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A TAXONOMIC REVISION OF SCUTELLARIA MULTICAULIS *(*LAMIACEAE) SPECIES COMPLEX IN IRAN

K. Safikhani, Z. Jamzad & H. Saeidi

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In a taxonomic revision of the genus *Scutellaria* in Iran, the specimens identified as *Sc. multicaulis* Boiss. in the herbarium of Research Institute of Forests & Rangelands (TARI) as well as specimens belonging to the newly collected materials were examined and sampled for DNA sequencing by using nrDNA ITS and cpDNA *trn*L-F as molecular markers. Morphological variations such as flowers color, leaf shape and indumentum were diagnostic characters in the studied specimens. Accordingly, specimens could be divided into two different morphological groups. Sampling of representatives of each morphological group was carried out for DNA sequencing. The results showed differences in sequences of determined groups. Based on the results of this study, three new taxa are recognized and described.

Keivan Safikhani, Department of Biology, Faculty of science, University of Isfahan, P.O. Box 81746-73441, Isfahan, Iran.- Ziba Jamzad, Research Institute of Forests & Rangelands, Agricultural Research, Education and Extension Organization (AREEO), P. O. Box 13185-116, Tehran, Iran.- Hojatolah Saeidi (correspondence ho.saeidi@sci.ui.ac.ir*), Department of Biology, Faculty of science, University of Isfahan, Isfahan, Iran*.

Key words: *Scutellaria;* Molecular phylogeny; nucleotide sequences; new species; Iran

مرور تاکسونومیکی گونه Scutellaria multicaulis در ایران

کیوان صفی خانی: دانشجوی دکتری سیستماتیک گیاهی دانشگاه اصفهان، گروه زیست شناسی، دانشکده علوم، اصفهان، ایران

زیبا جم زاد: استاد پژوهش مؤسسه تحقیقات جنگلها و مراتع کشور، سازمان تحقیقات، آموزش و ترویج کشاورزی، تهران، ایران

حجت اله سعیدی: دانشیار گروه زیست شناسی دانشگاه اصفهان

در مرور تاکسونومیکی جنس Scutellaria در ایران، نمونه‌های شناسایی شده تحت نام .Scutellaria multicaulis Boiss در هرباریوم مؤسسه تحقیقات جنگلها و مراتع کشور (TARI) و همچنین نمونه‌های مربوط به جمع‌آوری‌های جدید مورد بررسی قرار گرفتند و جهت تعیین توالی DNA با استفاده از مارکرهای مولکولی هسته‌ای ITS و کلروپلاستی trnL-F نمونه برداری شدند.

تنوع ریخت‌شناسی مانند رنگ گل‌ها و شکل برگ‌ها و نوع کرک‌ها در نمونه‌های مورد بررسی مشاهده گردید. بر اساس رنگ گل‌ها نمونه‌ها به دوگروه مختلف تقسیم شدند. تعیین توالی DNA نمایندگانی از هر گروه انجام شد و نتایج به دست آمده تفاوت‌هایی را در بین گروه‌های تعیین شده نشان داد. بر اساس نتایج حاصل سه آرایه جدید شناسایی و شرح داده شدند.

INTRODUCTION

Scutellaria L. is a sub-cosmopolitan genus of Lamiaceae subfamily Scutellarioideae which grows in both Old and New Worlds. The members of this genus are typically characterized by their calyx shape, with two undivided lips and the presence of a scutellum on the upper lip. The calyx shows variable characters in different species, and the scutellum may be absent, or

calyx may be inflated in the upper lip (Cantino et al. 1992).

With 425 currently recognized species, *Scutellaria* is one of the large genera within Lamiaceae, but when the possible synonyms are considered, the actual number of species is closer to 360 (Paton 1990a). In the Plant List database (http://www.theplantlist.org) there are 735 species names for *Scutellaria*, of which 465 are accepted species names. The Irano-Turanian Region, particularly Central Asia and Afghanistan, is the main center of diversity of the genus. However, Eastern Mediterranean and the Andes are the second centers of its speciation (Paton1990a). Many members of *Scutellaria* possess a wide range of pharmacological traits such as antitumor, anti-angiogenesis, hepatoprotective, antioxidant, anticonvulsant, antibacterial and antiviral activities (Shang et al. 2010), which makes this genus economically important.

The infrageneric classification of the genus has been done differently by different authors. Hamilton (1832) recognized three sections in the genus (sects. *Lupulinaria* A. Hamilton, *Stachymacris* A. Hamilton and *Galericularia* A. Hamilton). Bentham (1834) classified *Scutellaria* into five sections (sects. *Lupulinaria* A. Hamilton, *Heteranthesia* Benth., *Stachymacris* A. Hamilton, *Galericularia* A. Hamilton and *Maschalostachys* Benth.). Later, in 1876 he divided the genus into three sections (sects. *Lupulinaria* A. Hamilton, *Heteranthesia* Benth. and *Vulgares* Benth.). Briquet (1896) considered two subgenera in his classification: *Euscutellaria* Briq. with three sections (sects. *Lupulinaria* A. Hamilton, *Heteranthesia* Benth. and *Vulgares* Benth.) and *Scutellariopsi*s Briq. with no sectional division. Rechinger (1982) recognized four subgenera in his treatment of the genus: *Euscutellaria* Briq. (sects. *Lupulinaria* A. Hamilton, *Stachymacris* A. Hamilton, *Galericularia* A. Hamilton); *Anaspis* (Rech. f.) Paton; *Apeltanthus (*Nevskyi ex Juz.) Juz. emend Paton and *Cystaspis (*Juz.) Juz. He did not consider any sectional divisions for the last three subgenera. The New world taxa were classified by Epling (1942) into 18 sections.

In the latest taxonomic review of the genus, the infrageneric classification of *Scutellaria* has been revised by Paton (1990a). In a comprehensive study on *Scutellaria* and the allied genera (Paton, 1990a, 1990 b, 1992) the features of the inflorescence, calyx, corolla and nutlets were reported as the most important and taxonomically reliable characters. He considered a broad concept for this genus.

Forty species for *Scutellaria* has been reported in Flora Iranica including 22 species form Iran, of which ten taxa are endemics (Rechinger 1982). Members of this genus are distributed all over the country, mainly in mountainous regions but a few in wet places at aquatic habitats and in forests. On the base of Rechinger's classification, Iranian *Scutellaria* species were recognized in subgenus *Scutellaria* sections *Galericulata* A. Hamilton, *Stachymacris* A. Hamilton and *Lupulinaria (*A. Hamilton) Paton and subgenus *Anaspis (*Rech. f.) Juz.,. Based on Paton's classification, the Iranian *Scutellaria* species are classified in the subgenus *Scutellaria* sections *Scutellaria* Paton, *Anaspis* (Rech. f.) Paton and subgenus *Apeltanthus*, section *Lupulinaria* A. Hamilton.

The Paton's classification was followed by Jamzad (2012). She added five new taxa to the previous species list of Iran by Rechinger (1982), increasing the number of Iranian *Scutellaria* species to 27, of these 12 taxa are endemic to Iran. Most Iranian *Scutellaria* species belong to subgenus Apeltanthus section *Lupulinaria* (19 species), a taxonomically complicated species group. It is hard to define species boundaries and relationship.

The distribution pattern of *Scutellaria* species in Iran is very different. Some species are distributed in limited geographical areas such as *Sc. tournefortii* Benth., but there are species with wide range of distribution, i.e. *Sc. pinnatifida* A. Hamilt and *Sc. multicaulis* Boiss., the latter is distributed in south-east, center and west of Iran in Sistan va Baluchestan, Kerman, Yazd, Isfahan, Chaharahal-va-Bakhtiari, Kohglouyeh-va-Boyerahmad, Semnan, Qom, Markazi, Lorestan, Kermanshah and Hamedan Provinces (Rechinger 1982; Jamzad 2012).

The wide range of distribution of *Sc. multicaulis*, is accompanied with a high morphological variation. Three subspecies of this taxon were reported in Flora Iranica area (Rechinger 1982), including subsp. *loringensis* (Rech. f. & Fitz) Rech. f. endemic to Afghanistan, subsp. *multicaulis* endemic to Iran, subsp. *koelzi* (Rech. f.) Rech. f. endemic to Afghanistan and Pakistan. Hedge (1990) in Flora of Pakistan did not recognize any subspecific taxon for *Sc. multicaulis* Boiss.

The use of molecular markers in taxonomy has provided valuable data which help taxonomists to decide about circumscription of a species or genus (Duminil & Di Michel 2009, Duminil & al. 2011). Here we used differences in DNA sequences accompanied with morphology to circumscribe species boundaries and taxonomic status of different taxa in *Sc. multiaculis* complex. We could infer the morphological variations by comparing DNA sequences in different taxa. The work by Hosokawa and colleagues (2000, 2005) showed that it is possible

to define species by molecular markers. They separated nine closely related species in the genus *Scutellaria* with using cpDNA and RAPD markers.

In a phylogenetic study of the genus we examined the Iranian species of the genus as well as a few species from Afghanistan and Pakistan. Here we report the taxonomic status of *Sc. multicaulis sensu lato* related species on the basis of morphological and molecular phylogenetic analysis of the examined species.

MATERIALS AND METHODS

Morphological studies

We studied sixty three specimens of *Sc.multicaulis* complex in the herbarium of Research Institute of Forests and Rangelands (TARI). The quantitative and qualitative characters of vegetative and floral parts were measured under a stereomicroscope (Olympus, SZ60). Based on flower color they were defined in two morphological groups: yellow and violet floweres (tables 1 & 2).

Table 1. Names, voucher numbers, and localities for the taxa that have been sequenced in this study and GenBank accession numbers.

Taxa	Voucher number	Locality and collector (s)	GenBank accession numbers	
			ITS	TRNL-F
Sc. patonii Jamzad & Safikhani	91621 (TARI)	Iran, Chaharmahal-va-Bakhtiari: Zardkuh, Marbur valley, 32°, 24' N, 50°, 07' E, 2407m., Mohebi	ITS1: LC306890 ITS2: LC306893	LC306901
Sc. multicaulis Boiss. subsp. *multicaulis* var. *multicaulis*	91626 (TARI)	Iran, Chaharmahal-va-Bakhtiari: Zardkuh; near Ardal; 2034m. Mohebi	ITS1: LC306891 ITS2: LC306894	LC306899
Sc. multicaulis subsp. *multicaulis* var. *gandomanensis* Jamzad & Safikhani	72391 (TARI)	Iran, Chaharmahal-va-Bakhtiari: Gandoman, mountains S. of Boldaji, 1400m., Assadi	ITS1: LC306892 ITS2: LC306895	LC306900
Sc. *arakensis* Jamzad & Safikhani	105204 (TARI)	Iran, Markazi: N.W. of Arak. The Hills of Modar (Ajori) Mountain.1820 m., Safikhani	LC306887	LC306902
Sc. litwinowii Bornm. & Sint ex Bornm.	35409 (TARI)	Iran, Khorasan: 20 km from Sabzevar to neyshabur. 1300 m, Assadi and Mozaffarian	LC306886	LC306897
Sc. persica Bornm.	105472 (TARI)	Iran, Markazi: The Road of Farmahin to Shahrab village. Near to village heights. 34° 35' 14.8" N , 49° 53' 03.6" E, 2100m., Safikhani	LC306888	LC306896
Sc. pinnatifida A. Hamilt. subsp. *alpina* (Bornm.) Rech. f.	105469 (TARI)	Iran, West Azabaijan: Avajiqh to Bazargan, Baduli village. 39°,20',54.3" N , 44°,14', 33.9" E, 1970m., Safikhani	LC306889	LC306898

Molecular studies

Molecular studies results presented here are part of a molecular phylogenetic study of the genus *Scutellaria* in Iran (in preparation). We have used part of the results referring to the *Sc. multicaulis s.l.* and some related species in this paper.

Taxon sampling

Sampling of the leaf material of four representatives of identified morphological groups was carried out from herbarium specimens deposited at the herbarium of Research Institute of Forests and Rangelands (TARI).

DNA isolation and amplification, Sequencing and Sequence alignment

In this study, the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA (nrDNA) and *trn*L-F region (cpDNA) sequences were used as molecular evidence for taxonomic revision of *Sc. multicaulis* complex. Total genomic DNA was extracted from dry leaves following the modified 2×CTAB (cetyltrimethylammonium bromide) protocol (Doyle and Doyle, 1987). Amplification of nrDNA ITS region was performed using ITS5, ITS4 (for ITS region) ITS5, ITS2 (for ITS1 region) and ITS3, ITS4 (for ITS2 region) primers (White et al., 1990). PCR amplification of ITS follows Baldwin (1992). The *trn*L-F region amplification follows Taberlet et al. (1991). Then PCR products were sent to the Faza Biotech Company for sequencing and were sequenced with an ABI 3730 XL sequencer.

All sequences were aligned using the program Mega ver. 7.0.18 (Kumar S, Stecher G, Tamura K., 2016) and adjusted manually using Bioedit ver. 7.2.5 software (Hall, 1999). The sequence results were used to compare the nucleotides differences within identified morphological groups. Table 1 shows voucher numbers, localities and GenBank accession numbers of representative taxa of identified morphological groups and the relative species.

Phylogenetic analyses were performed on the aligned nrDNA ITS and *trn*L-F data matrices separately using the model suggested by MrModeltest 3.7 (Posada and Crandall, 1998; Posada and Buckley, 2004). The phylogeny was reconstructed based on Bayesian inference using the MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003), based on analysis results, the status of examined taxa were determined and their relationships together and to closely related taxa were defined.

RESULTS

Among the specimens identified as *Sc. multicaulis*, based on morphological data, two main groups were recognized: the specimens with yellow flowers, short retrorse hairs in lower and middle parts of stem, simple hairs intermixed with stipitate glandular patent hairs in inflorescence (group 1) and those with dark violet flowers with yellow spot in lower lip or yellow lower lip, short, simple antrorse hairs, intermixed with sessile glands in inflorescence (group 2).The measured morphological characters are summarized in table 2.

The nucleotides sequences of ITS and *trn*L-F markers represent differences among the taxa of the two groups (tables 3 & 4). The phylogenetic analysis of the genus in Iran revealed different taxa within *Sc. multicaulis s.l.* The examined species are inhabited in different clades in phylogenetic trees (manuscript in preparation). According to the analysis and based on the comparative morphological study, it was concluded that the specimens with yellow flowers, simple and glandular patent hairs in the inflorescence can be distinguished from the others and is describe as a new species.

On the other hand, according to table 2, in the dark violet flowers specimens (group 2), there are variations in some morphological characters such as flowers size, leaf margins and hairs on lower and upper surfaces of leaves. Furthermore, as seen in (tables 3 & 4), there are differences in nucleotide sequences of ITS and *trn*L-F. Considering morphological variations and sequencing results and comparing phylogenetic trees, a new species and one new variety are recognized in this group. In total two new species and one new variety are described.

New taxa

Scutellaria patonii Jamzad & Safikhani **sp. nov.** (fig. 1)

Perennial tufted, suffruticose herb with a thick woody rootstock, with remnants of previous year growth. Stems 25-45 cm high, erect, slender, round-quadrangular, much branched; lower and middle parts with short, simple retrorse hairs, upper part in inflorescence axes with patent simple and glandular hairs. Leaves lanceolate-ovate to ovate, 5-16 × 2.5-9 mm, crenate to subentire, cuneate, acute; upper surface with short, simple curved hairs intermixed with papillose glandulous hairs, lower surface with short appressed or short curved hairs and dense sessile glands. Petioles up to 8 mm, upper leaves sessile. Inflorescence 4-sided, lax, with simple and glandular patent hairs, verticillasters two flowered, distant; bracts equal to longer than calyx, 2-6 × 1-5mm, elliptic to ovate, entire or subentire, cuneate, acute, covered by dense glandular and simple hairs. Pedicels 2-4 mm. Calyx 2 mm, enlarging in fruit to 3 mm, with a small, 2 mm long scutellum, covered by hairs similar to inflorescence axis. Corolla 12-25 mm, yellow with violet lower lips, covered by dense glandular and non-glandular hairs; tube yellow, 13-17 mm. Nutlets smooth, covered with appressed grey stellate hairs, c. 1.5 × 1 mm long.

Holotype: Iran, Chaharmahal-va-Bakhtiari: Zardkuh, Marbur valley, 32°, 24' N, 50°, 07' E, 2407m., 2009/06/27, Mohebi 91621 (TARI).

Table 2. Diagnostic morphological characteristics of *Scutellaria* taxa examined in this study.

Characters \ Taxa	***Scutellaria patonii* sp. nov** Jamzad & Safikhani	***Sc. multicaulis* subsp. *multicaulis* var. *multicaulis***	***Sc. multicaulis* subsp. *multicaulis* var. *gandomanensis*** Jamzad & Safikhani **var. nov.**	***Sc. arakensis*** Jamzad & Safikhani **sp. nov**	***Sc. persica*** Bornm.	***Sc.*** *pinnatifida* A. Hamilt. **subsp.*alpina*** **(**Bornm.) Rech. f.	***Sc. litwinowii*** Bornm. & Sint.
Stem height (cm)	22-60	26-50	34-40	26-43	35	5-10	37-55
Indumentum of lower and middle part of stems	Short and simple retrorse hairs intermixed with sessile glands	Simple antrorse subvelutinous	Simple antrorse subvelutinous	Simple antrorse subvelutinous	Short simple retrorse	Hirsute-tomentose	Short pubescence
Indumentum of inflorescence	Patent simple and glandular hairs	Simple antrorse subvelutinous-puberulous	Simple antrorse subvelutinous-puberulous	Simple antrorse subvelutinous-puberulous	Short simple ±patent hairs	Hirsute-tomentose and glandular	Hispid-glandular
Leaf margins	Crenate to subentire	Sometimes entire or indistinctly dentate	With 3-5 distinct rounded teeth	With 3-5 obvious rounded teeth	Pinnatisect; with 5 segments; revolute margin	Pinnatisect, with 4-6 segments, with revolute margin	Indistinctly dentate, often ±entire
Leaf length (mm)	2-6	5-17	5-10	5-13	7-18	7-15	9-20
Leaf width (mm)	1-5	3-15	1.5-4	2-8	4-10	5-10	3.5-8
Flowers color	Yellow with violet lower lips	Violet with a yellow spot on the lower lip.	Violet with a yellow spot on the lower lip.	Violet with a yellow spot on the lower lip.	Yellow	Yellow	Yellow, lower lip purple-brown
Flowers size (mm)	12-25	16-23	14-15.5(-18)	18-22.5	25-30	30-35	20-30 mm
Indumentum of lower surface of leaf	Short appressed or curved hairs, chiefly along the nerves and for the rest dense sessile glands	Short appressed hairs	Short curved hairs on the surface,intermixed with sessile glands	Short curved hairs, chiefly along the nerves, dense sessile glands on the surface	Hirsute-villous, papillose-glandular + subssesile glands	Dense, lanate	Dense pubescens, with subsessile glands
Indumentum of upper surface of leaf	Short and simple curved intermixed with sessile glands	Chiefly with short curved hairs along the nerves, for the rest with dense sessile glands.	Short curved hairs	Short appressed hairs	Lax hairs	Long appressed hairs	Lax pubescens, with subsessile glands
Bracts length (mm)	2-6	5-7	2-3	2-4	6-8	6-8	6-9
Bracts width (mm)	1-5	3-4	1-2	2-3	3-4	3-4	3-4
Scutellum length/width in fruiting calyx	1-2 / 2-4	1.5 / 3	1.5 / 3	2-2.5 / 3.5-4.5	1.5 -2.5/3.5 -6.5	1.5 / 2	3-4 / 7-10

Table 3. ITS nucleotide sequence variations among examined species in *Sc. multicaulis* s. l. and the related species (only the variable sites are shown).

Nucleotide Number	68	85	118	120	121	125	128	130	133	175	176	181	183	189
Sc. multicaulis* subsp. *multicaulis* var. *multicaulis	C	-	C	A	G	C	T	C	C	-	A	G	G	-
Sc. multicaulis* subsp. *multicaulis* var. *gandomanensis	C	T	C	A	G	C	T	C	C	-	A	G	G	-
Sc. arakensis	C	-	C	A	G	T	T	C	C	-	A	G	G	C
Sc. litwinowii	T	-	G	G	A	T	C	T	T	-	C	G	G	-
Sc. patonii	T	-	G	G	A	T	C	T	T	-	T	G	G	-
Sc. persica	C	-	C	A	G	T	T	C	C	-	A	G	G	-
Sc. pinnatifida* subsp. *alpina	C	-	C	A	G	C	C	C	C	A	A	A	A	-

Nucleotide Number	197	201	205	211	213	218	224	228	230	236	243	244	248	259
Sc. multicaulis* subsp. *multicaulis* var. *multicaulis	A	T	T	C	G	G	A	T	T	C	A	C	G	T
Sc. multicaulis* subsp. *multicaulis* var. *gandomanensis	A	T	T	C	G	G	A	T	T	C	A	C	G	T
Sc.. arakensis	A	G	T	C	G	G	A	G	G	C	C	C	G	T
Sc. litwinowii	T	T	T	T	G	G	G	G	G	T	C	T	G	T
Sc. patonii	T	T	T	T	G	G	G	G	T	T	C	T	G	T
Sc. persica	A	T	-	C	C	A	C	G	G	C	C	C	C	G
Sc. pinnatifida* subsp. *alpina	A	G	T	C	G	G	A	G	G	C	C	C	G	T

Nucleotide Number	280	326	335	337	339	350	367	372	382	384	394	396	399	401
Sc. multicaulis* subsp. *multicaulis* var. *multicaulis	G	G	C	G	G	C	G	G	G	-	T	C	T	C
Sc. multicaulis* subsp. *multicaulis* var. *gandomanensis	G	G	C	G	G	C	G	G	G	-	T	C	T	C
Sc. arakensis	A	C	C	A	G	C	G	G	G	-	T	G	G	C
Sc. litwinowii	A	G	C	G	G	C	G	C	C	C	A	C	T	C
Sc. patonii	G	G	C	G	G	C	G	G	G	C	A	C	T	C
Sc. persica	A	G	G	G	A	G	C	C	C	-	T	G	G	G
Sc. pinnatifida* subsp. *alpina	A	G	C	G	G	C	G	C	C	-	T	G	G	C

Table 3. Continued.

Nucleotide Number	404	406	407	408	413	415	419	427	434	438	439	442	449	453
Sc. multicaulis* subsp. *multicaulis* var. *multicaulis	T	-	-	G	T	C	G	C	C	C	T	C	C	C
Sc. multicaulis* subsp. *multicaulis* var. *gandomanensis	T	-	-	G	T	C	G	C	C	C	T	C	C	C
Sc. arakensis	G	-	-	G	G	C	A	C	C	C	T	C	C	C
Sc. litwinowii	T	G	G	T	G	C	A	T	C	T	C	C	C	C
Sc. patonii	T	G	G	T	G	C	G	T	T	T	C	C	C	C
Sc. persica	T	-	-	G	G	G	A	C	G	C	T	G	G	G
Sc. pinnatifida* subsp. *alpina	G	-	-	G	T	C	A	C	C	C	C	C	C	C

Nucleotide Number	461	462	472	475	476	478	479	480	487	508	513	518	521	534
Sc. multicaulis* subsp. *multicaulis* var. *multicaulis	C	G	C	C	G	A	C	G	C	A	G	C	T	C
Sc. multicaulis* subsp. *multicaulis* var. *gandomanensis	C	G	C	C	G	A	C	G	C	A	G	C	T	C
Sc. arakensis	C	G	C	C	G	A	C	G	C	A	G	C	T	C
Sc. litwinowii	C	G	C	C	G	A	A	G	C	A	G	C	T	C
Sc. patonii	C	G	C	C	G	A	A	G	C	A	G	C	T	C
Sc. persica	G	C	G	A	C	C	C	C	A	C	C	G	G	G
Sc. pinnatifida* subsp. *alpina	C	G	C	C	C	A	C	G	C	A	C	C	T	C

Nucleotide Number	538	551	553	556	557	558	559	560	561	562	563	570	573	575
Sc. multicaulis* subsp. *multicaulis* var. *multicaulis	G	G	G	C	-	-	-	-	-	-	-	A	G	C
Sc. multicaulis* subsp. *multicaulis* var. *gandomanensis	G	G	G	C	-	-	-	-	-	-	-	A	G	C
Sc. arakensis	G	A	A	C	-	-	-	-	-	-	-	A	A	C
Sc. litwinowii	C	A	A	T	-	-	-	-	-	-	-	A	A	C
Sc. patonii	G	G	G	T	C	G	A	A	A	G	A	T	G	C
Sc. persica	C	A	A	A	-	-	-	-	-	-	-	A	A	A
Sc. pinnatifida* subsp. *alpina	G	A	A	C	-	-	-	A	A	G	A	A	A	C

Table 3. Continued.

Nucleotide Number	607	621	626	629	642									
Sc. multicaulis* subsp. *multicaulis* var. *multicaulis	-	T	C	G	G									
Sc. multicaulis* subsp. *multicaulis var. gandomanensis	-	T	C	G	G									
Sc. arakensis	-	T	C	G	G									
Sc. litwinowii	C	T	G	A	G									
Sc. patonii	-	T	C	G	G									
Sc. persica	-	G	G	G	A									
Sc. pinnatifida* subsp. *alpina	-	T	G	G	G									

Table 4. *trn*L-F nucleotides sequence variation among examined species in *Sc. multicaulis* and the related species (only the variable sites are shown).

Nucleotide Number	169	171	233	433	434	435	436	437	438	451	452	453	454	455
Sc. multicaulis* subsp. *multicaulis* var. *multicaulis	G	G	-	A	A	A	A	A	T	-	-	-	-	-
Sc. multicaulis* subsp. *multicaulis* var. *gandomanensis	G	C	-	-	-	-	-	-	-	C	C	C	C	C
Sc. arakensis	G	C	-	-	-	-	-	-	-	-	-	C	C	C
Sc. litwinowii	G	G	-	A	A	A	A	A	T	-	-	-	-	-
Sc. patonii	G	C	-	-	-	-	-	-	-	-	C	C	C	C
Sc. persica	A	C	A	-	-	-	-	-	-	-	-	-	C	C
Sc. pinnatifida* subsp. *alpina	G	C	-	-	-	-	-	-	-	-	-	-	C	C

Nucleotide Number	500	523	547	554	557	577	605	631	670	676	684	687	693	
Sc. multicaulis* subsp. *multicaulis* var. *multicaulis	T	T	T	G	T	G	A	G	G	A	A	-	C	
Sc. multicaulis* subsp. *multicaulis* var. *gandomanensis	T	A	G	A	T	A	A	A	A	A	C	-	G	
Sc. arakensis	T	T	T	A	T	A	A	G	A	A	C	-	G	
Sc. litwinowii	T	T	T	G	T	G	A	G	G	A	A	-	C	
Sc. patonii	A	T	T	G	A	G	C	G	G	C	C	-	G	
Sc. persica	T	T	T	G	T	G	A	G	G	A	C	A	G	
Sc. pinnatifida* subsp. *alpina	T	T	T	G	T	G	A	G	G	A	C	-	G	

Fig. 1. *Scutellaria patonii* Jamzad & Safikhani. A, flower; B, calyx and scutellum; C, leaves and leaf indumentum.

***Paratypes*:** Iran, Lorestan: 24 km from Shoulabad to Aligoodarz, 2100-2350 m., Mozaffarian & Sardabi, 42496 (TARI). Fars: Gardaneh Bizhan, Kuhe Dena, 3450 m., Riazi 9860 (TARI). Kohgiloyeh-va-Boirahmad**:** Between Yasuj & Dehdasht, Dilegoon, Kuh-e Saverz, 2200-3200 m., Assadi & Abouhamzehis, 46390 (TARI). Chaharmahal-va-Bakhtiari, Shahre Kurd, Chelgerd around Tunele Kuhrang, 2350-2500 m., Mozaffarian, 57701 (TARI). Isfahan, Natanz, Kuhe Karkas, Mazdeh, 2300 m., Shams and Janighorban, 14546 (TARI). Yazd, Deh-Balla, Shirkuh Mt., 2700 m., Foroughi & Assadi, 17936 (TARI). Kerman, 40 km. from Jiroft on the road of Kerman, Kuhe Sarzeh, 3000-3300 m., Assadi & Miller, 25359 (TARI). Baluchestan, Taftan Mt. region, Rocky Mt., N. of Anjirak, 2600-2800 m., Mozaffarian, 53038 (TARI). Qom: 63 km. S. Qom, 2000m., Amin & Bazargan, 18923 (TARI). Semnan, Shahrud, S. and foot of Kuh-e- Razeh (1.5 km above and N. of Razeh), 1400 m., Freitag & Mozaffarian, 28582 (TARI).

The new species differs from *Sc. multicaulis* subsp. *multicaulis* by having yellow flowers with a violet lower lip, (not violet flowers with yellow lower lip), retrorse hairs in lower and middle parts of the stem (not short velutino-puberulose) and patent simple and glandular hairs in inflorescence. The new species is close to *Sc. multicaulis* subsp. *loringensis* endemic of Afghanistan in flower color but differs from it in stem and inflorescence hairs, shape and margins of leaves. It differs from *Sc. multicaulis* subsp. *koelzi* endemic of Afghanistan and Pakistan (Rechinger 1982) in indumentum which is lax and often non-glandular and corolla which is dark purple-violet in subsp. *koelzii*. It is close to *Sc. litwinovii* Bornm. & Sint. ex Bornm. but differs from it in having smaller leaf, smaller flowers, bracts elliptic not broad ovate, scutellum small in fruiting calyx 2 mm wide (not 7-10 mm) and nutlet with short appressed stellate hairs (not long villouse hairs). The morphological differences of the new species with the close species are summarized in table 2. The new species also differs from the *Sc. litwinovii* in its nucleotides sequences with 21 substitutions and 18 insertion or deletions and from *Sc. multicaulis* subsp. *multicaulis* var. *multicaulis* with 45 substitutions and 20 insertion or deletions (tables 3 & 4).

Etymology: The new species is named in honor of Dr. Alan Paton, Royal Botanic Gardens Kew, who did an outstanding taxonomic review on *Scutellaria*.

Sc. arakensis Jamzad & Safikhani **sp. nov.** (fig. 2)

Perennial tufted, suffruticose herb with a thick woody rootstock. Stems 26-43 cm high, in all parts covered with short antrorse hairs intermixed with a few sessile glands. Leaves 5-13 × 2-8 mm, elliptic or ovoid-lanceolate, petioles 0-5 mm, in margins with 3-5 distinct rounded teeth, upper surface with appressed hairs, lower surface with short curved hairs, intermixed with sessile glands. Bracts 2-4 × 2-3 mm, broad ovate, acuminate, entire, covered by short appressed hairs and sessile glands. Calyx 1.5-2 × 1 mm, covered by short apprised hairs and sessile glands, in the margin of teeth ciliate with simple and stipitate glands. Scutellum 1 × 0.5 mm. Corolla 18-22.5 mm long, dark violet with a yellow spot on the lower lip, sometimes with partly yellow in lower part tube, covered by simple patent hairs. Nutlets covered by dense short appressed stellate hairs.

***Holotype*:** Iran, Markazi**,** Toureh, Besri, N. E slope of Kuh-e Aladagh, 2100-3100 m., Mozaffarian, 64069 (TARI).

***Paratypes*:** Markazi, N.W. of Arak. The Hills of Modar (Ajori) Mountain, 1820 m., Safikhani, 105204 (TARI). Lorestan**,** Dorud, neck Mt. between Saravand and Gahar Lake (SU1), 2300-3500 m., Mozaffarian & Sardabi, 42324 (TARI). Hamedan**,** Malayer, the road to Arak, Zangeneh-e Olya village, 1800 m., Safikhani & Kalvandi, 99070 (TARI).

The new species differs from *Sc. multicaulis* in indumentum, leaf size and shape and margin of leaf. It is closely related to *Sc. persica* Bornm. an endemic Iranian species distributed in central part of Iran. It differs from *Sc. persica* in leaf size which is 7-18 x 4-10 mm, leaf shape which is pinnatisect, flower which is yellow in *Sc. persica.* It differs from *Sc. pinnatifida* subsp. *alpina* in plant habit, leaf shape, flower color (ascending to prostrate stem, pinnatifid leaves, dense inflorescence, yellow flowers in *Sc. Pinnatifida* subsp. *alpina*), (table 2). The new species also differs in its nucleotide sequences from the *Sc. persica* with 44 substitutions and 5 insertions or deletions and from *Sc. pinnatifida* subsp. *alpina* in 16 substitutions and 7 insertions or deletions (tables 3 & 4).

Sc. multicaulis Boiss. subsp. ***multicaulis*** var. ***gandomanensis*** Jamzad & Safikhani **var. nov.** (fig. 3)

Perennial, tufted, suffruticose herb with a thick woody rootstock. Stems 34-40 cm high, thin, covered in all parts with short antrorse simple hairs intermixed with papillose glandulous indumentum. Laves 5-10× 1.5-4 mm, few, elliptic or oblong-lanceolate, indistinctly dentate or with 3-5 distinct rounded teeth, leaf internode 3.5-4 cm, petioles 0-4 mm, upper

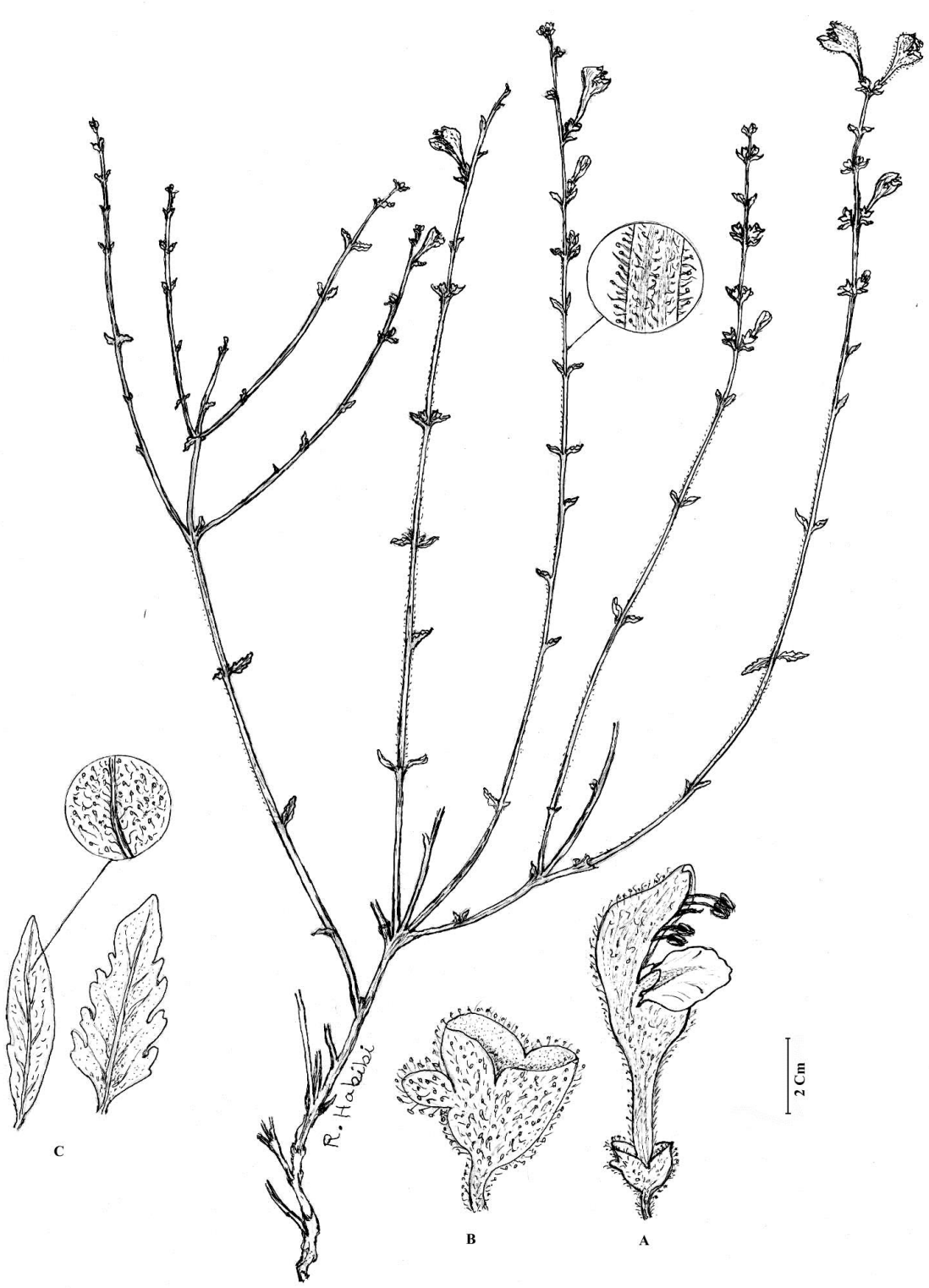

Fig. 2. *Sc. arakensis* Jamzad & Safikhani. A, stem indumentum; B, leaf; C, calyx & scutellum.

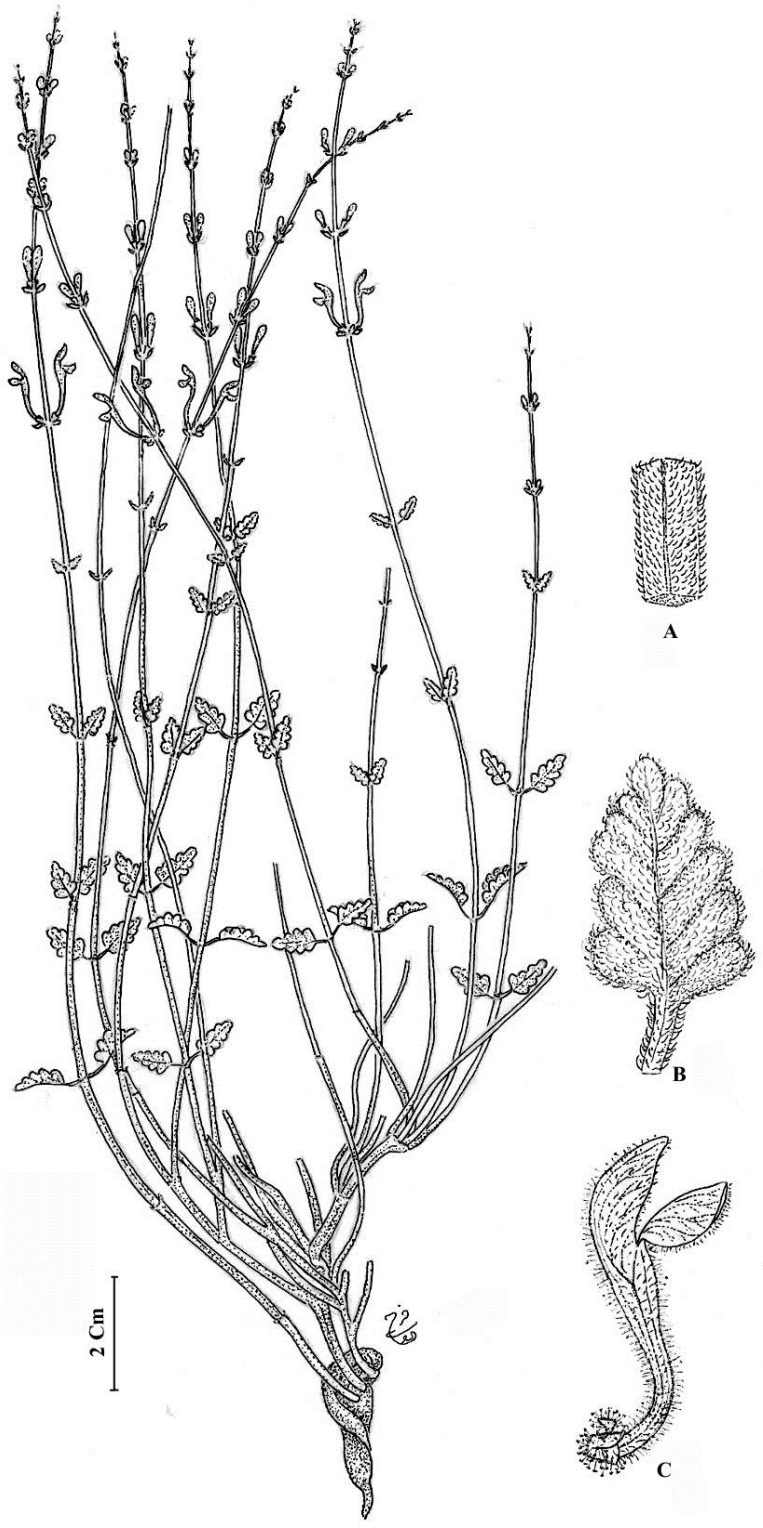

Fig. 3. *Sc. multicaulis* subsp. *multicaulis* var. *gandomanensis* Jamzad & Safikhani. A: flower; B: calyx & scutellum; C: leaves.

surface with short curved hairs, lower surface covered with short curved hairs all over the surface, intermixed with sessile glands. Inflorescence lax, verticillasters 2-flowered; flowers ±sessile. Bracts 2-3 × 1-2 mm. Calyx 1-2 × 0.5-1 mm, scutellum 0.5-1 × 0.5 mm. Corolla 14-15.5 (-18) mm long, dark violet with a yellow spot on the lower lip. Nutlets 1.2 x 1, ovate, rounded at apex, covered by short dense, appressed whit stellate hairs.

***Holotype*:** Chaharmahal-va-Bakhtiari**,** Gandoman, Mountains S. of Boldaji, 1400 m., Assadi, 72391 (TARI).

***Paratypes*:** Markazi, Arak, Haftad-Gholeh, Chekab, 2200 m., Mazhari & Serpooshan, 76883 (TARI). Isfahan, Ghameshloo Protected Area, Cheshmeh Zaloo, 2200 m., Yousefi, 99071 (TARI). Kermanshah**,** 78 km. Kermanshah to Amrolah Mt., 2150 m., Nemati & Mirabadi 99072 (TARI).

The new variety differs from the type variety by plant habit (having many slender stems and long internodes), smaller leaves and smaller flowers (14-15.5 mm (not 16-22 mm.). It differs from *Sc. patonii* in plant habit, stem and leaf indumentum, flower size and color and from *Sc. arakensis* in plant habit, stem indumentum and leaf size and shape. The morphological differences of the new variety from the related taxa are summarized in table 2. The new variety is also different in its nucleotides sequences with 9 substitutions and 12 nucleotide insertions or deletions from *Sc. multicaulis* subsp. *multicaulis* var. *multicaulis* (tables 3 & 4).

Distribution

The distribution pattern of the new taxa is presented in fig. 4. *Scutellaria patonii* has a wide range of distribution in 10 provinces from the southeast toward center and west of Iran, but *Sc. arakensis* has western distribution. *Scutellaria multicaulis* subsp. *multicaulis* is distributed in central and western provinces.

In spite of overlap in general distribution pattern of the studied taxa in some provinces, the co-occurrence of them in a single habitat has not been observed.

DISCUSSION

There are several species in Lamiaceae with wide distribution pattern and usually with high morphological variations, which can be a reflection of high adaptability to the environmental conditions. In the genus *Scutellaria* in Iran, *Sc. multicaulis* and *Sc. pinnatifida* are widely distributed, with high morphological variation. This variation resulted in taxonomical complexities which resulted different taxonomic treatments by different authors. In these cases, the decision on the taxonomic status of a taxon will be very difficult without applying supplementary biosystematic methods.

The morphological variations among populations of *Sc. multicaulis* were recognized as 3 sub-specific taxa in Flora Iranica area (Rechinger 1982). During detailed studies, we noticed more morphological variations which should be considered in the taxonomic treatment of *Scutellaria*. Rechinger (1982), in his treatment of the genus, reported only subsp. *multicaulis* from Iran, which is characterized by dark violet or dark purple flowers. On the other hand, he has reported yellow flowers specimens with violet lower lip and others diagnostic characters as subsp. *loringensis* from Afghanistan. Meanwhile, there are some yellow flowered specimens in the TARI which were identified as subsp. *multicaulis* by him.

Jamzad (2012) noticed both the yellow and dark violet flowers in the *Sc. multicaulis* but did not define them as distinct species regarding to their morphological characters.

Hedge & Paton in Flora of Pakistan (1990) have considered *Sc. multicaulis* and *Sc. edelbergii* as close species and noted that the differences between them are slight and often quantitative rather than qualitative (different in branches form). Despite this, Rechinger (1982), in Flora Iranica, has considered both species very different based on their inflorescence form. On the base of morphological data and considering the nucleotides sequences differences of ITS and *trn*L-F, the boundaries of the new taxa in *Sc. multicaulis* complex are inferred. This interpretation has been confirmed by the results of phylogenetic analysis of the genus *Scutellaria* in Iran (in preparation). *Scutellaria patonii* has a wide distribution from southeastern to the west of Iran with a wide range of environmental conditions. *Scutellaria multicaulis* subsp. *multicaulis* with a more limited distribution in Iran has its allies in Afghanistan and Pakistan.

CONCLUSIONS

In this study, we were able to interpret the observed morphological variations within *Sc. multicaulis* complex by using the nrDNA ITS and *trn*L-F data and identify new taxa. Our results showed that the use of molecular markers could help to the circumscription of taxa with a wide range of morphological variations in their quantitative and qualitative characters. It is hoped that futuer studies will benefit from the results presented here and that a comprehensive analysis of the entire genus, could unravel evolutionary patterns of the genus.

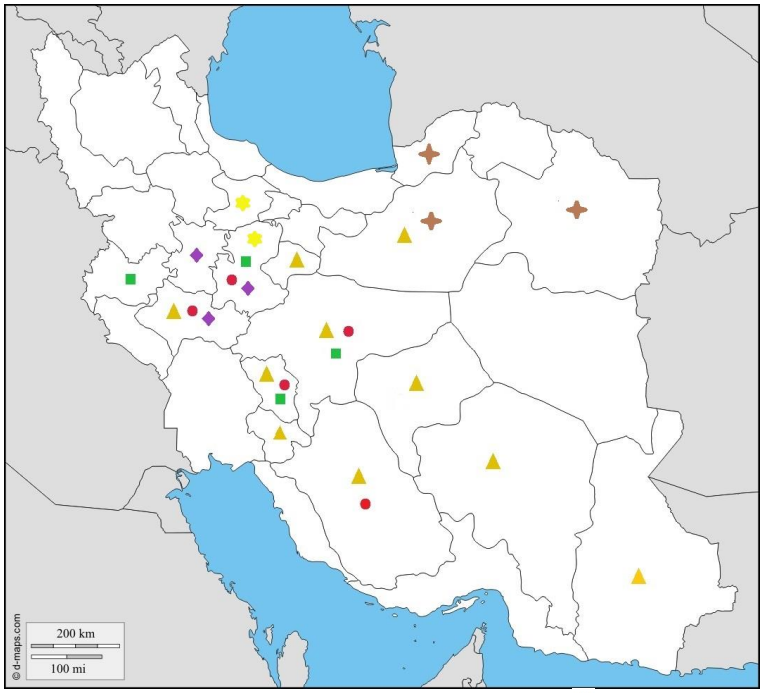

Fig. 4. Distribution map of studied *Scutellaria* species: ▲: *Sc. patonii*; ✹ :*Sc. persica*; ✚: *Sc. litwinowii*; ■: *Sc. multicaulis* subsp. *multicaulis* var. *gandomanensis* ●: *Sc. multicaulis* subsp. *multicaulis* var. *multicaulis*, ♦: *Sc. arakensis* in Iran.

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