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Tribe *Trifolieae*: Evidence from Seed Characters

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Abstract: Thirteen seed characters have been studied in thirty eight species distributed over five genera representing tribe *Trifolieae* (family Leguminosae). The results obtained supported the separation of both genera *Ononis* and *Trifolium*, each in a separate subtribe as proposed by previous works. The genera *Trigonella* and *Melilotus* have variable characters which can be used in the subscription of their species. Species of *Medicago* have characteristic seed of their own which enables the separation of the genus in a separate subtribe *Medicagonieae*. Keys to the studied species within the genera *Ononis*, *Trigonella*, *Melilotus* and *Trifolium* have been constructed.

Key words: Seed morphology, seed coat, ononodieae, trigonelleae, taxonomy

INTRODUCTION

Tribe *Trifolieae* is one of the major group belonging to subfamily Papilionoideae, family Leguminosae and considered by Debelle *et al.*^[1] as one of the Leguminous tribes forming the galepoid group. According to Berchtold and Presl^[2] and recently Polhill and Raven^[3] this tribe includes seven genera; *Ononis*, *Parochetus*, *Melilotus*, *Trigonella*, *Factorovskya*, *Medicago* and *Trifolium*. These genera are characterized by having stipules adnate to the petiole, except in *Parochetus*, trifoliolate leaves and small seeds with well developed radicle. Schulz^[4] found that the ovules within these genera differ and he separated the genera *Trifolium*, *Parochetus* and *Ononis* in a tribe called *Trifolieae* for having seeds with micropyle supra funiculum. He put the other four genera in another tribe, *Trigonelleae*, having seeds with micropyle infra funiculum. Hutchinson^[5] separated the genus *Ononis* in a monogeneric tribe, *Ononideae*, for having reduced leaves and monadelphous dimorphic androecium. Meanwhile, he kept the other six genera in tribe *Trifolieae*. Hutchinson treatment of the genera has been accepted^[6-9]. Small^[10], Small *et al.*^[11,12] accepted the separation of genus *Ononis* in a monogeneric tribe but he regrouped the other six genera in two subtribes; subtribe *Trifoliniinae* with the genus *Trifolium* only and subtribe *Trigonelliniinae* with the rest of the genera. This treatment of the genera has been supported by Lersten^[13] who found that genus *Ononis* has uniseriate, from one to eight

cells, suspensor and genus *Trifolium* has small spherical poorly developed suspensor while the rest of the genera has suspensors containing from one to four series of cells. This treatment of the genera is followed by most of the taxonomists till Chaudhary and Sanjappa^[14] when restudied this tribe and suggest the separation of genus *Parochetus* in a new subtribe *Parochetinae*. In spite of that, genera within tribe *Trifolieae* still in need to more taxonomic findings to assess these opinions. For that the seeds of five genera of them; *Ononis*, *Melilotus*, *Trigonella*, *Medicago* and *Trifolium* have been studied as first step to assess the relationships between them.

MATERIALS AND METHODS

Thirty eight species, from the Egyptian flora, are represented in this study as follow: *Ononis* six species, *Melilotus* five species, *Trigonella* eight species, *Medicago* eleven species and *Trifolium* eight species. A complete list of the species, their life forms, locality and collector are given in Table 1. Mature seeds from ten individuals belonging to each species are measured and subjected to stereozoom examination. Selected seeds were mounted in silver paste onto standard stubs, coated with gold and examined by JEOL JSM T-20 scanning electron microscope at 15 kv. The terminology used here is that of Barthlott^[15]. This study has been carried out during the period of 2000 to 2001.

Table 1: Taxa investigated, their life form, locality and collector

| Name of Taxon | L.F. | Source/ Locality/ Collector and Date |
|--|------|--|
| <i>Ononis natrix</i> L.v. <i>stenophylla</i> Boiss. | Per. | Alex.Univ./ Rafah,near the sea/ Boulos, 31-3-1977. |
| <i>O. vaginalis</i> Vahl. | Per. | Alex.Univ./ Matruh-Siwa road/ Ayyad et al., 21-3-1989. |
| <i>O. reclinata</i> L.v. <i>minor</i> Moris | Ann. | Alex.Univ./ Burg El-Arab/ El-ghazaly et al., 2-4-1982. |
| <i>O. sicula</i> Guss. | Ann. | Alex.Univ./ Matruh, wadi El-Ramla/ Tadros, 14-4-1948. |
| <i>O. pubescens</i> L. | Ann. | Alex.Univ./ Rafah / Ayyad et al.,14-4-1981. |
| <i>O. serrata</i> Forssk. | Ann. | Alex.Univ./ Rosetta/ Rizk et al.,28-3-1992. |
| <i>Trigonella arabica</i> Del. | Ann. | Alex. Univ./ El-Arish/ Boulos 18-3-1955. |
| <i>T. anguina</i> Del | Ann. | Alex. Univ./ Burg El-Arab/ Ayyad et al., 27-2-1988. |
| <i>T. occulta</i> Del. Ex DC. | Ann. | CAI / Helwan desert/ Ghabour & Mostafa, 13-3-1959. |
| <i>T. monspeliaca</i> L. v. <i>nuda</i> Sirj. | Ann. | Alex. Univ./ Mariut/ Zidan, 15-3-1994. |
| <i>T. stellata</i> Forssk. | Ann. | CAI / Sinai, El-Hasnaa/ Shamsa, 3-4-1988. |
| <i>T. laciniata</i> L.v. <i>subsessilis</i> Boiss. | Ann. | Alex. Univ./ Burg El-Arab/ Rizk et al., 24-3-1981. |
| <i>T. maritime</i> Del. | Ann. | Alex. Univ./ Siwa oasis/ Ayyad et al., 4-4-1978. |
| <i>T. hamosa</i> L. | Ann. | Alex. Univ./ El-Arish/ Boulos, 18-3-1955. |
| <i>Medicago marina</i> L. | Per. | Alex. Univ./ Burg El-Arab/ Ayyad et al., 3-4-1986. |
| <i>M. lupulina</i> L. | Ann. | Alex. Univ./Botanical garden of faculty of science/Ahmed, 10-8-1987. |
| <i>M. coronata</i> (L.) Bartal. | Ann. | Alex. Univ./ Matruh-El-sallum road/ Ahmed, 24-4-1993. |
| <i>M. orbicularis</i> (L.) Bartel. | Ann. | Alex. Univ./ El-Omayed / Zedan, 14-3-1988. |
| <i>M. arabica</i> (L.) Huds. | Ann. | Alex. Univ./ El-Omayed/ Zedan, 14-3-1988. |
| <i>M. truncatula</i> Gaertn. v. <i>longiaculeata</i> Urb. | Ann. | Alex. Univ. / El-Omayed/ Zedan, 14-3-1988. |
| <i>M. rigidula</i> (L.) All. | Ann. | Alex. Univ./ Burg El-Arab/ Zedan 1-4-1988. |
| <i>M. intertexta</i> (L.) Mull. v. <i>cillicaris</i> (L.) Heyn | Ann. | Alex. Univ./Burg El-Arab/Fakhry, 21-4-1991 |
| <i>M. minima</i> (L.) Bartal | Ann. | Univ./ Matruh-Siwa road/ Ahmed, 12-4-1987 |
| <i>M. aschersoniana</i> Urb. | Ann. | Alex. Univ./ Alex.-Matruh road/Zedan, 27-5-1991. |
| <i>M. sativa</i> L. | Per. | Kew/ Montpellier/ Tadros, 11-8-1948. |
| <i>Melilotus alba</i> Medic | Bi. | CAI/ Ras El-Hekma/ Tackholm et al., 30-4-1955. |
| <i>M. sulcata</i> Desf. | Ann. | CAI/ El-Sharkiya/ Amer, 8-2-1982. |
| <i>M. siculus</i> (Turra) B.D. Jacks. | Ann. | Alex. Univ./ El-Mansoura/ Boulos, 5-2-1981. |
| <i>M. elegans</i> Salzm ex Ser. | Ann. | Alex. Univ./Burg El-Arab/ Fakhry, 21-4-1991. |
| <i>M. indica</i> (L.) All. v. <i>tommassinii</i> Jord. | Ann. | Alex. Univ./ El-Bousily/ Rizk et al.,21-3-1989. |
| <i>Trifolium fragiferum</i> L. | Per. | Alex. Univ./ Burg El-Arab/ El-Ghazaly et al., 7-2-1981. |
| <i>T. resupinatum</i> L. | Ann. | Alex. Univ./ El-Bousily/ Rizk et al., 21-3-1989. |
| <i>T. tomentosum</i> L. v. <i>orientale</i> Borrm. | Ann. | Alex. Univ./ Matrouh/ Rashad, 20-3-1975. |
| <i>T. procumbens</i> L. | Ann. | Alex. Univ./ El-Omayed/ Zedan, 14-3-1988. |
| <i>T. alexandrinum</i> L. | Ann. | Alex. Univ./ Samouha/ Abdel Aziz, 6-1-1948. |
| <i>T. scabrum</i> L. | Ann. | Alex. Univ./ Matrouh-Siwa road/ Ayyad et al.,21-3-1989. |
| <i>T. stellatum</i> L. | Ann. | Alex. Univ./ Burg El-Arab/ Fakhry, 23-3-1992. |
| <i>T. desvauxii</i> Boiss et Bl. | Ann. | Alex. Univ./ Matrouh-Siwa road/ Ayyad et al.,12-4-1987. |

The arrangement of the genera and species is according to Tackholm

Abbreviations: L.F.= Life Form, Per.= Perennial, Ann.= Annual, Bi.= Biennial, Alex.Univ.= Alexandria University Herbarium, CAI= Cairo University Herbarium, Kew= Kew gardens

RESULTS AND DISCUSSION

The results are summarised in Table 2 and Fig. 1 to 68. These results revealed that the variation in the seeds of the studied taxa are quite obvious and can be of taxonomic use in the identification of certain taxa. Figure 1-12 show the seeds of the studied *Ononis* species which are mostly lenticular or global with papillate testa and superficial or sunken micropyle. Figure 23-36 show the variations in the seed shape within *Trigonella* species. From these Fig. 23-36 it can be observed that both *T. maritime* (Fig. 23 and 24) and *T. occulta* (Fig. 25-26) have lenticular seeds, while *T. anguina* (Fig. 35-36) and *T. hamosa* (Fig. 31-32) have globular seeds. Meanwhile, *T. arabica* (Fig. 37) and *T. laciniata* (Fig. 31-32) have bilateral seeds and *T. monspeliaca* (Fig. 27-28) has trigonal seeds. *Melilotus* species are illustrated in Fig. 13-22. From these Fig. 13-22 it can observe the great variations in seed shapes and testa ornamentations within

the studied species. *Medicago* species have mostly uniform seed shape and testa pattern as all of them have kidney shaped seeds with smooth testa and sunken micropyle (Fig. 38-52). The only variable character within the *Medicago* species is the arrangement of the seed coat cell and their anticlinal walls as illustrated in Fig. 39, 41, 42, 43, 44, 45, 46, 47, 49, 50, 51 and 52. *Trifolium* species are less uniform than *Medicago* species (Fig. 53-68) as they vary in shape from oval in *T. resupinatum*, (Fig. 59) *T. tomentosum* (Fig. 67), *T. procumbens* (Fig. 61) and *T. scabrum* (Fig. 53), to global in the rest of the studied species. The seed coat pattern are also variable from smooth in *T. tomentosum* (Fig. 68), *T. procumbens* (Fig. 62), *T. scabrum* (Fig. 54), *T. stellatum* (Fig. 56) and *T. desvauxii* (Fig. 64) to striate in *T. fragiferum* and *T. resupinatum* (Fig. 60 and 62) or even wrinkelled in *T. alexandrinum* (Fig. 66).

Also, it can observe that the arrangements of the seed coat cells has a considerable weight in the

Table 2: Seed characters as investigated by both stereo- and scanning electron microscopes

| Characters taxa | 1 | 2 | | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
|------------------------------------|---|------------------|-----------------|---|---|---|---|---|---|---|----|----|----|----|
| | | L. | W. | | | | | | | | | | | |
| <i>Ononis natrix</i> | 2 | 0.9-1.2 -1.1 | 0.6-0.8 0.65 | 2 | 2 | 2 | 3 | 1 | 2 | 1 | 1 | 3 | 2 | 2 |
| <i>O. vaginalis</i> | 1 | 1.5-1.7 -1.65 | 1.5-1.7 1.65 | 3 | 5 | 2 | 2 | 1 | 2 | 2 | 1 | 3 | 4 | 2 |
| <i>O. reclinata</i> | 2 | 0.9-1.2 -1 | 0.9-1.2 1 | 3 | 4 | 1 | 1 | 1 | 4 | 1 | 1 | 3 | 4 | 2 |
| <i>O. sicula</i> | 2 | 0.9-1.3 -1.2 | 0.7-1.0 0.95 | 2 | 4 | 2 | 2 | 1 | 3 | 1 | 1 | 1 | 4 | 2 |
| <i>O. pubescens</i> | 2 | 2.3-2.7 -2.4 | 2.3-2.7 2.4 | 3 | 1 | 1 | 2 | 1 | 1 | 2 | 1 | 1 | 2 | 2 |
| <i>O. serrata</i> | 2 | 1.2-1.5 -1.4 | 1.0-1.2 1.08 | 2 | 4 | 1 | 3 | 1 | 4 | 1 | 1 | 3 | 4 | 2 |
| <i>Trigonella arabica</i> | 2 | 0.2-0.5 -0.3 | 0.2-0.5 0.35 | 6 | 2 | 2 | 2 | 4 | 2 | 3 | 1 | 2 | 3 | 2 |
| <i>T. anguina</i> | 3 | 1.0-1.2 -1.08 | 0.5-0.8 0.65 | 2 | 2 | 2 | 2 | 4 | 2 | 3 | 3 | 2 | 3 | 2 |
| <i>T. occulta</i> | 2 | 4.0-4.6 -4.2 | 4.0-4.6 4.2 | 1 | 5 | 3 | 1 | 3 | 3 | 1 | 1 | 3 | 3 | 2 |
| <i>T. monspeliaca v. nuda</i> | 2 | 1.2-1.5 -1.3 | 0.6-1.1 0.9 | 4 | 2 | 3 | 1 | 2 | 1 | 1 | 1 | 3 | 2 | 2 |
| <i>T. stellata</i> | 2 | 1.1-1.4 -1.25 | 0.5-0.7 0.56 | 2 | 1 | 2 | 2 | 4 | 3 | 3 | 3 | 2 | 3 | 2 |
| <i>T. laciniata</i> | 3 | 0.5-0.7 -0.65 | 0.3-0.6 0.45 | 6 | 4 | 2 | 1 | 1 | 4 | 2 | 1 | 3 | 3 | 1 |
| <i>T. maritime</i> | 2 | 1.5-2.0 -1.65 | 1.5-2.0 1.65 | 1 | 5 | 2 | 1 | 1 | 1 | 2 | 2 | 3 | 1 | 2 |
| <i>T. hamosa</i> | 2 | 1.3-1.6 -1.5 | 0.9-1.2 1.1 | 2 | 5 | 3 | 1 | 1 | 4 | 1 | 1 | 3 | 5 | 1 |
| <i>Medicago marina</i> | 2 | 1.9-2.5 -2.35 | 0.8-1.0 0.95 | 5 | 1 | 2 | 2 | 1 | 2 | 1 | 1 | 3 | 3 | 2 |
| <i>M. lupulina</i> | 2 | 1.1-1.5 -1.3 | 0.5-0.7 0.6 | 5 | 1 | 2 | 3 | 4 | 1 | 2 | 1 | 1 | 4 | 2 |
| <i>M. coronata</i> | 2 | 1.2-1.5 -1.35 | 0.3-0.4 0.35 | 5 | 1 | 2 | 2 | 4 | 1 | 3 | 3 | 1 | 4 | 2 |
| <i>M. orbicularis</i> | 2 | 1.5-2.2 -1.8 | 0.9-1.2 1.15 | 5 | 1 | 2 | 2 | 3 | 2 | 3 | 3 | 2 | 3 | 2 |
| <i>M. arabica</i> | 2 | 1.9-3.0 -2.3 | 1.0-1.9 1.7 | 5 | 1 | 2 | 1 | 1 | 4 | 1 | 1 | 3 | 1 | 2 |
| <i>M. truncatula</i> | 2 | 1.8-2.2 -2 | 0.9-1.2 1.1 | 5 | 1 | 2 | 1 | 1 | 4 | 1 | 1 | 3 | 1 | 2 |
| <i>M. rigidula</i> | 2 | 1.6-2.1 -1.9 | 0.9-1.3 1.1 | 5 | 1 | 2 | 3 | 2 | 1 | 3 | 2 | 2 | 1 | 2 |
| <i>M. intertextata v. ciliaris</i> | 3 | 2.2-3.7 -3 | 1.3-1.8 1.65 | 5 | 1 | 2 | 3 | 2 | 1 | 2 | 1 | 3 | 2 | 2 |
| <i>M. minima</i> | 2 | 1.6-2.0 -1.8 | 0.9-1.4 1.2 | 5 | 1 | 2 | 1 | 3 | 4 | 1 | 1 | 3 | 1 | 2 |
| <i>M. aschersoniana</i> | 2 | 1.8-2.3 -2 | 1.0-1.4 1.2 | 5 | 1 | 2 | 2 | 3 | 2 | 1 | 1 | 2 | 3 | 2 |
| <i>M. sativa</i> | 3 | 0.7-1.5 -0.9 | 0.5-0.9 0.7 | 5 | 1 | 2 | 2 | 4 | 1 | 3 | 3 | 3 | 1 | 2 |
| <i>Melilotus alba</i> | 3 | 1.5-2.0 -1.8 | 1.5-2.0 1.8 | 1 | 1 | 2 | 2 | 3 | 2 | 3 | 3 | 2 | 1 | 2 |
| <i>M. sulcata</i> | 3 | 1.5-1.9 -1.75 | 1.1-1.4 1.2 | 2 | 4 | 1 | 1 | 1 | 4 | 1 | 1 | 3 | 3 | 2 |
| <i>M. sicula</i> | 3 | 3.0-3.5 -3.3 | 3.0-3.5 3.3 | 1 | 5 | 1 | 3 | 2 | 2 | 1 | 2 | 3 | 1 | 2 |
| <i>M. elegans</i> | 2 | 2.4-2.8 -2.6 | 1.6-2.0 1.85 | 2 | 5 | 1 | 1 | 2 | 1 | 3 | 3 | 1 | 1 | 1 |
| <i>M. indica</i> | 3 | 1.7-2.2 -1.9 | 1.1-1.6 1.3 | 2 | 3 | 1 | 1 | 2 | 1 | 3 | 3 | 1 | 1 | 1 |
| <i>Trifolium fragiferum</i> | 3 | 1.3-1.6 -1.5 | 1.3-1.6 1.5 | 1 | 1 | 1 | 3 | 4 | 3 | 2 | 1 | 1 | 2 | 2 |
| <i>T. resupinatum</i> | 2 | 1.4-1.8 -1.6 | 1.4-1.8 1.6 | 1 | 1 | 1 | 3 | 1 | 1 | 2 | 1 | 1 | 2 | 2 |

Table 2: Continued

| Characters taxa | 1 | 2 | | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
|-----------------------------------|---|------------------|-----------------|---|---|---|---|---|---|---|----|----|----|----|
| | | L. | W. | | | | | | | | | | | |
| <i>T. tomentosum v. orientale</i> | 2 | 0.9-1.2 -1 | 0.6-0.8 0.7 | 2 | 1 | 1 | 3 | 1 | 1 | 3 | 1 | 1 | 1 | 2 |
| <i>T. procumbens</i> | 2 | 0.9-1.3 -1.2 | 0.9-1.3 1.2 | 1 | 1 | 1 | 3 | 1 | 1 | 2 | 1 | 1 | 1 | 1 |
| <i>T. alexandrinum</i> | 3 | 2.0-2.4 -2.2 | 2.0-2.4 2.2 | 1 | 3 | 1 | 3 | 1 | 4 | 2 | 1 | 1 | 3 | 1 |
| <i>T. scabrum</i> | 2 | 1.5-1.8 -1.65 | 0.9-1.2 1.1 | 2 | 5 | 1 | 3 | 2 | 2 | 1 | 3 | 2 | 1 | 2 |
| <i>T. stellatum</i> | 3 | 2.0-2.5 -2.3 | 2.0-2.5 2.3 | 1 | 1 | 1 | 3 | 1 | 1 | 2 | 1 | 1 | 1 | 2 |
| <i>T. desvauxii</i> | 3 | 1.5-1.8 -1.65 | 1.5-1.8 1.65 | 1 | 1 | 1 | 3 | 4 | 1 | 2 | 2 | 1 | 1 | 1 |

Characters investigated: 1-Seed colour; 1-Yellowish-green 2-Light brown 3-Dark brown 2-Seed size in mm. (L.=Length, W.=Width), average of 10 readings; lowest-highest (mean) 3-Seed shape; 1-Lenticular 2-oval 3-global 4-trigonal 5-kidney-shaped 6-bilateral 4-seed-coat pattern; 1-smooth 2-wrinkled 3-striate 4-papillate 5-granulate 5-micropyle position; 1-superficial 2-sunken 3-grooved 6-arrangement of seed coat cells; 1-with elevated cells 2-with supercellular net-like pattern 3- with flat cells 7- outlines of cells; 1-isodiametric 2-elongated 3-irregular 4-rectangular 5-others 8-antidinal wall; 1-straight 2-irregular 3-curved 4-sinuate 9-relief of cell boundary; 1-channelled 2-superficial 3-raised 10-thickness of cell boundary; 1-thin 2-moderately thick 3- considerably thick 11-curvature of outer peridinal wall; 1-flat 2-concave 3-convex 12-secondary sculpture; 1-smooth 2-striate 3-wrinkled 4-reticulate 5-micropapillate 13-epicuticular secretion; 1-present 2-absent

Fig. 1 and 2: *Ononis serrata* (Show the variations of characters)

sumscription of the genera, then the micropyle position. These results are in accordance with Gutterman and Heydecker^[16] and La Sota^[17] who pointed to the importance of seed characters in the identification of species of *Ononis*. It can be observed that seed colour was of limited use as all the studied taxa have brown seeds except *O. vaginalis* which has greenish-yellow seeds (Table 2). The seed shape were mostly lenticular, oval or global except in *Trigonella monspeliaca* (Fig. 27) which is trigonal and in both *Trigonella laciniata* and *T. arabica* (Fig. 29 and 37) the seeds are bilateral or winged. All the studied *Medicago* species have kidney-shaped seeds which varies in size and seed-coat pattern only. The sizes of the seeds are almost small except in both

Trigonella occulta and *Melilotus sicula* the seeds are large. Seed-coat pattern or the micro-ornamentation on the surface of the outer cell wall can be considered of high taxonomic value in the identification of the species as indicated by Duke^[18], Dahlgren and Clifford^[19], Berthlott and Frolich^[20], Berchtold and Presol^[2] and Taia^[21]. According to this character it can easily distinguish the papillate seeds of *Ononis* (Fig. 1, 3 and 5) and the striate seeds in both *Melilotus elegans* and *M. indicus* (Fig. 17 and 19). *Medicago* species have almost smooth seeds which varies in the microornamentation of the outer cell wall surface (Fig. 38 to 52). Accordingly, it will be limited to use the seed characters in the circumscription of the *Medicago* species as previously mentioned by Ahmed

and Taia^[22]. Species of *Trifolium* have smooth seeds, as well, except both *T. alexandrinum* which has faintly striate seeds (Fig. 65) and *T. scabrum* which has granulate seeds (Fig. 53). The epicuticular secretions such as waxes and related substances were used in taxonomy by Barthlott and Frolich^[20], but here it is restricted to few species, *Trigonella laciniata*, *T. hamosa*, *Melilotus elegans*, *M. indica*, *Trifolium procumbens*, *T. alexandrinum* and *T. desvauxii*.

Thus, the results obtained from the seed characters support the separation of the genus *Ononis* in a monogeneric tribe *Ononideae* as proposed by Hutchinson^[5] and Larsten^[13]. The genera *Trigonella* and *Melilotus* have different seed shapes and ornamentations which can be used in the identification of their species. These variations do not reach to separate either of them in a separate subtribe. The genus *Medicago* has completely unique seed shape in the group which can be separated in a monogeneric subtribe, *Medicagoneae*, if this results supported by the other taxonomic tools. The genus *Trifolium* has characteristic seeds which can support^[10-12] in putting this genus in a separate subtribe *Trifoliinae*.

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