Morphological phylogenetic analysis of the genera *Fragaria* and *Duchesnea* in Iran

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Abstract

The phylogenetic relationships of the genera *Fragaria* and *Duchesnea*, and their closely related species are reconstructed using 17 morphological characters. Data matrix consisted of 11 taxa belonging to two species of *Fragaria*, two species of *Duchesnea*, two species of *Potentilla*, and also five species of *Drymocallis rupstris*, *D. poteriifolia*, *Geum urbanum*, *Rosa centifilia*, and *Filipendula vulgaris*. The two latter species were used as outgroups. The phylogenetic analysis was carried out using maximum parsimony (MP) and Bayesian analysis. The result showed that, *Fragaria* and *Duchesnea* are monophyletic taxa, and *Potentilla reptans* is the closest relative of *Duchesnea*. Two theca anthers, terminal to subterminal style and yellow petals are synapomorphic for *Duchesnea indica* and *Potentilla reptans*. In addition, six characters in *Fragaria* were genus specific. An identification key was also presented based on the studied morphological traits.

Keywords: Cladistics analysis, maximum parsimony, mock strawberry, related species, *Rosaceae*, strawberry

مطالعه فیلوژنتیک ریخت‌شناختی جنس‌های *Fragaria* و *Duchesnea* از تیره گل‌سرخیان در ایران*

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خلاصه

در این تحقیق، روابط فیلوژنتیکی دو جنس *Fragaria* و *Duchesnea* و گونه‌های خویشاوند وابسته نزدیک آن‌ها متعلق به تیره گل‌سرخیان با استفاده از ۱۷ صفت ریخت‌شناختی مورد بررسی قرار گرفت. ماتریس داده‌ها شامل ۱۱ آرایه متعلق به دو گونه *Fragaria*، دو گونه *Duchesnea*، دو گونه *Potentilla* و همچنین پنج گونه *Drymocallis rupstris*، *D. poteriifolia*، *Geum urbanum*، *Rosa centifolia* و *Filipendula vulgaris* می‌باشد. دو گونه اخیر، به عنوان برون‌گروه مورد استفاده قرار گرفتند. آنالیز داده‌ها به دو روش بیشینه پارسیمونی (MP) و بایزین مورد تجزیه و تحلیل قرار گرفتند. نتیجه نشان داد که *Fragaria* و *Duchesnea* جنس‌هایی تک‌نیا و گونه *Potentilla reptans* نزدیک‌ترین خویشاوند *Duchesnea* است. بساک دو حجره‌ای و خامه انتهایی تا تقریبا انتهایی از صفات مشترک پیشرفته برای دو گونه *Duchesnea indica* و *Potentilla reptans* هستند. همچنین، شش صفت تفکیک‌کننده جنس *Fragaria* تشخیص داده شدند. براساس صفات ریختی گونه‌های مورد مطالعه کلید شناسایی ارایه شده است.

واژه‌های کلیدی: بیشینه پارسیمونی، تحلیل کلادیستیک، توت فرنگی، توت فرنگی کاذب، گل‌سرخیان، گونه‌های خویشاوند

Introduction

Fragaria L. and *Duchesnea* Sm. are two representative genera of the tribe *Potentilleae* (Hutchinson 1964, Dobeš & Paule 2010, Soják 2008), under the subfamily *Rosoidea* (Morgan 1994, Eriksson *et al*. 1998, 2003, Potter *et al*. 2007) and the family *Rosaceae*. The two genera resemble each other on their common names (*Fragaria*: strawberry and *Duchesnea*: Mock strawberry, Indian strawberry or false strawberry) (Zare *et al*. 2007), and also taxonomic complexities bases in their history of classification. *Fragaria* comprises about 30 species (Soják 2008) world widely including herbaceous plants, distributed in the north temperate and holarctic zones (Rousseau-Gueutin *et al*. 2008). Primarily, Linnaeus (1753) introduced *Fragaria* as one of the five relevant genera (*Potentilla* L., *Comarum* L., *Fragaria* L., *Sibbaldia* L., and *Tormentilla* L.) (Eriksson *et al*. 1998). However, many changes were made to this classification. Adanson (1763) separated *Fragaria* and *Comarum* from the former group. Wolf (1908) isolated plant having white petal, ternate leaves in the genus *Fragaria.* The genus comprises three species (including: *Nubicola* Lind., *F. viridis* Weston and *F. vesca* L.) in both Flora Iranica (Schonbeck-Temesy 1969), and Flora of Iran (including: *Fragaria viridis*, *F. vesca*, and *F. × ananassa* (Weston) Duchesne ex Rozier (Khatamsaz 1993). Among them, *F. × ananassa* is a hybrid of *F. chiloensis* (L.) Mill. and *F. virginiana* Mill. (Purgar *et al*. 2017).

The genus *Duchesnea* consists of about six herbaceous representatives growing in East Asia and North America (Eriksson *et al*. 2003). These plants are similar to strawberry in terms of life form but differ by their inedible dry fruits. For this reason, Smith (1818) separated and classified them under the genus *Duchesnea*.

However, taxonomic delimitation of the genus is influenced by several controversies and often is in concurrent with that of *Fragaria* (Syn.: *Fragaria indica* Andrews 479: 1807), *Fragaria malayana* Roxb.

520: 1832), and *Potentilla* [Syn: *Potentilla trifida* Lehm. 263: 1851, *Potentilla indica* (Andrews) Wolf 661: 1904] (Wolf 1908, Bate-Smith 1968, Kalman 1988). Wolf (1908) placed *Potentilla indica* close to *P. reptans* under *Tormentillae* group. Juzepchuk (1941) and Schonbeck-Temesy (1969) classified it with short rootstock; creeping stems; ternate leaves, solitary flowers; toothed (3–5) episepals; yellow petals; 20–25 stamens; numerous ovaries, fruit not separating from hypanthium; lateral or nearly terminal styles in a distinct genus *Duchesnea*. In addition, Soják (2004) based on style position (terminal style) and anther structure (two-theca anther), placed *Duchesnea*, under the subtribe *Potentillinea*. His main aim of this study was to conduct a phylogenetic analysis of *Fragaria* and *Duchesnea* based on the morphological traits to identify important traits that can provide efficient identification key at generic and species level.

Duchesnea comprises one species in the Flora Iranica, reported from Afghanistan and Pakistan (Schonbeck-Temesy 1969). However, some researches confirm presence of *D. indica* in Mazandaran (Zare *et al.* 2003) and Guilan provinces (Pourebrahim *et al.* 2018) also. The most important researches conducted in the genus *Fragaria* are those of Bringhurst & Senanayake (1966), Harrison *et al.* (1997), Hancock (1999), Staudt *et al*. (2003, 2006), Darrow (1966), Oda & Nishimura (2009), Retamales *et al*. (2005), Rousseau-Gueutin *et al.* (2009), Olbricht *et al.* (2012), DiMeglio *et al.* (2014), and Dujmoví´c Purgar *et al*. (2017). However, regarding the genus *Duchesnea*, only a few researches have been performed (Parado *et al.* 2012, Naruhashi & Sugimoto 1996).

The present survey, however, tries to answer the following questions:
1. *Fragaria* and *Duchesnea*; are they monophyletic?
2. Which of the two genera *Fragaria* and *Potentilla* are the closest relatives of *Duchesnea*?

Materials and Methods

In the current survey, 17 morphological characters belonging to 11 taxa were used in the cladistics analysis. These evidences obtained through the current morphological analyses and several populations from different species were examined. This includes 23 population of the genus *Fragaria* (including 17 populations of *Fragaria vesca*, and six populations of *F*. *viridis*), two species of the genus *Duchesnea* (*Duchesnea indica* and *D*. *Chrysantha*), and *Potentilla reptans* and *P*. *micrantha* as their closely related species. To this, we added two species of *Drymocallis* (*Drymocallis rupstris* and *D*. *poteriifolia*), *Geum urbanum*, *Rosa centifolia*, and *Filipendula vulgaris*. The two latter species considered as outgroups. Taxa selection follows the sequences of Schonbeck Temesy (1969), Soják (2012), and previous molecular phylogenetic studies of Eriksson *et al*. (2003), Potter *et al.* (2007), Dobeš & Paule (2010), and Faghir *et al*. (2014). Both dry and freshly collected samples (during 2016–17) were used for morphological evaluation. The herbarium specimens deposited in the herbaria of the Guilan University (GUH), Natural Resources Research Center of Guilan province (GILAN), and Tehran University (TUH). The voucher specimens of newly collected materials were deposited in the Guilan University [GUH. For identification purpose, Schönbeck-Temesy (1969), Khatamsaz (1993), and Soják (2004, 2012) were used].

Species used in this survey are presented in table 1.

- Data analysis and cladogram construction

Phylogenetic reconstruction was inferred using both maximum parsimony (MP) and Bayesian methods.

- Maximum parsimony analysis (MP)

Phylogenetic analyses of the data matrix (Table 3) were carried out using maximum parsimony analysis (MP) incorporated in the PAUP Ver. 4.0b10 software program, installed in Windows (Swofford 2002). The polarity of characters was determined using the outgroup method (Maddison *et al*. 1984). Heuristic search settings followed by all characters 'unord' type, having equal weight. Starting trees obtained via stepwise addition, branch-swapping algorithm: tree-bisection-reconnection (TBR). Initial 'MaxTrees' setting = 100. 'MulTrees' option were turned on and steepest descent option were not affected. For character-state, optimization accelerated transformation (ACCTRAN) was performed. For improving, the trees' indices, successive re-weighting strategy (Farris 1969) were conducted. The reweighing of the traits continued until the trees' indices did not change in two separate analyzes after two replications. The bootstrap values (Felsenstein 1985) were calculated from 1000 and 20000 replicates. Re-scaled consistency index (RC), consistency index (CI), and retention index (RI) were also calculated.

Table 1. Plant samples used in the current study along with related data

Taxon	Collection data (all samples are from Iran)	Accession No.
1. *Fragaria*		
F. viridis	Mazandaran province: Haraz Road, 2.4.2011, Ebrahimzadeh	5779 (GUH)
F. vesca	Guilan province: Langeroud, Darvizhe, 13.4.2008	5794 (GUH)
2. *Duchesnea*		
Duchesnea indica	Guilan province: Pirbazar, 29.6.2015, Ashouri	5793 (GUH)
	Guilan province: Roodbar, 5.6.2011, Assadi	64085 (GILAN)
D. chrysantha	Tehran province: Campus of Tehran University, 9.8.2016, Hadirzadeh and Rahmani	46799 (TUH)
3. *Potentilla*		
Potentilla reptans	Guilan province: Siahkal, 16.6.2006, Faghir	36639 (TUH)
P. micrantha	Guilan province: Asalem to Khalkhal, 6.6.2006, Faghir	36602 (TUH)
4. *Drymocallis*		
Drymocallis rupestris	Mazandaran province: Firouzkooh, 12.6.2006, Attar	39000 (TUH)
D. poteriifolia	Chaharmahal-o-Bakhtiari province: Yasouj, 17.6.2006, Attar and Zamani	36674 (TUH)
5. *Geum urbanum*	Golestan province: 11 km to south Shahpasand, 10.5.1966, Papo	7521 (TARI)
6. *Rosa centifolia*	Guilan province: Rasht, 15.4.2017, Pourebrahim	7540 (GUH)
7. *Filipendula vulgaris*	Azerbaijan province: Kalibar, 21.6.1992, Ghahreman and Mozaffarian	17532 (TUH)

Table 2. Morphological characteristics used in the current cladistics analysis (abbreviations and character state are presented)

Character	Character state
1. Stem height	0 = Tall (over 20 cm) 1= Short (8–20 cm)
2. Stem type	0 = Erect, not stoloniferous 1 = creeping, stoloniferous
3. Leaf shape	0 = Pinnate, 1 = trifoliate, 2 = 5-foliate
4. Leaflets shape	0 = Middle leaflet oblong, acute, 1 = middle leaflet widely ovate, obtuse
5. Number of flower in each inflorescence	0 = Many, 1 = a few (2–4) , 2 = 1
6. Inflorescence type	0 = Not solitary (corymbiform, cyme) 1 = solitary
7. Direction of sepals in the mature fruits	0 = Ascending-erect, 1 = recurvate
8. Presence or absence of epicalyx (outer sepal)	0 = –, 1 = +
9. Epicalyx number of teeth	0 = Entire, 1 = forked, 2 = trifide
10. Receptacle enlargement after anthesis	0 = Receptacle protruding from calyx, 1 = receptacle not protruding from calyx
11. Receptacle type	0 = Dry or flesh but dries at ripening 1 = fleshy at ripening
12. Petal color	0 = White-yellow or pink, 1 = yellow
13. Pistil hairy or glabrous	0 = Hairy, 1 = glabrous
14. Anther type	0 = Single theca,1 = two theca
15. Filament type	0 = Not broadened, flat, 1 = broadened, flat
16. Style position	0 = Lateral, 1 = terminal
17. Fruit type	0 = Follicle, 1 = achene, 2 = fleshy aggregate

Table 3. Data matrix of studied species in the present study

Taxon	Character																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1. *Fragaria*																	
Fragaria viridis	1	1	1	0	1	0	0	1	0	1	1	0	1	0	1	0	2
F. vesca	1	1	1	0	1	1	1	1	0	1	1	0	0	0	1	0	2
2. *Duchesnea*																	
Duchesnea indica	1	1	1	0	2	1	0	1	2	0	0	1	1	1	0	1	1
D. chrysantha	1	1	1	1	2	1	0	1	2	0	0	1	1	1	0	1	1
3. *Potentilla*								1									
P. reptans	1	1	2	0	1	1	0	1	0	0	0	1	1	1	0	1	1
P. micrantha	1	0	1	0	1	0	0	1	0	0	0	0	0	1	0	1	1
4. *Drymocallis*																	
Drymocallis rupestris	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1
D. poteriifolia	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1
5. *Geum urbanum*	0	0	0	0	0	0	0	1	0	0	0	1	0	?	0	1	1
6. *Rosa centifolia.*	0	0	0	0	0	0	0	0	0	0	0	1	0	?	0	0	1
7. *Filipendula vulgaris*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0

- Bayesian method

Bayesian analyses were performed by MrModeltest Ver. 2.3 programs (Nylander 2004). The discrete approach to the morphological data matrix was adopted. All 17 characters were treated as discrete variables and treated as ordered (Table 1). Lewis (2001) bases the model used by MrBayes for morphological (standard) discrete data on the Markov model. Secondary probabilities were also determined. Analyses were repeated for several million generations and MrBayes performed two 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 linear phase (3 million generations), burn-in period samples (including 25% of initial trees sampled) were removed and a tree with maximum 50% (majority rule consensus tree) was plotted. The values of posterior probability (PP) were measured and the final tree was plotted.

Results

The phylogenetic analysis based upon equally weighted characters resulted 100 most-parsimonious trees of 26 steps with consistency index (CI) = 0.8889 and retention index (RI) = 0.9189, Homoplasy index (HI) = 0.1308 and Rescaled consistency index (RC) = 0.8069. All characters were parsimony-informative. The strict consensus tree of obtained from morphological characters was shown in figure 1. It comprises one independent clade of *Filipendulla vulgaris* and two lower small monophyletic groups (A and B) at the base of the tree. Group A includes *Rosa centifolia* and *Geum urbanum* and group B comprises two species of *Drymocallis* species. Species of *Fragaria*, *Duchesnea*, and *Potentilla* were united in a monophyletic group (O), from which two clades (O1 and O2) are derived. In clade O1 (with BP = 71%), *Duchesnea indica* and *D. chrysantha* formed a monophyletic group, and *P. reptans* and *P. micranhta* formed independent paraphyletic clades while clade O2 (with BP = 94%) comprised a monophyletic group of *F. viridis* and *F. vesca*.
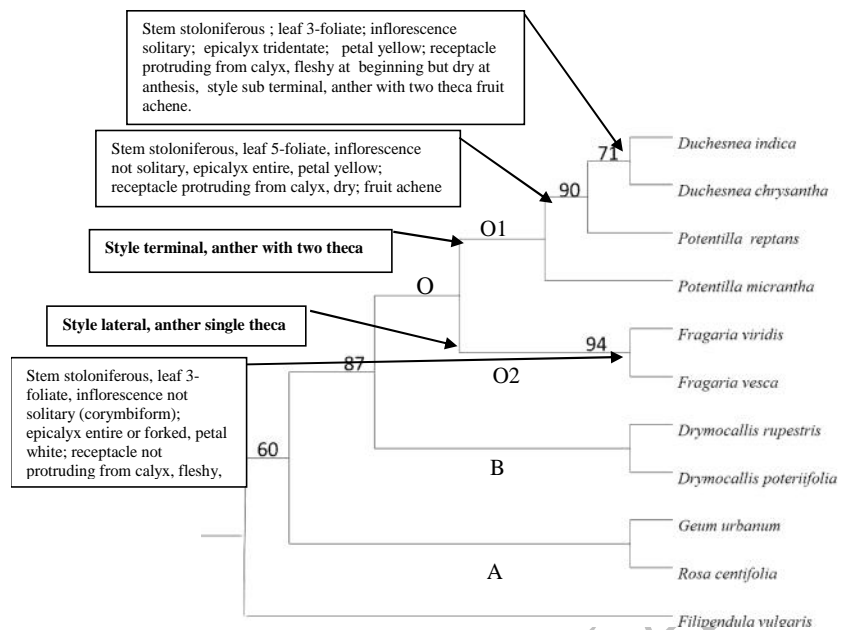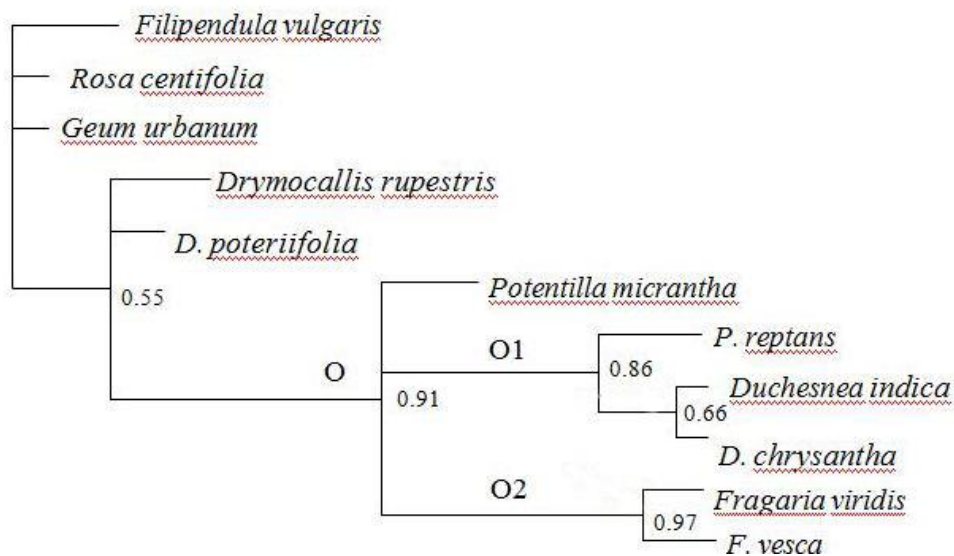
Fig. 1. A strict consensus tree obtained from 100 most parsimony trees of 17 morphological traits. Numbers above branches are bootstrap supports over 50%. Consistency index (CI) = 0.8889, and retention index (RI) = 0.9686. Clades characteristic features indicated in squares.

Fig. 2. Fifty percent majority-rule consensus tree obtained from a Bayesian analysis 17 morphological characters. Posterior probability (PP) indicated near each branch.

In the Bayesian analysis, 50% majority rule consensus tree was obtained (Fig. 2). At the base of this tree, *Filipendula vulgaris*, *Geum urbanum*, *Rosa centifolia*, *Drymocallis rupstris*, and *D. poteriifolia* formed paraphyletic independent branches while species of *Fragaria*, *Duchesnea*, and *Potentilla* units are in a monophyletic group (O).

The representatives of this group form a tritomy including three species of *Duchesnea indica*, *D. chrysantha*, and *Potentilla reptans* in group Q1, two

species of *Fragaria vesca* and *F. viridis* in group O2, and *P. micrantha* in an independent branch.

Discussion

In the present cladistics analysis, the two species of *Drymocallis*, three species of *Geum urbanum*, *Rosa centifolia* and *Filipendula vulgaris* formed either monophyletic groups (in MP tree, Fig. 1) or paraphyletic independent branches (in Bayesian tree, Fig. 2). This corresponds to synplesiomorphic morphological characters such as stem height and type, pinnate leaf, more number of flower, and inflorescent not solitary (characters Nos 1–7, 10–11, 14 and 15) shared between these species.

The present phylogenetic analysis based on morphological traits showed monophyly of the two genera *Fragaria* and *Duchesnea*. This result supports former molecular researches (Eriksson *et al.* 2003, Töpel *et al.* 2011). The two genera have identical stem height and type (characters Nos 1 and 2), leaf shape (character No. 3, state 1), and direction of epicalyx (character No. 7, state 1). Using these evidences, some previous studies (Schonbeck-Temesy 1969, Juzepchuk 1941) classified the two genera under subfamily *Rosoideae*. However, based on Soják's (2004, 2012) morphological survey, *Fragaria* and *Duchesnea* have been separated in to two distinct subtribes (*Fragarinea* and *Potentillinea*), for having two theca anther (character No. 14, state 1) and subterminal style (character No. 16, state 1). The current result is in agreement with former molecular researches (Dobes & Paule 2010, Faghir *et al.* 2014, Zhang *et al.* 2017, Xiang *et al.* 2016, Heo *et al.* 2019).

Fragaria is characterized by its six specific characters as following: 3-foliate leaf (character No. 3, state 1), inflorescence not solitary (corymbiform) (character No. 6, state 0), entire or forked epicalyx (character No. 9, state 1), white petal (character No. 12, state 0), receptacle protruding from calyx (character No. 10, state 1), and fruit fleshy aggregate (character No. 17, state 2). The genus composed of euploid chromosome numbers form diploid (2n=2x=14), tetraploid (2n=4x

=28), hexaploid (2n=6x =42), octoploid (2n=8x=56), and decaploid (2n=10x=70 (DiMeglio *et al.* 2014). Among them, *Fragaria vesca* is self-compatible, sympodial-running diploid, native from northern Europe and North American continent (Hummer *et al.* 2011). The later species, in Europe overlaps in distribution with another diploid, *F. viridis*, resulting a hybrid species (*F.* × *bifera*) (Staudt *et al.* 2003). However, direction of sepals in the mature fruits (character No. 7) and hairy or glabrous pistil (character No. 13), are importance characters isolating *F. vesca* from *F. viridis*.

In this analysis, *Duchesnea* with its terminal to subterminal style (character No. 16, state 1); two-theca anther (character No. 14, state 1), yellow petal (character No. 12, state 1), dentate epicalyx (character No.9, state 1), solitary flower (character No. 6, state 1), and positioned far from *Fragaria* and units with *Potentilla* species in O1 clades in both MP and Bayesian trees. The result supports previous molecular analyses based on both nrDNA ITS and chloroplast *trn*L-F sequence data studies (Eriksson *et al.* 2003, Faghir *et al.* 2014). Two theca anthers, terminal to subterminal style and yellow petals are the most informative characters that put the two species *D. indica* and *P. reptans* close to each other. These two characters are synapomorphic for both species. Adding *D. chrysantha* to the present analysis resulted a small monophyletic group composed of *Duchesnea indica* and *D. chrysantha*. The two morphologically species are very similar (Debes *et al.* 2011), but they can be recognized based on their receptacle enlargement (character No. 10), and middle leaflet shape (character No. 4). *Duchesnea indica*, is originated from India with 2n=84, while *D. chrysantha* has been reported in Japan, China, India, Korea, Taiwan, Philippines, and Indonesia, having chromosome number of 2n=14 (Naruhashi *et al.* 1999).

In conclusion, the current phylogenetic morphological based study revealed the phylogenetic relationship between two genera of *Fragaria* and *Duchesnea*. It was shown that, *Fragaria* and *Duchesnea* are monophyletic taxa and *Potentilla reptans* is the

closest relative of *Duchesnea*. Among the studied evidences, anthers structure, style positions are synaphomorphic traits that clearly separates the two genera *Duchesnea* and *Potentilla* from *Fragaria*.

Identification key based on studied morphological traits for *Fragaria* and *Duchesnea*, and their allied species

1. Anthers with two thecae, style terminal to subterminal …………….……………….…………….……..…..…..……………..… 2

   Anthers with one thecae, style lateral …………..…………….……………………..…..……………..……….……….…….. 5

2. Leaf trifoliate, episepals tridentate ………..…………….…………….…………………………….…………………………..… 3

   Leaf 3- and 5-foliate, episepals entire …………..….…………………….……………….…………………….… 4

3. Receptacle not protruding from calyx, middle leaflet -oblong, acute ….…..…….……….………..… *Duchesnea indica*

   Receptacle protruding from calyx, middle leaflet widely ovate, obtuse …………...…….….…………..… *D. chrysantha*

4. Stem not stoloniferous, leaves tri-foliate, petal white …………..…………….……….....….……….… *Potentilla micrantha*

   Stem stoloniferous leaves 5-foliate, petal yellow ………….…………..….…..………….……..…….……….………..… *P. reptans*

5. Sepals in mature fruit ascending-erect …………..………………….……...…..….………………..… *Fragaria viridis*

   Sepals in mature fruit recurved below …………..……………….…………….……………………….… *F. vesca*

Acknowledgments

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