

Reassessment of the taxonomic relationships between closely related taxa of Papilionoideae

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ABSTRACT

The present work was carried out to study some features of the family Papilionoideae. These features include seed protein profiles and morphology. These were used to reassess some taxonomic relationships between some closely related taxa of the Papilionoideae; identifying the similarities and dissimilarities between them based on the SDS-PAGE of seed protein characters; determination of the interrelationships and affinities between them based on current systems of classification by using numerical methods. Seed protein profiles produced by gel electrophoresis and the molecular mass in kilo-Dalton of their polypeptide subunits and the morphological features were recorded in 36 species belonging to 11 genera of the Papilionoideae (family: Fabaceae). Phenetic relationships of these species were established based on UPGMA-clustering applied to the produced 97 seed protein characters in combination with 173 morphological characters by using different modules of the NTSYS-pc 2.2 program package. The produced data were compared by measuring each of the tree distortion; from its relevant data matrix; and association to each other based on their cophenetic (ultrametric) correlation coefficients. The present data were useful in revealing new distinguishing characters, identifying the similarities/ differences and confirming the taxonomic interrelationships between the studied taxa based on current systems of classification.

Key words: SDS-PAGE, NTSYS, UPGMA, seed proteins, taxonomy, morphology.

Introduction

Papilionoideae (syn. Faboideae) is the largest, most diverse and widely distributed subfamily of Fabaceae *sensu lato* [1]. It comprises ca. 478 genera and 13,860 species and includes most of the familiar domesticated food plants, forage crops, genomic/genetic species and nitrogen fixation plants [2, 3]. It is well represented in semi-arid to arid habitats of the world, with center of diversity in the temperate and tropical regions of South America. However, it is noticeably poorly represented or completely absent in the cool temperate habitats as in alpine

regions and the understory of cool temperate forests [2].

Seeds of the subfamily contain about 50% protein from which the storage fraction represents nearly 50% of the total amount found in the seeds [4]. Storage protein is not sensitive to environmental fluctuations, being the third-hand copy of DNA and reflects the genetic make-up of the plant [5]. Seed protein banding patterns; produced by the polyacrylamide gel electrophoresis (PAGE) technique; constitute powerful attributes for plant taxonomy at various ranks specially

when combined with morphology [6]. This fact prompted a considerable number of studies, many of which explained the inter/ intraspecific variations in individual genera e.g. *Sesbania* and *Lathyrus* [7,8], *Onobrychis* [9], *Trifolium* [10], *Vicia* [11] and *Lathyrus* [12,13].

The subfamily-wide studies in general have relatively been rare [14,15], but there have been an increasing number of studies that have focused at tribal level, or on a number of tribes [16,17,18,19,20,21,22]. However, none of them had used the SDS-PAGE of seed protein criteria in relation to morphology. In the present study, such criteria are used with the aim of: refining the taxonomic relationships between some closely related taxa of the Papilionoideae; identifying the similarities and dissimilarities between them based on the SDS-PAGE of seed protein characters; determination of the interrelationships and affinities between them based on current systems of classification by using numerical methods.

Material and Methods

Seeds of 36 species belonging to 11 genera of the Papilionoideae were either collected from the Egyptian phytogeographic regions or provided by the Egyptian and German botanical gardens. The examined species, their source and taxonomic delimitation are given in Table 1. For the morphological examination, seeds were grown in a protected open area in the Botanical Garden of the Faculty of Education, Ain Shams University. Taxonomic identification and nomenclature of Egyptian species follow Täckholm (1974) [23] and Boulos (1999) [24], whereas those of the cultivated species follows Ball (1968) [25] and Bailey & Bailey (1976) [26]. Identification was further confirmed by matching against dried specimens in the Herbaria of the

Faculty of Science, Ain Shams University (CAIA) and that of the Faculty of Education, Ain Shams University. Morphological description of the species was made from living flowering plants and vouchers are kept at the Herbarium of Biological and Geological Sciences Department, Faculty of Education, Ain Shams University.

For the electrophoretic analysis, seed protein was extracted by mixing 0.5 g of mature seeds with an equal weight of clean, sterile fine sand and powdered using a mortar and pestle and homogenized with 1 M Tris-HCl buffer, pH 8.8 in clean Eppendorf tube and left in refrigerator overnight. The extract was centrifuged at 3.000 rpm for 10 min. The supernatant (protein extract) was transferred to new tubes and immediately used for electrophoresis or kept in deep-freeze until use. For electrophoresis, 10 µl of the extract were mixed with 5µl of a treatment buffer. Electrophoresis was carried out by the modified discontinuous sodium dodecyl sulfate PAGE (DISC SDS-PAGE) method (Laemmli, 1970) [27] using 12% (w/v) acrylamide separating gel (0.375 M TRIS-HCl buffer, pH 8.8) and 4% (w/v) acrylamide stacking gel (0.125 M TRIS-HCl, pH 6.8). The electrode buffer was TRIS-glycine (2.25 g TRIS and 10.8 g glycine per 750 ml buffer solution, pH 8.3) with 0.1% (w/v) SDS. Gels were then stained overnight in 0.1% (w/v) Coomassie Brilliant Blue-R250 solution containing 40% (v/v) methanol and 10% (v/v) trichloroacetic acid; destained in the same solution but without adding the dye. Gels were photographed, scanned and analyzed using Gel Doc 2000 Bio-Rad system (Bio-Rad Laboratories, Hercules Co. USA). Total bands in the produced electrophorogram were scored and their biochemical masses (Mr) in kilo-Dalton (kDa) were

calculated using standard protein marker.

For the numerical analyses, the data editor program NTedit 2.2; that is included in the program package of NTSYS-pc 2.2 (Rohlf, 2005) [28]; was used to prepare two sets of data matrices (proteins and morphology). Each of the studied species was considered as operational taxonomic unit (OTU) and numbered as indicated in Table (1). Multistate characters were transformed to two-state characters in coding (Sneath & Sokal, 1973) [29]. A cluster analysis for the seed protein data set in combination with that of morphological data was performed by using the program NTSYS-pc 2.2 (Rohlf, 2005) [28]; as follows: The raw data matrix was standardized with STAND module; dissimilarity matrix was generated by SIMINT module based on Jaccard similarity distance (J). Clustering was performed using unweighted pair group method, arithmetic average (UPGMA) and represented in phenogram. In order to test reliability of results, the correlation coefficient (r) value that measures the distortion between the produced phenogram and the relevant similarity matrix (Rohlf & Sokal, 1981) [30] was estimated by using the program NTSYS-pc 2.2 (Rohlf, 2005) [28] as follows: The cophenetic (ultrametric) value matrix of the phenogram was computed using CPH module and compared to the related distance matrix using MXCOMP module.

Result and Discussion

The photograph of the produced SDS-PAGE of seed protein profiles of the studied species is shown in Figure (1). The selected 270 characters (97 of protein and 173 of morphology); their codes and data matrix used in the numerical analysis are given in Appendix 1. UPGMA phenogram illustrating the relationships between the studied species based on each of

the seed proteins, morphological and combined data sets are given in Figure (2). This phenogram revealed that; at reference line of 0.2; three major clusters (A, B & C) are separated. The first cluster (A) comprises three minor groups (a_1 , a_2 & a_3) that are grouped together at the similarity level of 0.21. The first minor group (a_1) comprises *Anthyllis barba-jovis*, *Dorycnium hirsutum* 1&2, *Genista linifolia* and *Colutea arborescens* are distinguished at the level of 0.26. The second group (a_2) comprises *Hippocrepis multisiliquosa*, *Lotus corniculatus* 1&2, *L. pedunculatus*, *Lathyrus pratensis* and *L. aphaca* that are grouped together at the similarity level of 0.28 and clustered with the group a_1 at the 0.24 level. The third group (a_3) comprises *Vicia angustifolia*, *Vicia faba* 1, 2 & 3 that are grouped together at the similarity level of 0.56 and clustered with the groups a_1 & a_2 at the 0.22 level.

The second major group (B) is represented by a single phenetic line that includes only *Astragalus hamosus* and is distinguished at the level of 0.20. The third major cluster (C) comprises three minor groups (c_1 , c_2 & c_3). The first minor group (c_1) comprises *Medicago citrina*, *M. lupulina*, *M. vardanis*, *M. orbicularis* and *M. sativa* and is distinguished at the level of 0.47. The second minor group (c_2) comprises *Melilotus albus*, *M. graecus*, *M. indicus*, *M. messanensis* and is distinguished at the level of 0.51. The third minor group (c_3) comprises *Trifolium alexandrinum*, *T. arvense*, *T. campestre* 1&2, *T. lappaceum*, *T. scabrum*, *T. fragiferum*, *T. infamia-ponertii*, *T. pratense*, *T. rubens* and *T. pignantii* and is distinguished at the level of 0.39. The produced correlation coefficient (r) value of the reliability test for the used data set is 0.95 that reveals no

significant distortion between the produced phenogram and its related similarity matrix.

[31] reported that, apertures and ornamentation of the pollen of the genus *Anthyllis* were very similar to those of *Lotus* and it was difficult to distinguish all the species in the two genera by pollen morphology. [32,33] classified *Anthyllis* in subtribe Anthyllidinae of the tribe Loteae. Their data revealed a close relationship between such genus and the genera *Genista* and *Dorycnium*. [34] supported these data and reported the taxonomic instability of the genus *Genista* to the extent that, he divided it into two genera (namely *Teline* beside *Genista s. str.*). In the present study, the examined species of these genera, namely *Anthyllis barba-jovis*, *Dorycnium hirsutum* (tribe Loteae), *Genista linifolia* (tribe Genistae) and *Colutea arborescens* (tribe Galegeae) were clustered in group a_1 of the cluster A. This was morphologically due to the shrub habit, broadly triangular stipules, capitate raceme flowers and teeth shorter than calyx tube. They also were characterized by the presence of protein bands with Mr of 106.41 and 32.45 kDa (Character no. 178 and 225, Appendix 1), respectively; the absence of bands with Mr 117.71, 99.00 and 81.41 (Char. no. 174, 182 and 190), respectively. Thus, the present data partially support the conclusions of [32,33]. However, those of [34] needs a further study based on adequate number of samples. [35] reported the taxonomic similarities between *Anthyllis* with the related genera *Dorycnium* and *Lotus* for the first time; on the positive interaction with the associated root nodule bacteria which give a further support for the present conclusion.

[31] reported that, *Hippocrepis* was more similar to *Lotus* among taxa of the tribe Loteae in spite of their sharing

two different types of pollen ornamentation. [36,37] pointed out that, the genera *Lotus* and *Hippocrepis* were restricted to the Old World with a highest number of species in the Mediterranean region. Of these, *Lotus* has non-lomentaceous fruits conforming to the traditional concept of Loteae while *Hippocrepis* was among the core of another tribe (Coronilleae). In the present study, *Hippocrepis multisiliquosa*, and *Lotus corniculatus* L. *corniculatus*², *L. pedunculatus* were clustered in the group a_2 of the major cluster A. This was morphologically due to the herb habit, branched base stem and glabrous leaflets surface. They were characterized by the presence of protein bands with Mr of 106.41, 9.58 kDa (Char. no. 178 and 269), respectively; the absence of bands with Mr 25.89, 30.74 and 34.52 (Char. no. 237, 229 and 223), respectively. These data support the close relationship between both taxa and disagree with the conclusion of [36,37].

[38,39] indicated the close relationships between *Astragalus*, *Trifolium* and *Vicia* based on phylogenetic analysis. In the present study, species of the three genera were clustered in different groups' a_3 , B and c_3 , respectively. *Astragalus hamosus* was distinguished by linear bracts, tubular calyx shape and slightly curved pod and the presence of protein bands with Mr of 69.00, 85.76 and 63.22 kDa (Char. no. 196, 188 and 201), respectively; the absence of bands with Mr of 102.97, 66.54 and 36.55 kDa (Char. no. 180, 199 and 220), respectively. *Vicia angustifolia*, *Vicia faba* G461, *Vicia faba* L. Line2 and *Vicia faba* L. Line4 were clustered morphologically due to the annual longevity, glabrous stem surface, appressed hairy calyx teeth and dentate stipules margin. They were

characterized by the presence of protein bands with Mr of 12.60, 11.03 24.67 kDa (Char. no. 260, 265 and 239), respectively; the absence of bands with Mr 31.41, 14.32 and 10.50 (Char. no. 227, 257 and 267), respectively. *Trifolium alexandrinum*, *T. arvense*, *T. campestre*, *T. campestre*2, *T. lappaceum*, *T. fragiferum*, *T. infamia-ponertii*, *T. pignantii*, *T. pretense*, *T. rubens* and *T. scabrum* were clustered together due to the herb habit, branched base, pubescent leaflets upper surface and entire stipules margin. They were characterized by the presence of protein bands with Mr of 117.71, 45.69 and 21.50 kDa (Char. no. 174, 211 and 244), respectively; the absence of bands with Mr 105.10, 98.65 and 90.16 (Char. no. 179, 183 and 186), respectively. These data don't support the relationships reported by [38,39].

[40,41] classified *Melilotus* into two subgenera; one includes *M. albus* and the other

subgenus included two sections that comprise all the other investigated species. [42] explained the deviation of *M. albus* based on the taxonomic similarity between species of the genera *Melilotus* and *Medicago* that have always been questioned. [43] constructed the phylogenetic relationships of both genera based on morphological characters and pointed out that, all species were strongly

related to each other except for *M. albus* which was evolved early and that, its taxonomic position towards both genera was unclear. In the present study, *Melilotus* and *Medicago* were grouped in the same major cluster C. *Melilotus albus*, *M. graecus*, *M. indicus* and *M. messanensis* were clustered in the group c₂ morphologically due to the annual longevity, lanceolate stipules, obovate-oblong corolla and glabrescent leaflets lower surface. They were characterized by the presence of protein bands with Mr of 81.41, 36.55 and 14.32 kDa (Char. no. 190, 220 and 257), respectively; the absence of bands numbers with Mr 108.00, 100.91 and 53.81 (Char. no. 177, 181 and 204), respectively. *Medicago citrina*, *M. lupulina*, *M. orbicularis*, *M. sativa* and *M. vardanis* were clustered due to the adnate to petiole stipules, entire stipules and obovate leaflets. They were characterized by the presence of protein bands with Mr of 115.85, 45.69 and 15.51 kDa (Char. no. 175, 211 and 254), respectively; the absence of bands numbers 176, 184 and 188 with Mr 111.00, 97.66 and 85.76, respectively. These data in one hand revealed the unity of *Melilotus albus* with the remaining studied species of its genus *Melilotus*. On the other hand, don't confirm the conclusion of [42] regarding the relationship of *Melilotus* and *Medicago*.

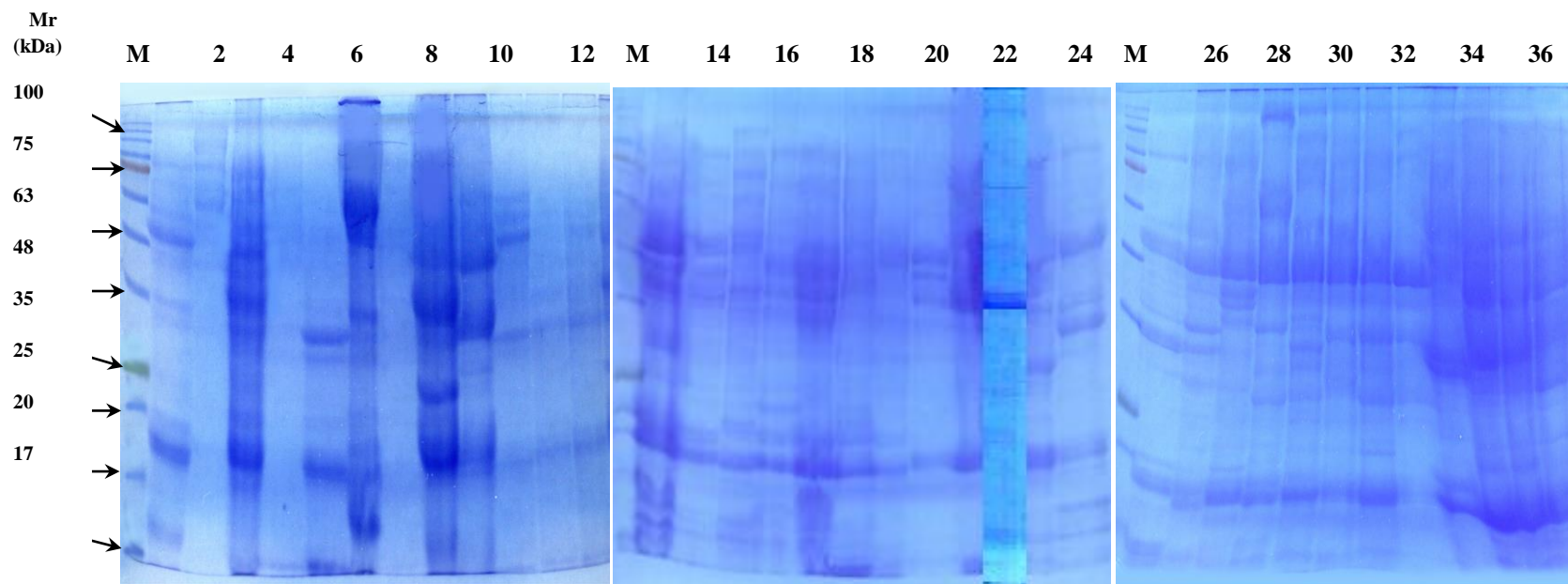
Table 1. The examined Papilionoid taxa in the present study; their source and taxonomic delimitation.

No.	Taxon	Source	Tribe
01	<i>Anthyllis barba-jovis</i> L.	BGBD	Loteae
02	<i>Astragalus hamosus</i> L. (= <i>Ankylobus hamosus</i> (L.) Steven; <i>Astragalus aegyptiacus</i> Mill.; <i>A. ancistrum</i> Pomel; <i>A. arnoceras</i> Bunge; <i>A. brachyceras</i> Ledeb.; <i>A. buceras</i> Willd.; <i>A. dorcoceras</i> Bunge; <i>A. embergeri</i> Jahand. & al.; <i>A. oncocarpus</i> Pomel; <i>A. pau</i> Pau; <i>A. stribrnyi</i> Velen.; <i>A. taekholmianus</i> Oppenh.; <i>A. volubilitanus</i> Braun-Blanq. & Maire; <i>Hamosa astragalus</i> Medik.; <i>Tragacantha brachyceras</i> (Ledeb.) Kuntze; <i>T. buceras</i> (Willd.) Kuntze; <i>T. hamosa</i> (L.) Kuntze)	WILD ¹	Galegeae
03	<i>Colutea arborescens</i> L. (= <i>Colutea arborea</i> "sensu auct., p.p., <i>Colutea breviaolata</i> Lange subsp. <i>gallica</i> forma <i>brevialata</i>).	BGBD	Galegeae
04	<i>Dorycnium hirsutum</i> (L.) Ser.(1) (= <i>Bonjeanea hirsuta</i> (L.) Rchb., <i>Lotus hirsutus</i> L., <i>Lotus affinis</i> Besser ex DC., <i>Lotus candidus</i> Mill., <i>Bonjeanea cinerascens</i> Jord. & Fourr., <i>Lotus hemorroidalis</i> Lam., <i>Bonjeanea hirta</i> Jord. & Fourr., <i>Bonjeanea italica</i> Jord., <i>Bonjeanea prostrata</i> Jord. & Fourr., <i>Bonjeanea sericea</i> (Sweet) Jord. & Fourr., Icon, <i>Dorycnium sericeum</i> Sweet, <i>Dorycnium tomentosum</i> G.Don, <i>Bonjeanea venusta</i> Jord. & Fourr., <i>Lotus intermedius</i> Loisel., <i>Lotus sericeus</i> DC.)	BGSA	Loteae
05	<i>D. hirsutum</i> (L.) Ser. (2)	BGBD	Loteae
06	<i>Genista linifolia</i> L. (= <i>Teline gomerae</i> (P.E.Gibbs & Dingwall) Kunkel, <i>Teline linifolia</i> subsp. <i>rosmarinifolia</i> (Webb & Berthel.) P.E.Gibbs & D, <i>Teline rosmarinifolia</i> Webb & Berthel., <i>Cytisus linifolius</i> , <i>Teline linifolia</i>)	GBGA	Genistae
07	<i>Hippocrepis multisiliquosa</i> L. (= <i>Hippocrepis ambigua</i> (Rouy) Bellot, <i>Hippocrepis confusa</i> Pau, <i>Hippocrepis multisiliquosa</i> subsp. <i>confusa</i> (Pau) Maire)	GBGA	Loteae
08	<i>Lathyrus aphaca</i> L. (= <i>Orobis aphaca</i> (L.) Doll., <i>Aphaca vulgaris</i> Presl., <i>Lathyrus segetum</i> Lam.)	WILD ²	Vicieae
09	<i>Lathyrus pratensis</i> L. (= <i>Orobis pratensis</i> .)	BGBD	Vicieae
10	<i>Lotus corniculatus</i> L. (= <i>Lotus ambiguus</i> Besser ex Spreng., <i>Lotus ambiguus</i> Spreng, <i>Lotus arvensis</i> Pers., <i>Lotus balticus</i> Miniaev, <i>Lotus carpetanus</i> Lacaita, <i>Lotus caucasicus</i> Kuprian, <i>Lotus caucasicus</i> Kuprian., <i>Lotus ciliatus</i> sensu Schur, <i>Lotus corniculatus</i> L. var. <i>crassifolia</i> Fr., <i>Lotus corniculatus</i> L. var. <i>kochii</i> Chrtkova, <i>Lotus corniculatus</i> L. var. <i>maritimus</i> Rupr., <i>Lotus corniculatus</i> subsp. <i>major</i> (Scop.) Gams, <i>Lotus corniculatus</i> var. <i>arvensis</i> (Pers.) Ser., <i>Lotus corniculatus</i> var. <i>glaber</i> Opiz, <i>Lotus corniculatus</i> var. <i>major</i> (Scop.) Brand, <i>Lotus corniculatus</i> var. <i>arvensis</i> (Schkuhr) Ser. ex DC., <i>Lotus filicaulis</i> Durieu, <i>Lotus frondosus</i> (Frey) Kuprian, <i>Lotus japonicus</i> (Regel) K.larson, <i>Lotus komarovii</i> Miniaev, <i>Lotus major</i> Scop., <i>Lotus olgae</i> Klokov, <i>Lotus peczoricus</i> Miniaev and Ulle, <i>Lotus ruprechtii</i> Miniaev, <i>Lotus tauricus</i> Juz., <i>Lotus ucrainicus</i> Klokov, <i>Lotus zhegulensis</i> Klokov)	BGBD	Loteae
11	<i>Lotus corniculatus</i> L. (= <i>Lotus ambiguus</i> Besser ex Spreng., <i>Lotus ambiguus</i> Spreng, <i>Lotus arvensis</i> Pers., <i>Lotus balticus</i> Miniaev, <i>Lotus carpetanus</i> Lacaita, <i>Lotus caucasicus</i> Kuprian, <i>Lotus caucasicus</i> Kuprian., <i>Lotus ciliatus</i> sensu Schur, <i>Lotus corniculatus</i> L. var. <i>crassifolia</i> Fr., <i>Lotus corniculatus</i> L. var. <i>kochii</i> Chrtkova, <i>Lotus corniculatus</i> L. var. <i>maritimus</i> Rupr., <i>Lotus corniculatus</i> subsp. <i>major</i> (Scop.) Gams, <i>Lotus corniculatus</i> var. <i>arvensis</i> (Pers.) Ser., <i>Lotus corniculatus</i> var. <i>glaber</i> Opiz, <i>Lotus corniculatus</i> var. <i>major</i> (Scop.) Brand, <i>Lotus corniculatus</i> var. <i>arvensis</i> (Schkuhr) Ser. ex DC., <i>Lotus filicaulis</i> Durieu, <i>Lotus frondosus</i> (Frey) Kuprian, <i>Lotus japonicus</i> (Regel) K.larson, <i>Lotus komarovii</i> Miniaev, <i>Lotus major</i> Scop., <i>Lotus olgae</i> Klokov, <i>Lotus peczoricus</i> Miniaev and Ulle, <i>Lotus ruprechtii</i> Miniaev, <i>Lotus tauricus</i> Juz., <i>Lotus ucrainicus</i> Klokov, <i>Lotus zhegulensis</i> Klokov)	BSST	Loteae

12	Lotus pedunculatus Cav. (= <i>Lotus granadensis</i> Zertova, <i>Lotus major</i> Smith, <i>Lotus trifoliolatus</i> Eastw., <i>Lotus uliginosus</i> Schkuhr)	BGBD	Loteae
13	Medicago citrina (Font Quer) Greuter (= <i>Medicago arborea</i> L. subspecies <i>citrina</i> Font Quer)	BGBD	Trifolieae
14	Medicago lupulina L. (= <i>Medicago appenina</i> Woods, <i>Medicago wildenowii</i> Merat, <i>Medicago lupulina</i> L. var. <i>cupaniana</i> (Guss.) Boiss, <i>Medicago lupulina</i> L. var. <i>glandulosa</i> Neilr.)	BGBD	Trifolieae
15	Medicago orbicularis (L.) Bartal. (= <i>Medicago orbicularis</i> (L.) All., <i>Medicago applanata</i> Hornem, <i>Medicago biancae</i> (Urban) Pinto da Silva, <i>Medicago cuneata</i> J. Woods, <i>Medicago marginata</i> Willd.)	BGBD	Trifolieae
16	Medicago sativa L. (= <i>Medicago afganica</i> (Bordere) Vassilcz; <i>M. grandiflora</i> (Grossh.)Vassilcz; <i>M. ladak</i> Vassilcz.; <i>M. mesopotamica</i> Vassilcz.; <i>M. orientalis</i> Vassilcz.; <i>M. polia</i> (Brand) Vassilcz.; <i>M. praesativa</i> Sinskaya; <i>M. sogdiana</i> (Brand) Vassilcz; <i>Trigonella upendrae</i> H.J. Chowdhery & R.R. Rao)	NRCD	Trifolieae
17	Medicago vardanis Vassilcz. (= <i>Medicago</i> × <i>varia</i> Martyn = <i>Medicago sativa</i> L. nothosubsp. <i>varia</i> (Martyn) Arcang.)	BGBD	Trifolieae
18	Melilotus albus Medik. (= <i>Melilotus argutus</i> Rchb., <i>Melilotus albus</i> var. <i>annua</i> Coe, <i>Melilotus leucanthus</i> W.D.J. Koch ex DC, <i>Melilotus melanospermus</i> Besser ex Ser., <i>Melilotus rugulosus</i> Willd., <i>Melilotus vulgaris</i> (Hayne) Willd., <i>Sertula alba</i> (Medikus) Kuntze, <i>Trifolium album</i> (Medikus) Loisel., <i>Trifolium vulgare</i> Hayne.)	BGBD	Trifolieae
19	Melilotus graecus (Boiss. & Spruner) Lassen (= <i>Trigonella graeca</i> Boiss. & Spruner)	BGBD	Trifolieae
20	Melilotus indicus (L.) All. (= <i>Melilotus bonplandii</i> Ten.; <i>M. indica</i> (L.) All.; <i>M. indicus</i> (L.) All. subsp. <i>permixtus</i> (Jord.)Rouy; <i>M. melilotus-indica</i> Asch. & Graebn.; <i>M. melilotus-indicus</i> Asch. & Graebn.; <i>M. officinalis</i> sensu Bojer; <i>M. parviflora</i> Desf.; <i>M. parviflorus</i> Desf.; <i>M. permixtus</i> Jord.; <i>M. tommasinii</i> Jord.; <i>Trifolium indica</i> L.; <i>T. indicum</i> L.; <i>T. indicus</i> L.; <i>T. melilotus-indica</i> L.)	WILD ³	Trifolieae
21	Melilotus messanensis (L.) All. (= <i>Melilotus sicula</i> (Turra) B.D. Jacks.; <i>M. siculus</i> (Turra) B.D. Jacks.; <i>Trifolium messanense</i> L.)	WILD ⁴	Trifolieae
22	Trifolium alexandrinum L. (= <i>Trifolium alexandrium</i> L.)	NRCD	Trifolieae
23	Trifolium arvense L. (= <i>Trifolium arenivagum</i> Boreau, <i>Trifolium brittingeri</i> Opiz, <i>Trifolium brachyodon</i> (Celak.)A.Kern., <i>Trifolium arvense</i> subsp. <i>gracile</i> (Thuill.)Nyman, <i>Trifolium arvense</i> var. <i>gracile</i> (Thuill.) DC., <i>Trifolium eriocephalum</i> Ledeb., <i>Trifolium gracile</i> Thuill., <i>Trifolium longisetum</i> Boiss. & Balansa, <i>Trifolium capitulatum</i> Pau, <i>Trifolium agrestinum</i> Boreau)	BGBD	Trifolieae
24	Trifolium campestre Schreb. (= <i>Trifolium erythranthum</i> (Griseb.)Halacsy, <i>Trifolium pseudoprocumbens</i> C.C.Gmel., <i>Trifolium thionanthum</i> Hausskn., <i>Trifolium campestre</i> var. <i>lagrangei</i> (Boiss.)Zohary, <i>Chrysaspis campestris</i> (Schreb.)Desv., <i>Trifolium agrarium</i> L. p. p., <i>Trifolium karatavicum</i> Pavlov, <i>Trifolium procumbens</i> var. <i>campestre</i> DC., <i>Trifolium lagrangei</i> Boiss., <i>Trifolium pumilum</i> Hossain, <i>Trifolium procumbens</i> L.)	BGBD	Trifolieae
25	T. campestre2	BGBD	Trifolieae
26	Trifolium lappaceum L. (= <i>Trifolium nervosum</i> C. Presl, <i>Trifolium rhodense</i> Pampanini, Boll. Soc., <i>Trifolium issajevii</i> Khalilov)	BGBD	Trifolieae
27	Trifolium fragiferum L. (= <i>Trifolium bonannii</i> Presl, <i>Trifolium neglectum</i> C.A. Mey, <i>Amoria fragifera</i> (L.) Roskov, <i>Galearia fragifera</i> (L.) C.Presl, <i>Galearia fragifera</i> Bobrov.)	BGBD	Trifolieae

28	<i>Trifolium infamia-ponertii</i> Greuter (= <i>Trifolium hybridum</i> , <i>Trifolium angustifolium</i> subsp. <i>gibellianum</i> Pignatti, <i>Trifolium angustifolium</i> subsp. <i>intermedium</i> (Gibelli & Belli) Arcang., <i>Trifolium angustifolium</i> var. <i>intermedium</i> Gibelli & Belli, <i>Trifolium intermedium</i> Guss.)	BGBD	Trifolieae
29	<i>Trifolium pignantii</i> Fauché & Chaub.	BGBD	Trifolieae
30	<i>Trifolium pratense</i> L. subsp. <i>Pratense</i> (= <i>Trifolium borysthenticum</i> Gruner, <i>Trifolium bracteatum</i> Schousb., <i>Trifolium lenkoranicum</i> (Grossh.) Roskov, <i>Trifolium pratense</i> var. <i>lenkoranicum</i> Grossh., <i>Trifolium ukrainicum</i> Opperman..)	BGBD	Trifolieae
31	<i>Trifolium rubens</i> L.	BGBD	Trifolieae
32	<i>Trifolium scabrum</i> L.	BGBD	Trifolieae
33	<i>Vicia angustifolia</i> L. (= <i>Vicia sativa</i> subsp. <i>nigra</i> (L.) Ehrh.)	BGBD	Vicieae
34	<i>Vicia faba</i> G461 (= <i>Faba bona</i> Medik.; <i>F. equina</i> Medik.; <i>F. faba</i> (L.) House; <i>F. major</i> Desf.; <i>F. minor</i> Roxb.; <i>F. sativa</i> Bernh.; <i>F. vulgaris</i> Moench; <i>Orobis faba</i> Brot.; <i>Vicia esculenta</i> Salisb.; <i>V. vulgaris</i> Gray)	EMAG	Vicieae
35	<i>Vicia faba</i> L. Line2	EMAG	Vicieae
36	<i>Vicia faba</i> L. Line4	EMAG	Vicieae

BGBD= Botanic Garden of Berlin-Dahlem, Germany; BGSA= Botanic Garden of Salzburg, Germany; BSST= Berlin-Spandau station, Germany; EMAG= Egyptian Ministry of Agriculture; GBGA= Botanic Garden of Gibraltar, Germany; NRCD= National Research Centre, Dokky, Giza, Egypt; WILD¹= Borg Al-Arab, Alexandria, Egypt; WILD²= Wadi Habes, Marsa-Matrouh, Egypt; WILD³= Tabia, Rashied, Egypt; WILD⁴= Boussaily, Rashied, Egypt.



11 **Fig. 1.** The produced banding patterns of seed protein analysis using SDS-PAGE technique. (M. Marker, 1. *Anthyllis barba-jovis*, 2. *Astragalus hamosus*, 3. *Colutea arborescens*, 4. *Dorycnium hirsutum*(1), 5. *Dorycnium hirsutum*(2), 6. *Genista linifolia*, 7. *Hippocrepis multisiliquosa*, 8. *Lathyrus aphaca*, 9. *Lathyrus pratensis*, 10. *Lotus corniculatus*(1), 11. *Lotus corniculatus*(2), 12. *Lotus pedunculatus*, 13. *Medicago citrina*, 14. *Medicago lupulina*, 15. *Medicago orbicularis*, 16. *Medicago sativa*, 17. *Medicago vardanis*, 18. *Melilotus albus*, 19. *Melilotus graecus*, 20. *Melilotus indicus*, 21. *Melilotus messanensis*, 22. *Trifolium alexandrinum*, 23. *Trifolium arvense*, 24. *Trifolium campestre*1, 25. *Trifolium campestre*2, 26. *Trifolium lappaceum*, 27. *Trifolium fragiferum*, 28. *Trifolium infamia-ponertii*, 29. *Trifolium pignanii*, 30. *Trifolium pratense*, 31. *Trifolium rubens*, 32. *Trifolium scabrum*, 33. *Vicia angustifolia*, 34. *Vicia faba*1, 35. *Vicia faba*(2), 36. *Vicia faba*(3).

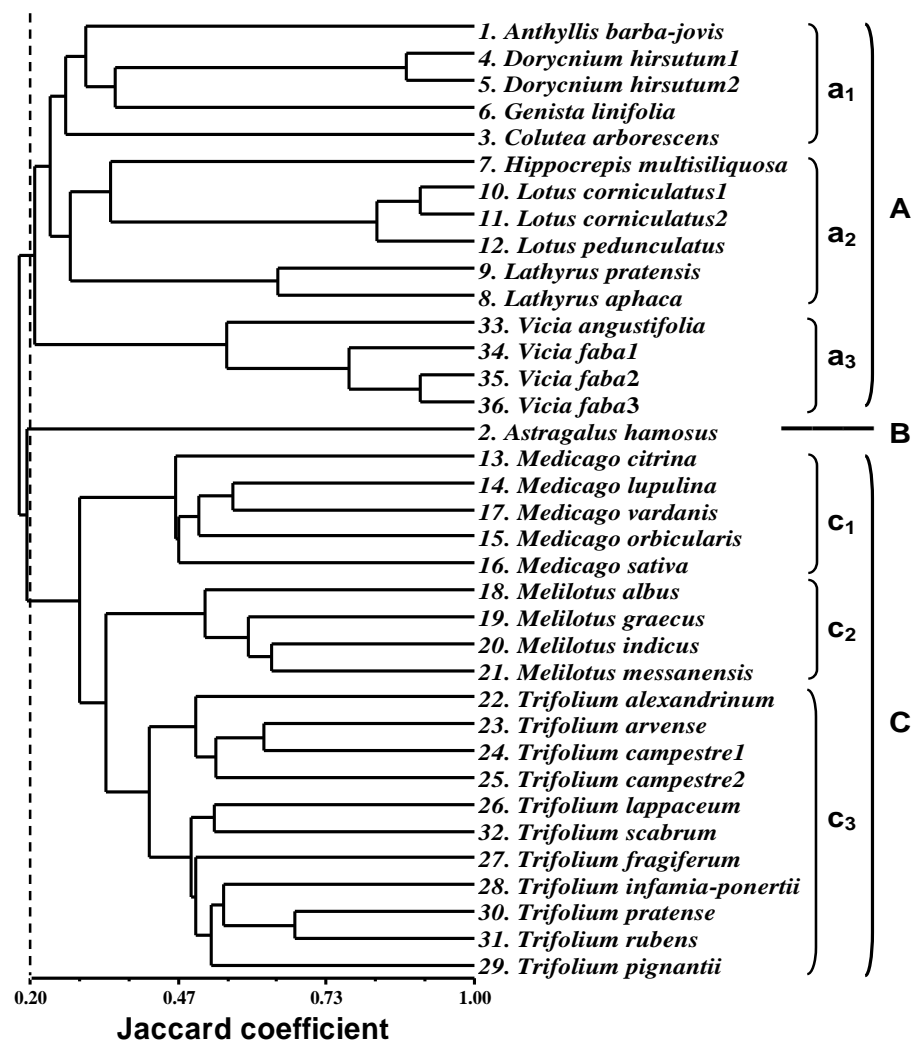


Fig. 2. The UPGMA phenogram showing clustering of the studied taxa based on combination of morphological and protein characters. Capital letters indicate the large groups, lowercase letters the subgroups.

Appendix 1. Basic data matrix of the 270 studied characters used in the phenetic analysis of Papilionoid taxa. Numbers correspond to the species in Table 1.

No.	Character \OUT's	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	3	3	3	3	3	3	3	3			
		1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6		
	Morphological Characters																																						
	Longevity:																																						
1	Annual	0	1	0	0	0	0	1	1	0	0	0	0	0	1	1	0	0	1	1	1	1	1	1	1	1	1	0	1	1	0	0	1	1	1	1	1		
2	Perennial	1	0	1	1	1	1	0	0	1	1	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0		
	Habit:																																						
3	Herb	0	1	0	0	0	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		
4	Shrub	1	0	1	1	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	Climbing:																																						
5	Tendrils	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	Stem Shape:																																						
6	crown arising from leaf axils	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
7	branched base	1	1	0	0	0	1	1	1	1	1	1	1	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
8	Woody base	0	0	1	1	1	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	Slightly hirsute	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
	Stem long:																																						
10	≥10 cm	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
11	10-30 cm	0	1	0	0	0	0	1	0	0	1	1	1	0	1	1	0	1	0	1	0	0	0	1	1	1	1	1	1	1	1	1	0	1	0	0	0	0	
12	30-100 cm	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	1	1
13	100-400 cm	1	0	1	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	< 400 cm	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Stem Surface:																																						
15	Glabrous	1	0	1	0	0	0	1	1	1	0	0	0	0	1	0	0	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	1	0	1	1	1	1
16	Pubescent	0	1	0	1	1	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	Hairy	0	0	0	0	0	1	0	0	0	1	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	1	0	0	0
	Stipules Shape:																																						
18	Adnate to petiole	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
19	Broadly triangular	1	1	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
20	Green-veined	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0
21	Lanceolate	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	1	1	1	1	1	0	0	0	0	1	1	0	0	1	1	0	0	1	1	0	0	0	0
22	Leafy	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23	Oblong	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24	Ovate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0	0	0	0
25	dark spots at the	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Pod Surface:																																															
152	Hairy	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0													
153	Veined	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1												
154	Pubescent	1	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0												
155	Smooth	0	0	0	0	0	0	1	1	0	1	1	1	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	1	1	1	1											
156	Pilose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0											
Pod Compression:																																															
157	Compressed	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1											
158	Deflexed	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0										
Pod Segment number:																																															
159	≥ 6	1	1	1	1	1	1	1	1	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1									
160	< 7-12	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0								
161	< 12	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0								
Seed Compression:																																															
162	Compressed	1	0	1	1	1	0	1	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1									
163	Not compressed	0	1	0	0	0	1	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0								
Seed Shape:																																															
164	Cylindrical	0	1	0	1	1	0	1	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0								
165	Flattened	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1								
166	Ovoid	1	0	0	0	0	1	0	1	1	0	0	0	0	0	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1								
167	Reniform	0	0	1	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0								
Seed Surface:																																															
168	Fine tuberculate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0								
169	Finely reticulate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0								
170	Glossy	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	0	1	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1								
Seeds Number:																																															
171	≥ 4	1	1	0	1	1	1	1	0	0	0	0	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	0							
172	< 4-8	0	0	1	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1						
173	< 8-15	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0						
Protein Characters*																																															
174	Mr. (kDa) 117.71	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	0	0	0	
175	115.85	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
176	111.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0

213	42.94	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0			
214	41.74	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0			
215	40.88	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0	1	1	1			
216	39.37	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0			
217	38.48	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0			
218	37.71	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0			
219	37.44	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0		
220	36.55	1	0	0	0	0	1	0	0	0	0	0	0	1	1	1	0	0	0	0	1	1	1	0	0	1	1	0	0	1	0	0	0	1	0	0	0	0		
221	35.40	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0		
222	35.00	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0		
223	34.52	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	1	1	1	1	0	0	1	1	0	1	1	1	1	0	0	0	0	0	0	0	0		
224	33.59	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0		
225	32.45	1	0	1	0	1	1	0	0	1	1	1	1	0	1	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0		
226	32.00	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0		
227	31.41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1	0	1	0	0	0	0	1	1	0	0	0		
228	31.00	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0		
229	30.74	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1		
230	30.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	
231	29.52	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1	0	0
232	28.74	0	0	0	0	1	0	0	0	0	1	0	0	0	1	1	0	0	0	1	0	0	1	1	1	1	0	0	0	0	1	0	0	1	0	0	1	0	0	0
233	27.71	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0
234	27.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	
235	26.62	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	1	0	0	1	0	0	0
236	26.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	
237	25.89	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	0	0	0	1	0	0	0	0	0	0	
238	25.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	
239	24.67	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	1	1	1	1
240	24.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	
241	23.68	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	1	1	0	1	0	1	0	0	
242	23.30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0	
243	22.42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	1	1	1	
244	21.50	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	1	1	0	0	1	1	0	1	1	1	1	1	1	0	1	0	0	0	1		
245	21.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	
246	20.66	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0	0	0	1	1	1	0	0	0	1	1	0	0	1	0	0	0	0
247	19.56	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	1	0	0	0	0
248	19.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	1	0	0	1	1	1

249	18.85	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0				
250	18.40	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0			
251	17.54	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0			
252	16.82	1	0	1	0	1	0	0	1	1	0	1	1	0	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0			
253	16.09	1	0	1	0	1	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
254	15.51	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	0	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0			
255	15.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	1	0	0	0			
256	14.70	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	1	1	0	0	0	0	0	0			
257	14.32	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	0	1	0	1	0	0	0	0	0	0	0				
258	13.52	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	1	0	1	1	1	0	0	0	
259	13.00	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0			
260	12.60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	
261	12.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1
262	11.95	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1
263	11.79	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
264	11.54	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
265	11.03	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	1	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	1	1	1	1	1	1	1
266	10.80	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	1	0	1	0	0	0	0	1	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1
267	10.50	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	0	0	0	0	0	0	0	1	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0
268	10.02	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	
269	9.58	0	0	0	1	1	0	1	0	0	1	1	1	0	0	0	1	0	0	1	1	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
270	9.42	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

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