

CHARACTERIZATION OF 7 *VICIA* L. TAXA FROM ALGERIA BASED ON EVIDENCE FROM MORPHOLOGY AND ECOGEOGRAPHY

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Abstract

Description of the subject: The genetic diversity of a vetch collection collected in Algeria was assessed by the variation of 78 plant morphological characters and 5 ecogeographic parameters of the collection stations. 37 accessions belonging to 7 taxa of the genus *Vicia* L. were the subject of this study

Objective: Characterize the accessions using two different markers and then correlate their results. In parallel, the results are used in the taxonomy and phylogeny of the populations studied

Methods: the hierarchical classification in clusters using a Euclidean distance matrix based on a UPGMA - principal component analysis - the Mantel test used to correlate the matrices of ecogeography and morphology

Results: Cluster analysis showed clear separation among accessions from their plant morphology but significant differences at subspecies level were observed regarding the results of investigated morphological traits. Important diversity in leaflet consistence, calyx hairness, stem form, leaflet abaxial hair density, leaflet hair elevation, seed colour, spongy inside legume and peduncle length was found in most accessions of the collection. The dendrogram obtained with ecogeographic parameters did not indicate clear division among accessions. A distribution map of each taxa was produced and data obtained can be used as a guide for future collecting missions.

Conclusion: inter and intraspecific differences were obtained by the morphological descriptors. The collected ecogeographic data can be used as a guide for future collection missions. Other markers should be used for the phylogeny of the collection.

Keywords: Algeria; diversity; ecogeography; morphology; *Vicia* L.

CARACTERISATION DE 7 TAXA DE *VICIA* L. COLLECTÉS EN ALGÉRIE, SUR LA BASE DE LA MORPHOLOGIE ET DE L'ÉCOGÉOGRAPHIE

Résumé

Description du sujet : La diversité génétique d'une collection de vesce collectée en Algérie a été évaluée par la variation de 78 caractères morphologiques et 5 paramètres écogéographiques des stations de collecte. 37 accessions appartenant à 7 taxa du genre *Vicia* L. ont fait l'objet de la présente étude

Objectifs : Caractériser les accessions en utilisant deux marqueurs différents puis corrélés leurs résultats. En parallèle, les résultats sont exploités dans la taxonomie et la phylogénie des populations étudiées

Méthodes : la classification hiérarchique en clusters en utilisant une matrice de distances Euclidiennes basée sur une UPGMA - une analyse en composantes principales - le test de Mantel pour corrélés les matrices de l'écogéographie et la morphologie

Résultats : L'analyse en clusters a montré une nette séparation entre les accessions sur la base de leur morphologie mais des différences significatives entre les sous-espèces ont été observées. Une diversité importante dans la consistance des folioles, la pilosité du calice, la forme de la tige, la densité des poils abaxiaux des folioles, l'élevation des poils des folioles, la couleur des graines, la spongiosité à l'intérieur des gousses et la longueur du pédoncule a été observée dans la plupart des accessions de la collection. Le dendrogramme obtenu avec les paramètres écogéographiques n'a pas indiqué de séparation claire entre les accessions. Une carte de distribution de chaque taxon a été produite

Conclusion : Des différences inter et intraspécifiques ont été obtenues par les descripteurs morphologiques. Les données écogéographiques collectées peuvent être utilisées comme guide pour les futures missions de collecte. D'autres marqueurs doivent être exploités pour la phylogénie de la collection.

Mots clés: Algérie; diversité; écogéographie; morphologie; *Vicia* L.

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INTRODUCTION

The taxonomy and nomenclature of *Vicia* species are very confused. The number of species recognized in the genus has varied significantly from about 150 accepted by [1] up to about 210 used by [2], which indicates differences in species delimitation and ranking in the genus, depending on the species concept and the criteria used for their delimitation. [3] pointed out that there had been 20 major classifications of the group since Linnaeus. These problems have, at least partially, resulted from the publication of national or regional floristic accounts, but also because of the conflicting results produced by morphological, cytological and molecular investigations [2]; [4]. Many characters vary considerably within the genus, but few prove useful for infrageneric grouping like characters of the morphology of the inflorescence, the flower, the leaf, the pod and the seed. Several not greatly different classifications were proposed for the genus on the basis of the above-mentioned character differentiations [2]. The genus has been subdivided into four sections (Ervum, Cracca, Vicia, Faba) by [5]. [1] has subdivided the genus into two subgenera (*Vicilla*, *Vicia*) which can be distinguished using stipule nectary, peduncle length, style type, keel shape, legume and canavanine. Subgenera *Vicilla* and *Vicia* have been further subdivided into 17 and 5 sections, respectively. The subgenus *Vicia* sensu [3] contains 9 sections including section Hypechusa composed of 14 species (incl. *V. lutea*) and section Narbonensis which comprises 7 species (incl. *V. narbonensis*). [6] indicated that *Narbonensis* should be considered a well-separated section which may be related to section *Vicia*. Moreover, [7] showed that *V. faba* clustered in the *Narbonensis* group with good support and treated the group as section Narbonensis. Section Hypechusa (subgenus *Vicia*) is based on the genus Hypechusa created by [8] who separated into it six *Vicia* species. Hypechusa is also treated as section by [1] and [3]. *V. lutea* was treated in a separate series Luteae B. Fedtch. by [9] and [10]. The isozyme evidence provides further support to this treatment, even suggesting sectional status for Luteae. According to [11], *V. lutea* differs distinctly from the other Hypechusa species by showing no cross-hybridization of genomic DNAs.

[12] placed *V. lutea* separately from a clade of series Hypechusa. The subgenus name Cracca (Dum.) Gams was rejected by [1] because it was published later than the subgenera proposed by [13]: *Vicilla*, *Ervoidea* and *Pseudoervoidea*. [14] has applied Cracca Peterm. as a correct name of the subgenus (published in 1847: Deutchl. Fl., 152). Section Cracca sensu [1] belongs to Subgenus *Vicilla* and is composed of 40 species including *V. monantha*, *V. tenuifolia*, *V. leucantha*.

In the case of the *Vicia* species, only a relatively minor proportion of the genepool is currently conserved and therefore available for utilization. There is a clear requirement for further study of the genepool, accurately targeted conservation (particularly seed collection), along with characterization and evaluation of the currently unavailable elements of the gene pool. The first step towards more efficient conservation is to undertake an ecogeographic study [15]. The distribution maps will be used to formulate conservation policy and specifically in the planning of future *ex situ* collecting missions.

In Algeria, the genus *Vicia* L. comprises 26 species according to the key used by [16] in the unique flora covering the entire country. Using the relative length of the flower, the presence or the absence of the peduncle and the number of flowers by inflorescence, the authors divided the genus into three series where *V. narbonensis* and *V. lutea* belong to the serie 1, *V. monantha* and *V. tenuifolia* are grouped in the serie 2 and *V. leucantha* belong to the serie 3. Since [16], no work has been undertaken to study the genus in Algeria. As the characterization of plant genetic resources is the first step towards improving their use and as effective exploitation of any crop germplasm must be preceded by efficient and comprehensive conservation of the gene pool, whether *ex situ* or *in situ*, the purpose of the present work was to study the diversity of *Vicia* L. taxa from Algeria on both an inter and intraspecific levels and evaluate species groupings and relationships in the genus. The paper describes variation of 78 morphological traits and 5 ecogeographic parameters among 7 *Vicia* taxa belonging to sections Hypechusa, Narbonensis and Cracca to improve the resolution of phylogenetic relationships and to assess the usefulness of plant morphology as diagnostic characters to discriminate vetch species and sections.

MATERIAL AND METHODS

1. Plant material and taxa identification

Thirty-seven populations of *Vicia* L. collected from their natural habitats in various bioclimatic conditions of north Algeria are used in the current study. Origins and field information are given in Table I. Localities of the studies taxa were shown in Figure I. Individual plants were randomly collected in each sampling site. Pods were shelled and the dry seeds were poured in

to separate paper bags at room temperature and the bags sealed tightly until their utilization. As the only taxonomic study of the whole genus *Vicia* in Algeria was undertaken by [16], the present study relies on their taxonomy. Taxonomic identification of accessions was verified by the morphology of plants grown from seeds. Germination and growing were carried out according to the same approach adopted in our previous study [17].

Table 1.Passport information and taxonomic identification of accessions investigated

| Species | Subspecies | Code | Date of collection | Province /Locality/Origin | Latitude | Longitude | Altitude (m) | | | |
|--------------------------|--|---------|--------------------|-----------------------------|---|--------------|--------------------------------|-------------|--------------|-----|
| <i>V. lutea</i> L. | | 1 | 28.5.14 | Skikda Ramdane Djamel | N36°45.977' | E006°53.432' | 42 | | | |
| | | 4 | 22.5.14 | Constantine University | N36°20.387' | E006°37.177' | 604 | | | |
| | | 58 | 27.5.14 | Constantine Didouche Mourad | N36°30.025' | E006°40.058' | 448 | | | |
| | | 82 | 28.5.14 | Guelma | N36°28.361' | E007°21.280' | 223 | | | |
| | | | 62 | 1st.6.14 | Skikda Azzaba | N36°43.531' | E007°04.708' | 110 | | |
| | | | 63 | 1st.6.14 | Skikda Ain Cherchar | N36°44.366' | E007°14.176' | 52 | | |
| | | | 79 | 1st.6.14 | El Tarf Ben M'hidi | N36°46.402' | E007°53.600' | 11 | | |
| | | | 87 | 30.5.14 | Jijel El Milia | N36°46.668' | E006°13.551' | 28 | | |
| | | | 90 | 1st.6.14 | Annaba | N36°49.980' | E007°34.092' | 24 | | |
| | | | | | 3 | 2.6.14 | Oum El BouaghiSigus | N36°04.485' | E006°48.867' | 822 |
| | | | | | 12 | 5.6.14 | Relizane | N35°43.689' | E000°24.265' | 105 |
| | | | | | 26 | 28.5.15 | Guelma | N36°14.816' | E007°03.045' | 757 |
| | 27 | 2.6.14 | | | Oum El Bouaghi | N35°51.459' | E007°06.377' | 887 | | |
| | 40 | 28.5.14 | | | Guelma | N36°16.276' | E007°05.751' | 711 | | |
| | 44 | 6.6.14 | | | Tlemcen | N34°52.088' | W001°11.698' | 843 | | |
| | 45 | 3.6.14 | | | Bordj Bou Areridj | N36°04.070' | E004°41.899' | 923 | | |
| | 49 | 28.5.14 | | | Constantine Ain Abid | N36°13.543' | E006°55.782' | 847 | | |
| | 60 | 2.6.14 | | | Khenchla | N35°33.685' | E007°02.177' | 860 | | |
| | 74 | 14.6.14 | | | Tébessa | N35°15.936' | E007°30.306' | 1078 | | |
| | 77 | 26.5.14 | | | Batna Ain Touta | N35°17.632' | E005°49.035' | 683 | | |
| | 78 | 20.5.14 | | | Constantine El Coudiat | N36°21.787' | E006°36.418' | 633 | | |
| | 84 | 10.6.14 | | | Constantine INATAA | N36°19.002' | E006°34.626' | 586 | | |
| | 98 | 14.6.14 | | | Tébessa Chria | N35°16.328' | E007°44.359' | 1087 | | |
| | 29 | 2.6.14 | | | Oum El BouaghiSigus | N36°04.485' | E006°48.867' | 822 | | |
| | <i>V. monanthasspinerea</i> (M.B.) Maire | | | | 18 | 3.6.14 | Bordj Bou Areridj Ain taghrout | N36°07.741' | E005°03.364' | 934 |
| | | | 46 | 3.6.14 | Bordj Bou Areridj El Achir | N36°04.017' | E004°40.525' | 944 | | |
| | | | 91 | 14.6.14 | Khenchla | N35°15.704' | E007°20.957' | 1222 | | |
| | | | 101 | 14.6.14 | Khenchla | N35°15.807' | E007°28.934' | 1077 | | |
| | <i>V. narbonensis</i> L. | | 23 | 30.5.14 | Constantine HammaBouziane (Chaabet El Medhbouh) | N36°26.391' | E006°33.282' | 425 | | |
| | | | 30 | 30.5.14 | Mila Messaoud Boudjriou | N36°29.748' | E006°25.530' | 325 | | |
| 34 | | | 27.4.14 | Constantine Didouche Mourad | N36°29.216' | E006°38.731' | 434 | | | |
| 55 | | | 27.5.14 | Constantine Didouche Mourad | N36°30.023' | E006°40.051' | 443 | | | |
| | | | 81 | 22.5.14 | Constantine University | N36°20.387' | E006°37.177' | 604 | | |
| | | | 56 | 6.6.14 | Ain Temouchent | N35°16.476' | W001°13.800' | 276 | | |
| | | | 89 | 6.6.14 | Sidi Bel Abbès Sidi Khaled | N35°06.59' | W000°44.238' | 543 | | |
| | | | 97 | 6.6.14 | Ain Temouchent Ain Tolba | N35°14.721' | W001°17.054' | 222 | | |
| <i>V. leucantha</i> Biv. | - | 100 | 10.6.14 | Constantine INATAA | N36°19.002' | E006°34.626' | 586 | | | |



Figure1.Geographical origin of the 37 Algerian populations studied

2. Morphological analyses

The variation of 78 morphological characters with two to five states was analyzed (Table 2) on 111 individuals using the same approach explained by [17].

Table 2. Morphological characters used for cladistic analysis

| | Symbol | Character name | Character state | | | |
|----------|---------------------------|-------------------------------------|------------------------------|----------------------------------|--------------------------------|-----------------------------|
| Stem | S.B | Stem branching | 0- unbranched | 1 - in the upper part | 2 - from the base | |
| | S.H | Stem height | 0 - small (up to 40cm) | 1 - high (over 40cm) | | |
| | S.F | Stem Form | 0- slender | 1 - rigid | | |
| | SEC | Stem edgescolor | 0- green | 1 - purple | | |
| | SP | Stem pubescence | 0- glabrous | 1 - sparse | 2 - dense | |
| | SHE | Stem hairerelevation | 0- ascending | 1 - adpressed | 2 - curled | |
| | SHL | Stem hairlength | 0- short | 1 - long | | |
| | SNC | Stem nodecolour | 0 - green | 1 - purple | | |
| Leaflets | LPL | Leaflets pairs per leaf | 0- 1-3 pairs | 1 - more than 3 pairs | | |
| | LRS | Leaflets relative shape | 0- all of same shape | 1 - different shapes | | |
| | LRS | Leaflets relative size | 0- all of same size | 1 - larger at leaf base | | |
| | LC | Leafletconsistence | 0- thin | 1 - normal | 2 - fleshy | |
| | LAC | Leafletabaxialcolour | 0- pale | 1 - same as adaxial | | |
| | LLP | Lowerleaflets position | 0- at base of rachis | 1 - higher | | |
| | LS | Leafletshape | 0- lineal | 1 - elliptical | 2 - obovate | 3 - ovate |
| | LBP | Leafletbroadest point | 0- at apex | 1 - in middle | 2 - at base | |
| | LAS | Leaflet apex shape | 0- acute | 1 - obtuse | 2 - truncate | 3 - emarginate |
| | LAD | Leaflet apex dentate | 0 - not | 1 - two teeth | 2 - more than two | |
| | LB | Leaflet base | 0- rounded | 1 - acute | | |
| | LM | Leafletmargin | 0 - entire | 1 - dentate | 2 - undulate | |
| | LMH | Leafletmarginhairness | 0- glabrous | 1 - ciliate | | |
| | LHE | Leaflethairelevation | 0 - ascending | 1 - adpressed | | |
| LAHD | Leafletabaxialhairdensity | 0 - glabrous | 1 - sparse | 2 - dense | | |
| Stipules | SS | Stipule size | 0- small (shorter than 6 mm) | | 1 - large (longer than 6 mm) | |
| | SSh | Stipule shape | 0- lanceolate | 1 - semi-hastatte | 2 - semi-sagittatte | 3 - ovate |
| | SNS | Stipule nectariferous spot | 0 - absent | 1 - dark | 2 - pale | |
| | SE | Stipule edge | 0- entire | 1 - dentate | 2 - 2 - 3-partite | |
| | Spa | Stipules of the pair | 0 - identical | 1 - different | | |
| Tendrill | TP | Tendrillpresence | 0- absent | 1 - present | 2 - in some leaves | |
| | TB | Tendrillbranching | 0- not branched | 1 - branched | | |
| | TL | Tendrilllength | 0- short | 1 - long | | |
| | THD | Tendrillhairdensity | 0- glabrous | 1 - sparse | 2 - dense | |
| Flower | NFI | Number of flowers per inflorescence | 0- one | 1 - 1 - 4 | 2 - 5 or more | |
| | FL | Flower length | 0 - up to 0.8(0.9) cm | 1 - 2.5 cm | 2 - over 2.5 cm | |
| | PcL | Pedicelllength | 0- shorter than calyx | 1 - equal or longer than calyx | | |
| | PL | Pedunclelength | 0- absent | 1 - very short | 2 - up to as long as leaf | 3 - longer than leaf |
| | SCP | Standard colour pattern | 0 - absent | 1 - differently coloured spot | 2 - differently coloured veins | |
| | SC | Standard colour | 0 - white | 1 - yellow | 2 - purple (bluish) | |
| | SP | Standard pubescence | 0- glabrous | 1 - pubescent at back | | |
| | SASh | Standard apex shape | 0- stronglyemarginate | 1 - slightlyemarginate | | |
| | WCP | Wing colour pattern | 0- absent | 1 - tip differently coloured | 2 - differently coloured veins | |
| | WC | Wing colour | 0- white | 1 - yellow | 2 - purple | |
| | WL | Wing length | 0- ¼ shorter than standard | 1 - little shorter than standard | 2 - longer than standard | |
| | KC | Keelcolour | 0- white | 1 - yellow | 2 - purple | |
| | KCP | Keelcolour pattern | 0- absent | 1 - dark tip | 2 - much darker | |
| | KL | Keellength | 0- shorter than wings | 1 - equal or longer than wings | | |
| | CBS | Calyx base shape | 0 - not gibbous | 1 - slightly gibbous | 2 - strongly gibbous | |
| | CMS | Calyxmouthshape | 0- straight | 1 - slightly oblique | 2 - strongly oblique | |
| | CH | Calyxhairness | 0- glabrous | 1 - clayx teeth only | | 2 - general coverage |
| | CHD | Calyxhairdensity | 0- sparse | 1 - dense | | |
| | CC | Calyxcolour | 0- green | 1 - purple at base | 2 - teeth purple | 3 - purple 4 - dark at base |
| | CTL | Calyxteethlength | 0- equal | 1 - unequal | | |
| | CUTL | Calyxupperteethlength | 0- longer than tube | 1 - shorter than tube | | |
| | CLTL | Calyxlowerteethlength | 0- longer than tube | 1 - shorter than tube | | |
| | CUTSh | Calyxupperteethshape | 0- triangular | 1 - lanceolate | 2 - subulate | |
| CLTSh | Calyxlowerteethshape | 0- triangular | 1 - lanceolate | 2 - subulate | | |

| | Symbol | Character name | Character state | | | |
|--------|--------|-------------------------|--------------------|--------------------------------|--------------------------------|----------------|
| Legume | LSh | Legumeshape | 0 - linear | 1 - lanceolate | 2 - rhomboid | 3 - oblong |
| | LCS | Legume cross-section | 0- rounded | 1 - slightly compressed | | 2 - compressed |
| | LP | Legume position | 0- erect | 1 - horizontal | 2 - hanging | |
| | LSt | Legumestipitate | 0 - not | 1 - stipitate | | |
| | Lpu | Legume pubescence | 0- glabrous | 1 - entire coverage | 2 - only on sutures | |
| | LSf | Legume surface | 0- smooth | 1 - slightly ridged with veins | 2 - strongly ridged with veins | |
| | LT | Legumetorulose | 0 - not | 1 - torulose | | |
| | LC | Legumecolour | 0- black | 1 - brown | 2 - yellowish brown | 3 - yellow |
| | LSC | Legume suture curvature | 0- suture parallel | 1 - sutures unparallel | | |
| | LSH | Legume suture hairness | 0- glabrous | 1 - hairy | 2 - ciliate | |
| | LBe | Legumebeak | 0- absent | 1 - short | 2 - long | |
| | LBSH | Legumebeakshape | 0- straight | 1 - curved up | 2 - curved down | |
| Seed | SNL | Seednumber per legume | 0 - 2 | 1 - 3 - 4 | 2 - over 5 | |
| | Ssha | Seedshape | 0- spherical | 1 - cubical | 2 - elliptical | |
| | SCS | Seed cross-section | 0- uncompressed | 1 - compressed | | |
| | Ssu | Seed surface | 0- smooth | 1 - wrinkled | | |
| | Sco | Seedcolour | 0- black | 1 - brown | 2 - red-brown | 3 - yellowish |
| | SCM | Seedcolourmottling | 0- absent | 1 - present | | |
| | HL | Hilumlength | 0 - long(70 - 80%) | 1 - intermediate(20 - 40%) | 2 - short(less than 20%) | |
| | HC | Hilumcolour | 0 - pale | 1 - seedcolour | 2 - dark | |
| | LSI | Legumespongyinside | 0 - not | 1 - spongy | | |
| | SSz | Seed Size | 0 - 3-5(6) mm | 1 - over 6 mm | | |

3. Ecogeographic parameters and climatic data corrections

A Global Positioning Systems (GPSGARMIN eTrex® model 30) was used to collect coordinates of sites investigated. Each sampling site was characterized by the five ecological factors (table 3) of Mediterranean climate as

described in [17]. Data of reference stations did not reflect the point of view ecogeographical actual bioclimatic conditions. For this reason, we have made correction of climate data based on extrapolations for different altitudinal point as reported by [17].

Table 3 Climatic characteristics of reference stations (2004-2014)

| Reference station | Latitude | Longitude | Alt. (m) | P (mm) | m (°C) | M (°C) |
|---------------------|----------|-----------|----------|--------|--------|--------|
| Jijel (airport) | 36°48 N | 05°53 E | 8 | 1066.1 | 6.8 | 31.5 |
| Skikda | 36°53 N | 06°54 E | 2 | 829 | 8.8 | 29 |
| Annaba | 36°50 N | 07°48 E | 3 | 684.4 | 6.7 | 31.5 |
| Relizane | 35°44 N | 00°32E | 95 | 352.5 | 5.3 | 38.6 |
| Constantine | 36°17 N | 06°37 E | 693 | 486.6 | 2.2 | 35.2 |
| Bordj Bou Areridj | 36°04 N | 04°46 E | 928 | 392.9 | 1.7 | 36.4 |
| Khenchla | 35°28 N | 07°05 E | 983 | 520.8 | 1.8 | 34.9 |
| Oum El Bouaghi | 35°52 N | 07°07 E | 889 | 410.4 | 1.1 | 35 |
| Tlemcen (Zenata) | 35°01 N | 01°28 W | 246 | 359.8 | 6.2 | 33.9 |
| Tébessa | 35°25 N | 08°07 E | 821 | 382.6 | 1.7 | 35.6 |
| Batna | 35°45 N | 06°19 E | 822 | 346.8 | 0.1 | 36.4 |
| Guelma | 36°28 N | 07°28 E | 227 | 622.3 | 4.5 | 36.4 |
| Mila* | 36°27 N | 06°16 E | 437 | 742 | 4.4 | 31.5 |
| Ain Temouchent* | 35°17 N | 01°08 W | 235 | 485 | 6.8 | 30.2 |
| El Tarf Ben M'hidi* | 36°46 N | 07°54 E | 6 | 707 | 7.1 | 31.2 |

Alt altitude, P annual rainfall, M and m are the of the average maximum temperature of the hottest month and the average of the minimum of the coldest month, respectively * Data from "Climate data org"

4. Calculation of the bioclimatic coefficient of Emberger (1955) and definition of the bioclimate

The pluviothermic Emberger quotient (Q2) is determined by the combination of three major climate factors. This quotient easy to interpret: the more Q2 values are larger and more stations are wet. Calculation details are reported in [17].

5. Data analyses

Cluster analysis of morphological characters and ecogeographic parameters were performed with Euclidean Distances Matrix based on the UPGMA. In the other side, a PCA was undertaken to clarify correlation between morphological characters and to identify characters which contribute the most in accessions distinction.

These analyses were carried out with Data analysis software (STATISTICA version 6.1 program). Finally, to display a possible correlation between morphological characters and ecogeographic parameters, a Mantel test [18] based on Pearson's correlation was used (XLSTAT Pearson edition, version 2014.5.03). The p-value was calculated from the distribution of $r(AB)$ using 10000 permutations.

RESULTS

1. Cluster analysis based on morphological characters

The dendrogram illustrated in Figure 2 shows the relationships between taxa, based on the variation in morphological characters. At a distance of 7.71, the dendrogram can be divided into 2 major clusters (I and II).

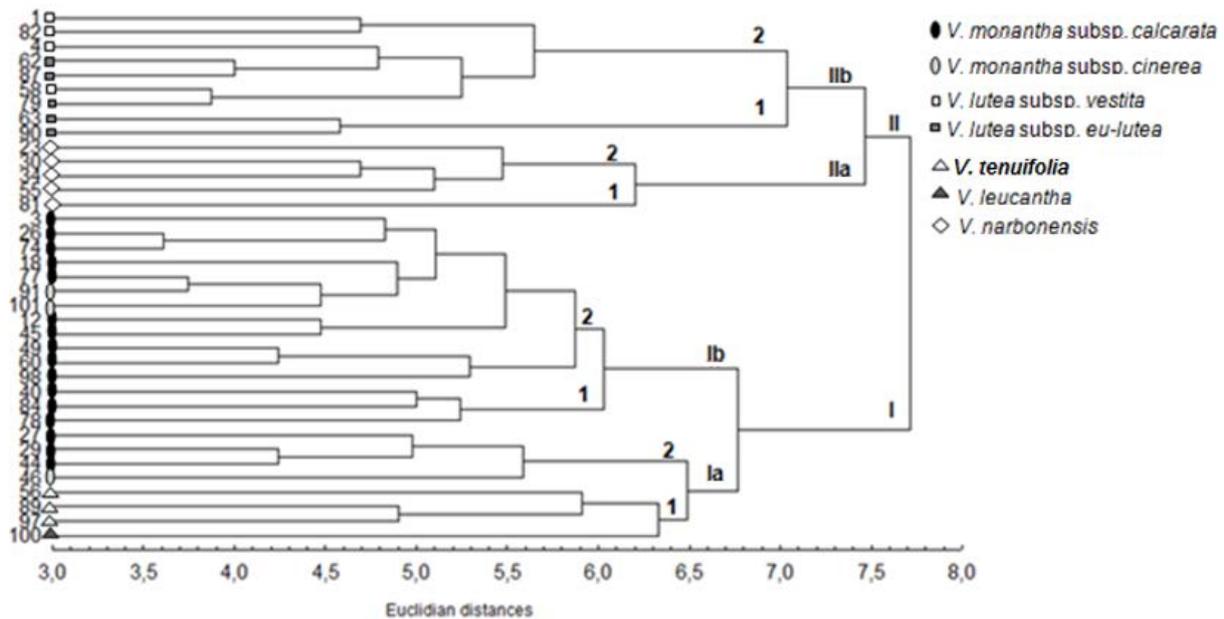


Figure 2. Dendrogram generated using UPGMA cluster analysis based on morphological diversity of 37 *Vicia L.* accessions

1.1. Interspecific polymorphism

The similarity matrix of all pairs of studied populations (Fig. 3) shows variability within and between accessions. The low distance value indicates high level of homology in the variation pattern of the morphological characters. Accessions 26 and 74 appears to be a sister taxa of *V. monantha* subsp. *calcarata*. This clade is strongly supported by the lowest distance ($d=3.61$). Differences between the two accessions concern only 13 characters on 78 used (SP-SHL-LB-LHE-TP-TL-THD-NFI-SCP-WCP-CBSh-CMSh-SNL).

The higher distance ($d=9.59$) is observed between 97 (*V. tenuifolia*) and 34 (*V. narbonensis*) which are different by 41 characters (SB-SEC-SP-LPL-LRSh-LRS-LC-LLP-LS-LAS-LB-LM-LHE-LAHD-SS-NFI-PL-WCP-WC-KC-CBSh-CMSh-CH-CC-CTL-CLTSh-LCS-LP-Lpu-LT-LC-LSH-LB-LBSh-SNL-Ssha-SCS-Ssu-SCM-HL-SSz).

1.2. Intraspecific variability

For each species, a distance matrix is obtained and used to construct the dendrogram between accessions of the same species (data not shown). The results of the dendrograms are used in the discussion.

2. Principal Component Analysis based on morphological data

Six characters (LAC-LBP-LAD-TB-WL-LBSh) were excluded from PCA as they are homologous between all accessions. The remaining 72 characters were used for the analysis. Two-dimensional (2D) plot was obtained using the first two PCs. The morphological characters which strongly contributed to the formation of PC1 are LC, CH, SF, LAHD, LHE, Sco, LSI and PL. Those which most contribute to the formation of PC2 are LSf, SCM, HL, LPL, LLP and LRSh. In the other side, characters which have a low loading in accessions distinction are SNL, NFI, FL, SB for PC1 and LSI, CC, PcL, SP and LC for PC2.

The strongest positive correlation ($r = 1.00$) was found between SP and PcLand also between SCM and LPL. The strongest negative one ($r = -0.89$) was obtained between LPL and LRSh, SCM and LRSH ($p < 0.05$).

In parallel, the projection of individuals (accessions) was done using the observation with the sum of the squared cosinus ≥ 0.00 . The results are shown in figure 5.

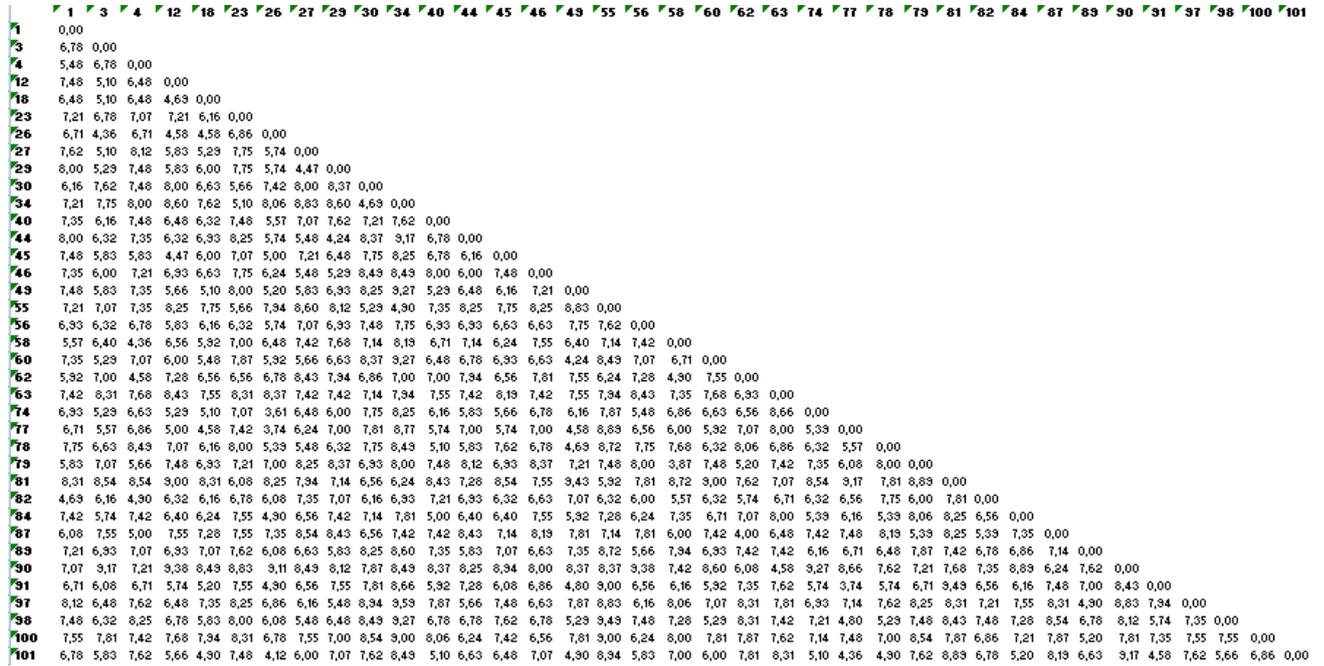


Figure 3. Similarity matrix of morphological characters

Figure 4. Principal Component Analysis of 72 morphological characters

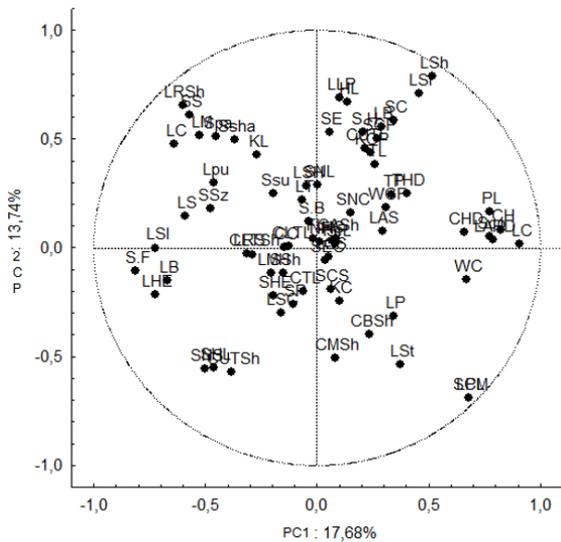
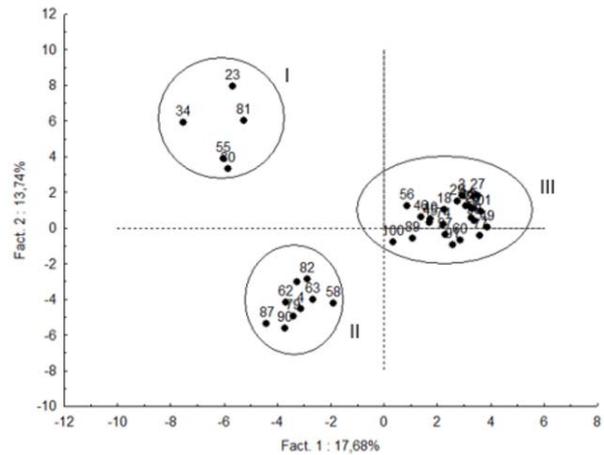


Figure 5. Principal Component Analysis of studied populations



3. Ecogeographic parameters

3.1. Data correction and bioclimate definition

The results of correction of the pluviometric and temperature data and calculation of Q2 were used to define the bioclimate of each site using

Emberger climagramm. All results are shown in table 4. The large difference between taxa in the range of GPS coordinates from which they were collected is reported in table 1.

Table 4. Corrected data of climatic characteristics and calculation of Emberger quotient of sampling sites investigated

| Code | Alt. (m) | K | P (mm) | m (°C) | M (°C) | Q2 | Bioclimate |
|------|----------|--------|---------|--------|--------|--------|-----------------------|
| 1 | 42 | 1,0386 | 860,99 | 8,64 | 28,72 | 147,07 | LH - warm winter |
| 4 | 604 | 0,9268 | 450,98 | 2,55 | 35,82 | 46,49 | HSA - cool winter |
| 58 | 448 | 0,7986 | 388,59 | 3,18 | 36,91 | 39,52 | MSA -temperate winter |
| 82 | 223 | 1,0025 | 611,62 | 4,51 | 36,52 | 65,54 | SH - mild winter |
| 62 | 110 | 1,1042 | 701,16 | 8,36 | 28,24 | 120,97 | SH - warm winter |
| 63 | 52 | 1,0482 | 868,95 | 8,6 | 28,65 | 148,65 | LH - warm winter |
| 79 | 11 | 1,0056 | 710,93 | 7,08 | 31,16 | 101,27 | SH - warm winter |
| 87 | 28 | 1,0150 | 1082,09 | 6,72 | 31,36 | 150,63 | LH - mild winter |
| 90 | 24 | 1,0245 | 701,16 | 6,61 | 31,35 | 97,21 | SH - mild winter |
| 3 | 822 | 0,9346 | 383,56 | 1,36 | 35,46 | 38,58 | MSA - cool winter |
| 12 | 105 | 1,0113 | 356,48 | 5,26 | 38,53 | 36,75 | LSA - mild winter |
| 26 | 755 | 1,3393 | 833,44 | 2,38 | 32,80 | 93,97 | SH - cool winter |
| 27 | 887 | 0,9980 | 409,57 | 1,108 | 35,014 | 41,43 | MSA -cool winter |
| 40 | 711 | 1,3111 | 815,89 | 2,56 | 32,80 | 92,54 | SH - cool winter |
| 44 | 843 | 2,3274 | 837,39 | 3,81 | 29,72 | 110,85 | LH - temperate winter |
| 45 | 923 | 0,9949 | 390,89 | 1,72 | 36,43 | 38,63 | MSA -cool winter |
| 49 | 847 | 1,1265 | 548,15 | 1,58 | 34,12 | 57,78 | HSA- cool winter |
| 60 | 860 | 0,9055 | 471,58 | 2,29 | 35,76 | 48,33 | HSA- cool winter |
| 74 | 1078 | 1,2686 | 485,36 | 0,67 | 33,80 | 50,25 | HSA -cold winter |
| 77 | 683 | 0,8396 | 291,17 | 0,65 | 37,37 | 27,20 | HA -cold winter |
| 78 | 633 | 0,9506 | 462,56 | 2,44 | 35,62 | 47,82 | HSA - cool winter |
| 84 | 586 | 0,9120 | 466,85 | 2,62 | 35,94 | 48,06 | HSA- cool winter |
| 98 | 1087 | 1,2780 | 488,96 | 0,63 | 33,73 | 50,67 | HSA- cold winter |
| 29 | 822 | 0,9346 | 383,56 | 1,36 | 35,46 | 38,58 | MSA - cool winter |
| 18 | 934 | 1,0061 | 395,29 | 1,67 | 36,35 | 39,10 | MSA - cool winter |
| 46 | 944 | 1,0162 | 399,26 | 1,63 | 36,28 | 39,52 | MSA - cool winter |
| 91 | 1222 | 1,1835 | 616,36 | 0,84 | 33,22 | 65,29 | SH - cold winter |
| 101 | 1077 | 1,0721 | 558,35 | 1,42 | 34,24 | 58,35 | HSA- cool winter |
| 23 | 425 | 0,7796 | 379,35 | 2,62 | 35,94 | 39,05 | MSA - cool winter |
| 30 | 325 | 0,9396 | 697,18 | 4,84 | 32,28 | 87,15 | SH - mild winter |
| 34 | 434 | 0,7870 | 382,95 | 3,23 | 37,01 | 38,88 | MSA- temperate winter |
| 55 | 443 | 0,7944 | 386,55 | 3,2 | 36,95 | 39,28 | MSA- temperate winter |
| 81 | 604 | 0,9268 | 450,98 | 2,55 | 35,82 | 46,49 | HSA- cool winter |
| 56 | 276 | 1,0338 | 501,38 | 6,63 | 29,91 | 73,87 | SH - mild winter |
| 89 | 543 | 1,0725 | 402,29 | 2,52 | 35,52 | 41,81 | MSA - cool winter |
| 97 | 222 | 1,0107 | 490,13 | 6,85 | 30,29 | 71,72 | SH - mild winter |
| 100 | 586 | 0,912 | 466,85 | 2,62 | 35,94 | 48,06 | HSA- cool winter |

K correction factor, Alt altitude, P annual rainfall, M and m the average maximum temperature of the hottest month and the average of the minimum of the coldest month, respectively, Q2 Emberger coefficient, SH subhumid, LH Less Humid, HSA Higher Semiarid, HA Higher arid, MSA Means semiarid, LSA Less semiarid

3.2. Cluster analysis based on ecogeographic data

The dendrogram illustrated in Figure 6 shows the relationships between taxa, based on the variation in ecogeographic parameters. At the distance of 754.84, the dendrogram can be divided into two major clusters (I and II). The distances matrix of all pairs of studied accessions (Figure 7) shows that the higher distance (d=1284) is obtained between 91 (*V. monantha* subsp. *cinerea*, Khenchla) and 87 (*V. lutea* subsp. *eu-lutea*, Jijel-El Milia) when the

lower distance (d=5) is observed between samples 55 (*V. narbonensis*, Constantine-DidoucheMourad) and 58 (*V. lutea* subsp. *vestita*, Jijel-El Milia).

4. Correlation between morphology and ecogeography

This test showed that the two matrices are not correlated ($r = -0.022$, $p = 0.576 = 57.61\%$, $\alpha = 0.05 = 5\%$).

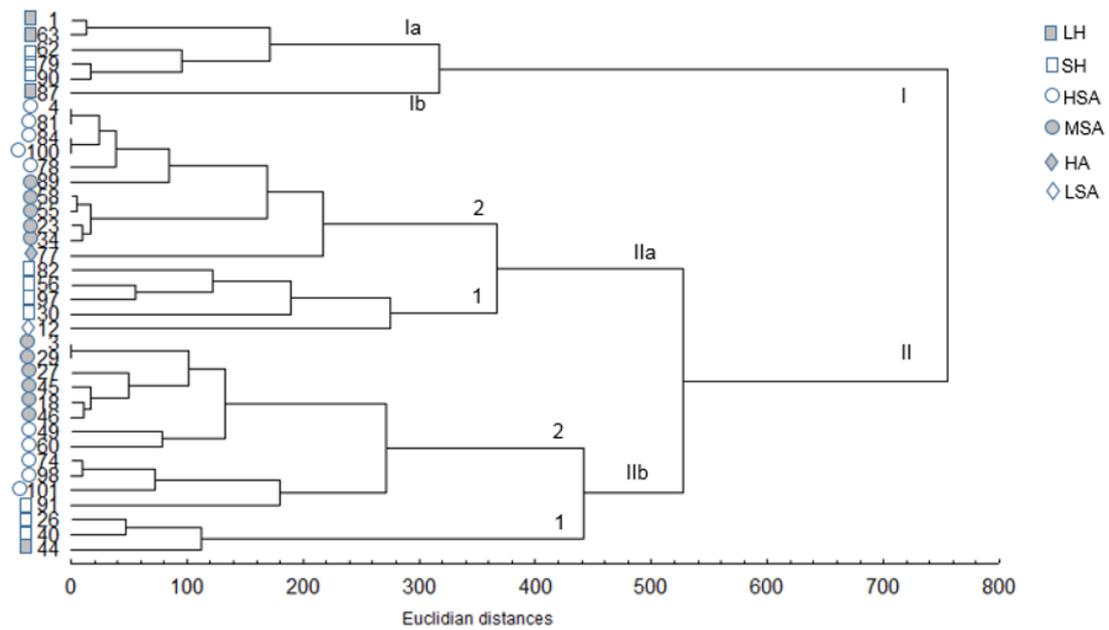


Figure 6. Dendrogram generated using UPGMA cluster analysis based on ecogeographic diversity of 37 *Vicia L.* accessions

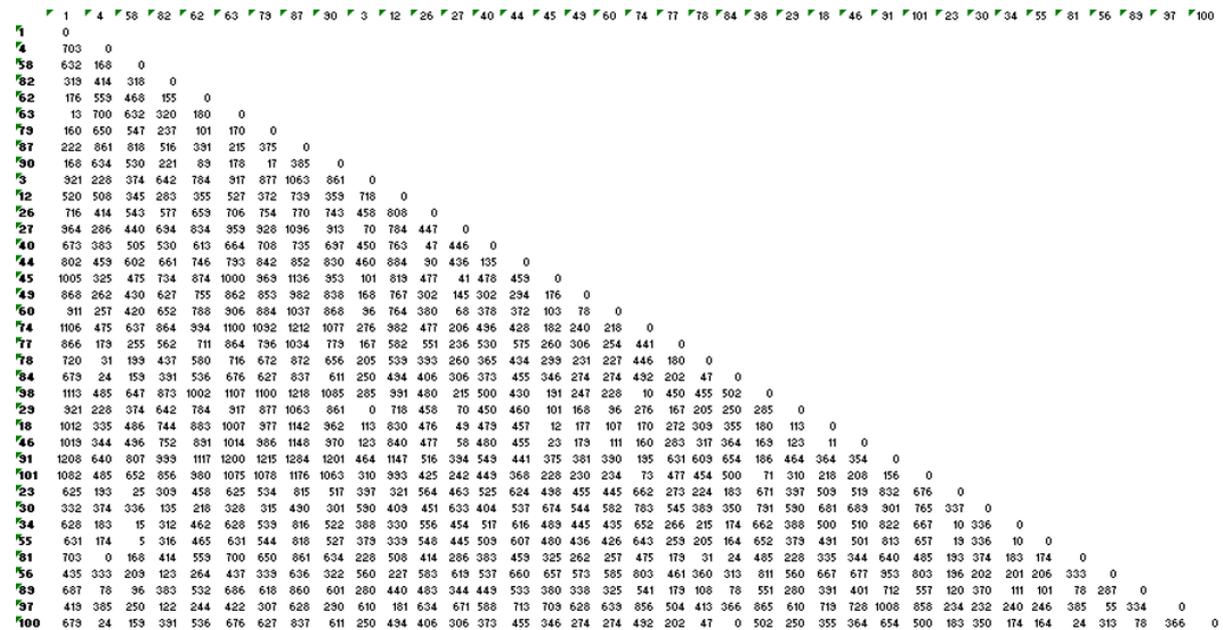


Figure 7. Similarity matrix of ecogeographic parameters

DISCUSSION

Morphological characters

Majority of the morphological characters used in different cladistic studies are binary, usually only some characters included have three or more states. In our morphological multistate matrix, 33 out of 78 characters used have two states and 45 of remaining many state characters are polymorphic. When the multistate characters were coded into presence/absence form, the need to code polymorphic states as new characters disappeared [19].

Our results have demonstrated that using morphological traits alone produce a stable grouping. The morphological resemblances which led [16] to place populations in the same species are correlated with the data of the 78 morphological traits used in our study. Indeed, accessions of the same taxon present a high homology level in morphological characters demonstrated by a low distance in the similarity matrix and by the dendrogram which places them in the same group.

Cluster analysis of morphological traits revealed two basic monophyletic groups, differing only in the relative position of some accessions in subclusters: I) populations of *V. monantha* in one subcluster linked to two other species (*V. tenuifolia* and *V. leucantha*) belonging to section Cracca of the subgenus *Vicilla* (sensu Kupicha) together with four accessions of *V. monantha*; II) all accessions of *V. narbonensis* (section Narbonensis) in one subcluster linked with samples of *V. lutea* (section Hypechusa) in a second subcluster. These results concord with those of [20] which found that species of section Hypechusa were unexpectedly nested within the NSC (subgenus *Vicia*) and section Cracca (subgenus *Vicilla*), respectively.

Sections Narbonensis and Hypechusa (cluster II)

All populations of *V. narbonensis* belong to the same subcluster (IIa). [16] have use 6 characters to rank samples in this species. Also, the utilization of the 78 characters of current study places them in the same subcluster. This observation shows that there are accessions which appear to belong to the same species on the basis of few characters while they have a lot of common characteristics. In parallel, a high distance can be obtained between accessions in this species as in the case of 30 and 81 showing morphological differences between accessions of *V. narbonensis*. This result can be explained by the fact that the key used by [16] did not consider the infraspecific level for this species while several others keys distinguished five or seven varieties. However, the distinction of each variety is based on characters that are difficult to discern in the field [21]. The subcluster IIb is composed of all accessions of *V. lutea*. These observations show that there is an overlap between accessions of *V. lutea* subspecies. Indeed, [16] used the color of flower to distinguish the two subspecies, but the utilization of characters of the present study shows a close relationship between the taxa when it is difficult to determine distinct groups which could be individually identified as *eu-lutea* or as *vestita*. [22] reported that subspecies have been established within species showing significant intra-specific variation. This is consistent with our results where differences at subspecies level can be observed between samples of *V. lutea* subsp. *eu-lutea* as in the case of 62 and 87.

The two populations differ by 8 flower and 5 legume traits, when the remaining characters were identical.

According to [16], *V. narbonensis* belongs to the same serie of *V. lutea* (serie 1). Also, in the present study, they are grouped in the same cluster and have a lot of common characteristics. In the other side, these two species, belonging to subgenus *Vicia*, section Narbonensis (*V. narbonensis*) and section Hypechusa (*V. lutea*) differ significantly from each other. Morphological differences are reflected in their placement in different subsections. The UPGMA results join the classifications of [1] and [3] who place *V. narbonensis* and *V. lutea* in the same sub-genus (*Vicia*). The apparition of the two sub-clusters (IIa and IIb) also joins these two authors who place the two species in different sections and series (*V. lutea* in the section Hypechusa, serie Hypechusa and *V. narbonensis* in the section Faba by Kupicha and section Narbonensis, serie Narbonensis by Maxted). Our results concord with those of [11] which showed the species of sections Hypechusa as sister to the clade of section Narbonensis. Moreover, in our study, the seed morphology confirms this division, since samples belonging to the two species can have identical seed characters: compressed seed cross-section, smooth seed surface, brown seed color, seed presenting color mottling, hilum length (20-40%), pale hilum, spongy legume inside and seed size 3-6mm. The section Hypechusa reported to be monophyletic based on several works [6]. A closer relationship between the NSC and section Hypechusa has been deduced by [20]. Our data revealed that subgenus *Vicia* is a well-separated subgenus and agreed with the traditional results based on morphology reported by [23] and with results on phylogenetic analyses [6]; [7]

Section Cracca (cluster I)

The results of the analysis of the section Cracca indicate that the species fall into two distinct groups. However, the division between these two groups is not as distinct as between section Hypechusa and section Narbonensis.

Species of section Cracca sensu Kupicha belong all to one group. Fifteen accessions of *V. monantha* are classified in the same subcluster (Ib). But, there are no clear groups defined as *cinerea* or as *calcarata* showing an overlap between the morphology of these subspecies.

An example can be given for samples 77 (*calcarata*) and 91(*cinerea*) distant by 3.74 which have 64 common characters when the key of [16] distinguish between the two subspecies by only two characters (flower size and legume size). Accessions belonging to the same subspecies present also differences. Samples 26 and 74 belonging to *V. monantha* subsp. *calcarata* are a good example in this case. In another side, the cluster II regroup samples of *V. monantha* (subsp. *calcarata* and subsp. *cinerea*) belonging to the series 2 together with the three accessions of *V. tenuifolia* (serie 2) and the unique accession of *V. leucantha* (serie 3). The subcluster Ia comprises populations of *V. tenuifolia*, *V. leucantha* and four populations of *V. monantha* showing also an overlap in morphological traits between these four accessions and the samples of *V. leucantha* and *V. tenuifolia*. The key of [16] classifies *V. tenuifolia* and *V. monantha* in the same series. They also have a lot of common characters according to the results of the present study. The unique accession of *V. leucantha* is classified by [16] in the series 3, when it has a lot of common characteristics with the samples of the serie 2. The species belonging to the cluster I are classified by [1] in the sub-genus *Vicilla*, section *Cracca*. Thus, the three species attributed to the section *Cracca* appear in a well-supported monophyletic group in the present work. *V. tenuifolia* and *V. leucantha*, form a subgroup of closely related species that revealed extensive homologous polymorphism with shared characters without any differentiation by species-specific traits. Seed morphology confirmed this division because both *V. tenuifolia* and *V. leucantha* can have similar characteristics, such as SB, SH, SF, SP, SHE, SHL; LPL, LRSh, LC, LAC, LS, LBP, LAS, LAD, LM, LMH, LHE, LAHD, SS, SSh, SNS, Spa, TP, TB, TL, THD, PcL, PL, SP, SASH, WC, WL, KC, KCP, KL, CH, CHD, CTL, LSh, LCS, LP, LSt, LSf, LT, LC, LSC, LBe, LBSh, SNL and all seed characters. However, both species also have different characteristics, such as SEC, SNC, LRS, LLP, SE, NFI, FL, SC, WCP, CBSH, CMSH, CC, CUTL, CLTL, CUTSh, CLTSh, Lpu, LSH. The largest section in the subgenus *Vicilla*, section *Cracca* (as interpreted by Kupicha), appears paraphyletic. In the present analysis, *V. leucantha*, the species transferred by [5] to his section *Ervum*, is in the same clade with the remaining *Cracca* species.

In spite of this, our analysis of the morphological characters supports Kupicha's placement of *V. leucantha* in section *Cracca* as was also done by [24]. These results join those of [7] in his study of phylogenetics of *Vicia* based on morphological data.

Principal Component Analysis

Among morphological characters which contribute the most in accessions separation, three are quantitative (PL, HL, LPL) and the majority is qualitative (LC-CHD-SF-LAHD-LHE-SC-LSI-LS-SCM...). Those which contribute least to samples separation are both qualitative (SB-CC-LSI-SP) and quantitative (SNL-NFI-FL-PL). As quantitative characters are essential at this taxonomic level, several quantitative characters were included in the present study according to recommendations of [25]. In the other side, there are many qualitative characters like color of seeds and hilum color, which usually are not used for taxonomy, but are important for cultivar identification. Information on the variability of these hereditary characteristic in populations from different geographical regions can also be useful for curators and collectors. Our data show that the qualitative variables allowed a clear separation of *Vicia* taxa and are good diagnostic taxonomic characters for the accessions. The same findings were reported by [26] in their study on several *Vicia* taxa in Southern Brazil where they underline a clear samples separation by corolla color, pod and seed color and pod constriction. Concerning characters that strongly contribute to the separation of the accessions, the range of variation does show overlap at subspecies level. PCA shows that morphological characters which strongly contribute in accessions distinction concern characters of leaflets, flower, stem, pod and seed showing the importance of each part of the plant in accession distinction. According to [24], *Vicia* species present considerable variations in almost every trait, but particularly in leaflet morphology. Our results show that, effectively, among characters which strongly contribute to samples distinction, six (LC, LAHD, LHE, LPL, LLP, LRSh) concern leaflets traits. In the present study, leaf and stem characters were measured at the first productive node. Leaflets were also measured at the lower part of the stems, so that heterophylly, an indicator of the difference in leaf shape at the bottom and the top of the plant, which has been used by [27].

The results of PCA show that there is a strong positive correlation between characters of the same part of the plant like standard pubescence and pedicel length or between characters which belong to different parts like seed color mottling and leaflets per leaf. In parallel, traits of the same plant part can have a strong negative correlation as in the case of leaflets relative shape and leaflets per leaf. In our results, no differences were observed for leaflet abaxial color, leaflet broadest point, leaflet apex dentate, tendril branching, wing length and legume beak shape. These characters are identical between the 37 *Vicia* populations and cannot be used for taxa identification. Morphological characters used in the present work allow good separation among accessions at species levels as shown by the use of the observation with the sum of the squared cosines (Figure 4) which determines three distinct groups which could be individually identified when no groups at subspecies level were generated. The first group includes all accessions of *V. narbonensis*. The second one contains samples of *V. lutea*. The third group includes all accessions of subgenus *Vicilla* section *Cracca* (*V. monantha*, *V. tenuifolia* and *V. leucantha*) showing even an overlap among these three taxa.

Ecogeographic diversity

The results of the ecogeographic survey elucidate each taxon geographic and ecological location, and thus which areas could most profitably be targeted for future collecting missions. The use of GPS in the present study is supported by the recommendations of [28]. According to [29], in the Mediterranean region, there is a need for more collection in Algeria, Egypt and Yemen.

The wide geographic ranges found in the present work should be taken into account both in conservation programs and in the genetic improvement of this economically important wild *Vicia* species as suggested by [30]. The UPGMA results show that accessions with high morphology differences and belonging to different taxa can have an identical bioclimate and close altitudes. On the other hand, the groups may comprise accessions from similar latitudes and climates. The same findings were reported by [31]. The dendrogram obtained with ecogeographic parameters did not indicate clear division among accessions based on their geographical locations, indicating that the association

between genetic similarity and geographical distance was less significant as it was reported by [32]

Considering all stations of the current work, the studied samples of *Vicia* L. occur from 11 to 1222m. Algeria has all the Mediterranean bioclimates from the perhumid in the north to the perarid in the south for the bioclimatic levels and from the cold to the hot for their temperature variants. Sites investigated belong to 6 different bioclimates (SH, SAS, SAM, SA, AS, SAI) and are characterized by cool, wild, warm or temperate winter with a minimum average temperature ranged from 0.63 to 8.64°C.

Samples of *V. narbonensis* were collected from sites receiving between 382.95 and 697.18 mm of precipitations and belonging to bioclimates characterized by cool or mild winter. This is consistent with findings of [28] who reported that taxa belonging to the *V. narbonensis* complex occur over a wide range of altitudes, from sea level to 3200m. According to [33], *V. narbonensis* shows adaptation in areas receiving 250–300 mm annual rainfall with low winter temperatures. Accessions belonging to *V. monantha* occur until 1222m and at these altitudes they require some frost tolerance as the temperatures can drop to 0.63°C. It is interesting to mention that it is from $m = 1$ and not $m = 0$, as the risk of frost become important. The patterns described above are not necessarily a true indication of the preferred altitude of each taxon but may reflect the number of collections that occurred at each altitude.

CONCLUSION

The present study is the first attempt to analyze phonetic relationships among some Algerian species of genus *Vicia* using cladistic analysis of 78 morphological characters and 5 ecogeographic parameters. Sections *Narbonensis* (*V. narbonensis*) and *Hypechusa* (*V. lutea*) were considered a separate group, while section *Cracca* was the most heterogeneous section. Significant differences at subspecies level were observed regarding the results of investigated morphological traits. Several studies have mapped the distributions of the *Vicia* species. Their results must be used in conjunction with those described in this study to provide further information on the ecogeography of *Vicia* species. To clarify the position of species, collections need to be made in Algeria and grown for further identification in the same location.

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