

# MORPHOLOGICAL CLADISTIC ANALYSIS OF CALLIGONUM AND PTEROPYRUM (POLYGONACEAE) IN IRAN

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The phylogenetic relationships in genera *Calligonum* and *Pteropyrum* (Polygonaceae) are reconstructed using 30 vegetative and reproductive morphological characters. The data matrix comprised 18 species of *Calligonum* and 3 species of *Pteropyrum* plus 2 *Atraphaxis* species representing outgroups. The analyses were conducted using maximum parsimony approach with characters treated both equally weighted and reweighted by rescaled consistency index. The results revealed that *Calligonum* is monophyletic. But *Pteropyrum* is appeared to be monophyletic after reweighting procedure. In both analyses *Calligonum* is composed of two clades, one is including the winged fruit species (sect. *Pterococcus*) and the second including the bristled fruit ones (sect. *Calligonum*) encompassing the monotypic sect. *Calliphysa* (*Calligonum junceum*). Of 30 characters analyzed, seven are synapomorphic for both genera. Six characters in *Calligonum* and one in *Pteropyrum* were genus specific. The remainder of characters was evolved homoplasiously (or ambiguously) among these taxa.

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## آنالیز کلادستیکی ریخت‌شناسی جنسهای *Calligonum* و *Pteropyrum* از تیره Polygonaceae

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۲۱ تاکسون شامل ۱۸ گونه *Calligonum* و ۳ گونه *Pteropyrum* بعنوان درون گروه به اضافه ۲ گونه از *Atraphaxis* بعنوان برون گروه با استفاده از ۳۰ صفت ریخت‌شناسی مورد مطالعه قرار گرفتند. در هر دو آنالیز بدون وزن‌دهی و با وزن‌دهی (توسط شاخص سازگاری تصحیح شده)، *Calligonum* و *Pteropyrum* تشکیل کلاد مجزا داده‌اند. هر دو بخشه *Pterococcus* و *Calligonum* (شامل بخشه تک گونه‌ای *Calliphysa*) از جنس *Calligonum* تک تبارند. از ۳۰ صفت آنالیز شده، ۷ صفت پیشرفته مشترک برای هر دو جنس می‌باشد. شش صفت مختص گونه‌های *Calligonum* و یک صفت ویژه *Pteropyrum* است. بقیه صفات بطور هم‌نما (یا مبهم) در میان این تاکسون‌ها تکامل یافته‌اند.

## INTRODUCTION

In most recent works, both *Calligonum* L. and *Pteropyrum* Jaub. & Spach have been classified in tribe *Polygoneae* (Haraldson 1978; Brandbyge 1993) and *Atraphaxideae* (Takhtajan 1997) of subfamily *Polygonoideae*, respectively. *Calligonum* possesses ca. 80 species with xeromorphic shrubby habit distributed from southern Europe through N. Africa to Western

Central Asia (Mabberley 1990). In the flora of Iran, 18 species including six endemics have been identified (Rechinger & Schiman-Czaika 1968; Mozaffarian 2005). According to fruit morphology *Calligonum* has been divided into three sections: *Calligonum* (with bristled fruit), *Pterococcus* (with winged fruit) and *Calliphysa* (with membranous saccate fruit) (Rechinger & Schiman-Czaika 1968). *Pteropyrum* has four to five

species in south west Asia and the Middle East, of which three species are distributed in Iran. The members of this genus like that of *Calligonum* are shrubby but have achenes with only three membranous wings.

Hitherto, no cladistic analyses using either morphology or DNA sequence data have been conducted to evaluate phylogenetic status of *Calligonum* and *Pteropyrum*. We performed both analyses in parallel. The present paper deals only with morphology based-phylogenetic analyses of these taxa to address the following questions:

1. Are *Calligonum* and *Pteropyrum* monophyletic? 2. are species-rich sections *Pterococcus* and *Calligonum* of *Calligonum* monophyletic? and 3. what is the position of the monotypic sect. *Calliphysa*?

## MATERIALS AND METHODS

### Taxon sampling

Eighteen species representing three currently recognized sections of *Calligonum* and three species of *Pteropyrum* were included as ingroup taxa in the analysis. Based on molecular phylogenies using chloroplast gene *rbcL* (Lamb Frye & Kron 2003) and nrDNA ITS and *trnL-F* (Tavakkoli 2007; Tavakoli & al. in preparation), two species of *Atraphaxis* L. were selected as outgroups.

### Characters and character states

Characters used in the cladistic analysis were obtained through examination of fresh materials in the field and herbarium specimens deposited at Central Herbarium of Iran (TARI) or adopted from appropriate references (Rechinger & Schiman-Czaika 1968; Pavlov 1970; Sabeti 1976; Haraldson 1978; Ronse Decraene & Akeroyd 1988; Hong 1995; Mozaffarian 2005; Ren & Tao 2004). Thirty informative characters with relevant character states used in present analysis are given in Table 1.

The polarity of characters was determined using the outgroup method (Maddison & al. 1984).

### Cladistic analyses

Phylogenetic analyses were performed on the data matrix (Table 2) using maximum parsimony method (MP) as implemented in the version 4.0b10 of PAUP\* (Swofford 2002) installed in a Macintosh computer. All characters were considered as equally weighted. The heuristic search option was selected using 100 replications of random addition sequence with ACCTRAN optimization and TBR (tree bisection reconnection) branch-swapping with MulTrees on and steepest descent off. Analyses were then conducted

using a successive reweighting strategy (Farris 1969) in order to improve the trees indices and decrease the effect of characters showing high homoplasy on tree topologies. Weights were assigned to characters using the "reweight characters" option based on the rescaled consistency (RC) index (Farris 1989) with a base weight of 1. When the tree length and consistency index (CI), retention index (RI) and RC remained unchanged in successive rounds, these trees were accepted as the successive reweighting trees. In both analyses, supports for clades were evaluated by bootstrapping (Felsenstein 1985) using 100 replications with the heuristic search option, simple addition sequence and TBR branch swapping. The evolutionary trend of the characters was further investigated using MacClade 4.08 (Maddison & Maddison 2005).

## RESULTS

The phylogenetic analysis based upon equally weighted characters yielded 923 most-parsimonious trees of 77 steps with CI of 0.662 and RI of 0.819. The strict consensus tree of these 923 trees was shown in Fig. 1. The three sampled *Pteropyrum* species did not form a common clade; whereas, *Calligonum* species formed a strongly supported clade (Bootstrap, BP= 100%) divided into two subclades: One, as weakly supported, BP <50%, solely composed of species of sect. *Pterococcus* (with winged fruit) and the second subclade, supported by 77% BP, containing the species of sect. *Calligonum* (with bristled fruit) as well as monotypic sect. *Calliphysa* (with membraneous saccate fruit). Species relationships within these sections were not properly resolved. The successive reweighting analysis, generated 16 most-parsimonious trees with Length= 44.5 steps, CI= 0.880 and RI= 0.943. The strict consensus tree of reweighting these is shown in Fig. 2. The general topology of the tree was the same as in Fig.1 except that *Pteropyrum* formed a weakly supported clade (BP=63% 0) and species relationships within *Calligonum* clade, in particular the bristled fruit species, are well resolved. Within the *Pteropyrum* clade, *P. olivieri* and *P. naufelum* were weakly allied taxa (BP= 53%) and *P. aucheri* was sister to them. *Calligonum* sect. *Pterococcus*, formed a weakly supported subclade (BP= 63%) and the relationships among its species were weakly resolved, while *Calligonum* sect. *Calligonum* made up a well supported and resolved subclade, in which, *C. laristanicum*, *C. junceum* (of sect. *Calliphysa*), *C. crinitum* and *C. turkestanicum* positioned as successive grades to the remaining seven species.

Table 1. The morphological characters used in the cladistic analysis.

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#1 Branch color: white (0); grey (1).
#2 Spines on branches: present (0); absent (1).
#3 Orientation of branches: straight (0); zigzag (1); flexuous (2).
#4 Status of branchlets: divergent (0); cluster (1).
#5 Leaf durability: persistent (0); persistent until fructification (1); deciduous (2).
#6 Leaf shape: ovate-rectangular (0); linear-subulate (1).
#7 Leaf arrangement: non-clustered (0); clustered (1).
#8 Leaf venation: distinct (0); indistinct (1).
#9 Inflorescence type: non-clustered (0); clustered (1).
#10 Perianth segments: tetramerous (0), pentamerous (1).
#11 Anticlinal walls of epidermal cells: irregular (0); straight (1).
#12 Tepal growth: achene included (0); achene exerted (1).
#13 Secondary ramification of the main vein of tepal: abundant and anastomosing (0); few and anastomosing (1).
#14 Cell shape of tepal epidermis: irregular-elongated (0); irregular-polygonal (1); regular-rectangular (2).
#15 Ovary shape: triangular (0); rectangular (1).
#16 Style number: 2 or 3 (0); 3 (1); 4 (2).
#17 Stamen number: 5 to 8 (0); 8 (1); 10 to 15 (2).
#18 Filament base: glabrous (0); hairy (1).
#19 Pollen sculpture: striate (0); perforate/foveolate (1); microreticulate (2).
#20 Fruit shape: triangular (0); cordate (1); ellipsoid-oblong (2); spherical (3).
#21 Exocarp: lignosus (0); semi lignosus (1) coriaceous (2);
#22 Exocarp thickness very thin, 25-30 $\mu\text{m}$ : (0); thin, 40-55 $\mu\text{m}$ (1); thick, 55-60 $\mu\text{m}$ (2).
#23 Fruit wing: non winged (0); 3 winged (1); simple 4 winged (2); double 4 winged (3); with bristles, free on apex (4); with bristles, connate at apex (5).
#24 Fruit meso- and endocarp thickness: <5mm (0); 5-10mm (1); 10-20mm (2).
#25 Fruit twisting: non twisting (0); little twisted (1); distinct twisted (2).
#26 Ratio of wing or bristle to achene: without wing or bristle (0); thinner than achene (1); the same width as achene (2); wider than achene (3.)
#27 Albumen: flourey (0); fleshy (1).
#28 Row of bristles: without bristles (0); 1 row in each side (1); 2 rows in each side (2).
#29 Bristle division at the base: without bristle (0); simple (1); divided to 2 (2).
#30 Bristle division at the apex: without bristle (0); simple (1); <10 division (2); >10 division (3).

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Table 2. Data matrix of morphological characters used in cladistic analyses.

	111111111122222222223
	123456789012345678901234567890
<i>Atraphaxis spinosa</i> L.	000000000000000001010000000
<i>A. suaedifolia</i> Jaub.& Spach	0000010000000?000001010000000
<i>Calligonum crinitum</i> Boiss.	012021011111001221131242231111
<i>C. junceum</i> (Fisch. & C.A. Mey.) Endl.	012021011111??1221131251021111
<i>C. leuocladum</i> (Schrenk) Bunge	011121011111121221121232031000
<i>C. tetrapterum</i> Jaub. & Spach	111121011111121221121222011000
<i>C. stenoptreum</i> Bunge ex Boiss.	011121011111121221121221011000
<i>C. bungei</i> Boiss.	1110010111111212211012a2021000
<i>C. schizopterum</i> Rech. f. & Schiman-Czeika	111021011111121221101222021000
<i>C. denticulatum</i> Bunge ex Boiss.	112021011111121221101222031000
<i>C. persicum</i> (Boiss. & Buhse) Boiss.	011121011111121221101231021000
<i>C. laristanicum</i> Rech. f. & Schiman-Czeika	111121011111101221131242031?11
<i>C. caput-medusae</i> Schrenk	112121011111101221131242131222
<i>C. arborescens</i> Litw.	011121011111101221121242131222
<i>C. amoenum</i> Rech. f. & Schiman-Czeika	01111011111101221121241221222
<i>C. paletzianum</i> Litw.	011121011111101221121242131222
<i>C. polygonoides</i> L.	012121011111101221121242221222
<i>C. intertextum</i> Rech. f. & Schiman-Czeika	011121011111101221131242131223
<i>C. comosum</i> L'Her.	012121011111101221131242131223
<i>C. turkestanicum</i> (Korov.) Pavlov	012121011111101221131241231223
<i>Pteropyrum aucheri</i> Jaub.& Spach	1100011011110?0111202011030000
<i>P. olivieri</i> Jaub.& Spach	01000010111110111202011030000
<i>P. naufelum</i> Al-Khayat	1100001011111?01112020110?0000

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? denotes that the character states were unavailable. a=1,2

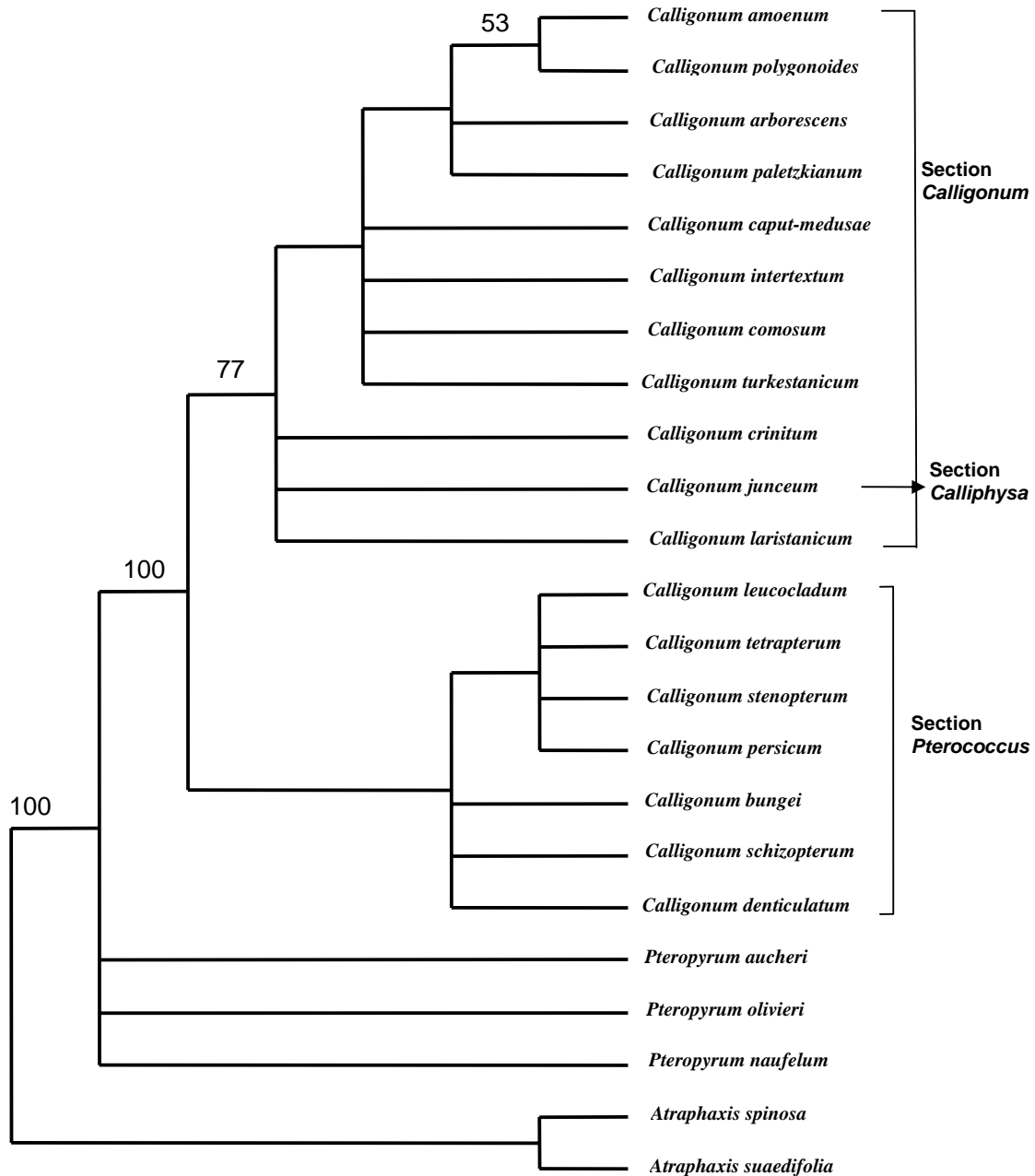


Fig. 1. Strict consensus tree of 923 most-parsimonious trees obtained from a morphological cladistic analysis with equal weighting (Length = 77 steps, CI= 0.662, RI= 0.819). Bootstrap values greater than 50% were shown above the branches.

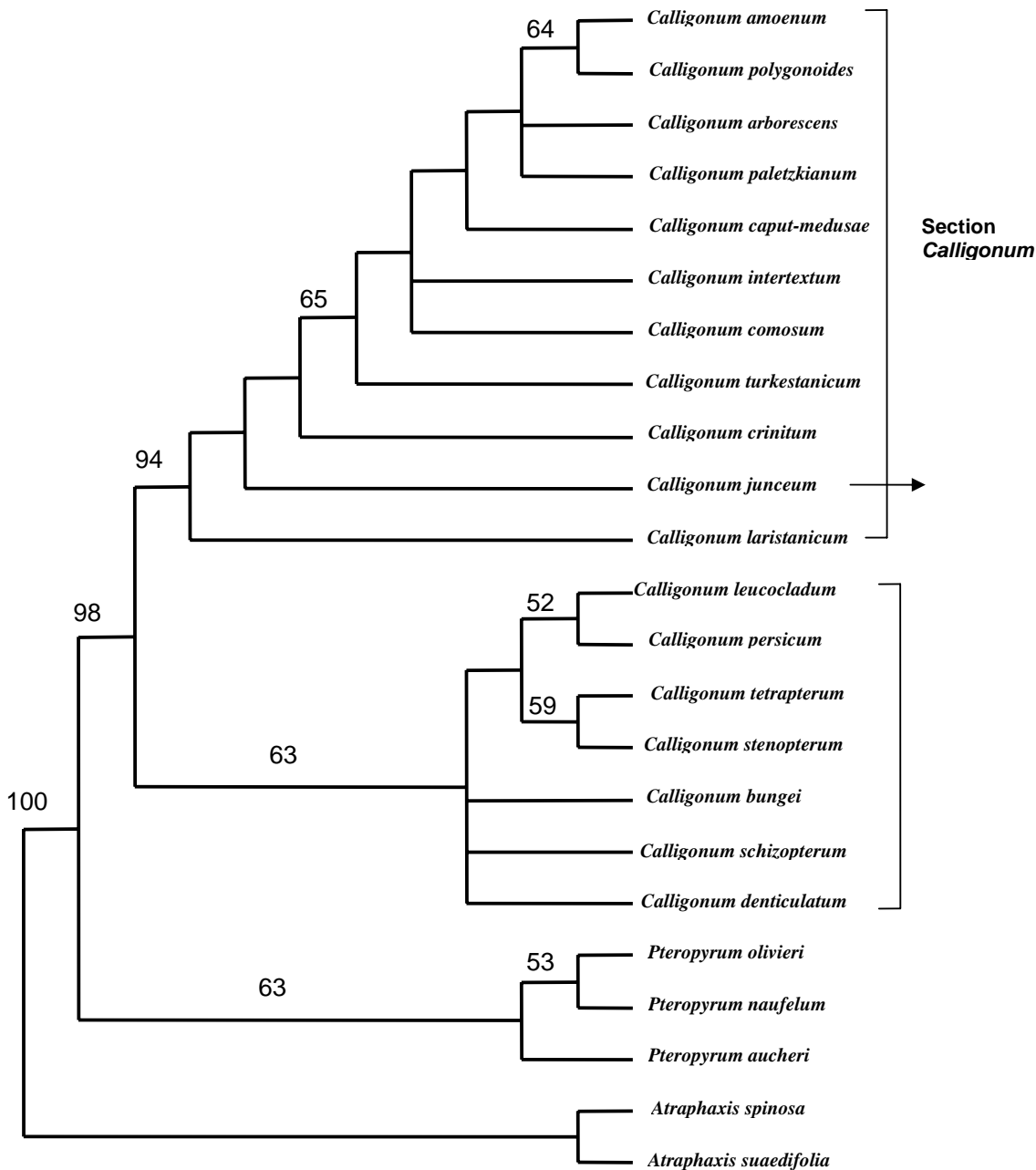


Fig. 2. Strict consensus tree of 16 most-parsimonious trees obtained from a morphological cladistic analysis after successive reweighting with RC (Length = 44.54206 steps, CI= 0.880, RI= 0.943). Bootstrap values greater than 50% were shown above the branches.

## DISCUSSION

### Monophyly and phylogenetic relationships of *Calligonum* and *Pteropyrum*

The present analysis based upon morphological features showed that both *Calligonum* and *Pteropyrum* are monophyletic taxa. Molecular analyses based on both nrDNA ITS and chloroplast *trnL-F* sequence data confirmed also the monophyly of these two genera as well as their close relationship (Tavakkoli 2007; Tavakkoli & al. in preparation). The previous studies using various evidence suggested that *Calligonum* and *Pteropyrum* are closest relatives. Some of the data considered in the previous studies are summarized below: vegetative morphology: polyachanthic life-form; stem anatomy: lignified secondary walls in collenchyma, (Haraldson 1978); floral characters: formation of commissural veins, non-fused petaloid tepals, stomata on the tepal surface of epidermis and presence of papillae at the base of stamen filament (Ronse-Decraene & Akeroyd 1988; Hong & al. 1998); fruit morphology: armed achenes with wings/bristles (Rechinger & Schiman-Czeika 1968; Brandbyge 1993); and pollen morphology: microreticulate/perforate exine sculpturing (Nowicke & Skvarla 1979, Hong 1995). However, some taxa such as, *Polygonum* s. str., *Oxygonum* Burchell ex Campdera *Fagopyrum* Miller and in particular *Atraphaxis* also share variously some of these features. But, as shown in Tavakkoli (2007), these genera are not closely related with them, indicating that these characters have evolved independently among the genera.

### Infrageneric relationships in *Calligonum* and *Pteropyrum*

As mentioned in the introduction, based on fruit morphology, *Calligonum* has been divided into three sections: *Calligonum* (with bristled fruit), *Pterococcus* (with winged fruit) and *Calliphysa* (with membranous saccate fruit) (Rechinger & Schiman-Czaika 1968). The resulting trees (Figs. 1, 2) show that the section *Pterococcus* is a weakly supported monophyletic taxon; whereas, section *Calligonum* with the inclusion of monotypic section *Calliphysa* (*Calligonum junceum*) forms a well supported clade (bootstrap values of 77% and 94%, respectively). As shown in the reweighted tree (Fig. 2), relationships within sect. *Calligonum* were resolved but statistically not well supported. Random amplified polymorphic DNA (RAPD) data analyses of 14 Chinese *Calligonum* species using UPGMA clustering method showed that *Calligonum junceum* is positioned far from the remaining species studied and species having bristled fruit were not grouped in a single cluster as do the winged fruit species (Ren &

Tao 2004). On the other hand, Zhang & Xi (1997) found reticulate-perforate/foveolate pollen exine surface (but see Hong 1995) among 11 species of *Calligonum* in China, but there were minor differences among the species. However, phylogenetic analyses of both nrDNA and chloroplast *trnL-F* sequences did not resolve relationships among sections of *Calligonum* (Tavakkoli 2007, Tavakkoli & al. in preparation).

In all classical taxonomic works (e.g., Rechinger & Schiman-Czeika 1968) *Pteropyrum aucheri* and *P. olivieri* are morphologically very similar to each other except that in the former, the shape of leaf is linear and in the latter spatulate. Both species are distributed in arid regions, but *P. aucheri* penetrates more to desert area (Mozaffarian 2005, and personal observations). However, the present cladistic analyses of morphological data and both nr DNA ITS and *trnL-F* phylogenies (Tavakkoli 2007, Tavakkoli & al. in preparation) did not put them close to each other. Instead, *P. olivieri* is allied with *P. naufelum*, a newly described species distributed in Iraq (Al-Khayat 1993) and south-west Iran (Akhani 2004; Mozaffarian 1994, 2005 reported as *P. noeanum*).

### Evolution of Characters in *Calligonum* and *Pteropyrum*

Among 30 characters analyzed here, only 18 ones were evolved unambiguously among species of *Calligonum* and *Pteropyrum* (Fig. 3). CI, RI, and RC of these characters are 1 (see Table 3.). Of which, character state of characters 2 (absence of spine on branches), 9 (clustered inflorescence), 10 (pentamerous perianth segments), 11 (straight anticlinal walls of epidermal cells), 12 (tepal growth), 18 (presence of trichomes on the filament base) and 26 (of the ratio of wing or bristle to achene) are shared by both genera. For instance, evolutionary trend of characters 11 and 12 in both genera are changed from undulating anticlinal walls of epidermal cells to straight anticlinal walls and tepal growth of achene included to achene exerted, respectively. Some binary characters including orientation of branches (no. 3, straight to zigzag/flexuous), leaf venation (no. 8, distinct to indistinct), ovary shape (no. 15, triangular to rectangular) and albumen (no. 27, floury to fleshy) evolved in *Calligonum* solely. Some species of sect. *Calligonum* (including *C. junceum* of sect. *Calliphysa*) are characterized by simple bristled fruits derived from winged fruits and then changed to basally divided bristle in the remainder species including *C. laristanicum* through *C. crinitum* (character 29). Likewise, Character 30, bristle division at the apex were undergone the same pattern. Character 7 (leaf arrangement from non-cluster to cluster) is unique to

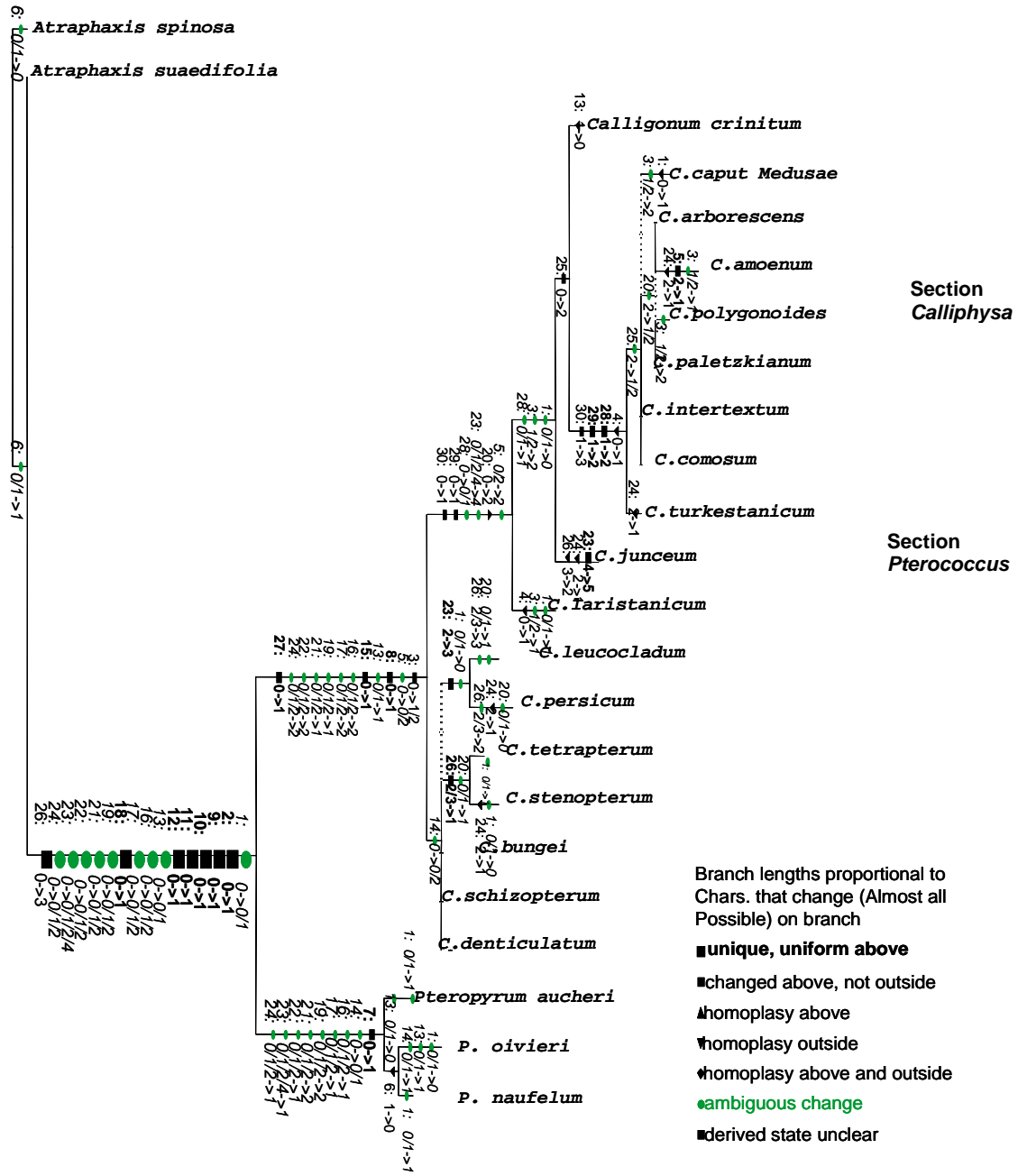


Fig. 3. MacClade reconstruction of character evolution among *Calligonum* and *Pteropyrum* species.

Table 3. Statistics of characters analyzed in the cladistic analysis of *Calligonum* and *Pteropyrum*.

Character	Tree steps	CI	RI	RC
1	6	0.167	0.286	0.048
2	1	1.000	1.000	1.000
3	6	0.333	0.600	0.200
4	3	0.333	0.778	0.259
5	3	0.667	0.800	0.533
6	2	0.500	0.500	0.250
7	1	1.000	1.000	1.000
8	1	1.000	1.000	1.000
9	1	1.000	1.000	1.000
10	1	1.000	1.000	1.000
11	1	1.000	1.000	1.000
12	1	1.000	1.000	1.000
13	3	0.333	0.333	0.111
14	2	1.000	1.000	1.000
15	1	1.000	1.000	1.000
16	2	1.000	1.000	1.000
17	2	1.000	1.000	1.000
18	1	1.000	1.000	1.000
19	2	1.000	1.000	1.000
20	4	0.500	0.833	0.417
21	2	1.000	1.000	1.000
22	2	1.000	1.000	1.000
23	5	1.000	1.000	1.000
24	7	0.286	0.375	0.107
25	3	0.667	0.857	0.571
26	7	0.429	0.429	0.184
27	1	1.000	1.000	1.000
28	2	1.000	1.000	1.000
29	2	1.000	1.000	1.000
30	3	1.000	1.000	1.000

*Pteropyrum*. Characters 1, 3, 4, 6, 20, 26 are evolved as parallelism/reversal among members of these two genera (see Fig. 3 and Table 3 for detail information). The remaining characters including 5, 13, 14, 16, 17, 19, 22, 23, 24, 25 and 28 were ambiguously changed among the taxa. Although, photosynthetic pathway character, C3/C4, were not included in the data matrix, it is evident that the C4 pattern, which has been known only for *Calligonum* species within *Polygonaceae*, clearly is a derived character state for the genus. Our anatomical study (Tavakkoli et al. unpublished data) showed that the *Pteropyrum* species examined have C3 photosynthetic pathway.

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