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Morphological Phylogeny of the Species *Phlomoides* Moench (*Lamiaceae*) Distributed in the Fergana Valley

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Abstract This article presents 22 morphological characters of the genus *Phlomoides* distributed in the Fergana Valley and the primary and progressive signs of evolution of root, leaf, calyx and crown color as the main taxa, their diagnostic analysis and phylogenetic tree. Phylogenetic analyzes were performed on the data matrix using the PAUP* v4.0b10 program using the maximal parsimonious method (MP). The results obtained and morphological characters are integrated into Mesquite v3.02.

Keywords Fergana Valley, Phlomoides, Phylogeny, Morphology, PAUP

1. Introduction

In recent years, a number of studies on molecular (Salmaki et al., 2012a, 2012b), morphological (Eyvazadeh and Salmaki, 2019) and seed micromorphology (Eyvazadeh and Salmaki, 2018), cytogenetic (Ranjbar et al., 2016) and taxonomy of the *Phlomoides* genus was carried out. These studies have been conducted in a regional and narrow framework and do not provide definitive comprehensive data on the genus. To date, many taxonomic studies have been conducted on a number of species (Adylov et al., 1986; Kamelin and Maxmedov, 1990; Salmaki et al., 2012b; Sennikov and Lazkov, 2013) but none of them is complete. Based on the above data, there is a need to conduct extensive taxonomic, geographical, ecological and phylogenetic research on the *Phlomoides* series in close collaboration with scientists from Iran, Central Asia and China.

We aim to shed light on the morphological phylogeny and their diagnostic analysis based on 22 morpho – characters of the genus species distributed in the Fergana Valley. This is the first attempt in Central Asia to determine the evolutionary morpho – characters of a species based on modern methods. We hope that our phylogenetic analyzes based on the morphological characteristics of the species of *Phlomoides* will serve in some way in revealing the place of the morphological features in the origin of the genus.

2. The Main Results and Findings

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Study area and plant material

The Fergana Valley covers less than 1% of Central Asia (22,000 km^2). It is 300 km long (east to west) and 80 – 100 km wide (north to south). The height of the valley is 3300 m in the eastern part of Kyrgyzstan and 1050 m in the western part (Khojand) (Kaparkar, 2019). The valley is surrounded by ridges that are part of the Western Tien Shan and Gissar – Alay mountain systems. It is surrounded by Qurama in the northwest, Chatkal in the north, Fergana and Otoynak in the northeast and east, Alay and Turkestan in the south and Mogultog in the west (National Encyclopedia of Uzbekistan, 2005). The mountains descend to the center of the valley and merge with the plains. The valley is politically and administratively located in the territory of three countries – Uzbekistan, Kyrgyzstan and Tajikistan. (Fig. 1).

Morphological evaluation

Our study was covered following a taxonomic classification published by Sennikov and Lazkov (2013). The classification recognizes 5 sections of the *Phlomoides* genus. 26 species of the genus *Phlomoides* and 22 morphological features of one outer group (*Phlomis hypoleuca*) were studied. The analyzed morpho – characters are represented by numbers from two to four (Table 1). Morphological features of species National Herbarium of Uzbekistan (TASH), Based on the analysis of samples collected in targeted field research for 2021-2022 and the botanical classification mentioned in the Lazkov monograph (2016). Primary and progressive morpho – characters of the species were described based on Makhmedov (1991) classification.

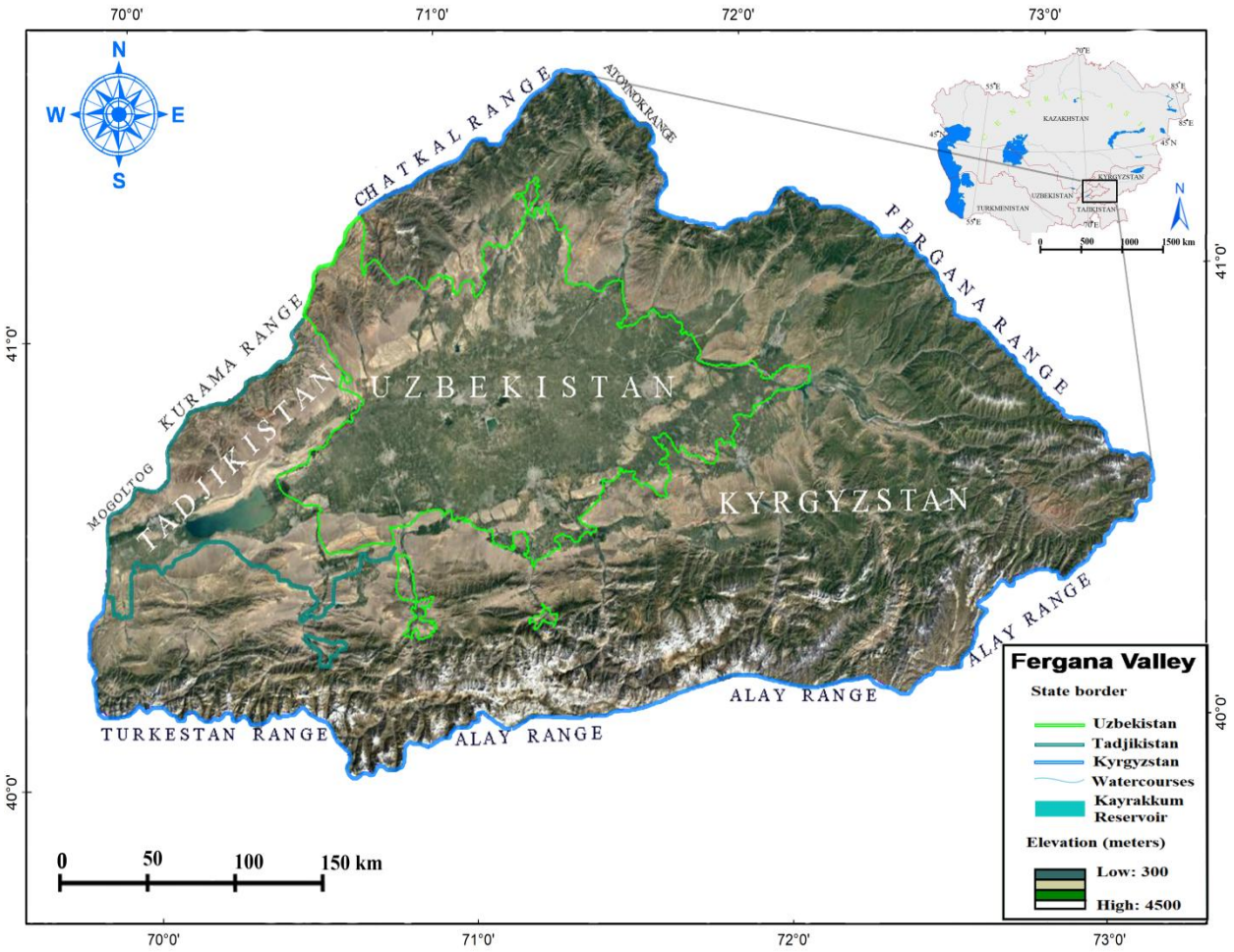

Figure 1. General topography of the Ferghana Valley in Central Asia

Phylogenetic analysis

Phylogenetic analyzes were performed on the data matrix (Table 2) using the PAUP* v4.0b10 (Swofford 2002) program using the maximal parsimonious method (MP). All characters were considered to have the same weight. The heuristic search option was selected using 100 replications of a random addition sequence with ACCTRAN optimization and replacement of TBR (splitting tree) branches. The reconnection limit is set to 8. We then applied a sequential retraction strategy to improve tree indices and reduce the effect of characters representing homoplasia on tree topologies (Farris 1969). Using equal weight symbols, the length of the first phylogenetic tree was determined to be 1.0 the consistency index CI = 0.462 the retention index RI = 0.731 and RC emained unchanged in successive rounds, these trees were accepted as the successive re-weighting trees. 100 trees were taken. In all analyzes, supports were revalued using the heuristic search option, simple addition sequence, TBR branch replacement and 10000 replications without MulTrees (Felsenstein 1985) and the maximum parsimonious heuristic search option retained the best single tree (Fig. 2). The relationship between the species of *Phlomoides* has not been resolved in this tree. The results and morphobes obtained in the PAUP program were integrated into Mesquite v3.02 (Maddison and Maddison, 2015) and the relationship between the characters was shown (Fig. 3).

The essence of the morphological method is to divide the primary and progressive characters into two opposite groups. The evolution of characters determines the evolution of taxa. If the original changes of morpho-characters in the adopted taxon are preserved, they will have to be placed in the lowest place in the system. To do this, the characters are identified by codes, which are used to determine the relative height of the level of development and the position of the species in the taxonomy (Makhmedov, 1991).

Based on the phylogenetic basis published by Salmaki (2012a) they presented an updated system of the *Phlomoides* genus. Sennikov and Lazkov (2013) *Phlomoides* genus into 5 section that were monophyletic. Including: *Phlomoides* (Bunge) Sennikov, *Eremostachys* (Bunge) Sennikov, *Filipendula* Kamelin & Mahmedov, *Moluccelloides* (Bunge) Sennikov and *Paraeremostachys* (Adylov, Kamelin & Makhm) Sennikov.

I Section *Phlomoides* (Bunge) Sennikov, Flora, 213 (2015) 40–48.

The section of *phlomoides* differs from other sections morphologically and biogeographically. This section is best

expressed in China, including Yunnan and Sichuan provinces. In our analysis, the section is represented by 8 species distributed in the valley. The leaves are simple, the leaf blade toothed or whole. The flowers are always the same color; purple, pink, yellow or white, upper lip arched.

II *S*ection *Filipendulina* Popov ex Kamelin & Machmedov in Bot. J. (Moscow & Leningrad) 75(2): 245 (1990).

The *Filipendula* section represents the largest and most diverse and controversial group in the *Phlomoides* genus. In our analysis, the section is represented by 14 species distributed in the valley. The leaves are pinnate (rarely - rarely the whole), the inflorescence is two different colors.

III *S*ection *Molucelloides* (Bunge) Sennikov, 2013, Memoranda Soc. Fauna Fl. Fenn. 89: 132. From a phylogenetic point of view, the representatives of this section are monophyletic (Salmaki et al., 2012a) and occupies a lower link compared to the other species in the genus.

In our analysis, the section is represented by 3 species distributed in the valley. The main root is turnip, the leaves are whole, the leaf blade is finely toothed or blunt. The calyx is funnel-shaped, the inflorescence is mostly yellow.

IV Section *Paraeremostachys* (Adylov, Kamelin & Machmedov) Sennikov, 2013, Memoranda Soc. Fauna Flora Fennica, 89: 133.

The results of molecular phylogenetic studies conducted on the genus *Phlomideae* (Salmaki et al., 2012a) suggest that there is no basis for recognizing *Paraeremostachys* as a separate tribe. Thus, Sennikov and Lazkov (2013) attached the level of this title to a section of the *Phlomoides* genus. In our analysis, the section is represented by 1 species distributed in the valley (calyx bell-shaped).

The discussion of the article cites the role of morpho-characters in the phylogenetic tree based on the structure of the root, leaf, calyx and corolla as the main taxonomic characters. The evolutionary role and significance of the remaining taxonomic characters, as well as comments on the genesis of the origin of the genus proposed by Popov (1940) and Makhmedov (1991) are not discussed in this article.

Table 1. List of morphological features of species of *Phlomoides* and their abbreviations

№	Characters	Characters states	Abbrevia-tion
1	Root	Rhizome with numerous pubescent buds (0) thick root (1) tuberous (2) radish-shaped (3)	R
2	Basal leaves	Ovate or oblong-ovate (0) oblong or broadly lanceolate, pinnately parted, pinnately divided or rarely entire (1) linear-lanceolate, lanceolate, oblong, oblong-ovate, obovate (2) ovate or broadly ovate, pinnately parted, punnately divided, shortly pinnately divided (3)	BL
3	Basal leaf length	Up to 10 cm (0) Up to 20 cm (1) Up to 40 cm	BLL
4	Basal leaves hairs	Multicellular and/or stellate (0) Multicel-lular and/or glandular (1) glabrous (2)	BLH
5	Cauline leaves	oblong — ovate or broadly lanceolate (0) oblong or lanceolate (1) oblong - ovate, ovate, rhombic (2)	CL
6	Inflorescence	(0) 3 — 9 (1) 4-8 (2)	I
7	Verticillasters flowers	Up to 10 (0) Up to 20 (1) Up to 20-50 (2)	VF
8	Bracts shape	Linear-lanceolate (0) linear- subulate (1) missing (2)	BS
9	Bracts length	Up to 10 mm (0) Up to 20 mm (1) no (2)	BL
10	Bracts hair	Multicellular, stellate and glandular (0) No (1)	BH
11	Calyx	Tubular (0) Tubular-campanulate (1) campanulate or broadly campanulate (2) campanulate (3)	C
12	Calyx length	Up to 15 mm (0) Up to 30 mm (1)	CL
13	Calyx hair	Multicellular, stellate or glandular (0), glabrous (1)	CH
14	Calyx teeth	Broadly oblong (0) round or triangular-ovate or triangular (1) short quadrangular or semiround (2) short (3)	CT
15	Corolla color	Pink and purple (0) pink-wite (1) white, yellow (2)	CC
16	Corolla length	Up to 20 mm (0) Up to 40 mm (1)	CL
17	Corolla tube	Up to 15 mm (0) Up to 25 mm (1)	CT
18	Lip	Upper lip longer than lower lip (0) lower lip longer than upper lip(1) Upper and lower lip equal (2)	L
19	High lip length	Up to 10 mm (0) Up to 18 mm (1)	HIL
20	Lower lip length	Up to 10 mm (0) Up to 20 mm (1)	LIL
21	Stamens	Spur-shaped (0) fimbriate appendages (1), Lamellar appendage (2) the outermost ones are longer (3)	S
22	Seed	Glabrous (0) hairs (1) glabrous or stellate and glandular (2)	S

Table 2. Data matrix used in phylogenetic analysis of *Phlomoides* Moench

Genus	Section	Subsection	Species	Morphological character code
Phlomoides Moench	*Phlomoides*	*Phlomoides*	*Phlomoides alaica* (Knorring) Adylov, Kamelin & Makhm	0010002000000000000000
			Phlomoides adylovii Lazkov	0000002100000010000000
		Anisostyleae	*Phlomoides oreophilla* (Kav.et Kir.) Adylov, Kamelin & Makhm.	0010101110010101110012
			Phlomoides canescens (Regel) Adylov, Kamelin & Makhm.	00101011100101111110002
			Phlomoides pratensis (Kar. & Kir.) Adylov, Kamelin & Makhm.	00201011100101011100000
		Isostyleae	*Phlomoides brachystegia* (Bunge) Adylov, Kamelin & Makhm.	1021111100000000010001
			Phlomoides osrtowskiana (Regel) Adylov, Kamelin & Makhm.	101111111001000010001
			Phlomoides ferganensis (Popov) Adylov, Kamelin & Makhm.	10111111100100000010001
	Filipendulina	*Labiosae*	*Phlomoides labiosa* (Bunge) Adylov, Kamelin & Makhm.	2110020100100021111101
		Fulgentes	*Phlomoides pulchra* (Popov) Adylov, Kamelin & Makhm.	1110020100100021111111
			Phlomoides ajdarovae Lazkov	22100201001000211111111
			Phlomoides speciosa (Rupr.) Adylov, Kamelin & Makhm.	2110020100110021111111
		Gymnocalyx	*Phlomoides kirghizorum* Adylov, Kamelin & Makhm.	211002010010002110111
			Phlomoides michaelis Adylov, Kamelin & Makhm.	211002010010001110111
		Nudae	*Phlomoides nuda* (Regel) Adylov, Kamelin & Makhm.	1220020221121021101110
			Phlomoides integior (Pazij et Vved.) Adylov, Kamelin & Makhm.	12220202211210211011110
		Integrifoliae	*Phlomoides Moench ebracteolata* (Popov) Adylov, Kamelin & Makhm.	2202010221120000110021
		Rhodanthae	*Phlomoides Moench lehmanniana* (Bunge) Adylov, Kamelin & Makhm.	1331011010110001111111
		Cordatae	*Phlomoides Moench cordifolia* (Regel) Adylov, Kamelin & Makhm.	101101101011001111111
			Phlomoides Moench stellate Lazkov	10110100101000111111101
			Phlomoides Moench kurpsaica Lazkov	1011011000100011111111
			Phlomoides urodonta (Popov) Adylov, Kamelin & Makhm.	1012010010110110110011
	Moluccelloides	-	*Phlomoides Moench isochila* (Pazij et Vved.) Salmaki	3011210110210121101111
			Phlomoides Moench codonantha Sennikov et Lazkov	301121010021012110101 1
			Phlomoides Moench hypoviridis Lazkov	30012101103101211010 11
	Paraere-mostachys	-	*Phlomoides Moench eriocalyx* (Regel) Adylov, Kamelin & Makhm.	2001220100110220111111
Phlomis L.	*Orientalis*	-	*Ph. hypoleuca* Vved. ex Knorr.	0210121110110301101131

The evolutionary role of taxonomic traits in morphological phylogeny (Fig. 3) is given and the primary and progressive morpho signs are given on the basis of Makhmedov (1991) classification. Including;

Non-thickened main and additional roots - the main root is not thickened, additional roots are thickened in the form of one or 2-3 turnips - additional roots are not thickened.

The whole leaf - patsimon carved or slightly trimmed - divided leaf

Calyx tubular - bell-shaped - funnel-shaped

Corolla color monochrome - heterochrome

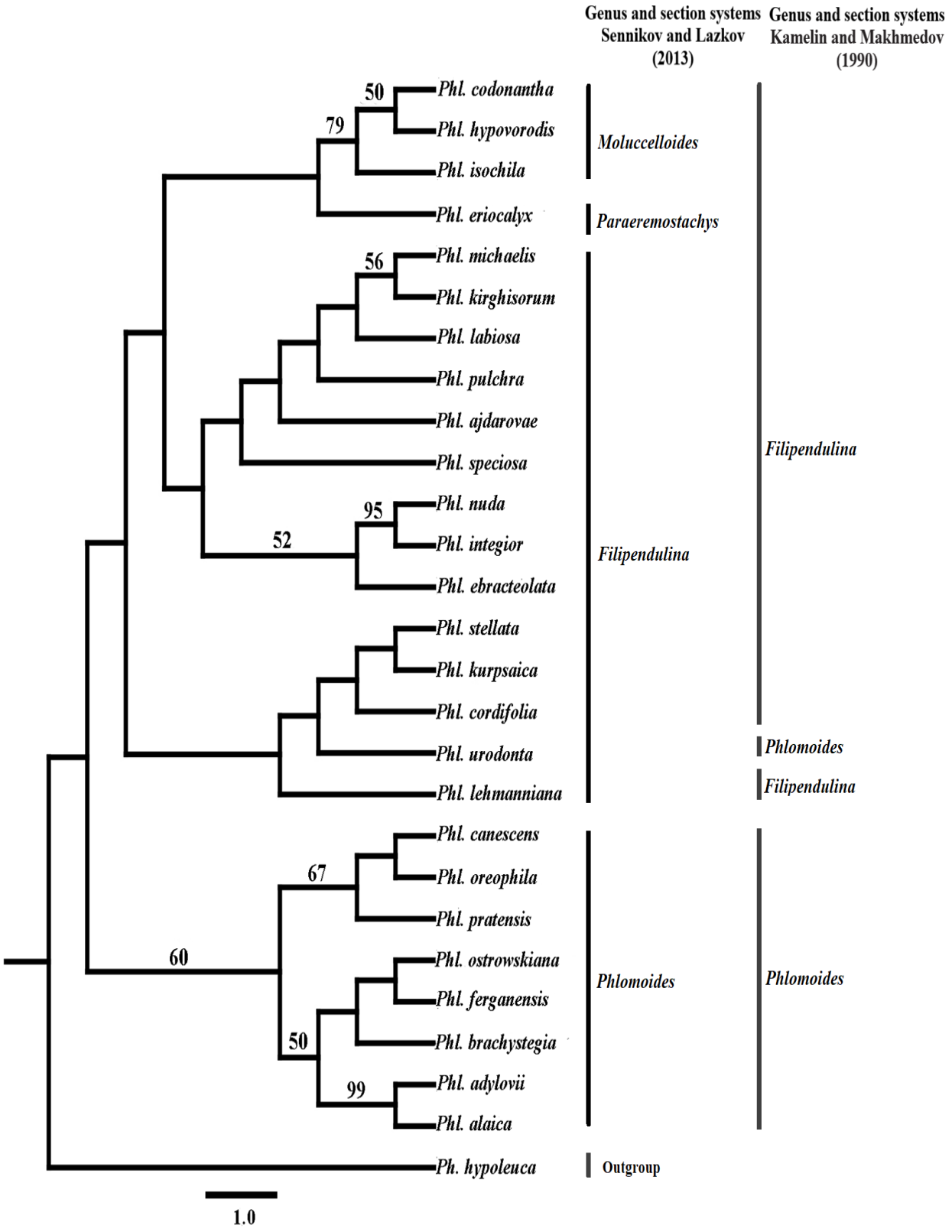

Figure 2. Strict consensus tree derived from the morphological data matrix after successive weighting by rescaled consistency index and the correspondence of the species sectional positions to taxonomic classifications of Sennikov & Lazkov (2013) and Kamelin & Makhmedov (1990). Numbers above branches are bootstrap values. Numbers <50% are not shown

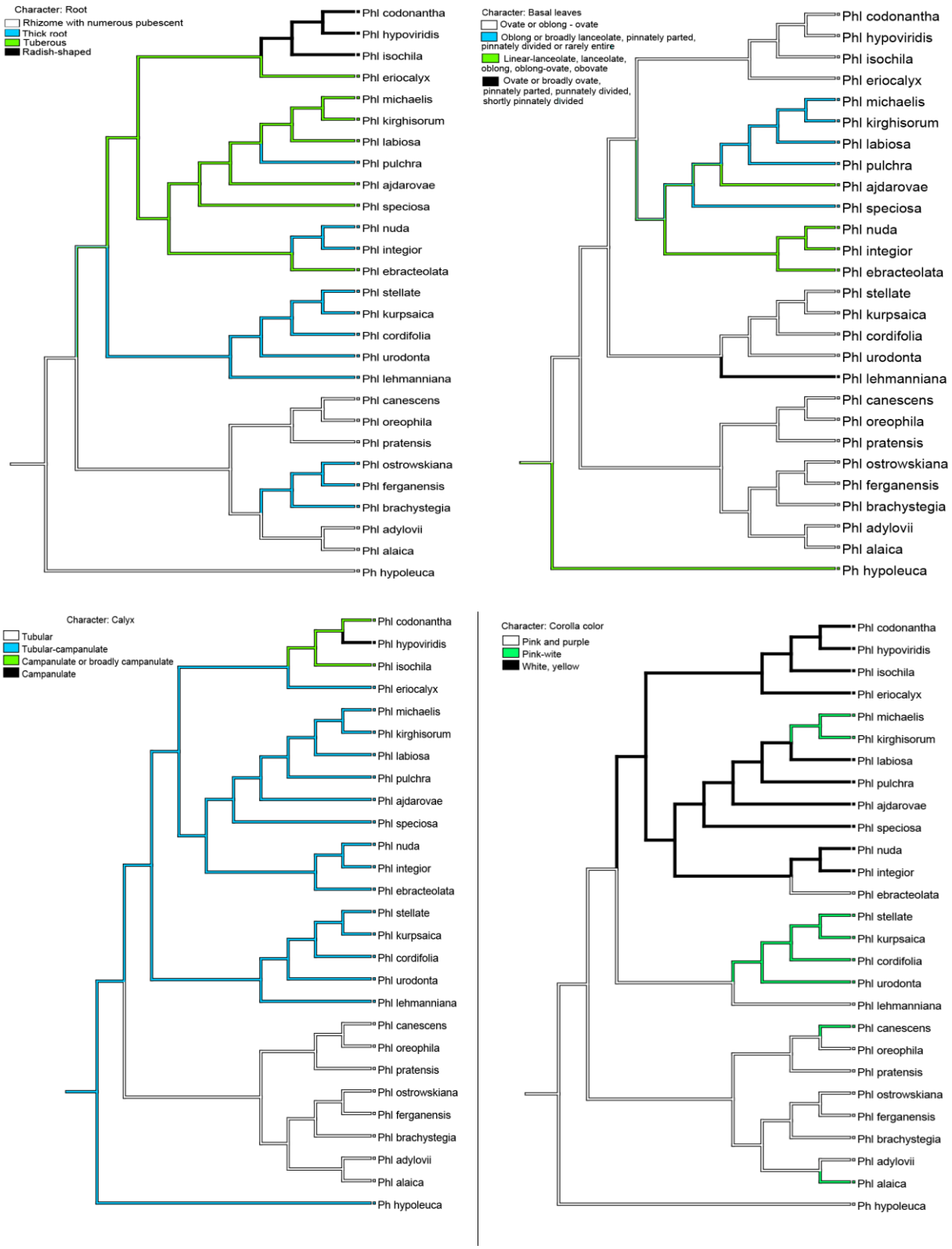

Figure 3. Reconstruction of the evolution of four morphological characters in *Phlomoides*: Root, basal leaves, calyx and corolla color

3. Conclusions

It can be said that the species of this series has undergone many morphological stratifications in its origin. On the one hand there are whole-leaved species prone to forest landscape, on the other hand there are species with cut or divided leaves adapted to xerophilous conditions. As a result of natural hybridization of the species distributed in the intersection areas, many intermediate forms were formed. This situation can be considered as a key factor in the

evolution of polymorphisms of species.

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