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TAXONOMIC IMPORTANCE OF FLOWER MORPHOLOGY IN PERENNIAL SPECIES OF TRIGONELLA L. (FABACEAE) IN IRAN

M. Aghaahmadi, H. Saeidi & M. Assadi

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Determining the boundaries of genera in subtribe Trigonellinae Small has been one of the most controversial taxonomic issues in this field in last 100 years. Proposing the many characters for separating these genera by various botanists can be considered as a reason for this claim. Characters of the flower parts as well as other vegetative traits have been used extensively. Here on the basis of 5 qualitative and 19 quantitative flower characters we separated *Trigonella* species (especially perennial section *Ellipticae* (Boiss.) Sirj.) from other genera of this subtribe and showed the results in dendrogram and scatter plot. Placement of the Medicagoid species of section *Bucerates* (Boiss.) Sirj. and *Medicago* L. in one branch and presence of *Melilotus indicus* (L.) All. along with other *Trigonella* species (except section *Ellipticae* as a distinct branch) are the main results of this investigation.

Majid Aghaahmadi & Hojjatollah Saeidi (correspondence <ho.saeidi@sci.ui.ac.ir>), Faculty of Science, University of Isfahan, Isfahan, P. O. Box 81746-73441, Iran. -Mostafa Assadi, Research Institute of Forests and Rangelands, Agricultural Research, Education and Extension Organization (AREEO).

Key words: Phenetic analysis; Fabaceae; Trifolieae; character evolution; PCO analysis; UPGMA method

اهمیت تاکسونومیکی ریخت‌شناسی گل در گونه‌های چند ساله شنبلیله (Trigonella) در ایران

مجید آقااحمدی: دانشجوی دکتری، گروه زیست‌شناسی، دانشکده علوم، دانشگاه اصفهان، اصفهان، ایران

حجت‌اله سعیدی: دانشیار، گروه زیست‌شناسی، دانشکده علوم، دانشگاه اصفهان، اصفهان، ایران

مصطفی اسدی: استاد، مؤسسه تحقیقات جنگلها و مراتع کشور، سازمان تحقیقات و آموزش و ترویج کشاورزی، تهران، ایران

تعیین محدوده سرده‌ها در زیرتباره Trigonellinae Small یکی از بحث برانگیزترین موضوعات تاکسونومیکی در این زمینه در حدود ۱۰۰ سال گذشته بوده است. طرح صفات جدا کننده متعدد توسط گیاه‌شناسان مختلف موید این ادعا می‌باشد. صفات مربوط به قطعات گل به خوبی دیگر ویژگی‌های رویشی به طور گسترده‌ای تاکنون مورد استفاده قرار گرفته است. در این مقاله براساس ۵ صفت کیفی و ۱۹ صفت کمی مربوط به قطعات گل گونه‌های متعلق به سرده Trigonella (به طور خاص گونه‌های چندساله متعلق به بخش Ellipticae) از دیگر سرده‌های این زیر تباره جدا شده است و نتایج به دو شکل طرح درختی و طرح پراکنده (Scatter plot) نمایش داده شده است. قرارگیری گونه‌های شبه یونجه (Medicagoid) مربوط به بخش Bucerates و یونجه در یک شاخه و حضور گونه Melilotus indicus به همراه سایر گونه‌های یکساله شنبلیله در یک شاخه مجزا از نتایج اصلی این مطالعه می باشند.

INTRODUCTION

Trifolieae as a tribe in Fabaceae family was introduced for the first time in 1865 by Bentham and is comprised of two subtribe Trifolinae (including of genus *Trifolium* L., *Ononis* L., *Parochetus* Buch.-Ham. ex D. Don) and subtribe Trigonellinae (consisting of *Trigonella*, *Medicago*, *Melilotus* Mill.). Nowadays this kind of division is current among the taxonomists. There are some disagreements about the relationship between the three genera of subtribe Trigonellinae. *Melilotus* was introduced under genus *Trifolium* by Linnaeus (1753). According to the shape of fruit (spiral

form for *Medicago* and non-spiral form for *Trigonella*), he placed 19 species in the first genus and 4 species in the second one. Sering (1825) put both genera *Melilotus* and *Trigonella* in section *Grammocarpus* Ser. of *Trigonella*. In addition to approving the standard of Linnaeus (shape of fruit) about delimitation between *Medicago* and *Trigonella*, also he introduced *Pocockia* Ser. ex DC. (with samara fruit) in this group. Trautvetter (1841) believed that no clear delineation between species of this group can be done. In a study on *Medicago*, he defined this genus as a plant which has fruit with many seeds, without wing and beak. Also in his opinion, *Pocockia* was defined as a genus that its fruits have membranous wings and few seeds and *Trigonella* has fruits with beak and without wings. But his boundary between *Medicago* and *Melilotus* was based only on the number of seed in the fruits (3 or more seeds in *Medicago*). Urban (1873) was the first who considered to a feature except of fruit's characters for separation of these genera. He proposed absence of swollen base of cotyledon (pulvinate) in *Medicago* and presence of this state in *Trigonella* and *Melilotus* as the deference between genera in this subtribe. Following the more extensive studies, Small (2010) accepted this criterion as a splitting tool and also he introduced the state of pulvinate cotyledon as primary character state which is disappeared in many species of *Medicago*. Sirjaev (1928-1934) as a main monographer of genus *Trigonella* accepted the comments of Urban about absence of pulvinate cotyledon in *Medicago*. He also placed some *Trigonella* species with similar flower structure to *Medicago* and pulvinate cotyledon in a special group under Medicagoid *Trigonella*. However, he was in agreement to transitional condition of this group but he refrained from commenting decisive in this respect.

According to phylogenetical studies on the basis of molecular marker, *Medicago* with Medicagoid species that have been placed in *Trigonella* as a monophyletic group has been separated from *Trigonella* and *Melilotus* but relationship between these two genera is still controversial (Bena 1998; Wojciechowski 2000; Steele & al. 2003; Wojciechowski 2003 & Khandani & al. 2016b).

In the following three genera in this subtribe, *Trigonella* with one section (near 40 species) and *Medicago* with 5 sections (near 20 species) comprise perennial members of this group. Current distribution of perennial species of *Trigonella* is in central Asia, Afghanistan and Iran. The Zagros Mountains in Iran, can be attributed as western limit of its distribution. While perennial species of *Medicago* (except *M. sativa* L. that has wide distribution), other annual and biennial species of this subtribe are distributed commonly in east of Mediterranean Sea, Black Sea and Caucasian Region (Greuter & al. 1984; Small 2010).

Because of various breeding system, in subtribe Trigonellinae several types of flowers are observed. All species of *Medicago* use the explosive tripping mechanisms for pollination, which this syndrome has been recognized as a distinguishing feature for this genus in Trigonellinae (Small 1990). In order to optimize the breeding system, numerous adaptations in flower structure have occurred and a set of characteristics associated to these mechanisms have been evolved.

In Trigonellinae a set of flower characters can be applied for separation of its genera and also these features are applicable for determining the position of problematic sections between genera *Trigonella* and *Medicago* (fig. 1). Some of these traits (fig. 2) and other characters of seeds and vegetative parts were applied in previous studies (Baum 1968; Small 1986 & 1989a, 2010; Small & al. 1987, 1989b, 1990 & Khandani & al. 2016a).

In the present study, a phenetic analysis were performed to determine the taxonomic value of flower characters for species and section delimitation in this subtribe with special emphasis on perennial section of *Trigonella*. Also some discussions about reproductive feature changes in this subtribe have been presented.

MATERIALS AND METHODS

Plant materials

Near the 100 individuals from 20 species were collected from various area of Iran during 2012-2016. According to available morphological keys the specimens were determined (Rechinger 1984; Boissier 1872). All of these samples are deposited in TARI (acronym according to Thiers 2015) and HUI (Herbarium of University of Isfahan). Also additional herbarium materials of these herbaria were studied.

Phenetic analysis

Seven species of section *Ellipticae* as main group, five annual species of *Trigonella* with seven species of *Medicago (*including *Medicago* and transferred Medicagoid species and one species of *Melilotus* as outgroup, were used as operational taxonomic units (OTUs). Twenty four qualitative and quantitative flower characters were analyzed (tab. 1). Generated data matrix was analyzed using NTSYS pc version 2.02 (Rohlf 2000) and MVSP version 3.1 software. The similarity matrix was used for cluster analysis and constructing a dendrogram using the unweighted pair group method (UPGMA) and principal coordinate analysis (PCO) to show the relationships among the taxa.

Fig. 1. Comparative shapes of flower parts in *Trigonella*, *Melilotus* and *Medicago.*
T. elliptica (I-a, II-a, III-a, IV-a & V-a); *T. spruneriana* Boiss. (I-b, II-b, III-c and V-d); *T. monspeliaca* L. (I-c, II-d, IV-c and V-c); *Medicago monantha* (C. A. Mey) Trautv. (I-e, II-e, III-f, IV-e and V-e); *Medicago lupulina* L. (II-g and III-d) and *Medicago persica* (I-f, II-h, III-e and V-f); *Medicago sativa* (I-g, II-f, III-g, IV-d and V-g) and *Melilotus indicus* (I-d, II-c, III-b, IV-b and V-d). (All presented shapes are schematic and without scale).

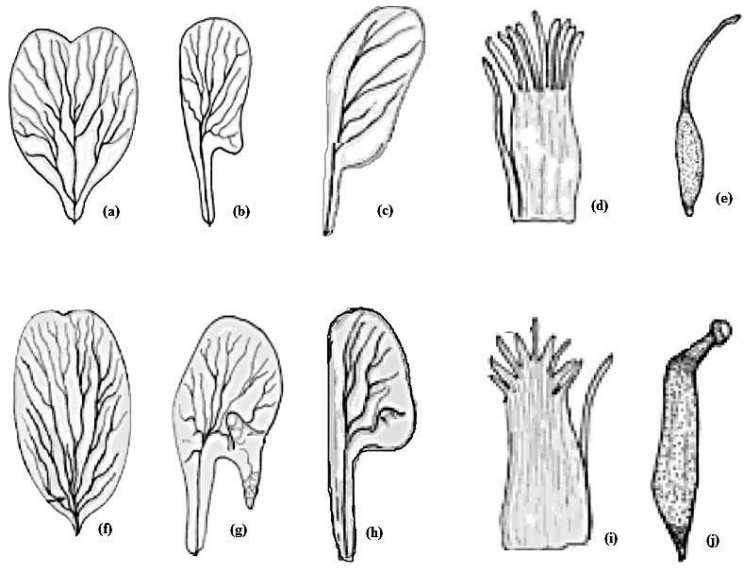

Fig. 2. Current shapes of flower parts in *Trigonella* and *Melilotus* (a-e) and *Medicago* (f-j). (According to Small, 2010 with modifications).

Table 1. List of qualitative and quantitative characters.

	Qualitative characters	
	Character	Character state
1	Type of standard venation	three main veins at the base of standard lead to cluster of veins in the top (1) / same distribution of veins (2)
2	Horn in wings	absence (1) / Presence (2)
3	Type of stamen tube tip	Trigonella type (1) / Medicago type (2)
4	Trichome on ovary	Presence (1) / Absence (2)
5	Type of stigma	Regular (1) / Fungoid (2)

	Quantitative characters*		
6	Standard length	16	Keel width
7	Standard width	17	Keel length/ width
8	Standard shape	18	Keel stalk length
9	Wing length	19	Keel length/stalk length
10	Wing width	20	Stamen length
11	Wing length/width	21	Pistil length
12	Wing stalk	22	Pistil stalk length
13	Wing length/Wing stalk	23	Style length
14	Wing trigger	24	Pistil / style length
15	Keel length		

* The measured values of the quantitative characters were normalized according to the standard formula () $\frac{xi - \min(x)}{\max(x) - \min(x)}$ between 0-1 and then acquired amounts were divided into four groups ($x < 0.25$, $0.25 \leq x < 0.5$, $0.5 \leq x < 0.75$ and $x \geq 0.75$) and then scored.

RESULTS

Character analysis of flowers

Continuous flower characters in Trigonellinae have important role in taxonomy of this group and in several studies have been proposed. In perennial species of *Trigonella* like other species of this genus, these features almost are repeated. The venation of standard with three main veins *vs*. same distribution of the venation, absence of the horn *vs*. presence of horn in the wing, staminal tube with truncate apex *vs*. arched or obtuse apex of the staminal tube, regular form of stigma *vs*. fungoid form of it, which all of them have significant roles in the explosive tripping mechanism of pollination (Small 1987), are the main aspects of differences among *Trigonella* and *Melilotus* to *Medicago*.

Standard

Despite to many features like color and diameter of the standard petal which can be detected, in this study three discrete characters (blade length, width and ratio between them) and two continuous traits (form of the standard blade and type of the standard venation) were analyzed. The standard venation and shape of the standard have significant role in the separation of *Trigonella* and *Medicago*. Average of these ratios in members of this group is near to each other. So that this quantity in section *Ellipticae* (SE), other *Trigonella* species (OTS) and *Medicago* species (MS) are 1.5319, 1.9098 and 1.8269 respectively. These results show the shape of the standard in SE is almost orbicular to sub-orbicular whereas in OTS and MS, standard is elliptical. Also variance of this character in SE (0.1652), OTS (0.2735) and MS (0.1415) indicates relative stability of it. The standard venation type is the other aspect of difference. In all species of *Trigonella* and *Melilotus* three main veins at the base of the standard lead to cluster of veins in the top, while in *Medicago* the venation is regular (fig. 2a & f).

Table 2. Average and variance amounts of quantitative characters of flower parts in three studied group; SE (*Trigonella* section *Ellipticae*), OTS (other *Trigonella* species (annual species)) and MS (*Medicago* species).

	SE		OTS		MS		All species
	Ave.	Var.	Ave.	Var.	Ave.	Var.	Var.
Standard							
Length of blade	8.5730	1.3319	4.1166	0.6847	5.8375	6.7023	4.1809
Width of blade	5.8250	1.5072	2.4333	1.1022	3.0875	1.2385	2.9460
Ratio of length to width of blade	1.5319	0.1652	1.9098	0.2735	1.8269	0.1415	0.1719
Wing							
Length of blade	8.2365	1.4934	4.00	1.040	4.65	3.9325	4.2523
Width of blade	1.7884	0.1406	0.75	0.0891	1.40	0.4075	0.2670
Ratio of length to width of blade	4.7779	1.0698	6.1960	7.5519	3.3520	0.6614	1.9350
Length of stalk	2.5173	0.2156	1.50	0.1514	2.2625	4.1198	0.7014
Length of trigger	0.9250	0.0426	0.20	0.0033	1.050	0.5975	0.1514
Keel							
Length of blade	7.6115	1.0306	3.0666	2.7422	4.1125	5.7410	3.0445
Width of blade	3.5173	0.4483	1.0166	0.3613	1.050	0.2575	1.2099
Ratio of length to width of blade	2.2293	0.2319	2.6095	1.7379	3.3224	2.4820	3.0611
Length of stalk	2.7173	0.2260	1.1833	0.3747	2.150	1.6425	0.4485
Staminal tube							
Length of tube	8.0403	1.4851	3.5333	1.3055	4.4857	5.7410	4.4270
Ovary							
Length of body	8.3961	1.6553	3.950	1.0458	4.7714	2.3755	4.6820
Length of stalk	0.9326	0.3564	0.0666	0.1222	0.1142	0.097	0.3954
Length of style	2.8346	1.2788	1.3333	1.3522	1.1428	0.5338	1.6266

Characters of the wing

Wing has key role in the pollination. Thereby one of the highest variations in the flower traits of Trigonellinae is related to characters of the wing. In this study six characters of the wing were evaluated. The blade length, width and ratio between them, which represents the shape of wing blade, have evolutionary and taxonomic values. Average quantity of the third

character in SE is (4.7779), in OTS is (6.1960) and in MS is (3.3520) which shows the elliptical or sub-orbicular form in *Medicago* and the oblong form in other members. Length of the wing stalk is the other significant feature. In SE average length of the wing stalk (2.5173 mm) is longer than OTS (1.50 mm) and MS (2.2625 mm). When these amounts are compared with the length of the wing blade, it can be favorable for separation of *Trigonella* and *Medicago*. Developed trigger is another main difference among this group that can divide it to three parts. The importance of trigger diameter can be understood when comparing to the wing blade diameter. According to this ratio, the largest trigger size is seen in MS and then in SE and OTS. The presence of the horn in *Medicago* and its allies is another very important character which is not seen in SE and OTS. This appendage led to attach the wing to the keel. Tall stalk of the wing like the presence of the horn and developed trigger are associated to the explosive tripping in *Medicago* species (Small 2010).

Characters of the keel

This part of the petal represents various adaptations in different groups of this subtribe. In this study four discrete characters have been analyzed including length and width of the blade, the ratio of length to width of the blade and length of the stalk. Length of the blade in SE has maximum amount of average with 7.6115 mm which is clearly different from the others (OTS with 3.0666 mm and MS with 4.1125 mm). This shows different shape of keel in perennial species of *Trigonella* and *Medicago*. Furthermore, the presence of deep incision in top of the keel in *Medicago* species is a diagnostic character which has a key role in pollination system of these plants (Small 2010).

Characters of the staminal tube

One of the main criteria for distinguishing *Trigonella*, *Melilotus* and *Medicago* is the staminal tube traits (Baum 1968; Small 2010). In this study two characteristic features are analyzed; length of the staminal tube with average amount in SE (8.0403 mm) more than twice as much as in OTS (3.5333 mm) and MS (4.4857 mm) and apical shape of the staminal tube separate *Trigonella*, *Melilotus* and *Medicago*. This feature is also related to explosive tripping in *Medicago* species.

Characters of the ovary

The features of ovary have determining role in separation of genera in this subtribe. Different aspects of these features can be considered. Here five characters are surveyed in which length and form of the style (straight form of the style in SE and OTS *vs*. spiral form of the style in MS), the form of stigma (regular stigma type in SE and OTS *vs.* fungoid stigma type in MS) have indicative design. Also length of the ovary body like length of the staminal tube in SE (with average 8.3961 mm) is more than twice as much as the others (0.0666 mm and 0.1142 mm in average amount in OTS and MS respectively).

DISCUSSION

Investigation of various markers in *Trigonella* offers several evidences for parallel evolution in this genus (Steele & Wojciechowski 2003; Steele & al. 2010; Gazara & al. 2001 & Dangi & al. 2016). Perennial species of *Trigonella* have sufficient characteristic traits for separation from the remaining annual species as a monophyletic section. In addition to distinctive speciation and diversity center and perennial habit, special inflorescence traits are seen in section *Ellipticae* which have been considered as basic character state in Trigonellinae. In this subtribe four inflorescence types can be noted: panicle raceme as ancestral state, solitary type in section *Gladiatae*, umbellate type and capitated type as an evolved state (Feng & al. 2011; Harris 1999).

Beside of these traits, some exclusive characters can be defined for this perennial group. Distinguishable average size of the standard blade, the wing blade, the keel blade, the stamen tube and body of the ovary with low ingroup variances show stability of these features for taxonomy of this group. While high variance of these traits in whole subtribe can be interpreted as diagnostic character of them.

In this regard PCO analysis represented three main groups with adequate distances (fig. 3). In this plot species of section *Ellipticae* are placed in farthest distance from *Medicago*. Other *Trigonella* species occupied a position between these two groups. Occurrence of Medicagoid species near *Medicago sativa* L. and *Melilotus indicus* in the neighboring of annual species of *Trigonella* confirm the molecular evidences (Steele & al. 2003, Wojciechowski & al. 2004, Dangi & al. 2016).

In other phenetic analysis, relationships among the species are evaluated by UPGMA in clustering method (fig. 4). Similar to PCO analysis, perennial species of *Trigonella* from a distinct branch but characters of the flower parts in this group are not sufficient for separation of perennial species of *Trigonella* from each other and it shows we need the other vegetative and pod features for this goal. Other annual species of *Trigonella* organize sister group for section *Ellipticae*. Other branch of dendrogram includes *Medicago sativa* and Medigoid species. Thereby transferring of

Medicagoid group to *Medicago* as well as close relationship between *Melilotus indicus* and *Trigonella* species are confirmed again.

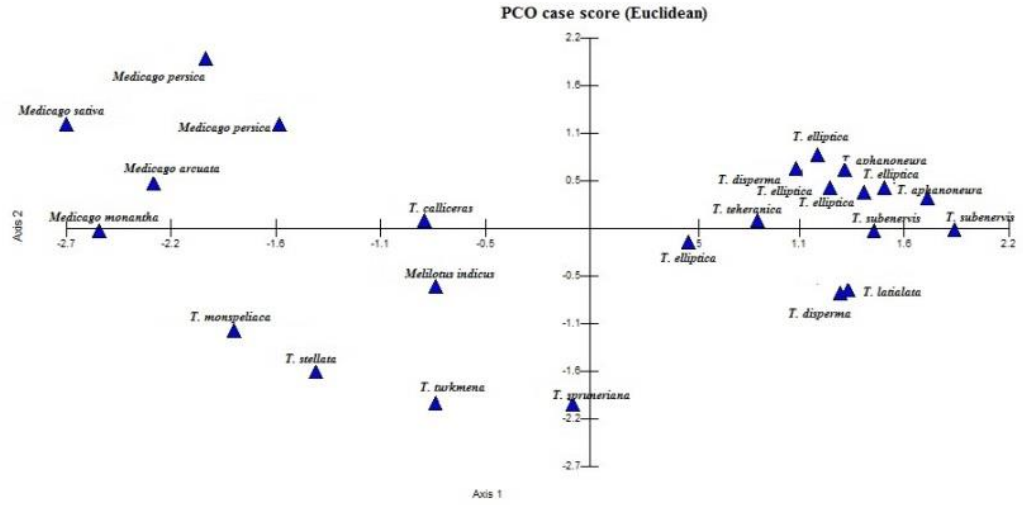

Fig. 3. PCO plot on the basis of flower characters.

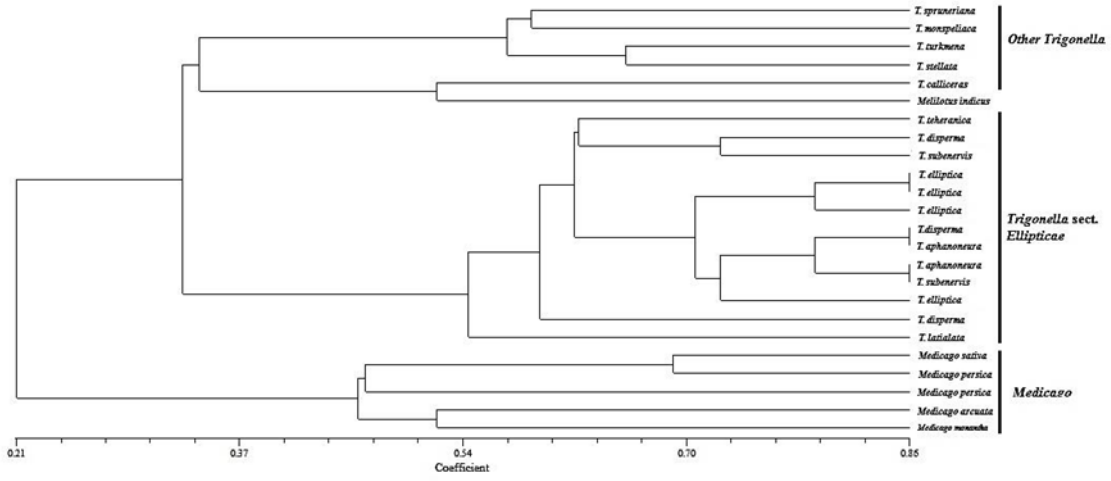

Fig. 4. UPGMA dendrogram according to the flower parts features.

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REFERENCES

Baum, B. R. 1968: A clarification of the generic limits of Trigonella and Medicago. -Canadian Journal of Botany. 46 (6), 741-749.

Bena, G., Jubier, M. F., Olivieri, I. & Lejeune, B. 1998: Ribosomal external and internal transcribed spacers: combined use in the phylogenetic analysis

of Medicago (Leguminosae). -Journal of Molecular Evolution. 46 (3): 299-306.

Bena, G. 2001: Molecular phylogeny supports the morphological based taxonomic transfer of the ‘‘medicagoid’’Trigonella species to the genus Medicago L.. -Plant Systematics and Evolution. 229: 217–236

Bentham, G., Hooker, J. D. 1865: Genera Plantarum. Vol. 1, part 2. -London.

Boissier, P. E. 1872: Trigonella. In: Boissier, P. E. (Ed.) Flora Orientalis 2: 74–91. –Genevae Basileae.

Dangi, R., Tamhankar, S., Choudhary, R. K. & Rao, S. 2016: Molecular phylogenetics and systematics of Trigonella L. (Fabaceae) based on nuclear ribosomal ITS and chloroplast trnL intron sequences. -Genetic Resources and Crop Evolution. 63 (1): 79-96.

Feng C. M., Xiang Q. A., Franks R. G. 2011: Phylogeny-based developmental analyses illuminate evolution of inflorescence architectures in dogwoods (Cornus s. l., Cornaceae). -New Phytol. 91:850–869.

Fenster, C. B., Armbruster, W. S., Wilson, P., Dudash, M. R. & Thomson, J. D. 2004: Pollination syndromes and floral specialization. -Annual Review.of Ecology, Evolution.and Systematics. 35: 375-403.

Gazara, M., Kamel, W. & Haider, A. 2001: Cladistic analysis of the genera: Trifolium, Trigonella and Melilotus (Fabaceae: Papilionaceae) in Egypt. Magda Gazara, Wafa Kamel and Ashraf Haider. – In Proceedings of the First International Conference (Egyptian British Biological Society, EBB Soc). Vol. 3: 161-170.

Greuter, W., Burdet, H. M. & Long, G. 1984: Med-checklist. Secrétariat Med-checklist. -Botanischer Garten & Botanisches Museum Berlin-Dahlem.

Harris, E. M. 1999: Capitula in the Asteridae: a widespread and varied phenomenon. -The Botanical Review. 65 (4): 348-369.

Khandani S., Assadi M., Nejadsattari T. & Mehregan I. 2016a: Phenetic analysis of genera Medicagoid Trigonella, Medicago and Melilotus (Fabaceae) on seed coat in Iran. -Biodiversitas vol 17, No. 1: 162-171.

Khandani S., Assadi M., Mehregan I. & Nejadsattari T. 2016 b: Phylogenetic analysis of Medicagoid Trigonella L. species based on ITS sequence data. -Ambient Science, vol. 3 (sp2)

Lavin, M., Herendeen, P. S. & Wojciechowski, M. F. 2005: Evolutionary rates analysis of Leguminosae implicates a rapid diversification of lineages during the Tertiary. -Systematic biology. 54 (4): 575-594.

Linnaeus, C. 1753: Species Plantarum. -Laurentius Salvius, Stockholm.

Rohlf, F. J. 2000: NTSYS-pc ver 2.11 T. Exter Software. Setauket, New York.

Rechinger, K. H. 1984: Trigonella. In Rechinger KH (ed.), Flora Iranica, vol 157: 207-253. -Akad.Druck- Verlagsanstalt, Graz.

Seringe, N.C. 1825: Medicago, Trigonella, Pocockia, A.P. Decandolle. -Prodromus Systematis Natwalis Regnivegetabilis. Paris. Vol. 2: 171-185.

Sirjaev, G. 1928-1934: Generis Trigonella L. Revisio Critica. -Publications of the Faculty of Science, University of Masaryk (Brno).

Small, E. 1986: A taxonomic study of the ‘‘medicagoid’’ Trigonella (Leguminosae). -Canadian Journal of Botany. 65:1199–1211

Small, E., Lassen, P. & Brookes, B. S. 1987: An expanded circumscription of Medicago (Leguminosae, Trifolieae) based on explosive flower tripping. –Willdenowia: 415-437.

Small, E. 1989a: Polythetic generic separation in the tribe Trifolieae subtribe Trigonellinae (Leguminosae). -Canadian Journal of Botany. 67:1480–1492

Small, E., Jomphe, M. 1989b: A synopsis of the genus Medicago (Leguminosae). -Canadian Journal of Botany. 67:3260–3294

Small, E., Brookes, B. & Lassen, P. 1990:.Circumscription of the genus Medicago (Leguminosae) by seed characters. -Canadian Journal of Botany. 68 (3): 613-629.

Small, E. 2010: Alfalfa and relatives: Evolution and classification of Medicago. -NRC Research Press.

Steele, K.P., Wojciechowski, M. F. 2003: Phylogenetic analyses of tribes Trifolieae and Vicieae, based on sequences of the plastid gene matK (Papilionoideae: Leguminosae). In Klitgaard B. B., Bruneau A. [eds.]. Advances in legume systematics, part 10: 355–370. -Royal Botanic Gardens, Kew, UK.

Steele, K. P., Ickert-Bond, S. M., Zarre, S. & Wojciechowski, M. F. 2010: Phylogeny and character evolution in Medicago (Leguminosae): evidence from analyses of plastid trnK/matK and nuclear GA3ox1 sequences. -American Journal of Botany. 97:1142–1155.

Thiers, B. 2015: [continuously updated] Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden’s Virtual Herbarium. http://sweetgum.nybg.org/ih/ (accessed 12 August 2015)

Trautvetter, E. R. 1841: Über die mit Trifolium verwandten Pflanzen gattungen. -Bulletin de

l'Academie imperial des sciences de Saint-Petersbouzg. 8:267-272.

Urban, I. 1873: Prodromus einer monographie der gattung Medicago L. -Verhandlungen des Botanischen Vereins für die Provinz Brandenburg. 15: 1-85.

Wojciechowski, M. F., Sanderson, M. J., Steele, K. P. & Liston, A. 2000: Molecular phylogeny of the ‘‘Temperate Herbaceous Tribes’’ of Papilionoid legumes: a supertree approach. In: Herendeen PS, Bruneau A (eds) Advances in legume systematics, vol 9: 277–298. -Royal Botanic Gardens, Kew.

Wojciechowski, M. F. 2003: Reconstructing the phylogeny of legumes (Leguminosae): an early 21st century perspective. In: Klitgaard BB, Bruneau A (eds) Advances in legume systematic, part 10: 5–35, higher level systematics. -Royal Botanic Garden, Kew.

Wojciechowski, M. F., Lavin, M. & Sanderson, M. J. 2004: A phylogeny of legumes (Leguminosae) based on analysis of the plastid matK gene resolves many well-supported subclades within the family. -American Journal of Botany. 91:1846–1862.