Turkish Journal of Botany

http://journals.tubitak.gov.tr/botany/

Research Article

Turk J Bot
(2014) 38: 417-429

doi:10.3906/bot-1303-67

Phylogeny of the genus *Potentilla* (Rosaceae) in Iran based on nrDNA ITS and cpDNA *trn*L-F sequences with a focus on leaf and style characters' evolution

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Received: 27.03.2013 • **Accepted:** 13.12.2013 • **Published Online:** 31.03.2014 • **Printed:** 30.04.2014

Abstract: The present survey reports maximum parsimony (MP) and Bayesian phylogenetic analyses of the genus *Potentilla* L. in Iran using the internal transcribed spacer (ITS) of nuclear ribosomal DNA and the *trn*L-F intergenic region of chloroplast DNA. A total of 25 taxa from 9 sections, 2 intersectional hybrids, and 4 related genera of Iranian taxa plus 40 previously sequenced data were analyzed for both *trn*L-F and nrDNA ITS regions. Individual and combined analysis of ITS and trnL/F data revealed monophyly of the genus *Potentilla.* The *Potentilla* core group was composed of monophyletic subclades of the sections *Persicae* (Th.Wolf) Juz., *Potentilla*, *Micranthae Sojak*., and *Speciosae* (Th.Wolf) Juz.; unresolved branches of the sections *Rivales* Poeverl., *Aureae* (Rydb.) Juz., *Terminales*, (Döll) Gren. & Godr., and *Pensylvanicae* Poeverl.; and the American genera *Ivesia* and *Horkelia*. The evolutionary trends of some diagnostic morphological traits were evaluated. Anther structure, and style shape and position evolved independently while multiple gains and parallel evolutions were inferred for leaf division.

Key words: *Potentilla*, phylogeny, cpDNA, nrDNA, Iran

1. Introduction

The generic circumscription of *Potentilla* L. was primarily discussed based on morphological characters, especially leaf shape, style position (Boiss, 1872), and indumentum types (Wolf, 1908). Boisser (1872) divided the genus into 2 large sections (*Fragariasterum* Koch. and *Eupotentilla* Koch.). Wolf (1908) classified the genus into 2 sections, a number of subsections, and groups of species. Following Wolf's classification, several authors (Juzepczuk, 1941; Schiman-Czeika, 1969; David, 1972) emphasized the diagnostic and taxonomic value of morphological characters in the genus (Soják, 1989, 2004, 2008; Eriksen and Yurtsev, 1999).

The systematic treatments of *Potentilla* using molecular data were reported primarily from broader studies on the subfamily Rosoidea of the family Rosaceae (Morgan, 1994; Eriksson et al., 1998, 2003; Potter et al., 2007). Dobeš and Paule's (2010) reconstruction of phylogenetic relationships of the genus revealed 2 major evolutionary lineages within the tribe *Potentilleae*. This result was congruent with an earlier report by Eriksson et al. (2003) and corresponded to the subtribes *Fragariinae* and *Potentillinae* proposed by Soják (1989). The subtribe *Potentillinae* consists of a well-supported monophyletic clade (Dobeš and Paule, 2010), composed of the Argentina group, *Potentilla* core group, members of the American genera *Ivesia* Torrey and Gray and *Horkelia* Cham. and Schltdl, *Tormentillae* Th. Wolf, and a group of taxonomically diverse but molecularly little diverged species of *Potentilla* (Eriksson et al., 1998; Dobeš and Paule, 2010).

Soják (2010) supported the previous molecular studies (Eriksson et al., 1998; Dobeš and Paule, 2010) and classified the Argentina group into a distinct genus (*Argentina* Hill), based on some morphological differences (especially ventral stipular auricles) (Soják, 2010).

Töpel et al. (2011) identified 6 major clades, namely *Anserina*, *Alba*, *Fragarioides*, *Reptans*, *Ivesioid*, and *Argentea*, within the genus *Potentilla* and confirmed both the chloroplast and the nuclear phylogenies (except for the clade Fragarioides) of Dobeš and Paule (2010).

This paper supplements the previous studies and reports the phylogenetic analyses of the internal transcribed spacer of nuclear ribosomal DNA (ITS) and the *trn*L-F region of chloroplast DNA for Iranian species of *Potentilla*, which were not included in previous molecular research (Eriksson et al., 1998, 2003; Dobeš and Paule, 2010; Töpel et al., 2011).

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The main objectives of this study were to achieve the following objectives: 1) comparing phylogenies derived from these data sets with those of previous studies, 2) determining the phylogenic relationships among Iranian species of *Potentilla*, and 3) evaluating characters' evolution in the context of the combined nrDNA ITS-*trn*L-F phylogeny.

2. Materials and methods

2.1. Taxon sampling

A total of 65, 60, and 58 taxa were included in this study respectively for nrDNA ITS, *trn*L-F, and combined analyses (Tables 1 and 2). We sequenced 25, 20, and 18 Iranian species of *Potentilla* (Table 1) and to this we added 41 taxa (sequenced by Eriksson et al. (1998, 2003)) (Table 2). Our sampling included 25 taxa from 9 sections, 2 intersectional hybrids, and 4 related genera, i.e. *Drymocallis* Fourr. ex Rydb. (*D. rupestris* (L.) Soják and *D. poteriifolia* (Boiss.) Soják), *Schistophyllidium* (Juz. ex Fed.) Ikonn (*Schi. bifurcum* (L.) Ikonn), *Argentina* Hill, (*A. lignosa* (Willd. in D.F.K. Schltdl.) Soják), and *Sibbaldia* L. (*S. parviflora* Willd.) (Table 2). Our main aim was to include at least one representative from each section. However, for larger sections, we used more species, e.g., section *Persicae* (Th. Wolf) Juz. (*P. kurdica* Boiss. and Hohen., *P. pannosa* Boiss. and Hausskn., *P. petraea* Willd. ex Schlecht., and *P. persica* Boiss. and Hausskn.) and *Rectae* (Poeverl.) Juz. (*P. adscharica* Sommier and Levier ex Keller, *P. iranica* (Rech.f.) Schiman-Czeika, and *P. recta* L.). We added one repeat for some taxa (e.g., *P. radiata* Lehmn. and *P. reptans* L., especially in nrDNA ITS analysis) in order to verify their polymorphism. *Rosa majalis* Herrm. and *Rosa*

Table 1. Samples included in cpDNA trnL-F and nrDNA ITS phylogenetic analyses.

			Genbank accession no.	
Taxa	Locality in Iran Province: Collector, Date	Accession no.	ITS	TrnL-F
Schistophyllidium bifurcum	Guilan: Asalem–KhalKhal road, Faghir, 6.6.2006	36600 (TUH)	AB894167	AB894187
Drymocallis poteriifolia 1	Fars: Abadeh, Termeh & Izadyar, 7.6.1969	36855 (IRAN)	AB894147	AB894170
Drymocallis poteriifolia 2	Chaharmahal bakhtiyari: Yasouj, Attar & zamanii, 17.6.2006	36674 (TUH)	AB894148	
Drymocallis rupestris	Mazandaran: Firouzkooh, Attar, 12.6.2006	39000 (TUH)	AB894149	AB894171
Sibbaldia parviflora	Guilan: Asalem–KhalKhal road, Termeh & Mousavi, 19.5.74	36839 (IRAN)	AB894166	AB894188
Argentina anserina	Mazandaran: Firouzkooh, Attar, 27.6.2005	39001 (TUH)	AB894145	AB894168
Potentilla argentea	Guilan: Siahkal, Faghir, 16.6.06	36585 (TUH)	AB894151	AB894173
Potentilla adscharica	Guilan: Siyahkal, Spili, Larikhani, Saidi, 13.5.93	18855 (TUH)	AB894150	AB894172
Potentilla recta	Guilan, Damash-Jirandeh, Faghir. 16.6.2006	36598 (TUH)	AB894160	AB894182
Potentilla	Ardabil: Sabalan mountains, Faghir	36616 (TUH)	AB894165	AB894186
Potentilla crantzii 1	Mazandaran: Ramsar, Samamous mountain, Faghir, 22.7.06	36631 (TUH)	AB894152	AB894174
Potentilla iranica	Guilan: Asalem–KhalKhal road, Faghir 16.6.06	36606 (TUH)	-	AB894175
Potentilla radiata 1	Guilan, Damash-Jirandeh, Faghir, 16.6.2006	36594 (TUH)	AB894158	AB894181
*Potentilla radiat*a 2	Azarbaijan: Kalibar, Attar & Dadjou, 17.7.93	17184 (TUH)	AB894159	-
Potentilla szovitsii	Guilan: Damash-Jirandeh, Faghir, 16.6.06	36593 (TUH)	-	AB894185
Potentilla supina	Qazvin: Alamout Ghahreman & Mozaffarian, 11.8.91	9963 (TUH)	AB894164	AB894184
Potentilla persica	Lorestan: Khoramabad, Attar & Mirtj, 27.7.88	24898 (TUH)	AB894156	AB894179
Potentilla kurdica	Kordestan: Sanandaj, Aral, Hanagalanvillage, Maroufi, 1.6.2007	8060	AB894153	AB894176
Potentilla pannosa	Hamedan: Alvand southern slope, Mozaffarian, 8.7.88	64940 (TUH)	AB894155	AB894178
Potentilla petraea	Mazandaran: Ramsar, Samamous mountain, Faghir, 22.7.2006	36632 (TUH)	AB894157	AB894180
Potentilla reptans 1	Guilan: Siahkal, Faghir, 16.6.2006	36639 (TUH)	AB894161	AB894183
Potentilla reptans 2	Mazandaran: Youshbaladeh, 18.5.2006	36626 (TUH)	AB894162	-
Potentilla speciosa	East Azarbaijan: Ormiyeh, Ghahreman & Mozaffarian, 29.6.97	20056 (TUH)	AB894163	-
Potentilla micrantha	Guilan: Asalem-Khalkal, Faghir, 6.6.2006	36602 (TUH)	AB894154	AB894177
Argentina lignosa	Tehran: Tehran–Shemshak road, Ghahreman & Mozaffarian, 20.7.88	6212 (TUH)	AB894146	AB894169

Table 2. Samples included in cpDNA trnL-F and nrDNA ITS phylogenetic analyses.

Species	DNA source (location, voucher)	Accession no.
1. *Acaena cylindristachya*		
nrDNA ITS	Hibbs 167 Andes	27524340, AJ512780.1
cpDNA *trn*L-*trn*F		27524339, AJ512775.1
2. *Acaena laevigata*		27524340, AJ512780.1
nrDNA ITS	RBGE; Falkland Islands	7524863, AJ512781.1
cpDNA *trn*L-*trn*F		27524862, AJ512776.1
3. *Agrimonia eupatoria*		
nrDNA ITS	Eriksson et al. 1998, Sweden, Uppland	U90798
cpDNA *trn*L-*trn*F	Eriksson and Smedmark 41 (SBT), HB; Germany	AJ512216
4. *Alchemilla alpina*		
nrDNA ITS	Eriksson et al. 1998 Sweden, Torne Lappmark	U90816, U90817
cpDNA *trn*L-*trn*F	Eriksson 805 (SBT) HB; Sweden, Torne Lappmark	AJ512217
5. *Alchemilla mollis*		
nrDNA ITS	Eriksson s.n. (SBT)	AJ511769
cpDNA *trn*L-*trn*F		AJ512218
6. *Aphanes arvensis*		
nrDNA ITS	Eriksson s.n. (SBT) Sweden, Uppland	AJ511770
cpDNA *trn*L-*trn*F		AJ512234
7. *Aremonia agrimonioides*		
nrDNA ITS	Eriksson et al. 1998 LD;	U90799
cpDNA *trn*L-*trn*F	Karlsson 94076 (LD) LD;	AJ512230, AJ512231
8. *Chamaerhodos erecta*		
nrDNA ITS	Eriksson et al. 1998 USA, Montana	U90794
cpDNA *trn*L-*trn*F	Norlindh and Ahti 10161A (S) Mongolia	AJ512219
9. *Fallugia paradoxa*		
nrDNA ITS	Eriksson et al. 1998 USA, New Mexico	U90805
cpDNA *trn*L-*trn*F	Smedmark and Eriksson 2002 USA, Colorado	AJ297331
10. *Filipendula vulgaris*		
nrDNA ITS	Eriksson 821 (SBT) Sweden, Uppland	AJ416467
cpDNA *trn*L-*trn*F		AJ416463
11. *Fragaria vesca*		
nrDNA ITS	Eriksson and Smedmark 43 (SBT) Sweden, Uppland	AJ511771
cpDNA *trn*L-*trn*F		AJ512232
12. *Otentilla multifida*		
nrDNA ITS	Eriksson 705 (SBT) Sweden, Torne Lappmark	AJ511776
cpDNA *trn*L-*trn*F		AJ512245
13. *Potentilla nivea*		
nrDNA ITS	Eriksson et al. 1998; Sweden, Torne Lappmark	U90814
cpDNA *trn*L-*trn*F		U90815, AJ512244
14. *Potentilla norvegica*		
nrDNA ITS	Eriksson et al. 1998; USA, Massachusetts	U90790
cpDNA *trn*L-*trn*F		AJ512246
15. *Comarum palustris*		
nrDNA ITS	Eriksson 659 (GH, S) Sweden, Uppland	AJ511777
cpDNA *trn*L-*trn*F		AJ512237

Table 2. (Continued).

Species	DNA source (location, voucher)	Accession no.
16. *Potentilla peduncularis*		
nrDNA ITS	Eriksson and Vretblad TE758 (SBT) GB; China, Yunnan	AJ511778
cpDNA *trn*L-*trn*F		AJ512239
17. *Potentilla salesowianum*		
nrDNA ITS	Eriksson and Vretblad TE751(SBT) GB; unknown	AJ511779
cpDNA *trn*L-*trn*F		AJ512228
18. *Potentilla stenophylla*		
nrDNA ITS	Eriksson and Vretblad TE763 (SBT) GB; China, Yunnan	AJ511780
cpDNA *trn*L-*trn*F		AJ512240
19. *Potentilla tridentata*		
nrDNA ITS	Eriksson et al. 1998 USA, New Hampshire	U90791
cpDNA *trn*L-*trn*F	Eriksson and Smedmark 40 (SBT) Canada, Nova Scotia	AJ512236
20. *Rosa majalis*		
nrDNA ITS	Eriksson et al. 1998; same DNA Sweden, Värmland	U90801
cpDNA tranL-F		AJ512229
21. *Rosa persica*		
nrDNA ITS	Eriksson and Smedmark 1 (SBT) Uppsala Bot. Garden; Iran or Afghanistan	AJ416468
cpDNA *trn*L-*trn*F		AJ416466
22. *Rubus chamaemorus*		
nrDNA ITS	Eriksson et al. 1998 Sweden, Torne Lappmark	U90803
cpDNA *trn*L-*trn*F	Eriksson 809 (SBT) Sweden, Västmanland	AJ416464
23. *Sanguisorba officinalis*		
nrDNA ITS	Helfgott et al. 2000	AF183533, AF183556
cpDNA *trn*L-*trn*F	Eriksson 804 (SBT) HB; unknown	AJ416465
24. *Sibbaldia procumbens*		
nrDNA ITS	Eriksson et al. 1998 Sweden, Torne Lappmark	U90820, U90821
cpDNA *trn*L-*trn*F	Eriksson 698 (SBT) Sweden, Torne Lappmark	AJ512235
25. *Fragaria virginiana*		
nrDNA ITS	Eriksson s.n. (SBT) Canada, Nova Scotia	AJ511772
cpDNA *trn*L-*trn*F		AJ512220
26. *Geum urbanum*		
nrDNA ITS	Eriksson et al. 1998 Sweden, Uppland	U90802
cpDNA *trn*L-*trn*F	Smedmark and Eriksson 2002 Sweden, Uppland	AJ297323
27. *Horkelia fusca*		
nrDNA ITS	Eriksson et al. 1998; USA, California	U90795
cpDNA *trn*L-*trn*F		AJ512247
28. *Ivesia gordonii*		
nrDNA ITS	Eriksson et al. 1998, USA, Utah	U90796
cpDNA *trn*L-*trn*F		AJ512221
29. *Leucosidea sericea*		
nrDNA ITS	Helfgott et al. 2000	AF183547, AF183524
cpDNA *trn*L-*trn*F	D.M. Helfgott Ben-3 1998 (TEX) Unknown	AJ512222
30. *Polylepis hieronymi*		
nrDNA ITS	Hibbs 133, 1998 Bolivia	AJ512774
cpDNA *trn*L-*trn*F		AJ512779

Table 2. (Continued).

Species	DNA source (location, voucher)	Accession no.
31. *Polylepis tarapacana*		
nrDNA ITS	Hibbs 163, 1999 Bolivia	AJ512773
cpDNA *trn*L-*trn*F		AJ512778
32. *Potentilla arguta*		
nrDNA ITS	Eriksson et al. 1998 USA, Washington	U90787
cpDNA *trn*L-*trn*F	Eriksson s.n. (SBT) Unknown AJ51222	AJ51222
33. *Potentilla chinensis*		
nrDNA ITS	Eriksson s.n. (SBT) HB; China, Beijing	AJ511774
cpDNA *trn*L-*trn*F		AJ512225
34. *Potentilla crantzii*	Topel, M. 2009	
nrDNA ITS	University of Gothenburg, Box 461, SE-40530, Gothenburg, Sweden	FN555609.1
35. *Potentilla dickinsii*		
nrDNA ITS	Eriksson et al. 1998 Korea	U90785
cpDNA *trn*L-*trn*F	Crompton, D’Arcy & Coke 139 (E) RBGE; Korea	AJ512243
36. *Potentilla fragarioides*		
nrDNA ITS	Eriksson et al. 1998 Japan	U90806, U90807
cpDNA *trn*L-*trn*F	Eriksson s.n. (SBT) HB; China, Beijing	AJ512226
37. *Potentilla fruticosa*		
nrDNA ITS	Eriksson et al. 1998 LD; Sweden, Öland	U90808, U90809
cpDNA *trn*L-*trn*F	Eriksson 806 (SBT) HB; Sweden, Öland	AJ512233
38. *Potentilla indica*		
nrDNA ITS	Eriksson s.n. (GH, SBT) HB; China, Gansu	AJ511775
cpDNA tranL-F		AJ512242
39. *Tetraglochin cristatum*	Eriksson T., Bergius Foundation, Royal	
nrDNA ITS	Swedish Academy of Sciences, Box 50017, SE-104 05	AJ512777
cpDNA trnL-trnF	Stockholm, Sweden	AJ512782
40. *Waldsteinia geoides*		
nrDNA ITS	Smedmark, J.E.	AJ302362
cpDNA trnL-trnF	Smedmark and Eriksson 2002 Stockholm University	AJ297348
41. *Sibbaldia parviflora*	Topel, M. 2008	
nrDNA ITS	University of Gothenburg, Box 461, SE-40530,	FJ356174.1
cpDNA trnL-trnF	Gothenburg, Sweden	FJ422302

persica Gmel. were selected as outgroup species based on previous phylogenetic studies of the genus (Eriksson et al., 1998, 2003; Dobeš and Paule, 2010).

2.2. DNA extraction

Total DNA was extracted from freshly collected specimens deposited in the Tehran University Herbarium (TUH), Iran. The extraction method was based on the modified CTAB procedure of Doyle and Doyle (1990). The 2 noncoding regions of cpDNA and nrDNA were amplified using the primers *trn*-C and *trn*-F of Taberlet et al. (1991), ITS4 and ITS5 of White et al. (1990), ITS5m of Sang et al. (1995), and AB101R and AB101F of Douzery et al. (1999).

2.3. PCR and DNA sequencing

The complete nrDNA ITS region was amplified using the primers ITS4 and ITS5 of White et al. (1990). The trnL-trnF intergenic spacer was amplified using the primers trn-c and trn-f of Taberlet et al. (1991). The total volume of amplification reactions was 20 µL, made up of 17.5 µL of deionized water, 0.5 µL of AccuPower PCRpre Mix buffer, 0.5 µL of each primer, and 1 µL of template DNA. The PCR cycles started with 2.5 min of predenaturation at 94 °C followed by 30 cycles of 30 s of denaturation at 94 °C, 30 s of primer annealing at 68 °C, 45 s of primer extension at 72 °C, and a final extension of 5 min at 72 °C.

Nucleotide sequences of PCR products were determined using cycle sequencing and an automated DNA sequencer by Gen Fanavaran Co. The same nrDNA ITS and cpDNA *trn*L-F primers were used for cycle sequencing reactions.

2.4. Sequence alignment

Sequences of *trn*L-F and nrDNA ITS data sets were edited by means of BioEdit ver. 7.0.9.0 (Hall, 2001). The alignment was carried out using ClustalX (Larkin et al., 2007) and manual adjustment. The alignment of both regions consisted of several uninformative and ambiguous gaps (Eriksson et al., 1998, 2003). The informative gaps were identified based on Eriksson et al.'s criteria (2003). The length of the indels varied from a single up to 10 (in positions 495–505) base pairs in the ITS data and from a single up to 182 (in positions 1128–1310) base pairs in the *trn*L-F data.

2.5. Phylogenetic analyses

2.5.1. Maximum parsimony

Phylogenetic analyses were carried out for separate and combined data sets. The data sets were analyzed using maximum parsimony (MP) criterion as implemented in PAUP (Swofford, 2002). The heuristic searches were performed using 100 replications of random addition sequence. Tree-bisection-reconnection (TBR) branch-swapping with MulTrees on and steepest descent off was performed. The maximum number of trees saved was set to 20,000 and these trees were allowed to swap to completion. In order to improve the trees' indices, a successive re-weighting strategy (Farris, 1969) was conducted. The rescaled consistency index (RC) (Farris, 1989), consistency index (CI), and retention index (RI) were measured. The bootstrap values (Felsenstein, 1985) were calculated from 20,000 replicates, simple sequence addition, and TBR branch swapping with 'Maxtrees' limit of 100 trees per bootstrap replicate.

2.5.2. Bayesian method

Appropriate evolutionary models for Bayesian inference analyses were selected using the program MrModeltest version 2.3 (Nylander, 2004) as implemented in MrMTgui based on the Akaike information criterion (AIC) (Posada and Buckley, 2004). The data sets were analyzed using the SYM + I + G models for nrDNA ITS, K81uf + I + G for cpDNA *trn*L-F, and GTR+ I + G for combined sequences. The program MrBayes version 3.1.2 (Ronquist and Huelsenbeck, 2003) was used for the Bayesian phylogenetic analyses. For partitioned and nonpartitioned data, substitution estimates and length branches were carried out independently. Secondary probabilities of the model parameters were determined using the initial default. Separate and combined analyses were repeated for several million generations. MrBayes performed 2 simultaneous analyses starting with different random trees (Nruns = 2). Four Markov chains were established for each tree and a tree from every 100 generations was sampled. After reaching the linear phase 3 million generations (3000 samples) were collected. After removal of burn-in period samples (including 25% of initial trees sampled) a tree with maximum 50% (majority rule consensus tree) was plotted. The values of posterior probability (PP) were calculated and the final tree was plotted using the Tree view software version 1.6.6 (Soltis and Soltis, 2003). To adjust the number of generations and to ensure stationary Markov chains the following points were considered: 1) a stable value of the log likelihood of the cold chain in 2 separate runs, 2) a value close to 0 for the standard deviation of split frequencies (0.005 for both runs), and 3) a value approaching 1.0 for the potential scale reduction factor (PSRF) for each parameter in the model. The Bayesian trees are not presented in the current paper.

3. Results

3.1. NrDNA ITS sequence data

The nrDNA ITS sequence data sets consisted of 65 taxa and 695 aligned DNA characters; of these, 318 were informative for parsimony analysis. Phylogenetic analysis resulted in a single most parsimonious tree (Figure 1).

The Bayesian analysis of the nrDNA ITS datasets resulted in 22,500 trees, after discarding 7500 initial trees as burn in. The topology of this tree is the same as that of the MP tree. In either of Bayesian and MP nrDNA ITS trees, clade A corresponds to the tribe *Potentilleae* (BP = 77%, PP = 9.97) and is composed of 2 large clades including the Potentillinae or "*Potentilla*" (C) (BP = 58%, PP = 1.00) and the Fragriinae clades (B) (BP = 66%, PP = 0.96) (Figure 1).

The "*Potentilla*" clade (C) in both MP and Bayesian trees consisted of 1) a well-supported (BP = 100%) but unresolved branch of *Potentilla fragarioides* L., 2) a large so-called *Potentilla* core group and *Horkelia fusca* Lindl/ *Ivesia gordonii* (Hook.) Torr. and A.Gray, (BP = 67 %, PP = 0.79) that formed 4 monophyletic subclades (C1–C4) (Figure 1). In the Bayesian tree, *Potentilla chinensis* Ser. and *P. nivea* L. formed a monophyletic subclade (PP = 0.92) within the C4B subclade (this is not found in the MP tree). *P. supina* L. and *P.* sp. 36616 formed 2 unresolved paraphyletic branches in the Bayesian and MP trees. The Fragriinae clades (B) of the MP and Bayesian trees are similar and composed of 2 main subclades, B1 (the Argentina group) and B2 (subtribe *Fragriinae*) (Figure 1).

In both trees the species of *Sibbaldia* (*S. parviflora*) and *Schistophyllidium* (*Schi. bifurcum*) are nested in a strongly monophyletic subclade (BP = 100 %, PP = 1.00) within the Fragriinae clade (clade F of Bayesian tree and clade B3 of MP tree). The 2 taxa of *S. parviflora* made their own subclade, while *S. tridentata* and *Schi. bifurcum*

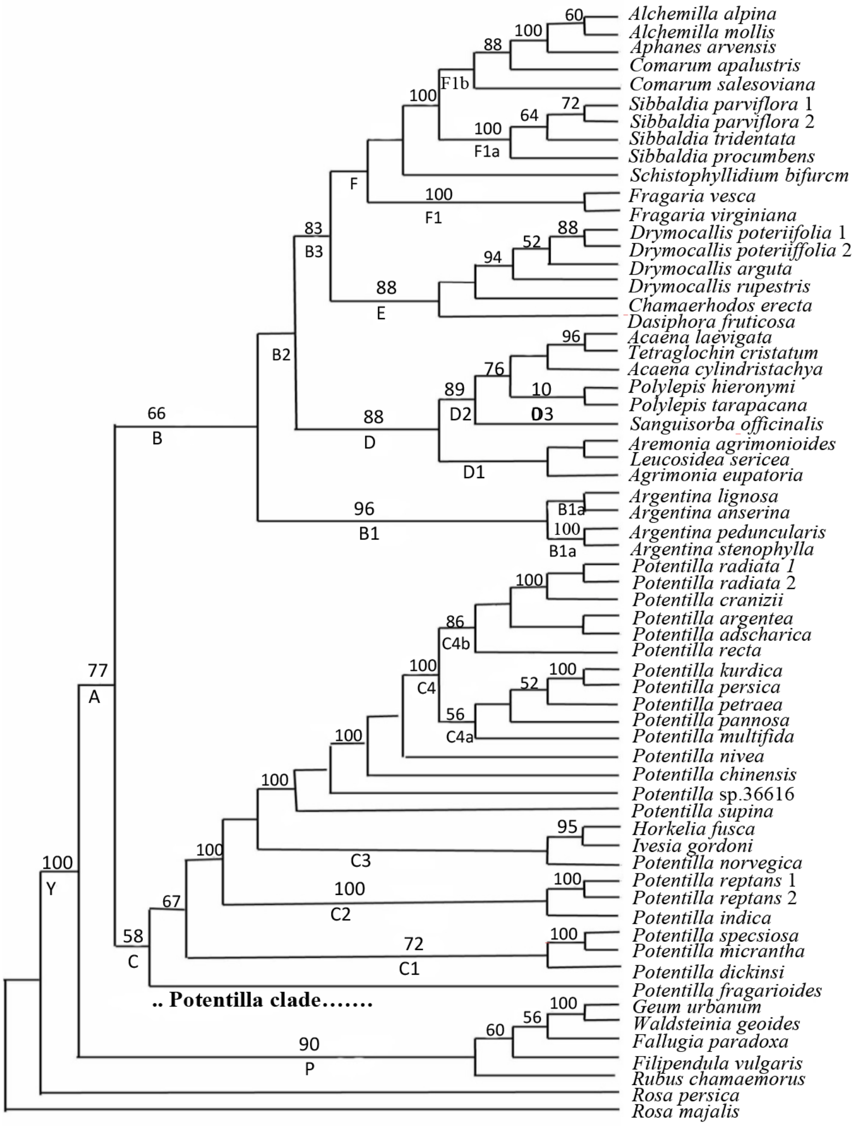

Figure 1. Strict consensus tree obtained from nrDNA ITS sequences. Numbers above branches are bootstrap values and clades are identified by letters. Consistency index (CI) = 0.4079, retention index relying on indicators (RI) = 0.7024, homoplasy index (HI) = 0.59), identical to that of the MP tree (not presented in this paper). Rescaled consistency index (RCI) = 0.286. Bayesian posterior probabilities are indicated below the branches.

formed separate unresolved branches (Figure 1, F1a in MP tree and B3a in Bayesian tree). In both Bayesian and MP nrDNA ITS trees, the species of *Sanguisorbeae* (D clade) and *Fragriinae*, *Colurieae* (clade P), *Potentillinae* (clade C), and *Fragriinae* clades (clade B) formed a sister group relationship.

3.2. TrnL-F sequence data

The *trn*L-F data set consisted of 60 taxa accessions with 1861 aligned DNA characters; of these, 337 were parsimony informative. A single most parsimonious tree was obtained (Figure 2). The Bayesian analysis of the *trn*L-F dataset resulted in 135,000 trees, after discarding 45,000 initial trees as burn in. In both the Bayesian (A 50% majority rule consensus tree) and MP cpDNA trees, clade A corresponds to the tribe *Potentilleae* (BP = 100%, PP = 1.00) including 2 subclades, Potentillinae or "*Potentilla*" (subclade B, BP = 100 %, PP = 1.00) and Fragriinae (subclade C, PP = 0.90) (Figure 2). The topology of the Bayesian and MP trees is almost the same except for 22 resolved species of subclade B7 in the MP tree. This relationship was collapsed as a trichotomy (B4, B5, and B6) in the Bayesian tree (this tree is not shown in this

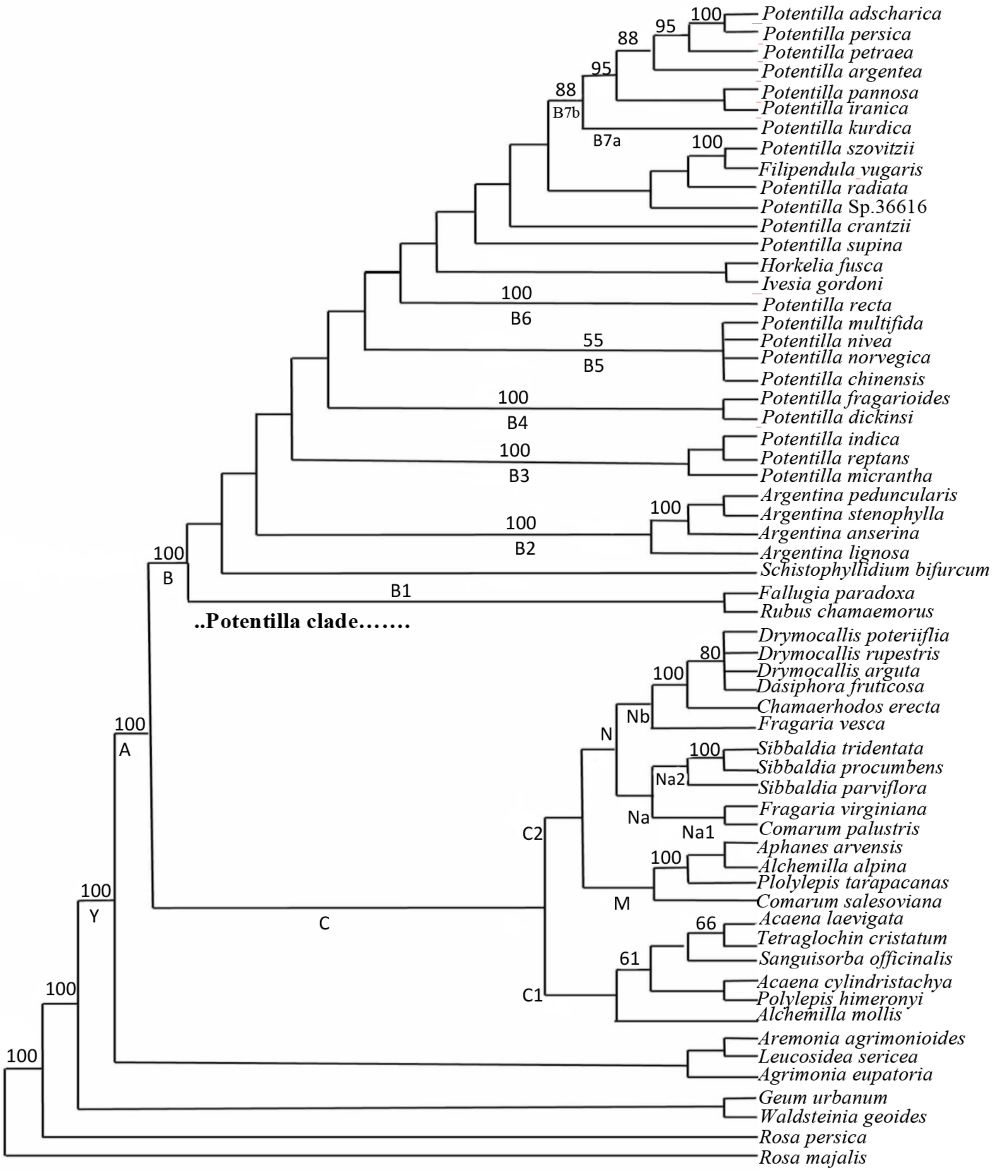

Figure 2. Strict consensus tree of most parsimonious tree resulting from phylogenetic analysis of the cpDNA *trn*L-F sequences. Numbers above branches are bootstrap values; clades are identified by letters. Consistency index (CI) = 0.5982, retention index (RI) = 0.8266, homoplasy index (HI) = 0.4018, and rescaled consistency index (RCI) = 0.495. Bayesian posterior probabilities are indicated below the branches.

paper). The Bayesian and MP trees share the following points: 1) the Potentilla clade consisted of a well-supported assemblage (BP = 100%, PP = 1.00) and 2 main subclades, a monophyletic *Rubus chamaemorus* L./*Fallugia paradoxa* (D.Don) Endl (PP=1.00) and a large Potentilla subclade including most of the *Potentilla* species, *Horkelia fusca*/*Ivesia gordonii* plus a branch leading to *Schistophyllidium bifurcum* (Figure 2); 2) the *Fragriinae* clade (C) is similar to that of ITS data sets except for some collapsed subclades (L and C1, C3) in the MP tree. It is divided into 2 main subclades, C1 and C2, plus the *Agrimonia* clade, which is sister to the Potentillinae, Fragriinae, and Sanguisorbeae clades (Figure 2).

3.3. The combined sequence data

The combined data matrix consisted of 58 species with 2164 DNA characters; of these, 645 were parsimony informative. The single most parsimonious tree is presented in Figure 3. The Bayesian analysis of the combined dataset resulted in 235,275 trees after discarding 7875 initial trees as burn in.

The general topology of the combined tree is a mixed topology of both the cpDNA and nrDNA ITS trees.

The Potentilla clade of the combined MP tree formed a strongly supported assemblage (bootstrap value of 100%) consisting of 2 subclades, 1) the so-called *Potentilla* s.s. (B2–B5), 2) a well-supported Argentina group (B1 subclade) and its Fragriinae clade (C) composed of 4 monophyletic groups (C1–C4) (Figure 3). The Potentilla clade of the combined Bayesian tree was similar to that of nrDNA MP trees and its Fragriinae clade (B) divided into 2 subclades, 1) the Argentina group (B1), 2) 2 subclades including *Sibbaldia* (B3a) and *Alchemilla* (B3b) plus unresolved branches of *Schistophyllidium bifurcum*, *Comarum* L. (*C. salesoviana* (Stephan) Ledeb. and *C. palusteris* L.) *Fragaria* L. (B4a), and *Drymocallis* (B4b) groups.

In the combined MP tree *Rubus* L. (clade O), *Sanguisorbeae* (clade Q), and *Geum* (clade P) formed 3 sister groups to the *Fragriinae* and *Potentilla* clades. However, in this Bayesian tree, the number of sister groups is reduced (including *Rubus*/*Geum* group (P) and *Sanguisorbeae* species) (clade D).

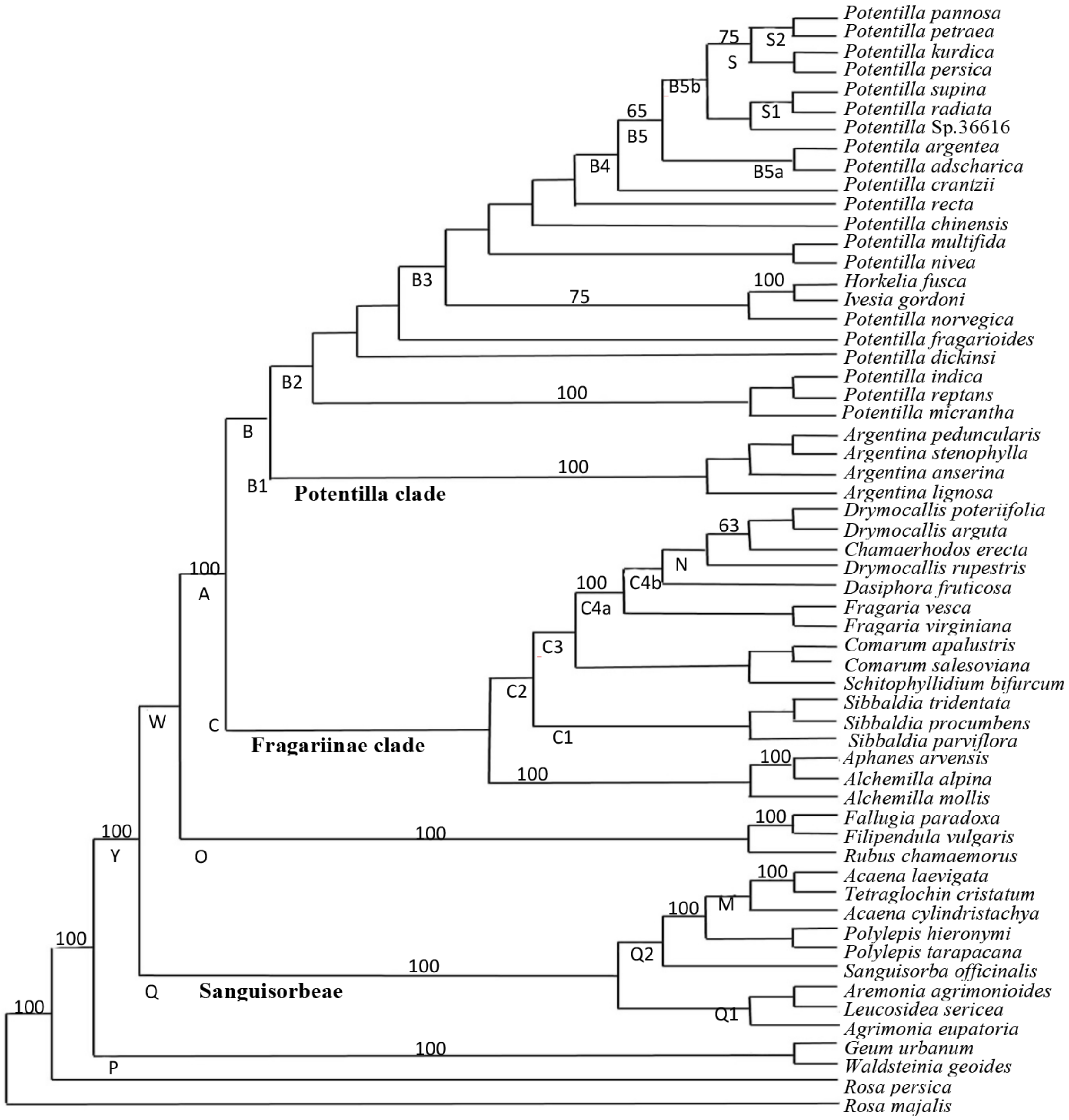

Figure 3. Fifty percent majority consensus tree derived from analysis of the combined plastid and ITS sequences. Numbers above branches are bootstrap values; clades are identified by letters. Consistency index (CI) = 0.4515, retention index (RI) = 0.7141, homoplasy index (HI) = 0.5485, and rescaled consistency index (RCI) = 0.3224. Bayesian posterior probabilities are indicated below the branches.

4. Discussion

The current survey revealed a major phylogenetic split, corresponding to 2 subtribes including *Fragariinae* (clade B in Figure 1, clade C in Figures 2 and 3) and *Potentillinae* (clade C in Figure 1, clade B in Figures 2 and 3). The well-supported monophyletic subtribe *Potentillinae* consisted of the so-called "Potentilla s.s." (including *Potentilla* core group and American genera *Ivesia/ Horkelia*) and Argentina subclades (including the Argentina group).

This confirms the previous phylogenetic studies by Eriksson et al. (2003), Potter et al. (2007), and Dobeš and Paule (2010) and supports the new taxonomic delimitation of the genus *Argentina* Hill proposed by Soják (2010).

The Potentilla core group comprised 20–22 species of *Potentilla* belonging to 9 sections and 2 intersectional hybrids. The species of 4 sections, namely *Persicae*, *Potentilla*, *Micranthae*, and *Speciosae*, formed monophyletic subclades. Four studied species of the section *Persicae* formed 2 monophyletic subclades (*Potentilla kurdica–P. persica* and *P. petraea–P. pannosa*). This supports their micromorphological characters and distribution pattern. The first 2 species are distributed in western Iran and covered by straight, sparse to moderately pilose and sericeous indumentum. The last 2 species are found in the west and northwest of the country and characterized by dense hirtellous hairs (Faghir et al., 2010). This result is in accordance with the previous studies by Schiman-Czeika (1969), Khatamsaz (1992), and Ertter and Attar (2007).

The phylogenetic reconstruction performed here showed a strong monophyletic Potentilla subclade (B2, Figure 3) and a close relationship between the 3 sections *Micranthae*, *Speciosae*, and *Potentilla*. The first 2 sections were placed in the subgenus *Fragariastrum* by Juzepchuk (1941), Khatamsaz (1992), and Schiman-Czeika (1969) because of their common morphological traits (e.g., woody caudex, ternate to digitate leaves, and white flowers).

In contrast, the representatives of the 5 remaining sections (*Rectae*, *Aureae*, *Terminales*, *Rivales*, and *Pensylvanicae*) formed unresolved branches, nested within the clade including the species belonging to other groups and produced multiple taxon polytomies, e.g., a weakly supported polytomy of *Potentilla multifida* L./*P. nivea* L./*P. norvegica* L./*P. chinensis* Ser. subclade, in the cpDNA PM tree (Figure 3).

The unresolved branches contain species, e.g., *Potentilla radiata* Lehm. (*P. argentea* × *P. thuringiaca*), with a hybrid origin (Soják, 2009, 2011; Faghir et al., 2010a) that usually participate in hybridization and polyploidization (Dobeš and Paule, 2010; Tople et al., 2011; Klingenberg et al., 2011), which in addition to poor congruence of morphological and molecular variation lead to inconsistencies in phylogeny of the genus. Our findings support the *Fragariinae* (clade E including the species of subtribes *Fragariinae*), the petalous *Agrimonia* (clade F including the species of subtribes *Sanguisorbae*) clades, and a clade containing apetalous groups of Eriksson et al. (2003).

4.1. Character evolution

The evolutionary trend of some morphological features was traced on the MP tree using combined data sets (Figures 4 and 5). These morphological features are considered to be characteristic and have been used for classification of the genus (Schiman-Czeika, 1969; Khatamsaz, 1992; Faghir et al., 2010a).

The 2 theca anther is a synapomorphy for the subtribe *Potentillinae*. It evolved from one theca anther of the subtribe *Fragariinae*. Anther structure was considered a characteristic trait for dividing the tribe *Potentilleae* into 4 subtribes (Sojak, 2008).

The Conostylae is a dominating style type in the Potentilla clade. It is a synapomorphy for the subtribe *Potentillinae*, derived from filiform Nematostylae (*P. dickinsi* Franchet and Savatier), club-shaped Gomphostylae (B2 clade, *Potentilla reptans*/*P. michranta*/*P. indica*), and slender leptostylae (Argentina group). The long Conostylae (style 2 to 3 times longer than matured achene) has been considered an important criterion at infrageneric level (e.g., sections) (Schiman-Czeika, 1969; Khatamsaz, 1992) (Figure 4).

Style with lateral to subbasal position is a synplesiomorphy for the subtribe *Fragariinae.* The terminal synapomorphic style in the subtribe *Potentillinae* [except for Argentina group (with lateral style)] is conspicuous and has been used for separating 4 subtribes (Sojak, 2008) (Figure 5).

Pinnate leaves are plesiomorphic states in Rosoideae subfamilies (Eriksson, 1998). However, parallel evolution of pinnate and triate leaves was obvious in *Fragariinae* (2 subclades (C2 and C4) (Figure 5). Our data revealed multiple gains and parallel evolution in pinnate, triate, digitate, and compound leaves (triate-pentate and digitate-pinnate). The species with similar leaves formed small monophyletic subclades, e.g., subclade C2 (triate), C3 (pinnate), C4 (C4a triate and C4b pinnate), B1 (pinnate), B2 (triate-pentate), B3 (triate-pinnate), B4 (digitate), and B5 (B5a and B5b digitate-pinnate) (Figure 5). This indicates that this character is useful for infrageneric grouping of the species. Presence of a compound leaf in subclades B2 (triate-pentate), B3 (triate-pinnate), and B5b (digitate-pinnate) reflexes the strong morphological integration discussed by Klingenberg (2012).

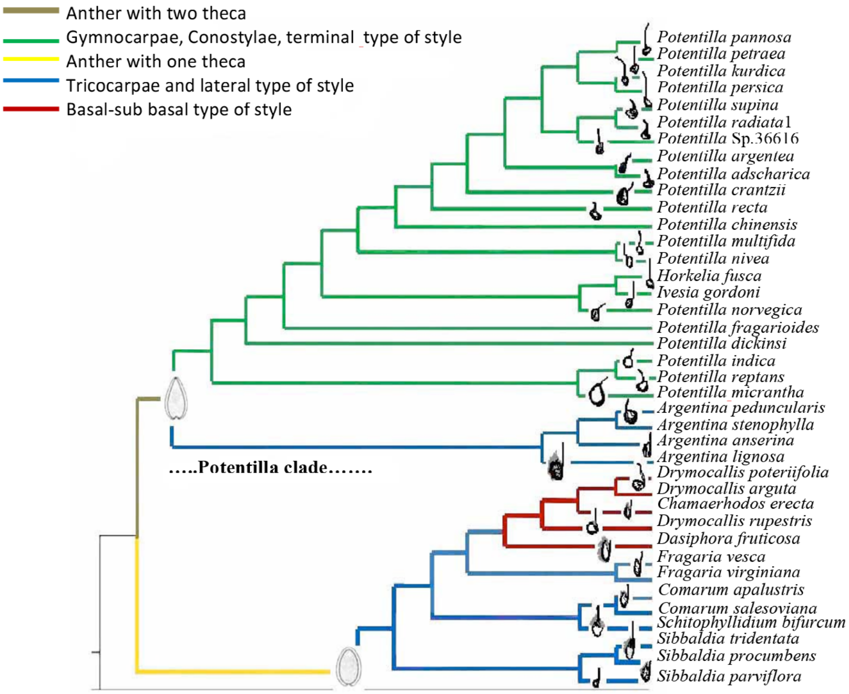

Figure 4. Character reconstruction (including: anthers structure, style position, length, and hairiness of achene, in *Potentillinae*, based on combined MP tree topology.

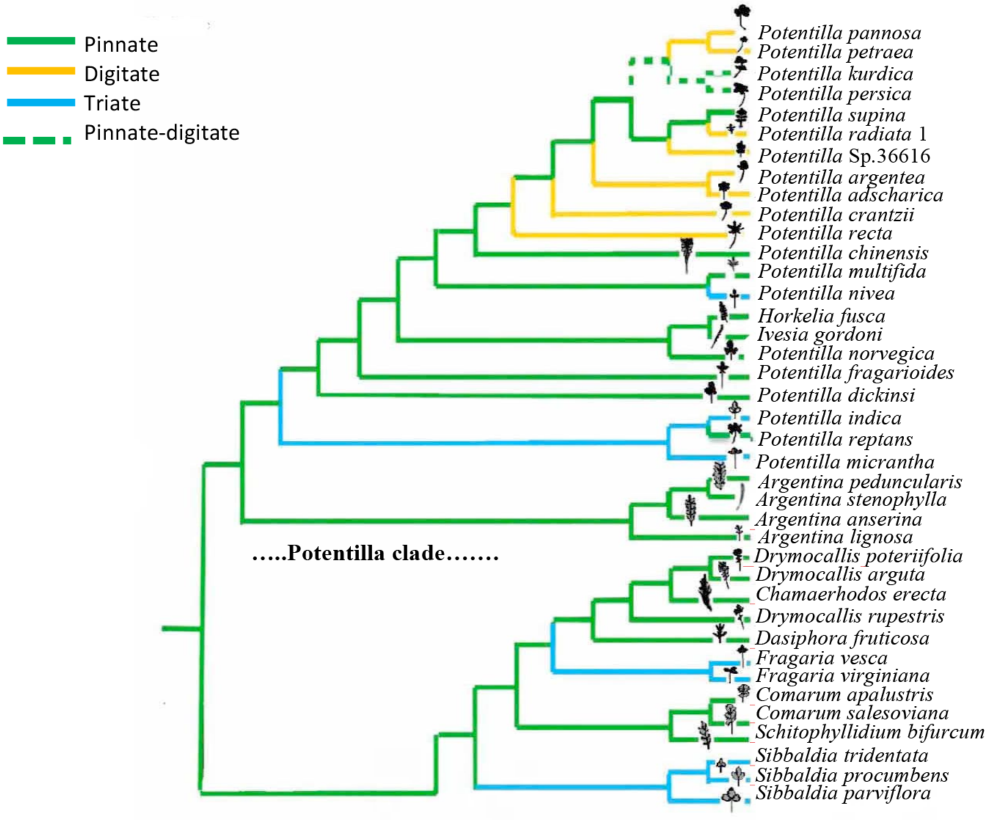

Figure 5. Leaf characters reconstruction in *Potentillinae*, based on combined MP tree topology.

In summary, our nrDNA and cpDNA phylogeny supports the major phylogenetic split within the tribe *Potentilleae*. It demonstrates that Iranian species of *Potentilla* are nested within the Potentilla core group, accompanied by 2 American genera, *Ivesia/Horkelia*, and the *Argentina* group. This confirms the previous molecular studies and supports taxonomic recognitions of some morphological traits that could be used for identification purposes at different taxonomic ranks. Independent and parallel evolutions were inferred for these diagnostic criteria.

Acknowledgments

The authors appreciate the support received from Guilan University authorities (University of Guilan, Rasht, Iran) to conduct this research. We acknowledge the contributions of SB Dalirsefat (Department of Sericulture, Faculty of Agricultural Sciences, University of Guilan, Rasht, Iran), P Potki (Department of Genomics, Agricultural Biotechnology Research Institute of Rasht, Guilan, Iran) and A Amirahmadi (Department of Plant Biology, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran) for their technical advice and help.

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