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Cluster analysis of leaf macro- and micro- morphological characteristics of *Vicia* L. (Fabaceae) and their taxonomic implication

Análisis de conglomerado de características foliares micro- y macro-morfológicas de *Vicia* L. (Fabaceae), y sus implicancias taxonómicas

Abozeid A[1,2], Y Liu[1], J Liu[1], ZH Tang[1]

Abstract. The genus *Vicia* L. belongs to the tribe Vicieae of the Fabaceae family. The genus includes about 190 species, from which about 40 species have economic importance. Some of them are food crops, but more than a dozen are forage plants. In this study, leaves of *Vicia* species from China, USA and Argentina were examined using stereo-microscopy and light microscopy. We determined macro- and micro-morphological characteristics that could be of taxonomic use. Forty eight characteristics of each taxon were determined including petiole and tendril length; leaflets number, length, width, shape, apex, base; blade surface, trichome shape, type, base and length; stipules shape, base, length, width and surface. Cluster analysis of these characteristics was used to construct a phenogram illustrating the relationships between the studied taxa, and to build a key to identify *Vicia* species.

Keywords: *Vicia*; Fabaceae; Stipules; Trichomes; Numerical taxonomy; Micro-morphology.

Resumen. El género que *Vicia* L. pertenece a la tribu Vicieae de la familia Fabaceae. El género incluye aproximadamente 190 especies, de los cuales alrededor de 40 especies tienen importancia económica. El género incluye algunos cultivos, y las plantas forrajeras más de una docena de especies. En este estudio, hojas de especies de *Vicia* provenientes de China, Estados Unidos y Argentina fueron examinadas usando microscopios para estudiar las características macro y micromorfológicos que podrían ser de utilidad taxonómica. Cuarenta y ocho características se extrajeron de cada taxón incluyendo: longitud del pecíolo y zarcillo; número de folíolos, longitud, ancho, forma, ápice, base; superficie foliar, tricomas forma, tipo, base y longitud; forma de estípulas, base, longitud, ancho y superficie. Un análisis de conglomerados de estas características fue utilizado para construir un fenograma, ilustrando la relación entre los taxones estudiados, y para construir una clave de identificación de las especies de *Vicia*.

Palabras clave: *Vicia*; Fabaceae; Estípulas; Tricomas; Taxonomía numérica; Micromorfología.

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INTRODUCTION

The micro-morphological features of different plant's parts are significant for their taxonomic consideration in Angiosperms (Ogundipe & Akinrinlade, 1998; Parveen et al., 2000; Prabhu et al., 2011). Taxonomic importance of the foliar morphological and micro-morphological characteristics has been well documented (Celka et al., 2006; Yasmin et al., 2009; Ogundipe & Kadiri, 2012; Fatihah et al., 2014; Trujillo-Moya et al., 2014). Leaf micro-morphological characters have systematic significance at different levels in the family Fabaceae (Luo & Zhang, 2004; Ren et al., 2007; Kadiri & Olowokudejo, 2008; Zou et al., 2008; Ogundipe et al., 2009; Mirzaei et al., 2015). Trichomes micro-morphologyis one of the most significant leaf micro-morphological characters studied in species of different genera (Eshratifar et al., 2011; Albert & Sharma, 2013; Grohar et al., 2016).

The genus *Vicia* L. belongs to the tribe Vicieae of the Fabaceae family. *Vicia* species are distributed throughout the temperate regions of Europe, Asia, and North and South America (Kupicha, 1976). The genus includes about 190 species (Bisby et al., 1999). About 40 species have economic importance. The genus includes some food crops, and more than a dozen forage species; the most important is common vetch, V. *sativa*, cultivated in many countries (Hanelt & Mettin, 1989).

Evaluating species delimitation between the important crops and their wild relatives has always been important, especially with the use of germplasm of wild species as a source of new characteristics to be introduced by wide crosses into the crop plants (Maxted et al., 2012; Yang et al., 2012). There have been few attempts for introducing promising wild vetch species as alternative crops or as a breeding material (Terziiski, 1986).

Taxonomic delimitation is also necessary to solve the overlapping between economic crops and harmful species. For example, Boissier (1872) treated *V. sativa* subsp. *nigra* as *V. angustifolia* subsp. *Pusilla*, while Gunn (1979) treated it as a subspecies of V. *sativa*. This kind of misidentification may be dangerous as *V. angustifolia* is a well-known economically important forage plant (Komarov et al., 1972). *Vicia sativa* ssp. *nigra* is possibly toxic as it contains gluco-alkaloids and neurotoxic cyano amino acids in the seed (Everist, 1974).

Cluster analysis has been used as a useful tool for clarifying relationships through different taxonomic levels (Van de Wouw et al., 2001; Khalik et al., 2002; Kendir et al., 2015; Turki et al., 2015). Morphological characters on leaves, especially the micro- morphological ones, which may provide information for understanding species relationships in the genus *Vicia*, have not been studied to date. The objectives of this work were using leaf features to identify taxa and to show the relationships among the studied taxa, and to evaluate their implications in the taxonomy of the genus.

MATERIALS AND METHODS

Seed source. *Vicia* species seeds in this study were obtained from the Desert Legume Program (DELEP) and the Germplasm Bank of Wild Species, Southwest China, as listed in Table 1.

Table 1. Examined *Vicia* species, and their corresponding sources and origin.
Tabla 1. Especies de *Vicia* examinadas, y sus fuentes y origen correspondientes.

Species	Source	Origin
Vicia amoena Fisch.	The Germplasm Bank of Wild Species, S.W. China	China
Vicia angustifolia L.	Desert Legume Program (DELEP)	USA
Vicia bijuga Hook. & Arn.	Desert Legume Program (DELEP)	Argentina
Vicia bungei Ohwi	The Germplasm Bank of Wild Species, S.W. China	China
Vicia costata Ledeb.	The Germplasm Bank of Wild Species, S.W. China	China
Vicia hirsuta (L.) Gray	The Germplasm Bank of Wild Species, S.W. China	China
Vicia japonica A. Gray	The Germplasm Bank of Wild Species, S.W. China	China
Vicia ludoviciana Nutt.	Desert Legume Program (DELEP)	USA
Vicia pilosa M. Bieb.	The Germplasm Bank of Wild Species, S.W. China	China
Vicia pulchella subsp. *pulchella* (Hemsl.)	Desert Legume Program (DELEP)	USA
Vicia sativa L. subsp. *nigra* (L.) Ehrh.	Desert Legume Program (DELEP)	Argentina
Vicia sativa L. subsp. *sativa*	The Germplasm Bank of Wild Species, S.W. China	China
Vicia tenuifolia Roth	The Germplasm Bank of Wild Species, S.W. China	China
Vicia tetrasperma (L.) *Schreb.*	The Germplasm Bank of Wild Species, S.W. China	China
Vicia villosa Roth subsp. *dasycarpa* (Ten.) Cavill	The Germplasm Bank of Wild Species, S.W. China	China
Vicia villosa Roth subsp. *varia* (Host) Corb.	Desert Legume Program (DELEP)	Argentina

Morphological examination. Seeds of each taxon were scarified by soaking them in 100% sulfuric acid for 20 minutes. This was to soften the hard seed coat and break their dormancy. It allowed them to get ready for germination following Ortega-Olivencia and Devesa (1997). Thereafter, they were washed 3 times with water and then allowed to germinate in perlite sand. Ten days after germination (seeds were considered germinated when their roots protruded from their envelopes), 50 young seedlings of each taxon were transferred to pots (ten seedlings per pot) containing peat moss soil. Sixty days later, 50 complete leaves were collected from each taxon; all leaves were collected from the fifth node.

A stereo-microscope was used for morphological measurements of the leaves such as petiole and tendril lengths, leaflets number, length, width, shape, apex and base as shown in Table 2 and Figures 1 and 2.

Lightmicroscopy was used for micro-morphological measurements of trichomes, and stipule characteristics such as trichome length, shape and base, and stipule length, width, shape, base and surface (Table 3, Table 4, Fig. 3, Fig. 4).

Cluster analysis. Forty eight leaf characteristics were coded as binary. Cluster analysis was performed with the standardized matrix (0,1 standardization) to investigate distances among each taxa based on the interval data distance similarity measure. This was made using the Hierarchical clustering (Sequential Agglomerative Hierarchical Nonoverlapping clustering- SAHN). The phenogram illustrating the relationships among the studied taxa was constructed using the NTsys 2.1 (Rohlf, 2000). We constructed a key to identify *Vicia* species based on the studied morphological characteristics.

RESULTS

The variation in *Vicia* leaf morphology was shown mainly in petiole and tendril lengths; leaflet number, length, width, shape, apex, base and blade surface; trichome shape, type, base and length and stipule shape, base, length, width and surface. The results are summarized in Tables 2 to 4, and in Figures 1 to 4. They show that the variation in leaf characteristics of the studied taxa can be of taxonomic value in the identification of certain taxa.

Leaf characteristics. Leaf without tendril was detected in *V. costata*; 0.3 to 0.9 cm tendril was observed in *V. bijuga, V. bungei* and *V. japonica* while 1.8 to 4.5 cm tendril was shown in the rest of the studied species. Leaf with 2 leaflets was determined in *V. amoena, V. bijuga, V. bungei*, *V. costata, V. ludoviciana, V. pilosa, V.*

Table 2. Leaf structure in species of *Vicia*.
Tabla 2. Estructura de las hojas en las especies de *Vicia*.

Characteristics / Species	Petiole length (cm)	Tendril length (cm)	Leaflets					
			Number	Length (cm)	Width (mm)	Shape	Apex	Base
Vicia amoena	0.5-0.8	3.5-4.5	2	1.9-2.2	4-5	Lanceolate	Acute	Obtuse
Vicia angustifolia	0.8-1.1	2-3.5	4	0.8-1.1	2-3	Oblong	Acuminate	Obtuse
Vicia bijuga	0.3-0.5	0.6-0.9	2	0.9-1.1	2-3	Lanceolate	Acuminate	Cuneate
Vicia bungei	0.5-0.8	0.3-0.5	2	0.9-1.2	7-9	Obovate	Obtuse	Cuneate
Vicia costata.	0.7-1.2	-	2	0.7-0.9	2-3	Oblong	Acuminate	Obtuse
Vicia hirsuta	0.5-0.7	2.5-3.5	4	0.7-0.9	2-3	Oblong	Truncate	Cuneate
Vicia japonica	0.3-0.5	0.6-0.9	4	0.8-1	3-4	Oblong	Obtuse	Cuneate
Vicia ludoviciana	0.6-0.9	2.2-3.5	2	0.9-1	1.5-2	Oblong	Acuminate	Obtuse
Vicia pilosa	0.5-1.5	3.5-4	2	1.5-2	3-4	Lanceolate	Acute	Obtuse
Vicia pulchella subsp. *pulchella*	0.3-0.6	2.5-4	4	1.2-1.5	1.5-2	Oblong	Acuminate	Obtuse
Vicia sativa subsp. *nigra*	0.7-1	2-3.5	2	1.5-1.7	3-4	Lanceolate	Acuminate	Obtuse
Vicia sativa subsp. *sativa*	0.8-1.2	1.8-2.5	2	1.2-1.4	4-5	Ovate	Acute	Obtuse
Vicia tenuifolia	0.7-1	3-4.5	2	1.3-1.5	4-5	Ovate	Acute	Cuneate
Vicia tetrasperma	0.7-1	2-3	2	0.7-0.9	2-3	Elliptic	Acute	Cuneate
Vicia villosa subsp. *dasycarpa*	0.5-0.8	2.5-3.6	4-8	0.8-1.1	2-3	Oblong	Acuminate	Cuneate
Vicia villosa subsp. *varia*	0.3-0.4	3-4.5	6	0.9-1.1	2-3	Lanceolate	Acuminate	Cuneate

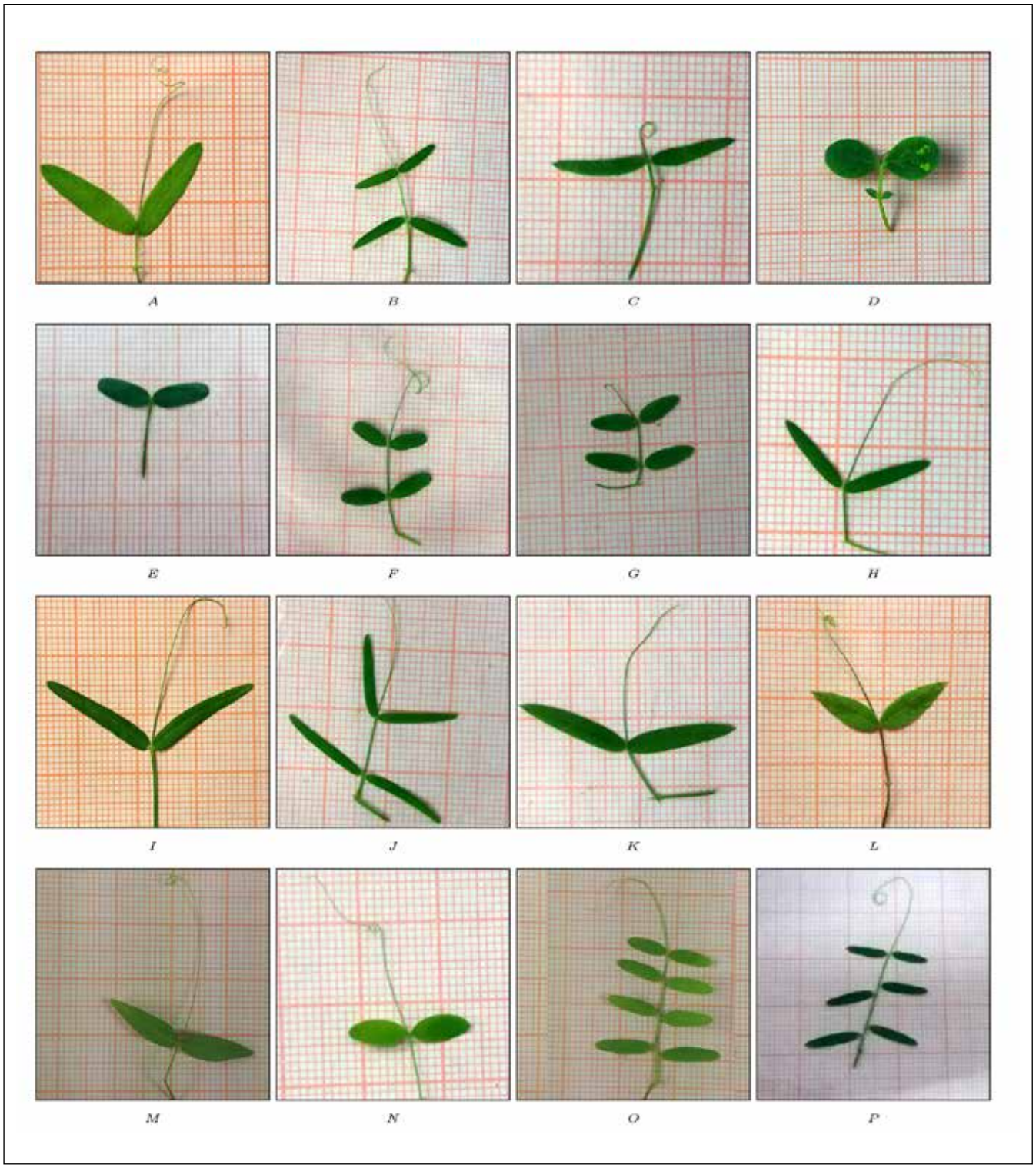

Fig. 1. Leaf structure in the studied **Vicia** species. (A) *V. amoena*, (B) *V. angustifolia*, (C) *V. bijuga*, (D) *V. bungei*, (E) *V. costata*, (F) *V. hirsuta*, (G), *V. japonica*, (H) *V. ludoviciana*, (I), *V. pilosa*, (J) *V. pulchella* subsp. *pulchella*, (K) *V. sativa* subsp. *nigra*, (L) *V. sativa* subsp. *sativa*, (M) *V. tenuifolia*, (N) *V. tetrasperma*, (O) *V. villosa* subsp. *dasycarpa*, (P) *V. villosa* subsp. *varia*.

Fig. 1. Estructura de las hojas en las especies de *Vicia* estudiadas. (A) *V. amoena*, (B) *V. angustifolia*, (C) *V. bijuga*, (D) *V. bungei*, (E) *V. costata*, (F) *V. hirsuta*, (G), *V. japonica*, (H) *V. ludoviciana*, (I), *V. pilosa*, (J) *V. pulchella* subsp. *pulchella*, (K) *V. sativa* subsp. *nigra*, (L) *V. sativa* subsp. *sativa*, (M) *V. tenuifolia*, (N) *V. tetrasperma*, (O) *V. villosa* subsp. *dasycarpa*, (P) *V. villosa* subsp. *varia*.

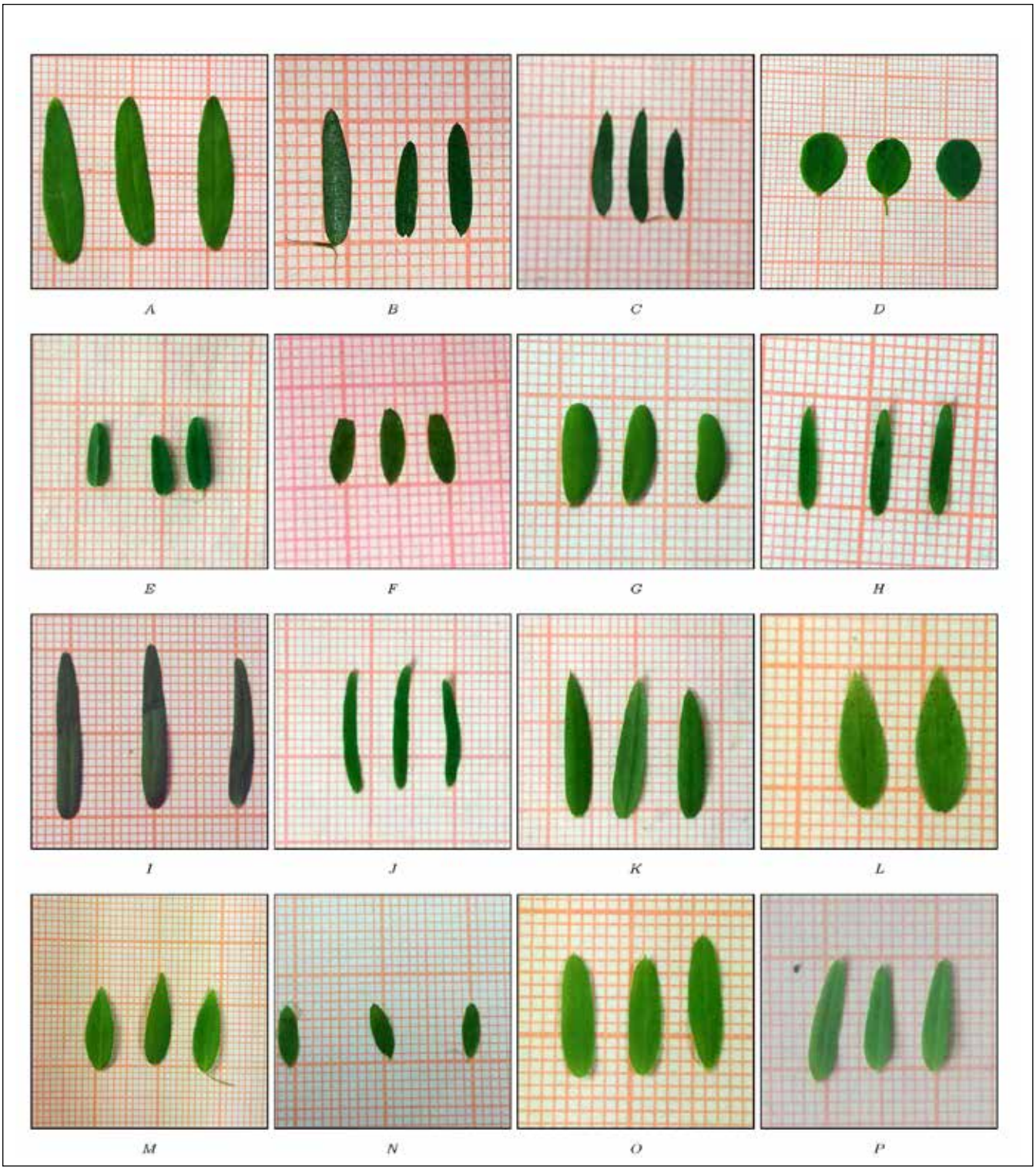

Fig. 2. Leaflets structure in the studied *Vicia* species. (A) *V. amoena*, (B) *V. angustifolia*, (C) *V. bijuga*, (D) *V. bungei*, (E) *V. costata*, (F) V. *hirsuta*, (G), *V. japonica*, (H) *V. ludoviciana*, (I), *V. pilosa*, (J) *V. pulchella* subsp. *pulchella*, (K) *V. sativa* subsp. *nigra*, (L) *V. sativa* subsp. *sativa*, (M) *V. tenuifolia*, (N) *V. tetrasperma*, (O) *V. villosa* subsp. *dasycarpa*, (P) *V. villosa* subsp. *varia*.

Fig. 2. Estructura de folíolos en especies de *Vicia*. (A) *V. amoena*, (B) *V. angustifolia*, (C) *V. bijuga*, (D) *V. bungei*, (E) *V. costata*, (F) *V. hirsuta*, (G), *V. japonica*, (H) *V. ludoviciana*, (I), *V. pilosa*, (J) *V. pulchella* subsp. *pulchella*, (K) *V. sativa* subsp. *nigra*, (L) *V. sativa* subsp. *sativa*, (M) *V. tenuifolia*, (N) *V. tetrasperma*, (O) *V. villosa* subsp. *dasycarpa*, (P) *V. villosa* subsp. *varia*.

Table 3. Blade hairiness in species of *Vicia*.
Tabla 3. Blade hairiness in species of *Vicia*.

Species \ Characteristics	Surface	Trichomes shape	Trichomes type	Trichomes length (mm)	Distance between trichomes (mm)	Trichomes base
Vicia amoena	Smooth	-	-	-	-	-
Vicia angustifolia	Hairy	Linear	Unicellular	1-2	0.1-0.3	Nonglandular
Vicia bijuga	Smooth	-	-	-	-	-
Vicia bungei	Smooth	-	-	-	-	-
Vicia costata	Smooth	-	-	-	-	-
Vicia hirsute	Smooth	-	-	-	-	-
Vicia japonica	Smooth	-	-	-	-	-
Vicia ludoviciana	Smooth	-	-	-	-	-
Vicia pilosa	Hairy	Acute	Multicellular	0.2-0.3	0.6-1.2	Glandular
Vicia pulchella subsp. *pulchella*	Smooth	-	-	-	-	-
Vicia sativa subsp. *nigra*	Hairy	Acute	Multicellular	0.2-0.4	0.1-0.5	Glandular
Vicia sativa subsp. *sativa*	Hairy	Acute	Multicellular	0.2-0.5	0.2-0.5	Glandular
Vicia tenuifolia	Hairy	Acute	Unicellular	1-2	0.6-0.8	Nonglandular
Vicia tetrasperma	Smooth	-	-	-	-	-
Vicia villosa subsp. *dasycarpa*	Hairy	Linear	Unicellular	1-2	More than 2	Nonglandular
Vicia villosa subsp. *varia*	Hairy	Acute	Unicellular	1-2	0.8-1.3	Nonglandular

Table 4. Leaf stipule characteristics in species of *Vicia*.
Tabla 4. Características de las estípulas de hojas en las especies de *Vicia*.

Species \ Characteristics	Shape	Base	Length (mm)	Width (mm)	Blade length (mm)	Base length (mm)	Surface	Hair length (mm)	Distance between trichomes (mm)	No. of trichomes in 1mm
Vicia amoena	Sagittate	Acute	1-1.2	0.2-0.3	0.5-0.6	0.5-0.6	Smooth	-	-	-
Vicia angustifolia	Sagittate	Acute	1.5-1.7	0.4-0.5	0.8-1	0.7-0.8	Hairy	0.16-0.4	0.08-0.1	12-16
Vicia bijuga	Lanceolate	Rounded	0.6-0.8	0.1-0.2	-	-	Smooth	-	-	-
Vicia bungei	Hastate	Acute	2.5-2.7	1.5-1.8	0.3-0.4	2.2-2.4	Smooth	-	-	-
Vicia costata	Sagittate	Acute	0.6-0.8	0.2-0.3	0.4-0.5	0.2-0.3	Smooth	-	-	-
Vicia hirsute	Sagittate	Forked	1.6-2	0.4-0.5	0.8-1	0.8-1	Smooth	-	-	-
Vicia japonica	Sagittate	Acute	1.8-2	0.4-0.5	1-1.2	0.8-0.9	Smooth	-	-	-
Vicia ludoviciana	Sagittate	Acute	0.8-1	0.1-0.2	0.6-0.7	0.2-0.3	Smooth	-	-	-
Vicia pilosa	Sagittate	Acute	3.3-3.5	0.2-0.3	1.7-1.9	1.6-1.8	Hairy	0.16-0.2	0.04-0.2	6-8
Vicia pulchella subsp. *pulchella*	Sagittate	Acute	1.8-2	0.4-0.5	1.3-.4	0.5-0.7	Smooth	-	-	-
Vicia sativa subsp. *nigra*	Sagittate	Acute	2.1-2.4	0.2-0.3	1.3-1.4	0.8-1	Hairy	0.08-0.16	0.008-0.16	5-7
Vicia sativa subsp. *sativa*	Sagittate	Forked	3.5-3.8	0.4-0.5	2.3-2.5	1.2-1.4	Hairy	0.16-0.28	0.12-0.36	7-9
Vicia tenuifolia	Sagittate	Acute	1.5-1.8	0.2-0.3	1.2-1.4	0.3-0.4	Smooth	-	-	-
Vicia tetrasperma	Hastate	Acute	1-1.2	0.2-0.	0.8-1	0.2-0.3	Smooth	-	-	-
Vicia villosa subsp. *dasycarpa*	Hastate	Acute	2-2.2	0.4-0.5	1.7-1.9	0.3-0.4	Smooth	-	-	-
Vicia villosa subsp. *varia*	Sagittate	Acute	1.3-1.5	0.4-0.5	1-1.1	0.3-0.4	Hairy	0.16-0.2	0.1-1	3-4

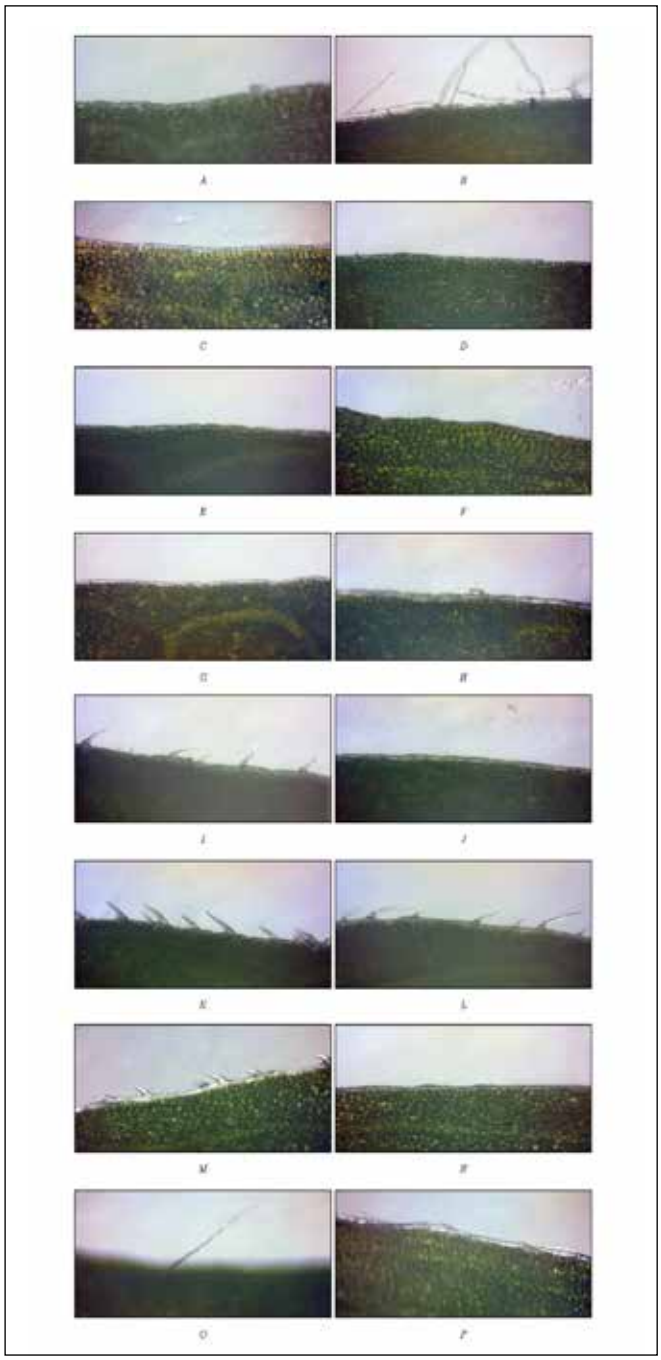

Fig. 3. Blade hairiness in the studied *Vicia* species. (A) *V. amoena*, (B) *V. angustifolia*, (C) *V. bijuga*, (D) *V. bungei*, (E) *V. costata*, (F) V. *hirsuta*, (G), *V. japonica*, (H) *V. ludoviciana*, (I), *V. pilosa*, (J) *V. pulchella* subsp. *pulchella*, (K) *V. sativa* subsp. *nigra*, (L) *V. sativa* subsp. *sativa*, (M) *V. tenuifolia*, (N) *V. tetrasperma*, (O) *V. villosa* subsp. *dasycarpa*, (P) *V. villosa* subsp. *varia*.

Fig. 3. Pilosidad de la hoja en especies de *Vicia*. (A) *V. amoena*, (B) *V. angustifolia*, (C) *V. bijuga*, (D) *V. bungei*, (E) *V. costata*, (F) *V. hirsuta*, (G), *V. japonica*, (H) *V. ludoviciana*, (I), *V. pilosa*, (J) *V. pulchella* subsp. *pulchella*, (K) *V. sativa* subsp. *nigra*, (L) *V. sativa* subsp. *sativa*, (M) *V. tenuifolia*, (N) *V. tetrasperma*, (O) *V. villosa* subsp. *dasycarpa*, (P) *V. villosa* subsp. *varia*.

Table 5. Numerical analysis, 0 1 codes, in the studied *Vicia* species: (A) *V. amoena*, (B) *V. angustifolia*, (C) *V. bijuga*, (D) *V. bungei,* (E) *V. costata*, (F) *V. hirsuta*, (G) *V. japonica*, (H) *V. ludoviciana*, (I) *V. pilosa*, (J) *V. pulchella* subsp. *pulchella*, (K) *V. sativa* subsp. *Nigra*, (L) *V. sativa* subsp. *sativa*, (M) *V. tenuifolia*, (N) *V. tetrasperma*, (O) *V. villosa* subsp. *dasycarpa*, and (P) *V. villosa* subsp. *varia*.

Tabla 5. Análisis numérico, códigos 0 1, en especies de *Vicia*: (A) *V. amoena*, (B) *V. angustifolia*, (C) *V. bijuga*, (D) *V. bungei*, (E) *V. costata*, (F) *V. hirsuta*, (G) *V. japonica*, (H) *V. ludoviciana*, (I) *V. pilosa*, (J) *V. pulchella* subsp. *pulchella*, (K) *V. sativa* subsp. *nigra*, (L) *V. sativa* subsp. *sativa*, (M) *V. tenuifolia*, (N) *V. tetrasperma*, (O) *V. villosa* subsp. *dasycarpa*, y (P) *V. villosa* subsp. *varia*.

Leaf part	Characteristics	Characters	01 Codes
			ABCDEFGHIJKLMNOP
Leaf structure	Tendril length (cm)	0	0000100000000000
		0.3 to 0.9	0011001000000000
		1.8 to 4.5	1100010111111111
	Number of leaflets	2	1011100110111100
		4-8	0100011001000011
	Leaflet width (mm)	Up to 5	1110111111111111
		7 to 9	0001000000000000
	Leaflet shape	Elliptic	0000000000000100
		Lanceolate	1010000010100001
		Oblong	0100111101000010
		Obovate	0001000000000000
		Ovate	0000000000011000
	Leaflet apex	Acute	1000000010011100
		Acuminate	0110100101100011
		Obtuse	0001001000000000
	Leaflet base	Truncate	0000010000000000
		Leaflet base	0011011000001111
		Obtuse	1100100111110000
Trichomes	Trichome shape	Smooth	1011111101000100
		Acute	0000000010111001
		Linear	0100000000000010
	Trichome type	Multicellular	0000000010110000
		Unicellular	0100000000001011
	Trichome length (mm)	Up to 0.5	0000000010110000
		1 2	0100000000001011
	Distance between trichomes (mm)	0.1 to 0.5	0100000000110000
		0.6 to 1.3	0000000010001001
		More than 2	0000000000000010
	Base	Nonglandular	0100000000001011
		Glandular	0000000010110000
Stipules Structure	Stipules Shape	Lanceolate	0010000000000110
		Sagitate	1100111111111001
	Base	Lanceolate	0010000000000110
		Sagittate	1100111111111001
		Acute	1101101111101111
		Forked	0000010000010000
		Rounded	0010000000000000

Length (mm)	Up to 2.7	1111111101101111
	3.3 to 3.8	0000000010010000
Width (mm)	Up to 0.5	1110111111111111
	1.5 to 1.8	0001000000000000
Blade to base length	Blade>base	0100101111111111
	Blade <=base	1001010000000000
Surface	Hairy	0100000010110001
	Smooth	1011111101001110
No. of trichomes in 1mm	3-4	0000000000000001
	5-9	0000000010110000
	12-16	0100000000000000

sativa, *V. tenuifolia* and *V. tetrasperma*. The remaining had leaves with 4 - 8 leaflets. Leaflet width was up to 5 mm in *V. amoena, V. angustifolia, V. bijuga, V. costata, V. hirsuta, V. japonica, V. ludoviciana, V. pilosa, V. pulchella*, *V. sativa*, *V. tenuifolia*, *V. tetrasperma* and *V. villosa*. Width of leaflets was from 7 to 9 mm in *V. bungei*. Shape of leaflets was elliptic in *V. tetrasperma*; lanceolate in *V. amoena, V. bijuga, V. pilosa, V. sativa* subsp. *nigra* and *V. villosa* subsp. *varia*; oblong in *V. angustifolia*, *V. costata, V. hirsuta, V. japonica, V. ludoviciana, V. pulchella* and *V. villosa* subsp. *dasycarpa*; obovate in *V. bungei* and ovate in *V. sativa* subsp. *sativa* and *V. tenuifolia*. Acute apexes were shown in *V. amoena, V. pilosa, V. sativa* subsp. *sativa*, *V. tenuifolia* and *V. tetrasperma*; acuminate

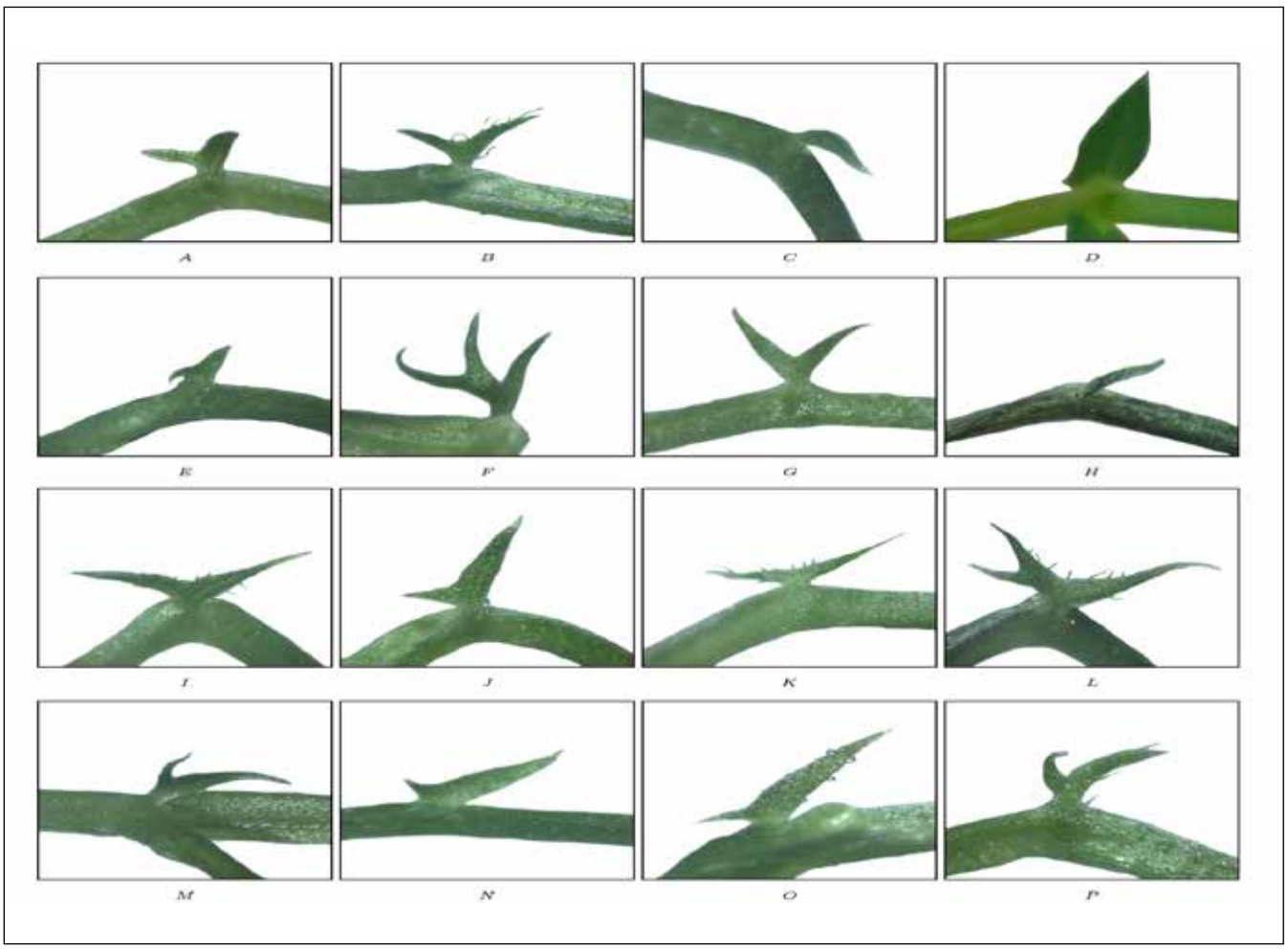

Fig. 4. Stipule structure in the studied *Vicia* species. (A) *V. amoena*, (B) *V. angustifolia*, (C) *V. bijuga*, (D) *V. bungei*, (E) *V. costata*, (F) V. *hirsuta*, (G), *V. japonica*, (H) *V. ludoviciana*, (I), *V. pilosa*, (J) *V. pulchella* subsp. *pulchella*, (K) *V. sativa* subsp. *nigra*, (L) *V. sativa* subsp. *sativa*, (M) *V. tenuifolia*, (N) *V. tetrasperma*, (O) *V. villosa* subsp. *dasycarpa*, (P) *V. villosa* subsp. *varia*.

Fig. 4. Estructura de las estípulas en especies de *Vicia*. (A) *V. amoena*, (B) *V. angustifolia*, (C) *V. bijuga*, (D) *V. bungei*, (E) *V. costata*, (F) *V. hirsuta*, (G), *V. japonica*, (H) *V. ludoviciana*, (I), *V. pilosa*, (J) *V. pulchella* subsp. *pulchella*, (K) *V. sativa* subsp. *nigra*, (L) *V. sativa* subsp. *sativa*, (M) *V. tenuifolia*, (N) *V. tetrasperma*, (O) *V. villosa* subsp. *dasycarpa*, (P) *V. villosa* subsp. *varia*.

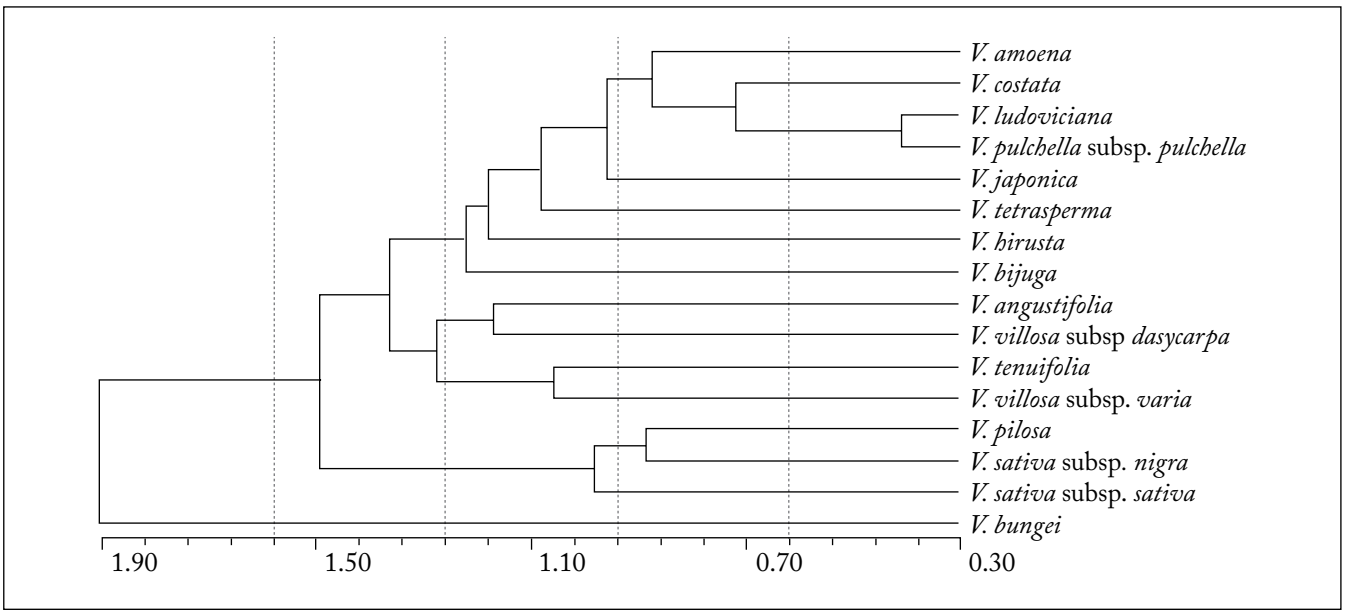

Fig. 5. Phenogram illustrating the relationships between the studied *Vicia* species based on the micro morphological characters of the leaf; the total number of the recorded characters was 48 in each taxon.
Fig. 5. Dendrograma que ilustra las relaciones entre las especies de *Vicia* en base a los caracteres micromorfológicos de la hoja, el número total de los caracteres registrados fue de 48 en cada taxon.

in *V. angustifolia, V. bijuga, V. costata, V. ludoviciana, V. pulchell*, *V. sativa* subsp. *Nigra* and *V. villosa*; obtuse in *V. bungei* and *V. japonica. Vicia hirsute* had truncate apexes. Leaflet base was cuneate in *V. bijuga, V. bungei*, *V. hirsuta, V. japonica, V. tenuifolia*, *V. tetrasperma* and *V. villosa*, and obtuse in *V. amoena, V. angustifolia, V. costata, V. ludoviciana, V. pilosa, V. pulchella* and *V. sativa*. Blade surface was hairy in *V. angustifolia, V. pilosa, V. sativa*, *V. tenuifolia* and *V. villosa*; while it was smooth in *V. amoena, V. bijuga, V. bungei*, *V. costata, V. hirsuta, V. japonica, V. ludoviciana, V. pulchella* and *V. tetrasperma*.

Trichomes. Trichomes were acute in *V. pilosa, V. sativa*, *V. tenuifolia* and *V. villosa* subsp. *varia* while they were linear in *V. angustifolia* and *V. villosa* subsp. *dasycarpa*. Multicellular trichomes were shown in *V. pilosa* and *V. sativa*, while they were unicellular in *V. angustifolia, V. tenuifolia* and *V. villosa*. Trichome length was up to 0.5 mm in *V. pilosa* and *V. sativa*, while it was from 1-2 mm in *V. angustifolia, V. tenuifolia* and *V. villosa*. Distance between trichomes was 0.1 to 0.5 mm in *V. angustifolia* and *V. sativa*; 0.6 to 1.3 mm in *V. pilosa, V. tenuifolia* and *V. villosa* subsp. *varia* and more than 2 mm in *V. villosa* subsp. *dasycarpa*. Trichome base was nonglandular in *V. angustifolia, V. tenuifolia* and *V. villosa*, and glandular in *V. pilosa* and *V. sativa*.

Stipules. Stipule shape was hastate in *V. bungei*, *V. tetrasperma* and *V. villosa* subsp. *dasycarpa*; lanceolate in *V. bijuga*, and sagittate in all other species. Stipules with acute base were observed in *V. amoena, V. angustifolia, V. bungei*, *V. costata, V. japonica, V. ludoviciana, V. pilosa, V. pulchella*, *V. sativa* subsp. *nigra*, *V. tenuifolia*, *V. tetrasperma* and *V. villosa*; forked in *V. hirsuta* and *V. sativa* subsp. *Sativa*, and rounded in *V. bijuga*.

Stipule length was up to 2.7 mm in *V. amoena, V. angustifolia, V. bijuga, V. bungei*, *V. costata, V. hirsuta, V. japonica, V. ludoviciana, V. pulchella*, *V. sativa* subsp. *nigra*, *V. tenuifolia*, *V. tetrasperma* and *V. villosa*, and from 3.3 to 3.8 mm in *V. pilosa* and *V. sativa* subsp. *sativa*. Width of stipules was up to 0.5 mm in *V. amoena, V. angustifolia, V. bijuga, V. costata, V. hirsuta, V. japonica, V. ludoviciana, V. pilosa, V. pulchella*, *V. sativa*, *V. tenuifolia*, *V. tetrasperma* and *V. villosa*, while it was 1.5 to 1.8 mm in *V. bungei*. Stipule blade was longer than the base in *V. angustifolia, V. bijuga, V. costata, V. japonica, V. ludoviciana, V. pilosa, V. pulchella*, *V. sativa*, *V. tenuifolia*, *V. tetrasperma* and *V. villosa*. In *V. amoena, V. bungei* and *V. hirsuta* stipule blade was equal to or shorter than the base. Hairy surface of stipules was in *V. angustifolia, V. pilosa, V. sativa* and *V. villosa* subsp. *varia*; while surface was smooth in all other studied species. Three to 4 trichomes were in 1 mm^2 of stipule surface in *V. villosa* subsp. *varia*; 5-9 trichomes in *V. pilosa* and *V. sativa*, and 12-16 trichomes in *V. angustifolia*.

DISCUSSION

Several authors have tried to provide a natural system to divide the genus *Vicia* into sections (Ball, 1968; Davis, 1970; Komarov et al., 1972; Kupicha, 1976; Gunn, 1979; Maxted, 1993; Anren et al., 1998; Leht, 2005). They based their studies on morphological characteristics such as stems, flowers, and fruits. In the present study a larger number of leaf characteristicss were used.

Based on the morphological characteristics, *V. hirsuta* has been moved in and out of the section Cracca in previous classifications. The phenogram in Figure 4 showed that *V. hirsuta* was more closely related to *V. tetrasperma*, a member of the section Ervum; however, embryo characters showed its close relation to section Cracca (Abozeid et al., 2017).

Komarov et al. (1972) arranged *V. amoena*, *V. costata* and *V. japonica* under the section Cracca. Kupicha (1976) separated *V. amoena* under the section Vicilla. Anren et al. (1998) rearranged the three species under the section Cassubicae. Leht (2005) placed *V. costata* back under the section Cracca. Our numerical analysis failed to separate the three species from other section than Cracca. The phenograh in Figure 4 showed that the three species were nested within the section Cracca. These results don't support their placement in the separate section Cassubicae, as it was reported by Anren et al. (1998).

Boissier (1872) treated *V. sativa* subsp. *nigra* as *V. angustifolia* subsp. *pusilla*; while Gunn (1979) treated it as a subspecies of *V. sativa*. Our phenetic analysis showed that *V. sativa* subsp. *nigra* was more closely related to *V. sativa* (0.2 - 0.5 mm, acute, multicellular trichomes with glandular base) than to *V. angustifolia* (1-2 mm, linear, unicellular trichomes with non-glandular base). Our results support the classification of Gunn (1979).

Taxonomic treatment. Based on leaf macro- and micro-morphological characteristics, and the results of the numerical analysis, a key was constructed for *Vicia* species as follows:

1a. Leaflets obovate, 7 to 9 mm width. Stipules 1.5 to 1.8 mm width..... ***V. bungei***
1b. Leaflets elliptic, lanceolate, oblong or ovate, up to 5 mm width. Stipules up to 0.5 mm width..... **(2)**
2a. Blade surface with multicellular trichomes, up to 0.5 mm length, with glandular base ..... **(3)**
2b. Blade surface smooth or with unicellular trichomes, 1 to 2 mm length, with nonglandular base ..... **(5)**
3a. Leaflets ovate. Stipules base forked ..... ***V. sativa* subsp. *sativa***
3b. Leaflets lanceolate. Stipules base acute ..... **(4)**
4a. Leaflet apex acute. Blade surface with 0.6 to 1.3 mm distance between trichomes. Stipules 3.3 to 3.8 mm length..... ***V. pilosa***
4b. Leaflet apex acuminate. Blade surface with 0.1 to 0.5 mm distance between trichomes. Stipules up to 2.7 mm length ..... ***V. sativa* subsp. *nigra***
5a. Leaflet blade hairy ..... **(6)**
5b. Leaflet blade smooth ..... **(9)**
6a. Leaflets oblong. Blade surface with linear trichomes ..... **(7)**
6b. Leaflets lanceolate or ovate. Blade surface with acute trichomes ..... **(8)**
7a. Leaflet base obtuse. Blade surface with 0.1 to 0.5 mm distance between trichomes. Stipules sagittate, with hairy surface ..... ***V. angustifolia***
7b. Leaflet base cuneate. Blade surface with more than 2 mm distance between trichomes. Stipules hastate, with smooth surface ..... ***V. villosa* subsp. *dasycarpa***
8a. Leaf with 2 leaflets. Leaflets ovate, with acute apex. Stipules with smooth surface ..... ***V. tenuifolia***
8b. Leaf with 4-8 leaflets. Leaflets lanceolate, with acuminate apex. Stipules with hairy surface ..... ***V. villosa* subsp. *varia***
9a. Stipules lanceolate, with rounded base ..... ***V. bijuga***
9b. Stipules hastate or sagittate, with acute or forked base ..... **(10)**
10a. Leaflets with truncate apex. Stipules with forked base ..... ***V. hirsuta***
10b. Leaflets with acute, acuminate or obtuse apex. Stipules with acute base..... **(11)**
11a. Leaflets elliptic. Stipules hastate…… ***V. tetrasperma***
11b. Leaflets lanceolate or oblong. Stipules sagittate……..... **(12)**
12a. Leaflets with obtuse apex and cuneate base ..... ***V. japonica***
12b. Leaflets with acute or acuminate apexes and obtuse bases……..... **(13)**
13a. Leaflets oblong, with acuminate apex. Stipule blade longer than the base..... **(14)**
13b. Leaflets lanceolate, with acute apex. Stipule blade equals the base in length ..... ***V. amoena***
14a. Leaf with tendril …..... **(15)**
14b. Leaf with no tendril ..... ***V. costata***
15a. Leaf with 2 leaflets..... ***V. ludoviciana***
15b. Leaf with 4-8 leaflets ..... ***V. pulchella* subsp. *pulchella***

CONCLUSIONS

Leaf macro- and micro-morphological characteristics are variable and helpful in distinguishing various species, and partially confirm sectional classification of the genus *Vicia*.

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Conflict of interest. The authors declare that they have no conflict of interest.

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