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New insights into the chemical biodiversity of *Minthostachys mollis* in Argentina

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ABSTRACT

Variations in the chemical composition of the essential oils of *Minthostachys mollis* Griseb. (*Lamiaceae*) collected in their natural habitat in Argentina were determined using GC-FID-MS analysis. Seventy five samples collected out of 40 wild plant populations in the Central and Northwestern Argentina during four years sampling, were analyzed. Principal Component Analysis was made on the chemical data showing that samples from Córdoba and San Luis belonged to the typical menthone – pulegone chemotype in accordance with previous reports; meanwhile samples from Tucumán, Salta and Catamarca showed the presence of different compositions, some previously unreported, mainly those detected in Tucumán province. In addition, cluster analysis was applied using Euclidean distance to measure the dissimilarity in the relative composition of samples from Tucumán, Salta and Catamarca, using Ward's method as the agglomeration technique. Five groups were retained with less than 10% of information loss: *i*) dihydrocarvone-carvone, *ii*) pulegone with absence of menthone, *iii*) carvacryl acetate-carvacrol, *iv*) limonene and *v*) linalool.
It is worth mentioning that the compositions of the different chemotypes remained constant throughout the four years of collection and despite the highly diverse pattern of compositions revealed in this search, the typical chemotype was not found present northwards Córdoba and San Luis provinces. This is the first report of a deep study on natural populations of peperina throughout the whole distribution area in Argentina with consecutive sampling repetitions of the same populations during several years.

1. Introduction

The genus *Minthostachys* Griseb. (*Lamiaceae*) is found in middle elevations along the Andes, from Venezuela to Argentina (Alkire et al., 1994; Epling and Játiva, 1963). This genus includes 17 species according to some authors (Schmidt-Lebuhn,

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Table 1
References and essential oils yields of the 40 populations collected in Argentina.

Population N°	Collection Site	Altitude (m.a.s.l)	Collection Date	Phenological stage	EO yields (% v/w)	Collector N° (Herbarium N°)
Province of Córdoba						
1	Icho Cruz	809	19/03/2007	Full bloom	3.09	Juárez 355 (BAB)
1	Icho Cruz	809	12/03/2008	Full bloom	3.75	Juárez 422 (BAB)
1	Icho Cruz	809	30/03/2009	Full bloom	2.59	Molina 6623(BAB)
2	Ambul	1127	20/03/2007	Full bloom	4.20	Juárez 369 (BAB)
3	Ambul – La Sierrita	1204	20/03/2007	Full bloom	3.40	Juárez 368 (BAB)
3	Ambul – La Sierrita	1204	28/11/2007	Beginning of bloom	2.93	Juárez 404 (BAB)
4	Tala Cañada	1225	20/03/2007	Full bloom	4.08	Juárez 363 (BAB)
4	Tala Cañada	1225	13/03/2008	Full bloom	3.99	Molina 6755(BAB)
4	Tala Cañada	1225	29/03/2009	Late bloom	3.51	Molina 6612(BAB)
5	Sagrada Familia	1206	20/03/2007	Full bloom	4.16	Juárez 366 (BAB)
5	Sagrada Familia	1206	29/11/2007	Beginning of bloom	3.54	Juárez 410 (BAB)
5	Sagrada Familia	1206	29/03/2009	Late bloom	3.22	Molina 6609(BAB)
6	Taninga	1101	29/11/2007	Beginning of bloom	2.87	Juárez 407 (BAB)
6	Taninga	1101	13/03/2008	Full bloom	3.46	Juárez 440 (BAB)
6	Taninga	1101	29/03/2009	Late bloom	2.32	Molina 6604(BAB)
7	Paso del Carmen	1065	13/03/2008	Full bloom	3.37	Juárez 436 (BAB)
7	Paso del Carmen	1065	29/03/2009	Late bloom	3.77	Molina 6591(BAB)
8	Río Pintos, Punilla	1085	01/04/2009	Late bloom	3.87	Molina 6637(BAB)
9	Characato, Cruz del Eje	1015	01/04/2009	Late bloom	3.69	Molina 6639(BAB)
Province of San Luis						
10	Merlo, El Pantanillo	930	18/12/2006	50% bloom	4.93	Juárez 344 (BAB)
10	Merlo, El Pantanillo	930	25/03/2007	Full bloom	4.24	Juárez 382 (BAB)
10	Merlo, El Pantanillo	930	17/03/2008	Full bloom	4.66	Juárez 462 (BAB)
11	Arroyo Pasos Malos	1110	18/12/2006	50% bloom	2.85	Juárez 347 (BAB)
11	Arroyo Pasos Malos	1110	25/03/2007	Full bloom	4.60	Juárez 386 (BAB)
11	Arroyo Pasos Malos	1110	24/11/2007	Beginning of bloom	4.92	Juárez 389 (BAB)
11	Arroyo Pasos Malos	1110	17/03/2008	Full bloom	4.74	Juárez 467 (BAB)
11	Arroyo Pasos Malos	1110	25/11/2008	Beginning of bloom	4.26	Molina 6314(BAB)
11	Arroyo Pasos Malos	1110	27/03/2009	Late bloom	4.35	Molina 6572(BAB)
12	Merlo, DamianaVega	960	27/03/2009	Late bloom	2.63	Molina 6584(BAB)
13	Merlo, Cerro de Oro	952	28/03/2009	Late bloom	2.83	Molina 6589(BAB)
14	Cortaderas	972	18/12/2006	50% Bloom	2.22	Juárez 352 (BAB)
14	Cortaderas	972	22/11/2007	Beginning of bloom	3.80	Juárez 382 (BAB)
14	Cortaderas	972	18/03/2008	Full bloom	4.00	Juárez 470 (BAB)
14	Cortaderas	972	28/03/2009	Late bloom	3.16	Molina 6585(BAB)
15	Carpintería	960	28/03/2009	Late bloom	2.64	Molina 6590(BAB)
Province of Tucumán						
16	Villa Padre Monti	870	19/11/2006	50% bloom	1.13	Juárez 272 (BAB)
17	Rio Nio A	1060	20/11/2006	50% bloom	0.94	Juárez 273 (BAB)
17	Rio Nio A	1060	08/04/2008	Late bloom	0.80	Juárez 476 (BAB)
17a	Rio Nio A	1060	08/04/2008	Late bloom	0.81	Juárez 475 (BAB)
18	Rio Nio B	1209	20/11/2006	50% bloom	0.84	Juárez 275 (BAB)
18a	Rio Nio B	1209	20/11/2006	50% bloom	0.96	Juárez 276 (BAB)
19	Rio Nio C	1212	08/04/2008	Full bloom	1.44	Juárez 477 (BAB)
20	Rio Nio D	1180	20/11/2006	50% bloom	0.67	Juárez 277 (BAB)
20a	Rio Nio D	1180	08/04/2008	Full bloom	1.07	Juárez 479 (BAB)
20	Rio Nio D	1180	08/04/2008	Full bloom	1.18	Juárez 478 (BAB)
21	La Florida	1030	20/11/2006	Full bloom	0.87	Juárez 280 (BAB)
21	La Florida	1030	08/04/2008	Full bloom	0.70	Juárez 481 (BAB)
22	Chorrillos	1113	20/11/2006	Full bloom	0.94	Juárez 281 (BAB)
22	Chorrillos	1113	08/04/2008	Full bloom	1.01	Juárez 482 (BAB)
23	El Cajón	1186	20/11/2006	Full bloom	1.04	Juárez 282 (BAB)
24	Tafi del Valle	2291	30/11/2006	Beginning of bloom	0.52	Juárez 318 (BAB)
24	Tafi del Valle	2291	18/04/2008	Late bloom	0.71	Juárez 532 (BAB)
24	Tafi del Valle	2291	30/04/2009	Late bloom	1.04	Molina 6715(BAB)
25	Tafí del Valle, La Ovejería	2285	30/04/2009	Late bloom	1.10	Molina 6716(BAB)
26	Las Juntas	1412	09/04/2008	Full bloom	0.59	Juárez 486 (BAB)
26	Las Juntas	1412	24/04/2009	Full bloom	0.61	Molina 6660(BAB)
27	Potrero Grande	1425	24/04/2009	Full bloom	1.11	Molina 6661(BAB)
28	Gonzalo A	1444	01/12/2006	Beginning of bloom	0.25	Juárez 323 (BAB)
28	Gonzalo A	1444	09/04/2008	Full bloom	0.67	Juárez 487 (BAB)
29	Gonzalo B	1454	24/04/2009	Full bloom	0.73	Molina 6662(BAB)
29a	Gonzalo B	1454	24/04/2009	Full bloom	0.46	Molina 6663(BAB)
30	Gonzalo C	1348	01/12/2006	Beginning of bloom	0.26	Juárez 324 (BAB)
30	Gonzalo C	1348	09/04/2008	Full bloom	1.01	Juárez 489 (BAB)
31	Gonzalo D	1350	24/04/2009	Full bloom	0.89	Molina 6664(BAB)
32	Siambón	993	01/12/2006	Beginning of bloom	0.26	Juárez 326 (BAB)

(*continued on next page*)

Table 1 (*continued*)

Population N°	Collection Site	Altitude (m.a.s.l)	Collection Date	Phenological stage	EO yields (% v/w)	Collector N° (Herbarium N°)
33	Las Tacanas	1537	09/04/2008	Full bloom	0.65	Juárez 492 (BAB)
33a	Las Tacanas	1537	09/04/2008	Full bloom	0.29	Juárez 491 (BAB)
33	Las Tacanas	1537	24/04/2009	Full bloom	0.65	Molina 6666(BAB)
34	Escaba	663	19/04/2008	Late bloom	1.00	Juárez 533(BAB)
35	Escaba de Abajo	805	19/04/2008	Late bloom	0.81	Juárez 534 (BAB)
36	Las Higuerillas	1157	19/04/2008	Late bloom	0.97	Juárez 537 (BAB)
Province of Catamarca						
37	Balcozna	1293	19/04/2008	Late bloom	0.56	Juárez 539 (BAB)
38	Cuesta Singuil	1393	19/04/2008	Late bloom	1.10	Juárez 540 (BAB)
39	Mutquin	908	02/05/2009	Late bloom	1.63	Molina 6726(BAB)
Province of Salta						
40	La Candelaria	1426	10/04/2008	Late bloom	0.55	Juárez 493 (BAB)

2008), while others suggest a polyploid complex and merged them into a unique polymorph species: *Minthostachys mollis* (Kunth) Griseb. (Epling and Játiva, 1963). This species or variants of a unique species are known along its natural distribution area with different common names and is used in traditional medicine to alleviate rheumatic pains, headache, heart palpitations and anemia, and also against illnesses of the respiratory and digestive systems (Alkire et al., 1994; Fournet et al., 1996; Schmidt-Lebuhn, 2008). Because of the extensive use as a medicine by local people in our country, it was codified in the Farmacopea Nacional Argentina (1978) as *M. mollis* (Kunth) Griseb. in agreement with Epling and Játiva (1963). The quality of the essential oil has been standardised as described in the Argentinean IRAM norm (IRAM 18606, 2003).

Though it was originally classified as *Minthostachys verticillata* (Griseb.) Epling (Epling, 1939), nowadays it is defined in the Flora of Argentina as *M. mollis* Griseb. (Zuloaga et al., 2008).

In Argentina, it is mainly known as "peperina" and is widely found in the provinces of Córdoba, San Luis, Tucumán and Catamarca. It is found in diverse environments, from the northwestern humid mountain forests, to moist and shady sites in the semiarid mountain woodlands of the Chaco Serrano in the Central region where it was found in greater

Table 2
Percentage composition of the main compounds of the essential oils obtained from collections in the central region of Argentina.

Population	Collection date	Limonene	Linalool	Menthone	Isomenthone	Pulegone	Piperitone	Piperitenone	Spathulenol
Province of Cordoba									
1	19/03/07	1.4	0	28.5	1.0	61.6	0.6	1.8	0.3
1	12/03/08	2.3	0.3	41.3	1.6	46.1	0.9	1.1	0.2
1	30/03/09	1.7	0.2	42.6	1.5	45.6	1.0	0.9	0.7
2	20/03/07	1.2	0.2	42.2	1.2	43.8	0.6	0.6	0.3
3	20/03/07	1.3	0.4	52.5	1.7	38.4	1.1	0.4	0.0
3	28/11/07	1.3	0.3	18.4	0.8	60.5	0.5	1.5	1.0
4	20/03/07	1.3	0.3	46.4	1.5	45.2	0.8	0.6	0.0
4	13/03/08	1.0	0.2	37.3	1.3	52.4	1.2	1.2	0.3
4	29/03/09	1.1	0.1	58.2	2.1	30.8	1.2	0.4	0.0
5	20/03/07	0.9	0.3	41.4	1.2	49.1	1.3	1.3	0.2
5	29/11/07	2.8	0.2	9.6	0.4	78.8	0.4	2.0	0.4
5	29/03/09	1.1	0.2	51.1	1.9	38.6	1.0	0.5	0.0
6	29/11/07	2.4	0.4	12.2	0.4	77.2	0.3	0.9	0.0
6	13/03/08	1.3	0.5	34.7	0.5	55.1	0.8	0.8	0.1
6	29/03/09	1.1	0.3	35.9	1.2	51.9	0.9	0.9	0.9
7	13/03/08	1.0	0.2	32.4	1.2	58.6	0.8	0.9	0.1
7	29/03/09	0.9	0.1	51.5	2.2	36.3	1.3	0.6	0.0
8	1/04/09	0.9	0	52.5	1.7	37.0	0.9	0.5	0.7
9	1/04/09	1.4	0.1	48.5	1.9	41.2	0.9	0.3	0.6
Province of San Luis									
10	18/12/06	2.4	0.2	23.8	0.8	65.3	0.4	1.0	0.4
10	25/03/07	1.8	0.1	38.8	1.1	51.6	0.7	0.9	0.6
10	17/03/08	1.5	0.3	30.0	0.9	59.5	0.8	1.3	0.3
11	18/12/06	2.5	0	16.3	0.6	73.6	0.4	1.2	0.6
11	25/03/07	1.2	0.2	28.6	1.0	61.8	0.7	1.2	0.2
11	24/11/07	2.4	0	8.2	0.3	81.2	0.4	1.3	0
11	17/03/08	1.4	0.2	36.1	1.2	53.2	0.7	0.9	0
11	25/11/08	0	0.	20.1	0.8	49.5	0.6	1.0	1.2
11	27/03/09	1.2	0	30.8	1.0	58.1	0.9	1.1	0.5
12	27/03/09	2.2	0.3	48.2	1.7	39.7	1.0	0.5	1.3
13	28/03/09	1.3	0.3	34.9	1.0	53.7	0.8	0.8	1.9
14	18/12/06	1.9	0.3	13.2	0.5	76.5	0.4	1.2	0.6
14	22/11/07	2.9	0.3	5.6	0.2	83.3	0	1.8	0.1
14	18/03/08	0.7	0.4	36.3	1.3	52.2	1.1	1.0	0.2
14	28/03/09	0.8	0.3	37.7	1.2	47.7	1.4	1.3	0.7
15	28/03/09	1.0	0.2	42.8	1.4	46.3	0.9	0.5	1.0

abundance. It is intensively exploited for the industries of herbal teas, bitter non-alcoholic beverages and phytotherapy (Bandoni et al., 2002; Elechosa et al., 2007), being also locally used by the people as an additive in the infusion named "mate".

Anatomical and morphological observations made on living and herbarium materials showed no significant differences among natural populations; some minor differences observed are attributable mostly to environmental conditions and phenological stages (Elechosa et al., 2007). Variability in yields and composition of the essential oils of peperina from Argentina has been reported (Bandoni et al., 2002; Elechosa, 2009; Elechosa et al., 2007; Mora et al., 2009; Zygadlo et al., 1996) as well as from other Andean countries (Alkire et al., 1994; Rojas and Usubillaga, 1995; Olivero-Verbel et al., 2010). Studies based on seasonal variations of the essential oil content and composition, established full bloom as the best harvesting time (Bandoni et al., 2002). The main objective of this study was to characterize the variability in yields and chemical composition of *M. mollis* essential oils, by means of a comparative study during four years sampling on wild plant populations in the provinces of Córdoba, San Luis, Tucumán, Salta and Catamarca.

2. Materials and methods

2.1. Field sampling

Samples of aerial parts from 30 to 50 plants per population were collected in different bloom stages during late spring (November–December) and beginning of autumn (March–April) from 2006 to 2009. Sampling was performed in different seasons of each year to test possible differences in the yields and compositions of essential oils. In addition, plants having different aromas detected *in situ* were sampled and labeled separately.

Geographical coordinates were measured for each population using a *Garmin Etrex Legend HCX*. Herbarium vouchers were taken from every site and stored in the "Instituto de Recursos Biológicos", INTA, Castelar (BAB) under collector number, as usual in *Florae* citation (Table 1). The species were validated using the description proposed by Epling and Játiva (1963).

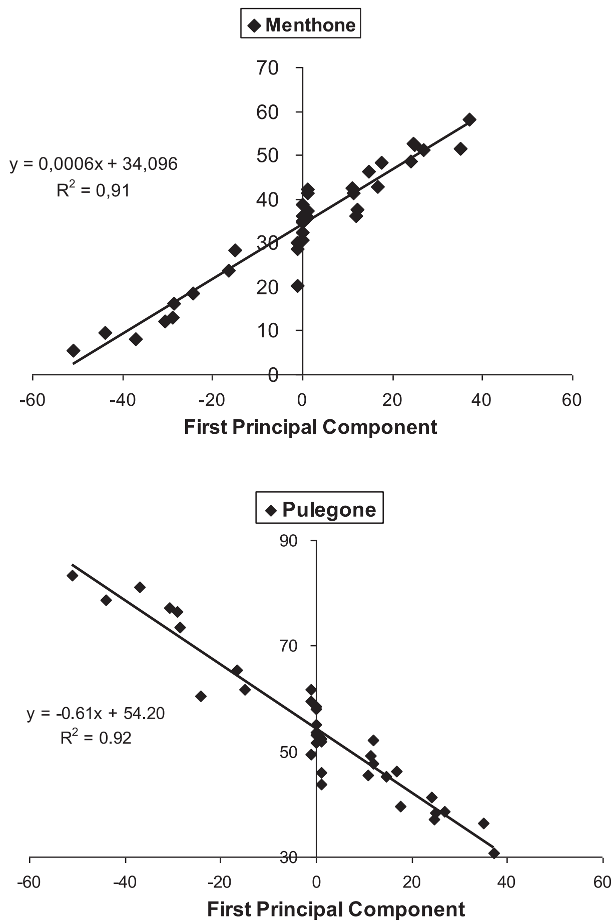

Fig. 1. Menthone and pulegone content variation according to first principal component, which is related to collecting season: from spring in the left to autumn in the right.

Seventy five samples out of 40 natural populations were studied. Each region is represented as follows:

1. Central Region: Province of Córdoba, 19 samples from 9 populations; Province of San Luis 16 samples from 6 populations.
2. Northwestern Region: Province of Tucumán, 36 samples from 21 populations; Province of Catamarca, 3 samples from 3 populations, and one sample from a single population in the Province of Salta (Table 1).

2.2. Essential oils extraction

The essential oils were isolated from natural air-dried material (12–15% final moisture), by hydrodistillation for 2 h using a *Clevenger*-type trap (IRAM 18729, 1996); remaining moisture was removed with anhydrous sodium sulfate and oils were stored at 2C° prior to analysis. The yields were expressed as V/W ratios.

2.3. Essential oils analysis

A GC-FID-MS system *Perkin Elmer Clarus* 500 was used with an autosampler injector (split ratio: 1:100) connected by a flow splitter to two capillary columns (*J&W*): a) polyethylene glycol MW *ca.* 20,000 and b) 5% phenyl-95% methyl silicone, both 60 m × 0.25 mm with 0.25 µm film thickness. The polar column was connected to a FID, while the non-polar column was connected to a FID and a quadrupolar mass detector (70 eV), by a vent system (*MSVent*™). Helium was used as mobile phase at a constant flow of 1.87 ml/min.

The oven temperature was programmed according to the following gradient: 90°C–240 °C at 3 °C/min, and then isothermal for 15 min. The injector and both FIDs were set at 255 °C and 275 °C, respectively. The injection volume was 0.5 µl

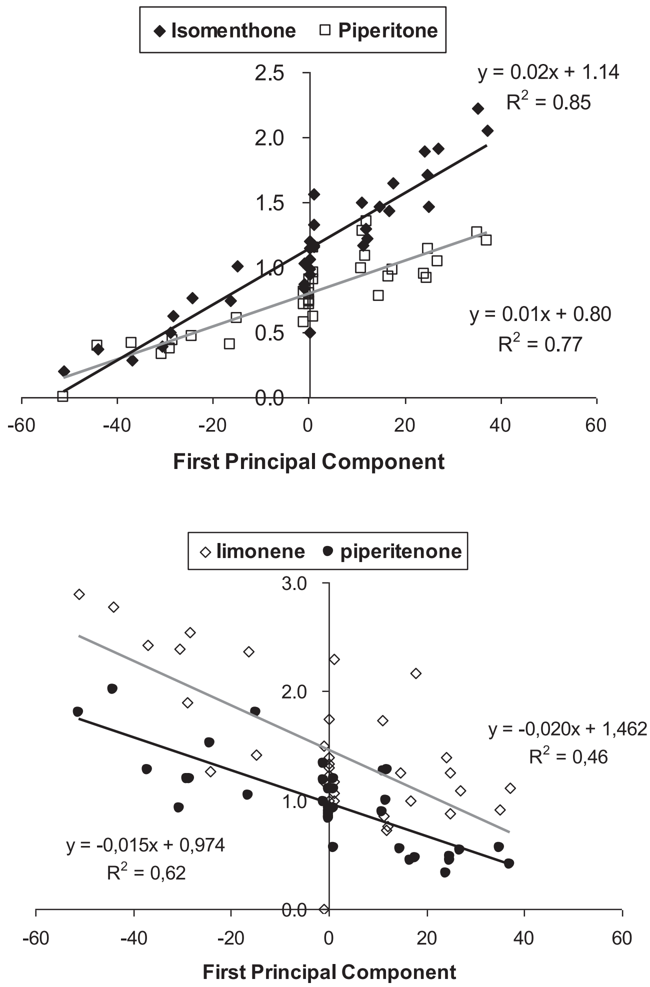

Fig. 2. Minor components variation according to first principal component, which is related to collecting season: from spring in the left to autumn in the right.

Table 3
Percentage composition of the main compounds of chemotypes detected in Northwestern Argentina.

Chemotype	Population N°	Collection date	Limonene	*cis*-β-Ocimene	γ-Terpinene	Linalool	*cis*-Dihhydrocarvone	Coahuilensol methyl ether[a]
1	25	30/04/09	0.4	0.2	0	0.6	57.2	0
	24	18/04/08	0	0	0	0.7	38.4	0
	24	30/04/09	0.2	0.2	0	0.6	49.9	0
	24	30/11/06	0.9	0	0	0.8	45.4	0
2	23	20/11/06	5.4	2.0	0.4	1.0	0	0.8
	16	19/11/06	6.8	1.3	0.1	0.9	0	0.4
3	22	08/04/08	0.4	0.2	4.5	0.2	0	0
	22	20/11/06	1.5	0.9	10.1	0.3	0	0.3
	17	08/04/08	0.3	0.2	1.1	0.4	0	0
	20a	08/04/08	1.5	0.5	0.9	0.5	0	0.3
	20	08/04/08	0.3	0.1	2.2	0.1	0.5	0
	19	08/04/08	0.4	0.3	1.5	0.3	0	0
	17	20/11/06	0.9	0.4	2.7	0.2	0	0
	18	20/11/06	1.4	0.3	6.2	0.3	0	0
	20	20/11/06	0.4	0.2	4.6	0.1	0	0
	32	01/12/06	3.7	0	3.3	13.4	0	0
4	40	10/04/08	43.3	0.42	0	6.0	0	0
	21	08/04/08	40.1	5.2	0	2.4	0	2.1
	21	20/11/06	35.7	5.0	0	1.3	0	15.0
5	28	09/04/08	0.2	0.6	2.3	56.1	0.1	0
	30	09/04/08	0.1	0.4	0	78.0	0	0
	29	24/04/09	0.1	0.3	0.2	64.9	0.1	0
	29a	24/04/09	0.1	0.7	0.3	71.6	1.0	0
	31	24/04/09	0.2	0.2	0	84.2	0.3	0
	28	01/12/06	0.2	0.4	0.2	71.7	1.2	0
	30	01/12/06	0	0.2	0	68.5	0	0
	26	09/04/08	0.3	0.2	0.1	60.2	0.5	0
	26	24/04/09	0.2	0.6	0.8	66.8	0.5	0
	27	24/04/09	0	0.8	0	76.7	0.4	0
	33	09/04/08	0	0.1	0	68.1	0.2	0
	33	24/04/09	0	0.1	0	66.8	1.0	0

[a] Tentatively identified by RI in the non polar column and mass spectrum (Adams, 2007).

of a 10% solution of oil in hexane. The temperature of the transference line and the ion source were 180 °C and 150 °C, respectively; the range of masses was 40–300 Da, 10 scan/sec.

Identification of the compounds was performed by comparison of the linear retention indices (relative to C8-C24 n-alkanes) obtained in both columns, with those of reference compounds or chemically well-known essential oils. Additionally, each mass spectra obtained was compared with those from usual libraries (Adams, 2007; Wiley/NIST, 2008) and from an own laboratory-developed mass spectra library built up from standards or components of known oils.

The relative percentage composition was achieved using the single area percentage method (FID), without considering corrections for response factors. The lowest response obtained from each column for each component was considered.

2.4. Statistical analysis

Multivariate analysis was performed using PC-ORD (vers. 5.0) (McCune and Mefford, 1999). Cluster analysis was applied using Euclidean distance and Ward's method as the agglomeration technique (Everitt, 2005; Quinn and Keough, 2002) for the 31 samples from the Northwestern region.

3. Results and discussion

Seventy five samples of *M. mollis* were collected in 40 natural populations in Central and Northwestern Argentina during four years sampling and analyzed for essential oils content and composition. The yields were very variable (0.25–4.93%) standing out those obtained from samples collected in populations of San Luis (2.63–4.93%) and Córdoba provinces (2.59–4.20%) while those from the Northwestern region (Tucumán, Catamarca and Salta) were significantly lower (0.25–1.63%), being most of them lower than 1%. In previous studies, full blooming was proposed as the best harvesting time for the typical menthone-pulegone chemotype present in Córdoba and San Luis provinces (Bandoni et al., 2002). However, in this study some non significant differences in yields were detected on regard to the blooming stage from replicated collections from different provinces, seasons and years, with the exception of the outcomes of Pasos Malos, San Luis Province (Table 1).

The essential oils obtained by hydrodistillation were analyzed by GC-FID-MS and results of the chemical compositions were analyzed using multivariate statistical methods.

Carvone	Pulegone	Piperitone	Carvacrol	Carvacryl acetate	β-Caryophyllene	Germacrene D	Bicyclogermacrene	Spathulenol
28.0	0	1.4	0	0	1.0	0	0.2	0
46.5	0.3	0	0	0	1.6	0.2	1.2	0.1
34.8	0	0.7	0	0.3	1.4	0	0.4	0
36.2	0.1	0.8	0.2	0	3.7	0.5	1.9	0.2
0	57.1	0	2.3	4.6	2.8	1.1	1.3	0.6
0	76.3	0	1.3	2.1	0.8	0.3	0.1	0.7
0.1	0	0	29.6	48.1	1.8	1.1	1.3	0.4
0	0.4	0	19.2	39.9	4.5	1.8	2.9	0.2
0.1	2.4	0	32.8	43.9	0.2	1.1	1.1	1
0	25.3	0	23.9	24.1	1.0	0.4	0	0.9
0.1	0.1	0	31.7	50.9	1.2	1.0	1.2	0.1
0	0	0	32.7	44.3	0.5	0	0	1.2
0	3.6	0	23.6	44.7	3.1	0.9	0.9	0
0	2.9	0	21.8	44.0	1.3	0.8	0.7	0.3
0	0.7	0	19.9	50.3	1.5	0.9	0.8	0.2
0	0	7.1	15.3	31.4	4.3	0.9	4.7	0
0.4	0	0	0.3	0	2.6	0.3	0	10.7
0.3	0.6	0.1	0.1	0	9.3	6.8	10.5	1.5
0	1	0	0.6	0.9	5.8	2.9	4.2	0.4
0.2	0	0	0.8	0.8	1.4	2.0	2.9	0.5
0	0	0.1	0	0	2.4	1.7	3.1	0.5
0	0	0	0	0	1.8	2.3	3.3	0.4
0.7	0	0	0	0	1.3	2.9	3.5	0.5
1.1	0	0	0	0	2.8	2.3	3.5	0.2
2	0.2	0	0.5	0.2	2.9	2.6	3.5	0.4
0	0.4	0	0	0	6.1	3.0	5.2	0.5
2.2	0	0.5	0.3	0.2	1.7	1.4	2.0	0.8
2.2	0	0.1	0.5	0.6	2.0	2.2	3.8	0.6
0	0	0.2	0	0	1.5	2.1	2.9	0.3
0.9	0.1	0	0.3	0	2.4	5.0	8.8	1.5
1.8	0	0.5	0	0	2.1	2.4	3.7	1.1

For a better understanding, the GC-FID-MS composition data obtained from 75 samples is reported separately in two regions as follows:

3.1. Central region: Córdoba and San Luis Provinces

Nine populations from Córdoba and 6 from San Luis (35 samples) were evaluated. The essential oils compositions were characterized by the contents of menthone and pulegone as is defined for the peperina Argentinean type (IRAM 18606, 2003), being limonene, isomenthone, piperitone and piperitenone minor compounds (Table 2). The sum menthone + pulegone remained about constant, ranging between 78.9 and 91.6%, thus confirming this chemotype as the most abundant in this geographical area in accordance with the results reported by Zygadlo et al. (1996).

Principal Component Analysis (PCA) was made on the chemical data set of the 35 samples collected in this region, using the presence/absence of 12 main compounds with contents above 2% as exclusion criteria and combining this data with the sampling sites and the date of collection. The first principal component explained 39.2% of the variability in the composition

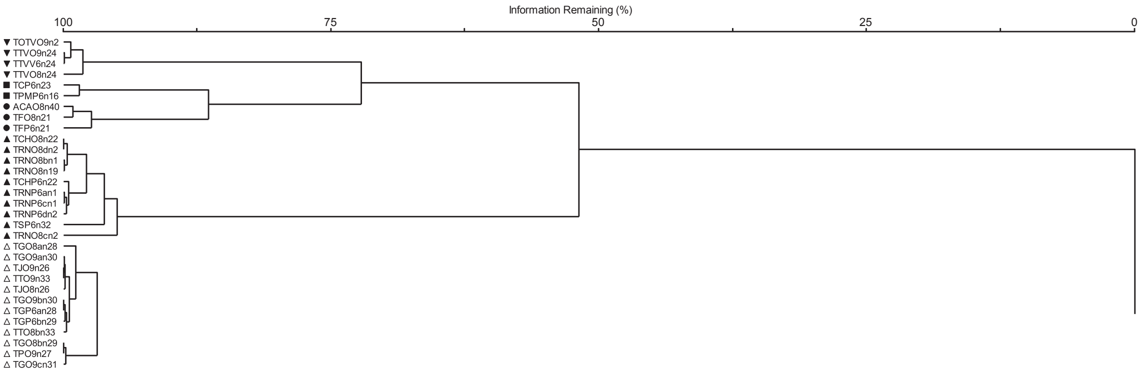

Fig. 3. Dendrogram showing the samples of Table 3 grouped in five clusters.

Table 4
Percentage composition of the main compounds of essential oils obtained from other collections from Tucumán and Catamarca provinces not yet assessed as chemotypes.

Population N°	Collection date	Limonene	Linalool	Menthen-3-ol	Menthone	Isomenthone	Neomenthol	Piperitenone	Isomenthol	Dihydro-carvone	Coahuilensol methyl ether[a]
17a	08/04/08	1.0	1.5	2.0	0.2	8.0	0	5.2	1.0	0	0
18a	20/11/06	4.3	1.5	13.6	0.1	10.2	t	0.3	2.8	0	0
34	19/04/08	0.8	0.8	2.5	14.7	12.6	3.5	0.2	4.6	0.5	0
35	19/04/08	1.1	1.3	4.0	9.4	25.6	2.8	0.1	11.6	0	0
36	19/04/08	2.8	1.3	8.3	8.3	8.3	2.8	8.3	0	0	0
33a	09/04/08	0	1.0	0	0	0	0	0	0	0.4	0
37	19/04/08	10.1	2.8	0	0	0	0	1.1	0	0	27.1
38	19/04/08	2.9	1.3	0	4.1	0.5	0.8	5.8	0	0	0
39	02/05/09	9.8	0.5	0	0	0	0	72.3	0	0	0

[a] Tentatively identified by RI in non polar column and mass spectrum (Adams, 2007).
[b] RI in non polar column: 1563; RI in polar column: 2135; MS (EI, 70 eV): m/z (%) = 152 [M + H$^+$] (3), 135(11), 134(89), 119(57), 109(11), 105(16), 95(10), 94(10), 93(32), 92(16), 81(12), 79(13), 69(9), 68(19), 67(12), 53(12), 43(100), 41(15).
[c] RI in non polar column: 1589; RI in polar column: 2079; MS (EI, 70 eV): m/z (%) = 205 [M + H$^+$] (5), 204(30), 189(9), 162(16), 161(100), 134(13), 133(19), 121(11), 119(40), 107(12), 105(62), 93(29), 91(41), 81(62), 79(27), 77(21), 55(20), 53(12), 43(27), 41(31).

and showed a clear association ($R^2 = 0.66$, p value < 0.0001) with the season of collection of the samples. Other components explained less than 20% each. The proportion of menthone and pulegone showed the strongest relationship with the first principal component (Fig. 1).

Other minor compounds showed a similar pattern: isomenthone and piperitone contents increased with blooming progress while limonene and piperitenone decreased through the same period (Fig. 2). These results are in agreement with the known biosynthetic pathways of these compounds. The dispersion of data for limonene may be explained because it is an in common precursor of the biosynthesis pathways of several of the main compounds.

In these collections, no significant differences have been detected in the *in situ* aroma, apart from the characteristic or typical menthone-pulegone scent, but in November a bitter initial note began to predominate, due to the high rate of pulegone.

3.2. Northwestern region: Tucumán, Salta and Catamarca

The PCA made on the chemical data set and collecting sites (figures not shown) of 31 samples from Tucumán and Salta provinces (Table 3), using the same exclusion criteria, showed 5 possible chemotypes as suggested previously (Elechosa et al., 2007) and confirmed with latter collections:

– Chemotype 1, populations 24 and 25: Tafí del Valle, predominantly with dihydrocarvone (38.4–57.2%) and carvone (28.0–46.5%);
– Chemotype 2, populations 16 and 23: El Cajón and Padre Monti, with high content of pulegone (57.1–76.3%), being menthone absent;
– Chemotype 3, populations 17, 18, 19, 20, 22, 32: Río Nío, Chorrillos and Siambón, with carvacryl acetate (24.1–50.9%) and carvacrol (19.2–32.8%) as the main compounds;
– Chemotype 4, populations 21 and 40: La Florida and La Candelaria (Salta), with high content of limonene (35.7–43.3%);
– Chemotype 5, populations 26, 27, 28, 29, 30, 31, 33: Gonzalo region from Las Juntas to Las Tacanas, with high content of linalool (56.1–84.2%).

In addition, cluster analysis was applied using Euclidean distance to measure the dissimilarity in the relative composition of 24 compounds in the 31 samples, using Ward's method as the agglomeration technique (Everitt, 2005; Quinn and Keough, 2002) (Fig. 3). Five groups were retained with less than 10% of information loss (McCune and Mefford, 1999).

Owing to the lack of replications, 9 samples (6 from Tucumán and 3 from Catamarca) were not submitted to statistical analysis (Table 4). Populations 17a, 18a and 33a presented different patterns to those typical for the site, as indicated in Table 3. The same was also valid for all the populations collected in Catamarca. Therefore, these preliminary results on the chemical composition of these collections allowed inferring the possibility of other chemotypes which have to be confirmed with future collections.

The typical aroma of this species was not detected *in situ* in these collections. In contrast, an important diversity of aromas was found, for instance "spearmint" scent (carvone) in Tafí del Valle, several "resembling savory" (carvacrol) populations in Rio Nío zone, "lemon" scent (limonene) in La Florida and several "coriander smelling" (linalool) populations between Gonzalo and Las Tacanas.

Essential oils of this species have been extensively studied in the Andean countries, and different compositions were found, some of them were also detected in this study.

Banchio et al. (2007) found quantitative differences in plants of peperina with the typical menthone – pulegone chemotype, as a response to herbivory or mechanical damages. This fact could be the explanation of the variability among the

Carvone	Pulegone	Piperi-tone	Menthyl acetate	Thymol	Carvacrol	Carvacryl acetate	β-Caryophyllene	Germacrene D	Bicyclogermacrene	Unknown [b]	Unknown [c]	Spathulenol
6.5	43.4	0	2.6	0.6	6.2	7.2	0.5	0	0.2	0	0	2.3
0	45.8	0.1	12.6	0	t	t	1.6	0.5	0.6	0	0	0.6
0.5	29.8	0.3	5.6	0	0	0	0	0	0	0	0	2.1
0	21.6	0.6	9.5	0	0	0	0.9	0	0	0	0	0.6
0.4	2.8	31.2	2.8	0	0	0	0.6	0	0	0	0	1.2
2.8	0	0	0	0.4	0	0	11.8	12.7	27.4	13.3	8.6	4.8
0	0	19.7	0	3.3	0.2	0	2.5	4.5	5.1	0	0	5.2
0	0	65.2	0.3	1.2	0	0	1.5	1.1	1.9	0	0	0
0.1	0	2.6	0	0	0	0	1.0	0.5	1.0	0	0	0

individuals of a same chemotype. However, all the specimens analyzed in this study were selected taking in account a healthy appearance, and no attacks by insects or mechanical injuries were visually checked.

In summary, the essential oil yields and chemical compositions of 75 samples collected during 2006–2009 in 40 populations in the Central and Northwestern Argentina were variable. This is the first report of a deep study of natural populations of peperina throughout the whole distribution area in Argentina with consecutive sampling repetitions of the same populations during several years. The menthone – pulegone chemotype, characterized by the presence of these compounds as well as their sum and ratio, seemed to be the only present in Central Argentina, but also the most widespread in South America. On the contrary, none of the populations from Tucumán belonged to this chemotype, and at to the extent and depth of this study, this allows inferring it is not present northwards Córdoba province.

It is worth mentioning that the compositions of the different chemotypes remained constant throughout the four years of collection. The wide variety of potential chemotypes recognized in this study supports the necessity for a careful selection of the best materials for introduction in future crops. The agreement between the different scents detected *in situ* and the CG-FID-MS compositions demonstrated the usefulness of a trained nose in the field collection team.

It is suggestive that our group has repeatedly found in Northwestern Argentina a singular phytochemical variability in three aromatic species: *M. mollis*, *Aloysia citriodora* and *Tagetes minuta*. This seems not to be random, but probably might be due to two features: a) contrary to other geographical regions in Argentina, the Central region presents a high phytogeographic and climate homogeneity, meanwhile Northwestern Argentine covers a remarkable ecological variability and this could influence the genome of the species that grow in this region. It is known that aromatic plants possess a high heritability, so they have more dependency on endogenous factors (genetic) than on the exogenous ones. The possible interference of ecological factors on the flora, which would result in the presence of ecotypes, was dropped with this study because the biodiversity of each chemotype remained constant in successive years (*Minthostachys* case). In other cases, we got independent of exogenous variables (soil, climate) through the cultivation of materials under the same conditions (as in *Aloysia* and *Tagetes*) (Gil et al., 2000, 2007). It is important to point out that the variations found in these studies are expressed in the chemical composition and not in the phenotype of each species; b) the second probable cause arises from the record that Northwestern Argentina is part of the last foothills of the Amazonian forest, the so-called Tucumanian-Bolivian jungle (Cabrera and Willink, 1973), second global source of floristic biodiversity. For this reason, it is likely that Northwestern Argentina is a center of genetic diversification of different species, if not the center of origin (Di Leo Lira et al., 2013).

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