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Biosystematic and Chemosystematic studies in five south american species of *Conyza* (Asteraceae)

JUAN D. URDAMPILLETA[1], ANÍBAL G. AMAT[1], CLAUDIO J. BIDAU[2,3] and NICOLÁS KOLB KOSLOBSKY[4]

Summary: The composition of the essentials oils of five species of *Conyza* Less from Argentina was determined by Gas Chromatography-Mass Spectrometer. This composition is associated to morphological and cytogenetic characters. The monoterpenes constitute more than 60% of the essential oils in *C. blakei*, *C glandulitecta*, *C. sumatrensis* var. *sumatrensis* and *C. sumatrensis* var. *floribunda*, in which limonene is the predominant compound. In *C. bonariensis* and *C. primulaefolia* the monoterpene content constitute less than the 40%. *C. bonariensis* presents only 13% limonene, while in *C. primulaefolia* it is absent. The similarity analysis of monoterpenes showed a relationship between the morphological and cytogenetic analysis, and the monoterpene content character seems to be important in biosystematic studies of the group studied. In general, *C. sumatrensis* var. *sumatrensis* is the species with more ancestral characters, while *C. bonariensis* and *C. primulaefolia* show more derived ones.

Key words: essential oils, chemosystematics, morphology, cytogenetics, *Conyza*, Asteraceae.

Resumen: Estudios biosistemáticos en cinco especies sudamericanas de *Conyza* (Asteraceae). Fueron determinados mediante Cromatografía Gaseosa - Espectrómetro de Masa los componentes de los aceites esenciales de seis especies de *Conyza* Less. de Argentina. Esta composición es asociada a caracteres morfológicos y citogenéticos. Los monoterpenos constituyen mas de 60% de aceites esenciales en *C. blakei*, *C glandulitecta*, *C. sumatrensis* var. *sumatrensis*, *C. sumatrensis* var. *floribunda*, en las cuales *limoneno* es el componente predominante. En *C. bonariensis* y *C. primulaefolia* el contenido de monoterpenos constituye menos de 40%. *C. bonariensis* presenta solo 13% limoneno, mientras que en *C. primulaefolia* este compuesto esta ausente. El análisis de similitud utilizando monoterpenos muestra relación con el análisis dado por caracteres morfológicos y citogenéticos, por lo que el carácter contenido de monoterpeno resulta importante en estudios biosistemáticos en el grupo estudiado. En general, *C. sumatrensis* var. *sumatrensis* es la especie que presenta un mayor numero de caracteres ancestrales, mientras que *C. bonariensis* y *C. primulaefolia* revelarían caracteres derivados.

Palabras clave: aceites esenciales, quimiosistemática, morfología, citogenética, *Conyza*, Asteraceae.

Introduction

The genus *Conyza* Less. (tribe Astereae) in Argentina comprises 22 species, seven of which were reported for Misiones Province (Zuloaga & Morrone, 1999). *Conyza* species usually grow in disturbed environments, and are considered weeds or invasive species for their colonizing ability (Thebaud & Abbott, 1995). This genus includes highly related species groups, some of them very polymorphic, and its taxonomic treatment results insufficient when it is only based on morphological characters. Cytogenetic studies of species from Misiones Province show predominance of hexaploid species (only one is octoploid), with similar relatively symmetrical karyotypes (Urdampilleta *et al.*, 2005).

Essential oils are very important in Asteraceae and are made up mainly of mono- and sesquiterpenes. These compounds have no taxonomic value by themselves, but taken as a whole, added to their biosynthetic pathway, reflects a particular genetic structure or "chemotype" (Tétényi, 1986; Gil *et al.*, 2000). The mono- and

[1]Departamento de Biología, Cátedra de Biología Vegetal, Facultad de Ciencias Exactas Químicas y Naturales, Universidad Nacional de Misiones.
[2]Departamento de Genética, Cátedra de Genética Evolutiva, Facultad de Ciencias Exactas Químicas y Naturales, Universidad Nacional de Misiones.
[3]Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina.
[4]Laboratorio Central, Facultad de Ciencias Exactas Químicas y Naturales, Universidad Nacional de Misiones.
E-mail: *juanurdampilleta@hotmail.com*

sesquiterpene compounds are synthesized by terpene-synthetases in different topographic spaces (plastids and cytoplasm, respectively) of the cell, these enzymes are encoded by nuclear genes (Bohlman *et al.*, 1998).

Cronquist (1943) transferred the *Coenotus* section of the genus *Erigeron* L. to *Conyza*, and recognized one transition form *Erigeron* to *Conyza* through this section. *Conyza* possesses a tendency toward a higher number of female flowers and a shortening of the ligule. Nesom (1990) redefined the genus after a complete separation from *Laennecia* Cass. Since delimitation of genera and species are conflicting, new and more intensive biosystematic studies are required. The purpose of this study is to carry out a comparative analysis of *Conyza* species from Misiones, based on karyotypical, morphological and chemical characters.

Material and Methods

The *C. blakei* (Cabrera) Cabrera, *C. bonariensis* (L) Cronquist, *C. glandulitecta* Cabrera, *C. primulaefolia* (Lam.) Cuatrec. & Lourteig, *C. sumatrensis* (Retz.) E. Walker var. *sumatrensis* and *C. sumatrensis* (Retz.) E. Walker var. *floribunda* (Kunth) J. B. Marshall specimens were collected from urban and suburban areas of Southwestern Misiones Province. Voucher specimens were deposited in LP, MNEF and SI herbaria.

Aerial parts were immediately taken to processing. 50g of fresh aerial parts were cut in small pieces and submitted to hydrodestillation for 2hs. in a clevenger-type apparatus. The analysis of the essential oil was made in a Gas Chromatograph (HP 5890 Series II Plus), coupled with a Mass Spectrometer (HP 5972) (GC-MS). A fused silica column HP-5 MS (30m x 0.25mm i.d.; film thickness 0.25µm). The operating conditions were as follows: injector temperature, 220°C, detector temperature, 240°C; carrier gas He (1 ml/min), oven temperature program, from 60°C at 3°C/min to 240°C. The oil components were identified by comparing their mass spectra with those of the computer library and confirmed by comparing their retention indices with data published in the literature (Adams 1995).

The cytogenetic and morphological characters analyzed were taken from Urdampilleta *et al.* (2005) and new characters related to pubescence were added. The matrix (Appendix) was standardized according to Crisci *et al.* (1983). For cluster analysis the Euclidian distance was calculated and UPGMA was used as amalgamation method. Cluster analysis was performed with the Statistica 4.3 software, using 48 continuous and discontinuous characters, of which 15 were morphological, 5 cytogenetic and 28 chemical (See Appendix). The different terpenic compounds are mentioned in the matrix with their percentage value in relation to total quantity of the corresponding group (mono- or sesquiterpene).

Material examined (all from Argentina, Prov. Misiones)

C. blakei. *Dpto. Capital*, Miguel Lanús, Urdampilleta 31-I-2000 (LP, SI), Urdampilleta 20-III-2000 (LP).

C. bonariensis. *Dpto. Capital*, Miguel Lanús, Urdampilleta 7-XI-1999 (MNEF), Urdampilleta 16-XI-1999 (MNEF); Posadas, Urdampilleta 8-VIII-1999 (LP, SI).

C. glandulitecta. *Dpto. Capital*, Miguel Lanús, Urdampilleta 7-XI-1999 (MNEF), Urdampilleta 16-XI-1999 (MNEF), Urdampilleta 30-I-2000 (SI, MNEF), Urdampilleta 31-I-2000 (LP, MNEF); *Dpto. L. N. Alem*, 2 Arroyos, Urdampilleta 23-I-2000 (LP, SI).

C. primulaefolia. *Dpto. Capital*, Miguel Lanús, Urdampilleta 7-XI-1999 (LP, SI), Urdampilleta 16-XI-1999 (MNEF), Urdampilleta 30-I-2000 (MNEF); *Dpto. Concepción*, Concepción de la Sierra, Urdampilleta 28-II-2000 (MNEF).

C. sumatrensis var. *sumatrensis*. *Dpto. San Ignacio*, Corpus, Urdampilleta III-1999 (SI, LP); *Dpto. Capital*, Miguel Lanús, Urdampilleta 18-X-1999 (MNEF), Urdampilleta 7-XI-1999 (MNEF), Urdampilleta 20-XI-1999 (SI); Posadas, Urdampilleta 30-I-2000 (LP). *Dpto. Candelaria*, Santa Ana, Amat & Urdampilleta 10-III-2000 (MNEF); *Dpto. L. A. Alem*, Dos Arroyos, Urdampilleta 23-I-2000 (MNEF); *Dpto. Concepción*, Concepción de la Sierra, Urdampilleta 28-II-2000 (MNEF).

C. sumatrensis var. *floribunda*. *Dpto. Candelaria*, Santa Ana, Amat & Urdampilleta 10-III-2000 (SI, MNEF); Santa Ana, Amat & Urdampilleta IV-2000 (SI, LP, MNEF).

Results and Discussion

The species of *Conyza* showed differentiation in the inflorescence topology and the structural features of the capitulum. This morphological variation is associated to karyotypical differences, both in ploidy level and chromosome structure (Urdampilleta *et al.*, 2005). Other source of variation is the indumentum, which is

determinant in the synthesis and secretion of secondary metabolites (Wise *et al.*, 1998).

The stem and leaf cover trichomes are heterogeneous in *C. sumatrensis* var. *sumatrensis* and *C. bonariensis*, one set of uniform abundant short addressed hair (strigulose) in both face of the leaves, and another of few rigid long (hirsute) in the rib of the stem and margin of the leaf, both types are pluricellular uniseriate hair, called conic simple type (Ramayya, 1962) (Table 2). The species possess a pubescence strigulose in the phyllary, and few biseriate glandular hairs in the corolla apical region of the hermaphrodite and female flowers. In *C. bonariensis* specifically conical simple trichomes in the corolla apical region of the hermaphrodite flowers are observed.

C. blakei and *C. sumatrensis* var. *floribunda* possess a hirsute pubescence with few trichomes above the rib of the stem and margin of the leaf, few trichomes in the central nervation of the phyllary and biseriate glandular hair in the corolla apical region of both types of flowers. *C. sumatrensis* var. *floribunda*, in particular, presents short addressed hair in both faces of the leaf.

C. glandulitecta has a heterogeneous pubescence in the stem and leaf, one set of a few rigid long (hirsute) -conical simple type- in the rib of the stem and margin of the leaf, and another set of uniform short glandular hair. In the phyllaries it possesses a glandular pubescence and few rigid trichomes in the central nervation; in the corolla apical region few biseriate glandular hair of both flowers types are present.

The stem of *C. primulaefolia* has only one set of uniform abundant short erect hair (hispid), and uniform abundant short addressed hair (strigulose) in both faces of the leaf. This species possesses a strigulose pubescence in the phyllary and few biseriate glandular hair in the corolla apical region of both flower types and conical simple trichomes in the corolla apical region of the hermaphrodite flowers.

Table 1. Analysis of essential oils, 1-9 monoterpenic compound, and 10-28 sesquiterpene. B = *C. bonariensis*, Bk = *C. blakei*, G = *C. glandulitecta*, P = *C. primulaefolia*, SS = *C. sumatrensis* var. *sumatrensis* and SF = *C. sumatrensis* var. *floribunda*.

	Compound	RI Kovats*	A	B	Bk	F	G	P
1	α-Pinene	*939*	0.90	0.00	0.19	0.61	0.37	0.00
2	Sabinene	*975*	0.72	0.00	0.00	0.00	0.17	0.00
3	β-Pinene	*979*	6.31	0.04	2.13	6.08	0.15	0.00
4	Myrcene	*991*	1.64	0.52	2.72	2.51	2.15	0.00
5	*dl*-Limonene	*1029*	47.79	13.52	79.29	63.15	56.37	0.00
6	(Z)-β-Ocimene	*1037*	0.27	0.48	0.52	0.86	0.70	0.36
7	(E)-β-Ocimene	*1050*	8.66	13.25	1.78	7.10	15.33	18.79
8	*p*-Mentha-1,5,8-Triene	*1097*	0.00	1.02	0.00	0.46	0.45	0.48
9	*p*-Mentha-1,3,8-Triene	*1110*	0.00	5.22	0.00	2.12	2.59	2.42
10	(E)-Caryophyllene	*1410*	4.36	3.31	1.84	3.96	0.94	6.58
11	α-Humulene	*1455*	0.49	0.92	0.19	0.32	0.31	0.68
12	β-Farnesene	*1457*	0.83	4.76	0.00	0.85	0.33	0.00
13	Germacrene D	*1485*	7.54	14.57	1.61	3.03	1.00	22.92
14	Ni	*1491*	0.09	1.66	0.00	0.00	0.13	0.00
15	Bicyclogermacrene	*1500*	5.29	6.62	3.14	2.50	1.89	17.56
16	δ-Cadinene	*1523*	3.54	1.72	2.59	0.00	0.91	0.34
17	Ni	*1542*	0.37	0.70	1.36	0.00	6.99	0.00
18	Trans-Nerolidol	*1562*	1.02	0.76	0.23	0.64	0.68	0.42
19	Germacrene D4-ol	*1576*	0.91	0.00	0.65	0.00	1.36	0.00
20	Spathulenol	*1578*	1.64	3.79	0.45	1.31	0.00	5.42
21	Caryophyleno oxide	*1583*	2.06	2.73	0.57	2.70	0.65	5.13
22	Ni	*1593*	1.03	0.61	0.00	0.00	0.00	0.43
23	Humulene epoxide	*1608*	0.00	1.06	0.00	0.00	0.11	0.36
24	Ni	*1623*	0.00	0.85	0.00	0.38	0.07	1.23
25	Epi-α-Muurolol	*1642*	0.61	0.96	0.20	0.00	0.75	1.32
26	α-Cadinol	*1654*	0.81	2.04	0.36	0.33	1.31	2.51
27	Ni	*1685*	0.78	1.69	0.00	0.59	0.11	3.88
28	Manool	*2057*	0.00	0.00	0.00	0.00	1.10	0.00

* Kovats Retention Index

Table 2. Basic data matrix. 1-15, Morphological characters; 16-20 Cytogenetic characters; 21-29 monoterpenes; 30-48 sesquiterpenes. B = *C. bonariensis*, Bk = *C. blakei*, G = *C. glandulitecta*, P = *C. primulaefolia*, SS = *C. sumatrensis* var. s*umatrensis* and SF = *C. sumatrensis* var. *floribunda.*

Characters \ Spp.	B	Bk	G	P	SS	SF
1*	226.40	83.40	231.50	548.00	142.00	57.40
2*	0.07	0.08	0.10	0.06	0.11	0.13
3*	1	0	1	1	0	0
4	1	0	0	1	0	0
5	0	2	1	0	0	2
6*	0	0	0	1	0	0
7*	2	1	0	1	0	0
8	0	0	1	0	0	0
9	1	0	0	1	1	0
10	1	1	1	0	1	1
11	0	0	1	0	0	0
12	0	0	0	1	0	0
13	1	0	0	0	1	0
14	1	1	1	0	1	1
15	0	0	0	1	0	0
16*	1	1	1	2	1	1
17*	104.45	103.03	98.19	122.11	86.68	96.07
18*	0.17	0.29	0.29	0.29	0.17	0.29
19*	0.2729	0.2734	0.2988	0.2943	0.2343	0.2855
20*	0.2414	0.2080	0.2006	0.2177	0.1879	0.1840
21	0.0	0.2	0.5	0.0	1.4	0.7
22	0.0	0.0	0.2	0.0	1.1	0.0
23	0.1	2.5	0.2	0.0	9.8	7.3
24	1.5	3.1	2.8	0.0	2.5	3.0
25	37.1	91.5	72.0	0.0	71.5	76.2
26	1.4	0.6	0.9	1.6	0.4	1.0
27	39.5	2.1	19.6	85.2	13.4	8.6
28	3.3	0.0	0.6	2.2	0.0	0.6
29	17.1	0.0	3.3	11.0	0.0	2.6
30	6.9	14.0	5.0	9.6	13.8	23.8
31	1.9	1.4	1.7	1.0	1.5	2.0
32	9.8	0.0	1.8	0.0	2.6	5.1
33	30.2	12.2	5.4	33.3	23.3	18.2
34	3.4	0.0	0.7	0.0	0.3	0.0
35	13.8	23.8	10.1	25.5	16.8	15.1
36	3.6	19.6	4.9	0.5	12.5	0.0
37	1.5	10.3	37.5	0.0	1.3	0.0
38	1.5	1.7	3.6	0.6	3.2	3.9
39	0.0	4.9	7.3	0.0	2.9	0.0
40	7.4	3.4	0.0	7.9	5.2	7.9
41	5.3	4.3	3.5	7.5	6.5	16.3
42	1.2	0.0	0.0	0.6	3.3	0.0
43	2.2	0.0	0.6	0.5	0.0	0.0
44	1.7	0.0	0.4	1.8	0.0	2.3
45	2.0	1.5	4.0	1.9	1.9	0.0
46	4.2	2.7	7.0	3.7	2.5	2.0
47	3.5	0.0	0.6	5.6	2.4	3.6
48	0.0	0.0	5.9	0.0	0.0	0.0

* Data from Urdampilleta *et al.* (2005)

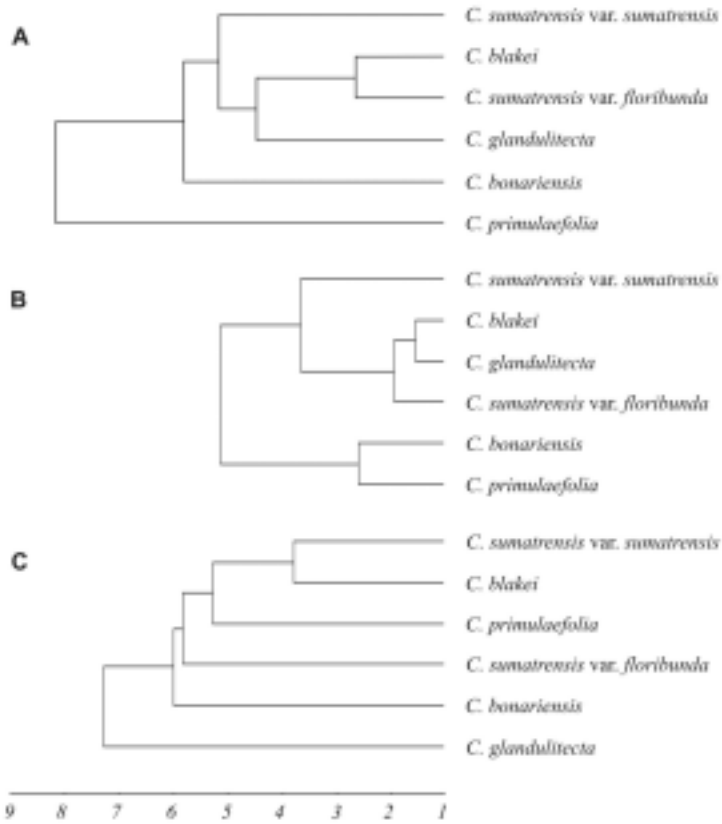

Fig. 1. Phenograms obtained from different sources of variation. A, Morphological and cytogenetic characters. B, Monoterpenes. C, Sesquiterpenes.

Mono- and sesquiterpenes constitute the essential oils in *Conyza*. The monoterpene fraction constitutes more than 60% of the essential oils in *C. blakei*, *C glandulitecta*, *C. sumatrensis* var. *sumatrensis*, and *C. sumatrensis* var. *floribunda* of which limonene is the predominant compound, showing a maximum of 79% in *C. blakei*. *C. bonariensis* and *C. primulaefolia* show a decrease in the monoterpenic level, which is lower than 40%. *Conyza bonariensis* presents only 13% limonene, while *C. primulaefolia* presents none. Another characteristic monoterpenic compound with relatively high levels in *C. bonariensis* (13.25%), *C. glandulitecta* (15.33%), and *C. primulaefolia* (18.79%) of (E)-ocimene; on the other hand, a noticeable decrease occurs in *C. blakei* (1,78%). *Conyza sumatrensis* var. *sumatrensis* and *C. sumatrensis* var. *floribunda* possess about 6% b-pinene, while *C. blakei* shows 2% and it is practically absent in *C. bonariensis*, *C. glandulitecta* and *C. primulaefolia* (Table 1).

In *C. bonariensis* and *C. primulaefolia*, sesquiterpenes comprise most of their essential oils, both quali- as quantitatively. Sesquiterpenes are the most diverse group of compounds in all species, reflecting the complexity of their biosynthetic route (Bohlman *et al.*, 1998). Germacrene D and biciclogermacrene are characteristic in the pool of sesquiterpenes; in *C. primulaefolia* they represent 22.92% and 17.56%, respectively; while *C. glandulitecta* presents a high level (6.99%) of an unknown compound (Table 1).

With respect to the indumentum, *C. glandulitecta* is unique with glandular hairs covering the vegetative organs of the plant. This character is correlated to the presence of an unknown compound of the sesquiterpenic fraction. Likewise, the complete loss of limonene biosynthesis is correlated with particularities in the indumentum of *C. primulaefolia*.

According to Urdampilleta *et al.* (2005), *C. blakei*, *C. glandulitecta*, *C. sumatrensis* var. *sumatrensis* and *C. sumatrensis* var. *floribunda* are derived hexaploids with 2n = 6x = 54 and relatively

symmetric karyotypes. *C. primulaefolia* and *C. bonariensis* present derived karyotypes: *C. bonariensis* has the highest degree of karyotypic asymmetry, and *C. primulaefolia* is a derived octoploid with 2n = 8x = 72 (Table 2). The latest species shows a tendency to the reduction and specialization in the typology of the inflorescence. Morphological and phytochemical characters support this evolutionary scheme. *C. sumatrensis* var. *sumatrensis* is the species with most ancestral character, and it is closely related to *C. blakei*, *C. glandulitecta* and *C. sumatrensis* var. *floribunda*. Assuming that the *C. sumatrensis* var. *sumatrensis* pubescence type is the ancestral condition within the group studied, *C. blakei*, *C. glandulitecta* and *C. sumatrensis* var. *floribunda* would show modifications from the primitive state by reduction and specialization; the same process could be observed for other morphological characters in *C. bonariensis* and *C. primulaefolia*. This morphological and cytogenetic variation is associated with a reduction in the relative amount of monoterpene in essential oils, particularly in *limonene* biosynthesis.

The cluster analysis of monoterpenes (Fig. 1B) showed, in general, a similar dendrograma to that obtained from the morphological and cytogenetic characters (Fig. 1A); two basic groups, one formed by *C. primulaefolia* and *C. bonariensis*, and the other, by *C. blakei*, *C. glandulitecta*, *C. sumatrensis* var. *sumatrensis* and *C. sumatrensis* var. *floribunda* are observed. *C. blakei* and *C. glandulitecta* show the highest similarity in monoterpenes. The dendrograma based on sesquiterpenes (Fig. 1C) does not indicate any relationship with the other characters. The use of the essential oils in taxonomic analysis requires discrimination of the compounds according to biosynthetic topographic space. The monoterpenes are synthesized within chloroplasts and are relevant to the biosystematic analysis in the group studied.

The monoterpene compounds are useful characters in systematic studies. Owing to their high volatility, monoterpenes are important in the interaction with pollinizators, which have great relevance in the evolution of Asteraceae (Leppik, 1977). Quali- and quantitative monoterpene variation seems to be correlated to chromosomal and morphological changes, which show an evolutionary tendency in the group studied of *Conyza* species (Urdampilleta *et al.*, 2005).

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Bibliography

ADAMS, R. P. 1995. *Identification of essentials oils components by Gas Chromatography/ Mass Sprectroscopy.* Allured Publ. Corp., Carol Stream, Il.

BOHLMAN, J., G. MEYER-GAUEN, & R. CROTEAU. 1998. Plant terpenoid synthases: Molecular Biology and phylogenetic analysis. *Proc. Natl. Acad. Sci, USA.* 98: 4126-4133.

CRISCI, J. V. & M. F. LÓPEZ AMENGOL. 1983. *Introducción a la teoría y práctica de la taxonomía numérica.* Monografía 26, Serie Biología. O.E.A., Washington, D. C.

CRONQUIST, A. 1943. The separation of *Erigeron* from *Conyza*. *Bull. Torr. Bot. Club* 70: 629-632.

GIL A., C. M. GHERSA, & S. LEICACH. 2000. Essential oils yield and composition of *Tagetes minuta* accessions from Argentina. *Biochem. Syst. Ecol.* 18: 405-407.

LEPPIK, E. L., 1977. The evolution of capitulum types of the Compositae in the light of insect-flower interaction. *In*: HEYWOOD, V. H., J. HARBORNE, & B. L. TURNER (eds.), *The biology and chemistry of the Compositae*, pp. 62-87. Academic Press, New York.

NESOM, G. L. 1990. Further definition of *Conyza* (*Astereae*: *Asteraceae*). *Phytologia* 68: 229-233.

RAMAYYA, N. 1962. Studies on the trichomes of some *Compositae*. I. General Structure. *Bull. Bot. Survey (India)* 4: 77-188.

TÉTÉNYI, P. 1986. Chemotaxonomic aspects of essential oils. *In*: CRAKER L. E. & J. E. SIMON (eds.). *Herbs, Spices, and Medicinal Plant: Recent advances in Botany, Horticulture, and Pharmacology*, pp. 11-32. Oryx Press, Phoenix.

THEBAUD, C. & R. J. ABBOTT. 1995. Characterization of invasive *Conyza* species (*Asteraceae*) in Europe: Quantitative trait and isozyme analysis. *Amer. J. Bot.* 82: 360-368.

URDAMPILLETA, J., A. G. AMAT & C. J. BIDAU. 2005. Karyotypic studies and morphological analysis of some reproductive features in five species of *Conyza* Less. (Astereae: Asteraceae) from Northeastern Argentina. *Bol. Soc. Argent Bot.* 40.

WISE, M., J. THOMAS, E. KALAHARI, & R. CRATE. 1998. Monoterpene synthases from common sage (*Salvia officials*). *J. Biol. Chem.* 273: 14891-14899.

ZULOAGA, F. & O. MORRONE. 1999. Catálogo de las Plantas Vasculares de la República Argentina II. *Dicotyledonieae*. *Monogr. Syst. Bot. Missouri Bot. Gard.* 74 [*Conyza*]: pp. 154-158.

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Appendix. List of characters and states used for phenetic analysis.

Morphological characters (15)
1-Flowers in the capitulum (mean)
2-Sex ratio (mean)
3-Anthocyanic pigments (0=absent, 1= present).
4-Corolla pubescence. (0= secretory hairs, 1= secretory hairs + cover hairs, 2= cover hairs).
5-Phyllaries pubescence. (0=strigulose, 1=glandular hairs, 2=erect cover hairs).
6-Inflorescence aspect. (0=frondose, 1=bracteate).
7-Inflorescence outline. (0=pyramidal, 1=cylindrical, 2=pseudocorimbose).
8-Glandular pubescence in leaves. (0=absent, 1=present).
9-Strigulose (uniform) pubescence in leaves. (0=absent, 1=present).
10-Erect (robust) cover hairs in central rib and margin of leaves. (0=absent, 1=present).
11-Glandular pubescence in stems. (0=absent, 1=present).
12-Hispid (uniform) pubescence in stems. (0=absent, 1=present).
13-Strigulose uniform pubescence. (0=absent, 1=present).
14-Robust cover hairs in stem ribs. (0=absent, 1=present).
15-System of root ramification. (0=clearly monopodial, 1=sympodial).

Cytogenetic characters (5)
16-Chromosome number (1= 54, 2= 72)
17-Total karyotypic length (TKL)
18-Submetacentric/metacentric chromosome ratio.
19-Intrachromosomal asymmetry ratio, A1.
20-Interchromosomal asymmetry ratio, A2.

Chemical characters (28), Ni: non identified compound.
21-*a*-Pinene
22-Sabinene
23- *b*-Pinene
24- Myrcene
25- *dl*-Limonene
26- (Z)-*b*-Ocimene
27- (E)- *b*-Ocimene
28- 1,5,8-*p*-Menthatriene
29- 1,3,8-*p*-Menthatriene
30- (E)-Caryophyllene
31- *a*-Humulene
32- *b*-Farnesene
33- Germacrene D
34- Ni
35- Bicyclogermacrene
36- *d*-Cadinene
37- Ni
38- Trans-Nerolidol
39- Germacrene D4-ol
40- Spathulenol
41- Caryophyleno oxide
42- Ni
43- Humulene epoxide
44- Ni
45- Epi-a-Muurolol
46- *a*-Cadinol
47- Ni
48- Manool