 2).

Nutlet evaluation of the S. *kurdica* taxa under LM has shown that their surface, light-brown to dark-brown, was usually obovate, ventral sides flat, rooflike



Fig. 3. Epidermal surface of stem (A), lamina (B), calyx (C), and corolla (D) under SEM. *S. kurdica* var. *kurdica* (A1, A3, A4, B1, B3, C1, C2, D1, D2), *S. kurdica* var. *brevidens* (A2, B2, B4, C3, C4, D3, D4).

			Б	-1	11.		1	(T		-)								G	land	lular	tric	nome	es					
ar.	Egiandular tricnomes (B type)						Capitate (C type)									Peltate (P type)												
laxa		B	1		B 2 B 3			C1 C2						C3														
	S	L	С	Cr	S	L	С	Cr	S	L	С	Cr	S	L	С	Cr	S	L	С	Cr	S	L	С	Cr	S	L	С	Cr
var. kurdica	+	±	-	-	+	+	+	+	±	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+
var. brevidens	+	±	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	_	+	+	+

Table 2. Trichome types observed on epidermal sufrace of the examined taxa.

S: stem, L: lamina, C: calyx, Cr: corolla

B1 TYPE: single cell, base large, cubic shaped

B2 TYPE: 1–5 cell, large base, cuticular micropapil, plain or slightly curled feathers

B3 TYPE: multicellular (3-8), thin, weak, flattened and weakly micropapil

C1 TYPE: stalk is a cells, head is pear or globuler in shaped, 1 or 2 cells???

C2 TYPE: stalk 2-celled, a short neck and pear- or globular-shaped head, 1 or 2 cells

C3 TYPE:stalk 3-5 celled, a short neck and usually pear-shaped head, 1 or 2 cells

P TYPE: basal epidermis cell, neck cell and expanded 8-16 cell head cells???

with rib, apex rounded, winged,  $1.8-2.2 \times 1.3-1.5$  mm. Surface faintly granulate, wrinkly, glabrous (e.g Fig. 4; Table 3).

The results have shown no systematically significant difference between epidermal surfaces and nutlets of the examined varieties of *S. kurdica*.

### **Karyological results**

In the present study, the results in two varieties of *S. kurdica* were evaluated. Chromosome numbers, karyological features, asymmetry index, karyograms, and ideograms were determined for the studied taxa. The

results were new for science and very close between the two varieties: *S. kurdica* var. *kurdica* and *S. kurdica* var. *brevidens*.

## Stachys kurdica var. kurdica

Our study has shown that the chromosome number of the S. *kurdica* var. *kurdica* was new for science: 2n = 34 (Fig. 5a). The shortest chromosome length was 0.95 µm, the longest 1.51 µm, and the haploid chromosome length was 19.73 µm. Chromosome arm ratios were measured as 1.18–1.97. Relative lengths



**Fig. 4.** Nutlet surface and general view of the examined varieties.

Taxa	Nutlet size (mm)	Nutlet shape	Apex	Wings	Colour	Cell	Anticlinal	Periclinal	Hilum width	Surface
						size	cell walls	cell walls	(µm)	ornamentation
var. kurdica	1.9–2.2 × 1.3–1.5	obovate to oblong	rounded	winged	brown	17–42	raised, flat	concave	162–195	reticulate
var. <i>brevidens</i>	1.8–2.1 × 1.3–1.5	obovate to oblong	rounded	winged	brown	18-32	raised, wrinkled	concave	245-320	reticulate



**Fig. 5.** a. Photographs of somatic metaphase chromosomes, b. ideogram, c. karyogram of *S. kurdica* var. *kurdica*.

varied from 4.79 to 7.66. The karyotype formula of this taxon consisted of twelve median pairs and five submedian pairs. The ideogram was based on the centromeric index and arranged in a decreasing 5order (Fig. 5b) and a karyogram was given (Fig. 5c).

As to karyotype asymmetry, the karyotype of this taxon was classified according to the symmetry classes of Stebbins as 2A. The other karyotype asymmetry indices were: 40%, 60%, 67, 77, 0.20, 0.32 and 0.13 for TF %, As K %, Syi, Rec, A, A1 and A2 (Table 4).

Table 4. Values of asymmetry indices in S. kurdica var. kurdica.

Stebbins' classification	TF%	As K %	Syi	Rec	Α	A1	A2
2A	40	60	67	77	0.20	0.32	0.13

#### Stachys kurdica var. brevidens

Our study has shown that the chromosome number of the *S. kurdica* var. *brevidens* was new for science: 2n = 34 (Fig. 6a). The shortest chromosome length was 0.99 µm, the longest 1.51 µm, and the haploid chromosome length was 20.14 µm. The chromosome arm ratios were measured as 1.14–1.97. The relative lengths varied from 4.91 to 7.50. The karyotype formula of this taxon consisted of fifteen median pairs and two submedian pairs. The ideogram was based on the centromeric index and arranged in a decreasing order (Fig. 6b), a karyogram was given (Fig. 6c). As to karyotype asymmetry, the karyotype of this taxon was classified according to the symmetry classes of Stebbins as 2A. The other karyotype asymmetry indices were: 43 %, 57 %, 75, 78, 0.15, 0.25 and 0.13 for TF %, As K %, Syi, Rec, A, A1 and A2 (Table 5).

Table 5. Values of asymmetry indices in S. kurdica var. brevidens.

Stebbins' lassification	TF%	As K %	Syi	Rec	A	A1	A2
2A	43	57	75	78	0.15	0.25	0.13

# Discussion

In this study, *S. kurdica* was comprehensively studied for the first time in terms of its morphological, micromorphological and karyological properties. The obtained morphological data was compared with the earlier studies (Bhattacharjee 1982; Rechinger 1982) in countries, where the species were found. The description of the species was expanded.

*Stachys kurdica* belongs to the subsection *Multibracteolatae* of section *Fragilicaulis* and is widely found in Southeast Turkey, North Iraq and Northwest Iran. The infraspecific taxa of *S. kurdica* share the same habitats in Hakkari, Southeast Turkey. Thus, these taxa were evaluated as a variety in this study.

At first glance, the morphological characters of var. *kurdica* and var. *brevidens* show some similarities.



**Fig. 6.** a. Photographs of somatic metaphase chromosomes, b. ideogram, c. karyogram of *S. kurdica* var. *brevidens*.

Calyx characteristics such as the length and rate of teeth are taxonomically significant for delimiting the taxa of *S. kurdica*. Therefore, var. *brevidens* differs from var. *kurdica* by broadly triangular calyx teeth and 1/5-1/4 x tube. These varieties can be easily delimited in their habitats from each other by the rate of calyx teeth. *S. kurdica* is closely related to *S. ballotiformis*, from which it differs by sparsely retrorse-pubescent stems, usually remote verticillasters, and broadly triangular to oblong-lanceolate calyx teeth.

Examination of the trichome structures of the taxa does not show any significant differences. More or less, all trichome types were found on the stem and leaf surfaces. Other trichome types, except the B type, were observed on the corolla and calyx. Peltate types were found in all other organs, except on stem. Capitate types were amply seen on calyx and corolla, seldom observed on the stem, and sparsely on leaves. No C3 type trichome could be found on the stem (Table 2).

The most important difference between the nutlets of the two taxa is seen on the anticline walls. The anticline walls of var. *kurdica* are flat and distinct, while the anticline walls of var. *brevidens* are slightly wrinkly and not evident in some places. The periclinal walls of both taxa are concave (Fig. 4). The other characters on the nutlets of the taxa presented in Table 3 are similar.

Salmaki & al. (2012) emphasized that the nutlets of *S. kurdica* var *kurdica* were narrowly winged, broadly obovoid,  $3.0-3.3 \times 2.5-2.7$  mm, and scalariform minutely reticulate on the surface. In the present study, it was observed that the nutlets of var. *kurdica* were smaller ( $1.9-2.2 \times 1.3-1.5$ ), obovate to oblong in shape, winged at apex and lateral edges, and distinctly reticulate on the surface.

The conducted karyomorphological study has determined the chromosome morphology of two *Stachys* taxa. The chromosome morphology has shown an important similarity between *S. kurdica* var. *kurdica* and *S. kurdica* var. *brevidens*. The shortest chromosome length (0.95  $\mu$ m) was observed in the taxon of *S. kurdica* var. *kurdica*. In contrast, the longest (1.51  $\mu$ m) one was observed in both varieties of *S. kurdica*. When the two varieties were compared according to the haploid chromosome length, *S. kurdica* var. *brevidens* (20.14  $\mu$ m) proved longer than *S. kurdica* var. *kurdica* (19.73  $\mu$ m). The smallest arm ratio was observed in *S. kurdica* var. *brevidens* (1.14) and the largest was observed in both varieties of *S. kurdica* (1.97). The smallest and the biggest relative length value were measured in *S. kurdica* var. *kurdica* (4.79 and 7.66). The methaphase chromosome pairs were usually of the median and submedian type. In the present study, the karyotype formulae were obtained as 15m+2sm for *S. kurdica* var. *kurdica* var. *brevidens* and 12m+5sm for *S. kurdica* var. *kurdica*.

When the two varieties were compared according to Stebbins (1971) classification for karyotype asymmetry in Stachs, both taxa were classified according to the Stebbins' symmetry classes as 2A and indicated the same symmetrical karyotypic features. It could be maintained that 3A class was more asymmetrical than 1A class, or that 2B class was more symmetrical than 2C class, but it could not be determined which of the 2A classes had higher symmetry. This means that Stebbins' classification did not clarify the issue, nor could it determine the most symmetrical or asymmetrical karyotype. Thus we resorted to other indices. The TF% and Syi-Rec values decreased with increasing asymmetry, while the As K%, A1-A2 and A values increased with increasing asymmetry (Zuo and Yuan 2011; Eroğlu & al. 2013). When the two varieties were compared by these indices, S. kurdica var. brevidens had the most symmetrical karyotype. Accordingly, TF %, Ask %, Syi, A1, and A indices were clearer than Stebbins' classification.

Such a detailed study including morphological, micromorphological and karyotype analyses allowed a more thorough understanding of the varieties of this species. Data on the trichome and nutlet micromorphology proved important for the taxonomy of these taxa. Therefore, the morphological differences were supported by data obtained from the chromosomal and micromorphological studies.

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