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Species delimitation in the *Aloysia gratissima* complex (Verbenaceae) following the phylogenetic species concept

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The *Aloysia gratissima* complex is a group of 22 accepted, specific and infraspecific taxa, the boundaries of which are controversial. Various different authors have chosen different characters as criteria for delimiting taxa in this complex, which has been reflected in the variable number of accepted species. To resolve the taxonomy of this group, a modified population aggregation analysis, based on the phylogenetic species concept, was employed. As a result, the *Aloysia gratissima* complex is here circumscribed as compromising only four species, *A. decipiens*, *A. gratissima s.s.*, *A. oblanceolata* and *A. schulziana*. These species are sustained by a different combination of four characters, of the 16 qualitative attributes analysed. A new approach based on cluster analysis is proposed here delimiting varieties. As a consequence two varieties, supported by significant differences in quantitative characters, are recognized here: *A. gratissima* var. *gratissima* and *A. gratissima* var. *sellowii*. A complete taxonomic revision of the accepted taxa is presented and six new synonyms are proposed. A key, illustrations and geographical distribution maps are included. © 2015 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2016, **180**, 193–212.

ADDITIONAL KEYWORDS: population aggregation analysis – taxonomy.

INTRODUCTION

Aloysia Palau is the third largest genus of tribe Lantaneae (Verbenaceae), following *Lippia* L. and *Lantana* L. *Aloysia* is distinguished from the other two by its long racemose inflorescences versus head-like inflorescences with a brief rachis in *Lantana* and *Lippia*. Fruits are dry in *Aloysia* (except *A. ovatifolia* Moldenke) and *Lippia* and fleshy in *Lantana*.

Recent phylogenetic studies of Lantaneae (Marx *et al.*, 2010; Lu-Irving & Olmstead, 2013) have indicated that *Aloysia*, as traditionally circumscribed, is polyphyletic. As a consequence, Lu-Irving *et al.* (2014) proposed the inclusion of most species of *Acantholippia* Griseb. [except *A. seriphioides* (A.Gray) Moldenke] and *Xeroaloysia* Tronc. in *Aloysia* and the exclusion of several North American *Aloysia* taxa to make the genus monophyletic. In this current circumscription, *Aloysia* includes *c*. 30 mostly South American species, with only two species, *A. wrightii* (A.Gray) Heller and *A. macrostachya* (Torr.) Moldenke, being endemic to North America (O'Leary *et al.*, 2016). Lu-Irving *et al.* (2014) suggested at least two dispersion events from South America to North America in tribe Lantaneae.

Sanders (2001: 310) suggested that *Aloysia* is supported by two synapomorphies: deciduous floral bracts and involute calyx lobes. However, Lu-Irving *et al.* (2014) mentioned the presence of a four-lobed calyx as the character that defines the new circumscription of *Aloysia*, in agreement with previous studies (O'Leary *et al.*, 2012).

ALOYSIA GRATISSIMA COMPLEX

The *A. gratissima* (Gillies & Hook. ex Hook.) Tronc. complex is a group of specific and infraspecific taxa, the limits of which are controversial. Various authors (Troncoso, 1964; Botta, 1979; Siedo, 2006; O'Leary

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Table 1. Taxa belonging to the *Aloysia gratissima* complex

1.	*Verbena gratissima* Gillies & Hook. ex Hook., Bot. misc. 1: 160. 1829. *Aloysia gratissima* (Gillies & Hook. ex Hook.) Tronc., Darwiniana 12. 527. 1962.
2.	*Aloysia lycioides* Cham., Linnaea 7: 237. 1832. *Lippia lycioides* (Cham.) Steud., Nomencl. Bot. 2: 62. 1841.
3.	*Aloysia floribunda* M.Martens & Galeotti, Bull. Acad. Brux. xi. II: 320. 1844.
4.	*Lippia sellowii* Briq., Annuaire Conserv. Jard. Bot. Genève 4: 21. 1900. *Aloysia sellowii* (Briq.) Moldenke, Revista Sudamer. Bot. 4: 15. 1937. *Aloysia gratissima* var. *sellowii* (Briq.) Botta, Darwiniana 22: 85. 1979.
5.	*Lippia pulchra* Briq., Ark. Bot. 2(10): 18. 1904. *Aloysia pulchra* (Briq.) Moldenke, Phytologia 1: 95. 1934.
6.	*Lippia ligustrina* (Lag.) Britton var. *lasiodonta* Briq., Annuaire Conserv. Jard. Bot. Genéve 7–8: 305.1904.
7.	*Lippia ligustrina* (Lag.) Britton var. *paraguariensis* Briq., Annuaire Conserv. Jard. Bot. Genève 7–8: 305. 1904. *Aloysia ligustrina* var. *paraguariensis* (Briq.) Moldenke, Phytologia 1: 167. 1935. *Aloysia lycioides* var. *paraguariensis* (Briq.) Moldenke, Phytologia 2: 464. 1948. *Aloysia gratissima* var. *paraguariensis* (Briq.) Moldenke, Phytologia 9: 500. 1964.
8.	*Lippia ligustrina* var. *schulziae* Standl., Field Museum Pub. Bot. 4: 256. 1929. *Aloysia lycioides* var. *schulziae* (Stand.) Moldenke, Phytologia 1: 95. 1934. *Aloysia gratissima* var. *schulziae* (Stand.) Moldenke, Phytologia 9: 500. 1964.
9.	*Aloysia schulziana* Moldenke, Lilloa 5: 381. 1940. *Aloysia gratissima* var. *schulziana* (Moldenke) Botta, Darwiniana 22: 87. 1979.
10.	*Aloysia meyeri* Moldenke, Lilloa 5: 378. 1940.
11.	*Aloysia looseri* Moldenke, Lilloa 5: 377. 1940. *Lippia looseri* (Moldenke) Looser, Revista Univ. (Santiago) 26, no. 2: 141. 1941.
12.	*Aloysia chacoensis* Moldenke, Lilloa 5: 373. 1940. *Aloysia gratissima* var. *chacoensis* (Moldenke) Botta, Darwiniana 22: 89. 1979.
13.	*Aloysia casadensis* Hassler ex Moldenke, Phytologia 3: 107. 1949.
14.	*Aloysia lycioides* var. *revoluta* Moldenke, Phytologia 3: 108. 1949. *Aloysia gratissima* var. *revoluta* (Moldenke) Moldenke, Phytologia 9: 500. 1964.
15.	*Aloysia oblanceolata* Moldenke, Phytologia 3: 108. 1949.
16.	*Aloysia chacoensis* var. *angustifolia* Tronc., Darwiniana 13: 630. 1964. *Aloysia gratissima* var. *angustifolia* (Tronc.) Botta, Darwiniana 22: 89. 1979.
17.	*Aloysia gratissima* var. *oblanceolata* Moldenke, Phytologia 15: 462. 1968.
18.	*Aloysia gratissima* fo. *macrophylla* Moldenke, Phytologia 29: 75. 1974.
19.	*Aloysia beckii* Moldenke, Phytologia 52(1): 18. 1982.
20.	*Aloysia lomaplatae** Ravenna, Onira 10(19): 60. 2006.
21.	*Aloysia famatinensis* Ravenna, Onira 11(4): 15. 2007.
22.	*Aloysia decipiens* Ravenna, Onira 11(4): 14. 2007.

Name with an asterik (*) could not be measured for all attributes.

et al., 2016) have assigned different significance to individual characters in delimiting taxa in the complex, resulting in a variable number of species and varieties recognized over time (Table 1). In a taxonomic revision of *Aloysia* for Argentina, Botta (1979) recognized five varieties of *A. gratissima*, based on differences in corolla pubescence, inflorescence density, calyx teeth shape and foliar traits. Botta (1979) considered *A. lycioides* Cham. to be a synonym of *A. gratissima* and accepted *A. pulchra* (Briq.) Moldenke as a distinct taxon, differing from *A. gratissima* in its leaf morphology and the calyx teeth shape. Siedo (2006), in a complete systematic study of *Aloysia*, recognized four varieties of *A. gratissima* based on differences in corolla pubescence, the relationship between corolla and calyx length, and leaf morphology. Siedo (2006) mentioned the presence of intermediate forms between *A. gratissima* var. *gratissima* and *A. gratissima* var. *angustifolia* (Tronc.) Botta, in the Argentinean provinces of Santiago del Estero and Córdoba. Furthermore, Siedo mentioned hybrid taxa in North America in the contact area between the distributions of *A. gratissima* var. *gratissima* and *A. gratissima* var. *schulziae* (Standl.) Moldenke. Siedo (2006) recognized *A. lycioides* Cham. as distinct from *A. gratissima*, and accepted two varieties: *A. lycioides* var. *schulziana* (Moldenke) Siedo (i.e. considering *A. schulziana* Moldenke as a variety of *A. lycioides*) and var. *lycioides*, including in its synonymy *A. pulchra*, which was accepted as a taxon by Botta (1979). Siedo (2006) differentiated *A. gratissima* and *A. lycioides* based on leaf morphology. Moldenke (1949: 108) described *A. oblanceolata* Moldenke and, many years later (Moldenke, 1968: 462), he transferred this taxon to *A. gratissima* as *A. gratissima* var. *oblanceolata* (Moldenke) Modenke. Siedo (2006) accepted *A. oblanceolata* and treated *Aloysia gratissima* var. *oblanceolata* as a synonym of *A. lycioides*. Recently, O'Leary *et al.* (2016) recognized four varieties of *A. gratissima*, based on differences in leaf size and morphology and density and length of inflorescences. O'Leary *et al.* (2016) considered *A. lycioides* as a synonym of *A. gratissima* and accepted *A. pulchra*, differentiating it from *A. gratissima* by its woodier habit and different habitat. These authors considered *A. gratissima* var. *sellowii* (Briq.) Botta as a synonym of *A. pulchra*, whereas Botta (1979) accepted it as a variety of *A. gratissima* and Siedo (2006) treated it as a synonym of *A. lycioides*. The authors who have worked on the group clearly considered different taxonomic criteria in trying to define taxa within the *Aloysia gratissima* complex (Table 2).

Phylogenetic species concept

The phylogenetic species concept (PSC) as defined by Nixon & Wheeler (1990: p. 217) is 'the smallest aggregation of population (sexual) or lineage (asexual) diagnosable by a unique combination of character states in comparable individuals'. Population aggregation analysis (PAA) is the method used for the identification of phylogenetic species (Davis & Nixon, 1992); it searches for fixed differences among local populations, followed by successive rounds of aggregation of populations (or previously aggregated population groups) that are not distinct from each other. The PSC–PAA approach distinguishes between characters and traits. A character is an attribute that is invariant in a terminal lineage and its constancy within populations provides evidence of fixation and lack of reticulation. A trait is an attribute that occurs in some but not all representatives of a terminal lineage, and it provides evidence of intersection and lack of hierarchy (Luckow, 1995). Phylogenetic species are distinguished by character states, not traits. Populations are the fundamental units of analysis in the PSC–PAA method and the method requires an a priori placement of specimens in a population. Henderson (2004, 2005a) included some modifications to allow PAA to be applied to herbaria material. Henderson proposed the scoring of specimens (instead of populations) for all attributes (traits and characters) and using successive cluster analyses to distinguish traits from characters at the same time that groups of specimens are delimited. Finally, groups of specimens with unique combinations of character states are found and considered as phylogenetic species. Luckow (1995: 000) discussed the extension of the PSC to the infraspecific level and stated that 'groups of populations that can be distinguished by differences in mean value would be recognized as subspecies or varieties'.

Table 2. The *Aloysia gratissima* complex according to the most complete taxonomic treatments

Botta (1979)
Taxonomic criterion: corolla pubescence, florescence density, shape of calyx teeth, and foliar traits.
Two species and five varieties recognized.
(*A. oblanceolata* Moldenke, 1949 not treated).
1a. *A. gratissima* (Gillies & Hook. ex Hook.) Tronc. 1962 var. *gratissima*
=*A. lycioides* Cham. 1832
1b. *A. gratissima* var. *angustifolia* (Tronc.) Botta 1979
1c. *A. gratissima* var. *chacoensis* (Moldenke) Botta 1979
1d. *A. gratissima* var. *schulziana* (Moldenke) Botta 1979
=*A. meyeri* Moldenke 1940
1e. *A. gratissima* var. *sellowii* (Briq.) Botta 1979
2. *A. pulchra* (Briq.) Moldenke 1934
Siedo (2006)
Taxonomic criterion: corolla pubescence, calyx/corolla relative length, and foliar traits.
Three species and six varieties recognized.
1a. *A. lycioides* Cham. var. *schulziana* (Moldenke) Siedo 2006, ined.
=*A. meyeri* Moldenke 1940
1b. *A. lycioides* Cham. var. *lycioides* 1832
=*L. sellowii* Briq. 1900
=*L. pulchra* Briq. 1904
=*L. ligustrina* (Lag.) Britton var. *paraguariensis* Briq.1904
=*L. ligustrina* var. *lasiodonta* Briq. 1904
=*A. gratissima* var. *oblanceolata* Moldenke 1968
2a. *A. gratissima* (Gillies & Hook. ex Hook.) Tronc. 1962 var. *gratissima*
=*A. floribunda* M.Martens & Galeotti 1844
=*A. gratissima fo. macrophylla* Moldenke 1974
=*A. beckii* Moldenke 1982
2b. *A. gratissima* var. *angustifolia* (Tronc.) Botta 1979
2c. *A. gratissima* var. *chacoensis* (Moldenke) Botta 1979
=*A. casadensis* Hassler ex Moldenke 1949
2d. *A. gratissima var. schulziae* (Standl.) Moldenke 1964.
3. *A. oblanceolata* Moldenke 1949
O'Leary *et al.* (2016)
Taxonomic criterion: density and length of florescences and foliar traits.
Three species and four varieties recognized.
1a. *A. gratissima* var. *angustifolia* (Tronc.) Botta 1979
=*A. decipiens* Ravenna 2007
1b. *A. gratissima* var. *chacoensis* (Moldenke) Botta 1979
=*A. casadensis* Hassler ex Moldenke 1949
1c. *A. gratissima* (Gillies & Hook. ex Hook.) Tronc. 1962 var. *gratissima*
=*A. lycioides* Cham. 1832
=*A. floribunda* M.Martens & Galeotti 1844
=*L. ligustrina* var. *lasiodonta* Briq. 1904
=*L. ligustrina* var. *paraguariensis* Briq. 1904
=*A. beckii* Moldenke 1982
=*A. famatinesis* Ravenna 2007
1d. *A. gratissima* var. *schulziana* (Moldenke) Botta 1979
=*A. meyeri* Moldenke 1940
=*A. looseri* Moldenke 1940
=*A. lomaplatae* Ravenna 2006
2. *A. oblanceolata* Moldenke 1949
=*A. gratissima* var. *oblanceolata* Moldenke 1968
3. *A. pulchra* (Briq.) Moldenke 1934
=*L. sellowii* Briq. 1900
=*A. lycioides* var. *revoluta* Moldenke 1949

The aim in this contribution is to delimit accepted species and varieties within the 22 recognized taxa belonging to the *A. gratissima* complex (Table 1), by means of a modified PAA. Morphological descriptions, geographical distribution, habitat, synonymy and other relevant notes are also included.

MATERIAL AND METHODS

Specimens

Herbaria F, MO, NY, SI and US, holding important collections of taxa of the *A. gratissima* complex, were consulted (herbaria acronyms follow Thiers, 2014). However, the statistical analysis was based on specimens from NY and SI. Morphological observations were conducted using a Leica ocular microscope. Type specimens from worldwide herbaria and digital images (http://plants.jstor.org/) were studied. Geographical and ecological data were obtained from specimen labels. Studied representative specimens are presented for each taxon, including one specimen from each main political division of each country.

Sampling

More than 500 specimens belonging to the 22 taxa (Table 1) of the *A. gratissima* complex were studied. To reflect all the morphological variation in the complex, specimens were obtained from all the geographical areas that have been reported in the literature for taxa, thus ensuring that all morphological variation in the complex is included. Morphologically similar specimens from the same geographical area were discarded. Thus, 262 of the 500 specimens were selected for the statistical analysis as many are from the same geographical area and lack morphological variation (Appendix 1). Only *A. lomaplatae* Ravenna was not measured for all attributes, as no original material or images were available; it was studied from the protologue descriptions.

Data matrix and analysis

Qualitative and quantitative attributes were explored from the entire plant (Table 3). Sixteen qualitative and five quantitative attributes were scored with no missing values for the 262 included specimens. All qualitative characters were binary coded, except character 19, which is multistate.

Quantitative attributes did not show gaps that could allow delimitation of discrete character states. Several attributes related to the flower (calyx length, calyx teeth length and corolla length) were measured and then removed as they all show low variation

Table 3. Attributes; quantitative attributes (*in italics*) reflect the median of three different measures taken from each specimen

Related to leaves
1. Phyllotaxis: one or two leaves per node (0), more than two leaves per node (verticillate) (1)
2. *Leaf blade length (mm)*
3. *Leaf blade width (mm)*
4. Leaf blade apex: acute (0), obtuse (1)
5. Leaf blade margin: entire or partially serrate–dentate (0), completely serrate–dentate (1)
6. Revolute leaf blade border: absent (0), present (1).
7. Leaf lateral venation: conspicuous (0), inconspicuous (1)
8. Type of leaf venation: craspedodromous (0), brochidodromous (1)
9. Abaxial leaf surface: strigose–puberulous (0), sclerous–puberulous (1)
10. Leaf blade obovate, mucronate apex and revolute border: absent (0), present (1).
11. Leaf blade elliptic, acute apex, serrate–dentate margin and conspicuous craspedodromous venation: absent (0), present (1).
12. Petiole: absent (0), present (1).
13. *Petiole length (mm)*
14. Leaf blade length/width ratio: ≥ 2 (0), ≤ 2 (1).

Related to inflorescence
15. Number of inflorescences per node: sometimes one (0), always two or more (1)
16. *Inflorescence length (cm)*
17. *Inflorescence peduncle length (cm)*

Related to flower
18. Floral bract form: elliptic–ovate (0), linear (1)
19. Floral bract pubescence: absent (0), hispid (1), strigose (2)
20. Calyx pubescence: hispid (0), strigose (1)
21. Corolla tube pubescence: glabrous to subpilose (0), densely pilose (1)

between specimens; they were not considered in the analyses. Bract length was analysed and then removed as it is difficult to score, mostly on type specimen images, as bracts are often ephemeral. Thus, qualitative variables were employed for species delimitation and quantitative variables for varietal identification.

Delimitation of specimen groups

Species delimitation: Henderson's (2004, 2005a) modifications to allow PAA to be used with herbarium material were followed. Cluster analysis (CA) was carried out based on similarity matrices obtained with the simple matching coefficient. A hierarchical clustering method was used and the average linkage criterion was applied. Groups were identified based on previous knowledge of the species and the recognition of type specimens conforming congruent morphological groups, which were thus identified as candidates in each round of CA.

All qualitative attributes were used in the first CA iteration. A profile from each cluster was made in each round; attributes were recognized as 'characters' or as 'traits', depending on whether they were constant or non-constant between the specimens. In each successive instance (iterative round) of CA those qualitative attributes that behaved like traits were removed, and the analysis was performed again until the groups were defined by unique combinations of character states (Henderson, 2005b). Each group of specimens with a unique combination of constant character states was considered a good species under PSC.

Varieties recognition: Within previously recognized species, the presence of specimen subgroups that could be considered varieties were studied, employing the quantitative attributes scored. Luckow's (1995) taxonomic concept for the recognition of infraspecific taxa, considering groups of specimens instead of populations, was followed here.

Previous studies (Henderson, 2004, 2005a,b; O'Leary *et al*., 2012) used principal components analysis (PCA) and discriminant analysis (DA) and varieties were recognized when subgroups with one or more significantly different character values could be distinguished. This method has the disadvantage that characters are assumed to be normal multivariate because procedures are relatively insensitive to deviations of normality. As a consequence, results had to be viewed as approximate. To solve this problem, a new approach is proposed here: CAs, as in species delimitation. This is an unbiased method that allows ordering and classifying of specimens in accordance with their phenetic similarity.

As three of the five quantitative attributes scored are related to leaves and two are related to inflorescence (Table 3, *italics*), it is likely that there would be association between these variables. This is a problem related to CA that can generate spurious results. Thus, the association between variables was studied by calculating the Pearson correlation coefficient between pair of attributes. For association between variables, PCA will have to be used to summarize information on a new independent synthetic variable.

The presence of outliers is another problem associated with CA that can also generate spurious groups. To recognize outliers, two criteria were used: univariate outliers were identified by box-plots and removed, whereas multivariate outliers were identified by the position of the specimens in the dendrogram. In this last case groups formed by only one specimen were removed and the analysis was performed again. If the dendrogram differed with and without the specimen then the specimen was labelled as an outlier and it was removed from the matrix, given this specimen generated conflict in the matrix. At the end of the study, all outliers were analysed and positioned within the resulting accepted taxa, or found to belong to different taxa (outside the *A. gratissima* complex).

A hierarchical CA applying the average linkage criterion and the Euclidean distance, due to the quantitative nature of the attributes, was used. Standardized quantitative variables were examined. Groups were identified following the same criterion used to delimit species. An ANOVA test was performed to determine whether the groups obtained by CA differ significantly from each other.

Each group of specimens with significant differences was considered a variety under Luckow's (1995) PSC with the modifications proposed by Henderson (2004, 2005a) to allow the use of herbarium material.

Quantitative characterization of recognized groups and subgroups: Species and varieties defined in this study were characterized as a function of the original quantitative variables. A one-way ANOVA test was performed for each variable; a generalized linear model (GLM) (Nelder & Wedderburn, 1972) approximation was performed for each variable that did not meet assumptions required for the ANOVA; this approach also allows the problem of imbalance within the recognized groups (here species or varieties) to be taken into account.

Given that the different variables are measured from the same specimen, they may be highly correlated, so the multiple tests may not be independent of each other. To preserve the global error of the tests, a Bonferroni correction was conducted, and the significance level of each test was adjusted (i.e. the error was divided by the number of variables used). All analyses were performed using the Infostat software (Di Rienzo *et al*., 2013), with a level of significance of 5% ($\alpha = 0.05$).

Taxonomic status

Groups and subgroups were compared with type material and original descriptions to assign the correct name, according to nomenclatural rules (McNeill *et al*., 2012). Traditional descriptions are given under the Taxonomic Conclusions section, including new taxon circumscriptions, synonymy and distributional data. Ranges (minimum–maximum) are given for plant structures and character statistics are shown by box-plots.

RESULTS

Species delimitation

Four of the 16 qualitative attributes were detected as characters by successive rounds of CA (5, leaf blade margin entire or partially serrate-dentate vs. completely serrate-dentate; 10, presence or absence of leaf blade obovate, mucronate apex and revolute border; 11, presence or absence of leaf blade elliptic, acute apex, serrate-dentate margin and conspicuous craspedodromous venation; and 12, presence or absence of petiole). The resulting dendrogram (Fig. 1) established four major clusters, groups A–D, each group characterized by a unique combination of four qualitative characters (Table 4, Fig. 2), and considered hereafter as accepted species under the extension of the PSC proposed by Henderson (2004, 2005a).

Variety recognition

The study of subgroups was not possible within groups A and C, due to the low number of individuals in each group. In group D no subgroups were found, probably due to the high homogeneity registered for the quantitative variables, confirming that no varieties can be distinguished within this group.

However, for group B, study of subgroups could be carried out and subgroups were found. Quantitative variables related to the inflorescence [average length of the inflorescence (16) and peduncle length (17)] and leaf petiole length (13) show low variation between specimens. Variables related to the leaf blade [length (2) and width (3)] show wide variation and these two were considered for the study of subgroups. However, as explained in the Materials and Methods, these two variables are associated (Pearson correlation coefficient: 0.84), so a PCA was carried out and a new synthetic variable (PC1), that contains 92% of the total variation, was retained and used in the CA.

The CA within group B was performed on 94% (197/209) of the specimens as 6% had to be removed as outliers. The CA indicated two subgroups, B1 and B2, defined by PC1 in the dendrogram obtained (Fig. 3). The ANOVA indicated significant differences ($P < 0.001$) between subgroups B1 and B2 for PC1; these are therefore considered as accepted varieties under the extension of the PSC proposed by Henderson (2004, 2005a).

Quantitative characterization of recognized groups and subgroups

Accepted species were characterized as a function of the original quantitative variables and this showed that blade width (attribute 3) is the only quantitative variable that allows the differentiation of the four species (Fig. 4B). Although the remaining four quantitative variables do not discriminate between the four recognized species, these variables have taxonomic relevance because they discriminate species groups significantly different from other species groups. The subgroups were characterized as a function of the original quantitative variables and this showed that the recognized varieties differ significantly (one-way ANOVA, $P < 0.001$) from each other for each of the five measured variables. Groups and subgroups found in the analysis and their assigned taxonomic name are shown in Table 5.

DISCUSSION

The methodology used proved to be valuable for taxonomic studies based on herbarium specimens. It is a useful tool that objectively detects characters that allow taxa (species or varieties) to be distinguished when the study involves many specimens and many variables. Thus, multivariate analysis can help to identify solid characters that distinguish each taxon.

The low morphological variation in the *A. gratissima* complex becomes clear as only four of the 16 attributes were retained as characters. The study shows that the leaf is the only organ that allows delimitation of taxa within the complex. These results are congruent with previous taxonomic studies performed on the group (Botta, 1979; Siedo, 2006; O'Leary *et al.*, 2016) in which the leaf was also defined as the taxonomic criterion to differentiate taxa.

The new methodological approach here proposed to identify subgroups, based on quantitative attributes, employing CA, has been shown to be precise compared with the previously employed approach, employing PCA (Henderson, 2004, 2005a,b; O'Leary *et al*., 2012). PCA is a useful methodology for sorting

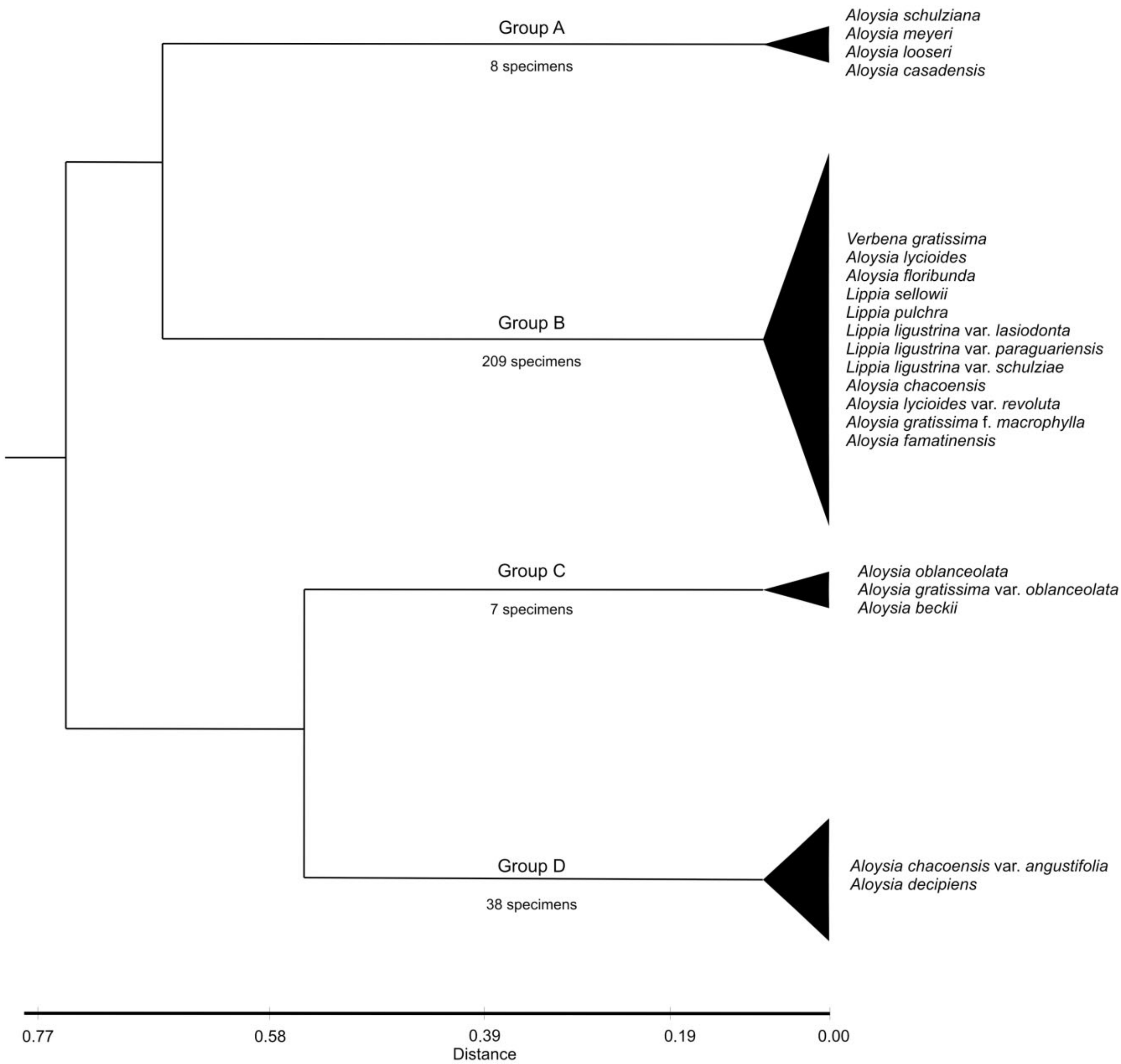

Figure 1. Dendrogram obtained by CA of qualitative attributes showing the four recovered groups, A–D. Type specimens included in the analysis are shown in the cluster they joined.

objects in a multivariate space, but when employed to identify groups it becomes a biased approach as there are no objective criteria for delimiting groups within the scatter plots. Instead, the CA is a unbiased tool for delimiting groups based on their phenetic similarity because it allows us to explicitly define groups according to the position of type specimens.

Group A corresponds to *A. schulziana*. In our analysis this group of specimens is distinguished by the combination of the following character states: plants with petiolate leaves; and blades with a serrate–dentate margin, an elliptic, acute apex and conspicuous craspedodromous venation. Quantitative characters show this species can be distinguished by having longer inflorescence peduncles (Fig. 4E), with the highest mean values, *c.* 0.5 cm difference from the remaining species and the largest leaves (mean 26.2 × 11.4 mm) (Fig. 4A, B).

This group includes type specimens of five names (Table 5). Among them, *A. schulziana*, *A. looseri* Moldenke and *A. meyeri* Moldenke are the oldest; all were described by Moldenke in 1940 and consequently all have the same priority (Art. 11, McNeill *et al.*, 2012). The name *A. schulziana* is chosen because it has been

Table 4. Major groups found in the CA and the combination of character states they present

	Leaf blade margin: entire or partially serrate–dentate (0), completely serrate–dentate (1) (*attribute* 5)	Leaf blade obovate, mucronate apex and revolute border: absent (0), present (1) (*attribute* 10)	Leaf blade elliptic, acute apex, serrate–dentate margin and conspicuous craspedodromous venation: absent (0), present (1) (*attribute* 11)	Petiole: absent (0), present (1). (*attribute* 12)
Group A (*A. schulziana*)	1	0	1	1
Group B (*A. gratissima*)	0	0	0	1
Group C (*A. oblanceolata*)	0	1	0	0
Group D (*A. decipiens*)	0	0	0	0

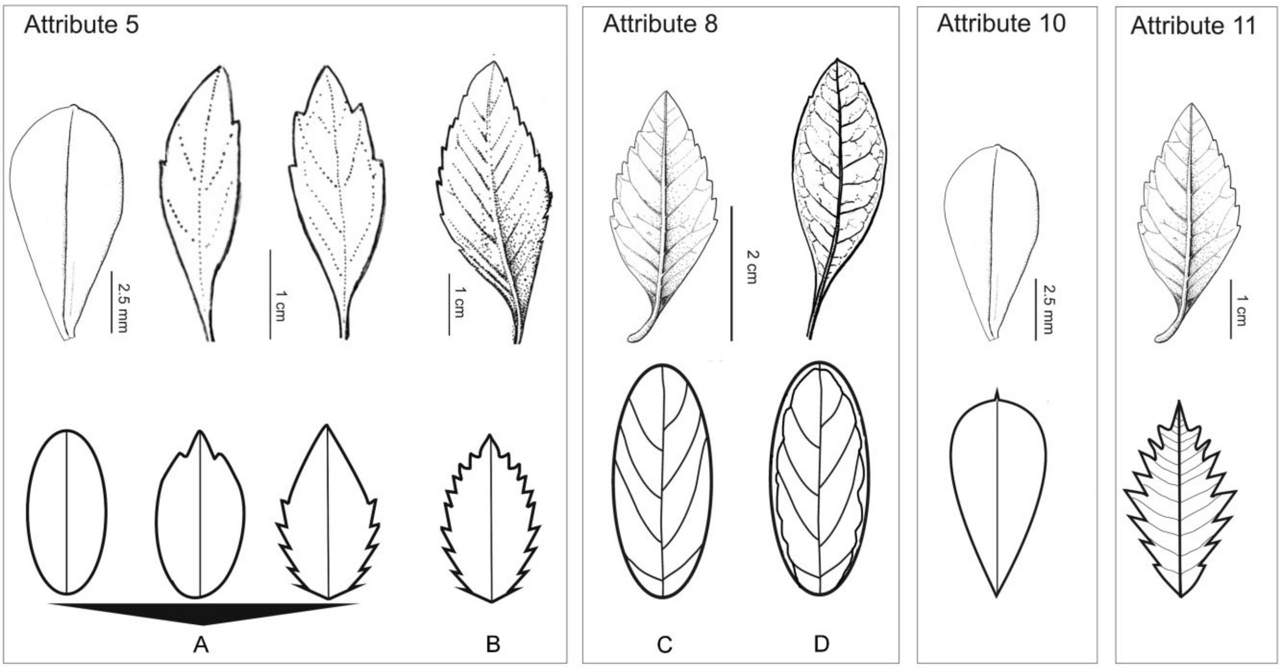

Figure 2. Illustration and schematic representation of important attributes. Attribute 5, different type of leaf margin: A, entire or partially serrate–dentate; B, completely serrate-dentate. Attribute 8, different type of leaf venation: C, craspedodromous, a unique central vein, with secondary veins originated from it and ending in the leaf margin; D, brochidodromous, a unique central vein, with secondary veins reunited in a lateral marginal vein. Attribute 10, leaf blade obovate, mucronate apex and revolute border. Attribute 11, leaf blade elliptic, acute apex, serrate–dentate margin and conspicuous craspedodromous venation.

used most frequently. The synonymy of these three taxa has been suggested by O'Leary *et al.* (2016).

Type material of *A. lomaplatae* Ravenna was not found. The description in the protologue (Ravenna, 2006) indicates that it belongs to this group given that the author described the leaf blades as 'lobate-dentate'. Furthermore, specimens determined as *A. lomaplatae* by Ravenna (e.g. *Maranta 1104*, Argentina, Formosa, BA) were analysed and corroborated as belonging to this group.

Group B corresponds to *A. gratissima*. In our analysis this group of specimens is distinguished by the combination of the following character states: plants with petiolate leaves; and blade margins partially serrate–dentate. This species is difficult to distinguish from the rest of the complex based on quantitative characters (Fig. 4). Leaf length and width in *A. gratissima* have high dispersion values (Fig. 4A, B), allowing us to differentiate two subgroups (varieties) within this taxon based on these features. *Aloysia*

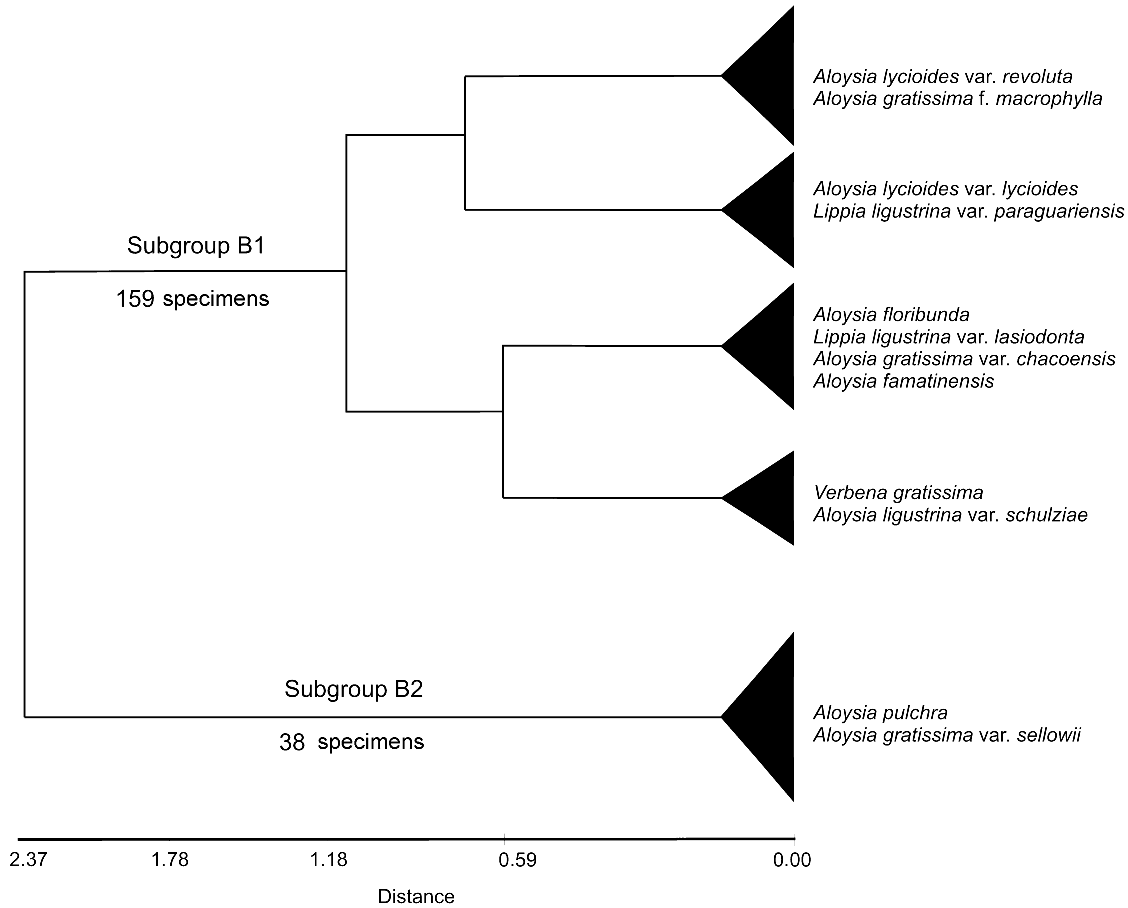

Figure 3. Dendrogram obtained by CA of PC1 within group B, showing the two subgroups, B1 and B2, found. Type specimens included in the analysis are shown in the cluster they joined.

gratissima and *A. schulziana* cannot be distinguished from each other based on mean petiole length (Fig. 4C).

Aloysia gratissima is a variable species, with the widest geographical distribution, being found from North America to South America, and as a result there are many different names found for this species. All the type specimens of the names reunited under this group were analysed and are synonymized under *A. gratissima*.

Siedo (2006) distinguished *A. lycioides* from *A. gratissima* by leaf morphology, but this character has been analysed in 14 different attributes, and none differentiated the taxa. The taxonomic conclusions reached by O'Leary *et al.* (2016) in relation to the species treated in this work lack the statistical support provided here, given it is a huge taxonomic revision of the whole genus *Aloysia*, based only on qualitative morphological traits. The authors recognized *A. chacoensis* Moldenke as a variety of *A. gratissima*. Here it is considered a synonym of *A. gratissima* var. *gratissima*, as it is nested in that taxon. *Aloysia lycioides* var. *revoluta* Moldenke is also nested here in this group, although O'Leary *et al.* (2016) considered it as a synonym of an accepted *A. pulchra*.

The name *Lippia sellowii* Briq. was chosen as a basionym for the variety, given the combination *A. gratissima* var. *sellowii* already existed. *Lippia pulchra* Briq. is considered a synonym of this variety.

Group C corresponds to *A. oblanceolata* Moldenke. In our analysis this group of specimens is distinguished by the combination of the following character states: sessile leaves; and blades obovate, with mucronate apex, entire margin and revolute border. This species is difficult to distinguish from the other species in the complex by quantitative characters (Fig. 4). However, with *A. decipiens* Ravenna, it has the smallest leaves (i.e. blade length and width) and it is impossible to differentiate these taxa based on these measures (Fig. 4A, B).

Siedo (2006) and O'Leary *et al.* (2016) considered *A. beckii* Moldenke as a synonym of *A. gratissima*. However, the type specimen of *A. beckii* has sessile

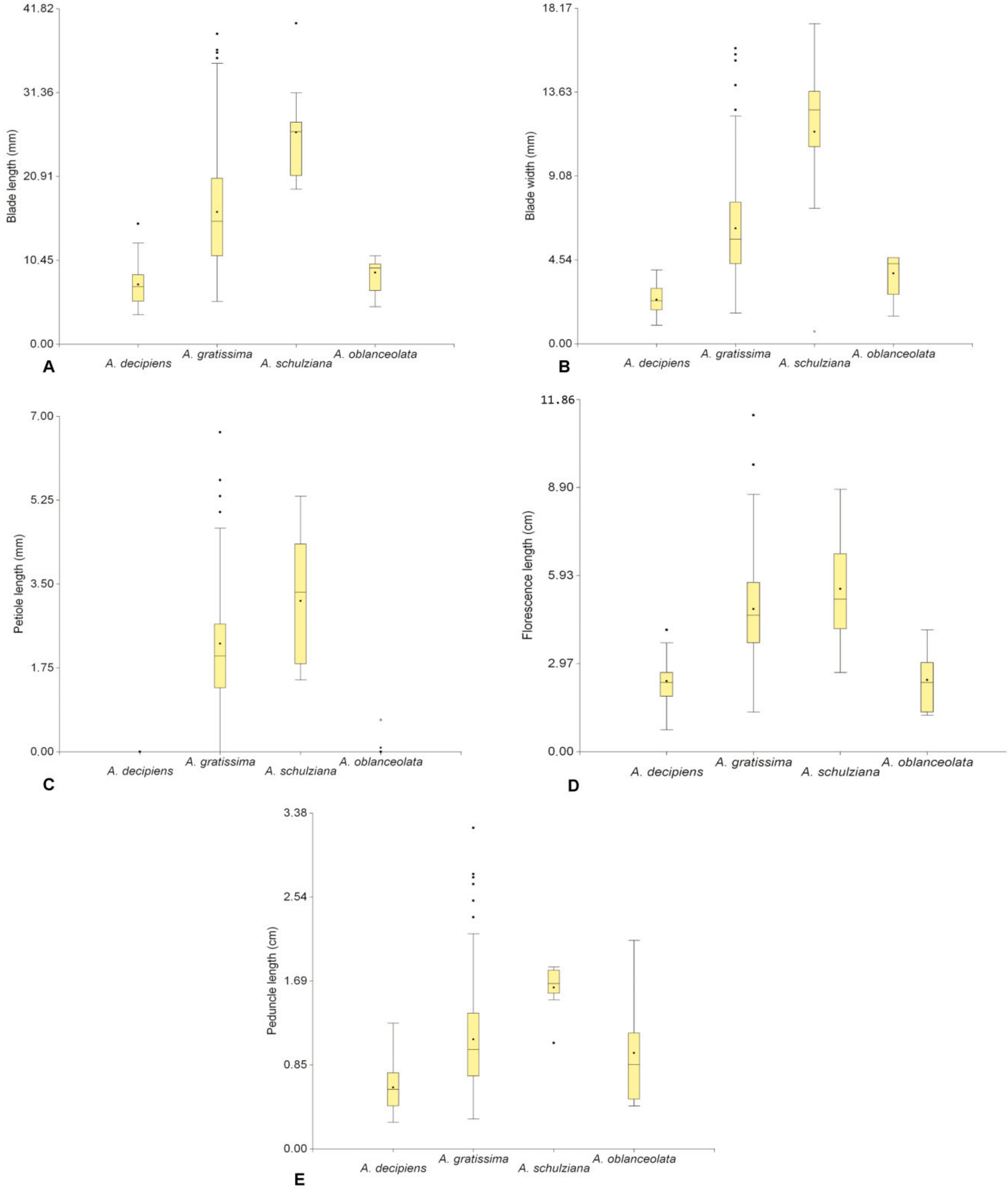

Figure 4. Box-plots of quantitative characters for the four species accepted in the *Aloysia gratissima* complex. Boxes incorporate 50% of values; horizontal line in box indicates median value; dark points within vertical line indicate mean value; dark points outside vertical line indicate outlier values; open circles indicate far outlier values.

Table 5. Taxonomic classification of taxa of the *Aloysia gratissima* complex

Group	Subgroup	Taxa accepted in this study	Synonymy
A		*Aloysia schulziana* Moldenke 1940	*Aloysia meyeri* Moldenke 1940 *Aloysia looseri* Moldenke 1940 *Aloyisa casadensis* Moldenke 1949 *Aloysia lomaplatae* Ravenna 2006
B	B 1	*Aloysia gratissima* (Gillies & Hook. ex Hook.) Tronc. var. *gratissima*	*Aloysia lycioides* Cham. 1832 *Aloysia floribunda* M. Martens & Galeotti 1844 *Lippia ligustrina* var. *lasiodonta* Briq. 1904 *Lippia ligustrina* var. *paraguarienses* Briq. 1904 *Lippia ligustrina* var. *schulziae* Standl. 1929 *Aloysia chacoensis* Moldenke 1940 *Aloysia lycioides* var. *revoluta* Moldenke 1949 *Aloysia gratissima* fo. *macrophylla* Moldenke 1974 *Aloysia famatinensis* Ravenna 2007
	B 2	*Aloysia gratissima* var. *sellowii* (Briq.) Botta 1979	*Lippia pulchra* Briq. 1904
C		*Aloysia oblanceolata* Moldenke 1949	*Aloysia gratissima* var. *oblanceolata* Moldenke 1968 *Aloysia beckii* Moldenke 1982
D		*Aloysia decipiens* Ravenna 2007	*Aloysia chacoensis* var. *angustifolia* Tronc. 1964

leaves, with obovate blades, mucronate apex and revolute border, and it is grouped under *A. oblanceolata* here. The synonymy of *A. gratissima* var. *oblanceolata* and *A. oblanceolata* has been suggested by O'Leary *et al.* (2016).

Group D corresponds to *A. decipiens*. In our analysis this group of specimens is distinguished by the combination of the following character states: sessile leaves; and entire blade margin. This species is difficult to distinguish from the other species of the complex by quantitative characters (Fig. 4). However, as stated before, with *A. oblanceolata*, it has the smallest leaves. Inflorescence length can be used to distinguish *A. decipiens* and *A. oblanceolata* (with smaller mean values) from *A. schulziana* and *A. gratissima* (with higher mean values) (Fig. 4D); the mean difference between the groups for this character is *c.* 2.8 cm.

This taxon had not previously been recognized as a species, always being treated as a variety. Therefore, the correct name for this group comes from a species described by Ravenna (2007). Ravenna stated that *A. decipiens* is closely related to *A. beckii*, but, as noted before, that taxon has been grouped under *A. gratissima* here, given that the type specimen has petiolate leaves; leaves are sessile in the definition of *A. decipiens* used here.

TAXONOMIC CONCLUSIONS

The *A. gratissima* complex is redefined to include four species and two varieties, all distributed in South America except *A. gratissima* var. *gratissima*, which is also found in southern North America.

1. *Aloysia decipiens* Ravenna Figure 5

Onira 11(4): 14. 2007. *Type*: Argentina. Santiago del Estero: Dpto. Robles, colonia Jaime, 19.xi.1948, *Luna Ruiz s.n.* (holotype BA 53388!)

Aloysia chacoensis var. *angustifolia* Tronc., Darwiniana 13: 630. 1964. *Aloysia gratissima* var. *angustifolia* (Tronc.) Botta, Darwiniana 22: 89. 1979, **syn. nov.** *Type:* Argentina. Buenos Aires: Campana, 28.x.1934, *A. Burkart 6695* (holotype SI!; isotype SI!).

Shrubs or suffruticose plants, 1.0–2.5 m high, stems glabrous. Leaves more than two per node, exceptionally one or two per node, sessile, blade elliptic, (3.7–) 7.4 (–15.0) × (1.0–) 2.4 (–4.0) mm, acute apex, sometimes obtuse, acute base, central vein conspicuous, lateral venation not conspicuous, adaxial surface strigose, abaxial surface strigose–puberulous, occasionally sclerous, entire margin. Axillary inflorescences two or more per node, occasionally one, (0.7–) 2.4 (–4.1) cm long, peduncle (0.3–) 0.6 (–1.3) cm long. Flowers white or yellowish, perfumed, slightly pedicellate, floral bracts ovate–elliptic, strigose or hispid, (0.6–) 1.1 (–1.8) mm. Calyx (1.4–) 2.2 (–3.1) mm long, hispid, with four acute or triangular teeth, (0.5–) 0.9 (–1.3) mm long, corolla tube (2.0–) 3.0 (–3.7) mm long, externally subglabrous to pilose, occasionally densely pilose. Nutlets two, 1.5–1.8 mm long, glabrous.

Synonymy: The type material of the new synonym *A. chacoensis* var. *angustifolia* was included in the data matrix, and it was nested in this group in the CA.

KEY TO THE SPECIES AND VARIETIES OF THE *ALOYSIA GRATISSIMA* COMPLEX

1 Sessile leaves, all the leaves with entire margin........2
2 Leaf blade obovate, mucronate apex and revolute border........***A. oblanceolata***
2′ Leaf blade elliptic, acute apex and non-revolute border........***A. decipiens***
1′ Petiolate leaves, leaves generally with non-entire margin........3
3 Leaf blade margin completely serrate–dentate, blade elliptic, acute apex and conspicuous craspedodromous venation........***A. schulziana***
3′ Leaf blade margin partially serrate–dentate, blade elliptic, acute or obtuse apex and craspedodromous or brochidodromous venation........4
4 Leaves larger, blade (17.7–) 20.7–32.4 (–38.7) × (5.7–) 7.7–13.0 (–16.0) mm........***A. gratissima*** var. ***sellowii***
4 Leaves′ smaller, blade (5.3–) 9.9–19.0 (–28.3) × (2.0–) 3.6–7.3 (–11.0) mm........***A. gratissima*** var. ***gratissima***

Distribution and habitat: Aloysia decipiens is endemic to Argentina, where it grows in the north and central part of the territory (Fig. 9A). It is found in sandy, clay-halophilous soil and is abundant in the provinces of Chaco, Córdoba and Santiago del Estero.

Representative specimens examined: ARGENTINA. Buenos Aires. Otamendi, 26.x.1928, *Perez Moreau 13577* (NY). Catamarca. La Paz, El Lindero, 28.ii.1947, *Brizuela 882* (NY). Chaco. San Fernando. Fontana, 1930, *Meyer 277* (SI). Córdoba. Quebrada de Las Rosas, 22.iii.1956, *Lanfranchi 1206* (SI). Entre Ríos. Paraná, Paracao, 31.x.1962, *Burkart 23805* (SI). Formosa. Pirané, Casco-Cue, 12.ii.1944, Morel 913 (NY). La Pampa. Lihuel-Calel, xii.1981, *Cabrera 32798* (SI). La Rioja. Los Llanos, La Jarilla, 12.ii.1940, *Castellanos 33892* (NY). Salta. Anta, San Javier, 27.xii.1988, *Saravia Toledo 1752* (SI). San Luis. Belgrano, entre San Pedro y San Antonio, 20.iv.1980, *Rotman 296* (SI). Santa Fe. 9 de Julio, Ea. Las Marías, 29.xii.1944, *Krapovickas 781* (SI). Santiago del Estero. Espada, 28.xii.1938, *Argañarás 61* (SI). Tucumán. Trancas, 8.xii.1913, *D. Rodriguez 1175* (SI).

2. *ALOYSIA GRATISSIMA* (GILLIES & HOOK. EX HOOK.) TRONC. FIGURE 6

Darwiniana 12. 527. 1962. *Verbena gratissima* Gillies & Hook. ex Hook., Bot. misc. 1: 160. 1829. *Type*: Argentina. Mendoza: 1829, *G. Gillies s.n.* (holotype K 470994!; isotypes BM 643773!, GH 69316!).

SHRUBS or suffruticose plants, to 3 m high, stems glabrous. Leaves two per node, exceptionally one per node, or more than two per node, petiole (0.7–) 2.3 (–6.7) mm, blade elliptic, (5.3–) 16.5 (–38.7) × (1.7–) 6.3 (–16.0) mm, acute or obtuse apex, acute base, lateral venation conspicuous or not, craspedodromous or brochidodromous, adaxial surface strigose–puberulous, abaxial surface sclerous–puberulous, occasionally strigose, margin partially serrate–dentate, border not revolute. Axillary inflorescences two or more per node, occasionally one, (1.3–) 4.8 (–11.3) cm long, peduncle (0.3–) 1.1 (–3.2) cm long. Flowers white, perfumed, slightly pedicellate, floral bracts ovate–elliptic, occasionally linear, strigose or hispid, (0.5–) 1.2 (–2.6) mm. Calyx (1.5–) 2.4 (–3.3) mm long, hispid, with four acute or triangular teeth, (0.3–) 0.8 (–1.3) mm long, corolla tube (2.3–) 3.5 (–5.1) mm long, externally subglabrous to pilose. Nutlets two, 1.5 mm long, glabrous.

Notes: Floral bracts are ovate–elliptic, occasionally linear (e.g. *Fernández Casa 284*, NY, Paraguay). Two varieties are found, based on leaf size differences.

Distribution: Aloysia gratissima is the most widely distributed *Aloysia* sp., being found in South and North America (Fig. 9B, C). The two varieties of *A. gratissima* are differentiated morphologically, but not geographically. They grow sympatrically in southern South America, sharing the same ecological niches. *Aloysia gratissima* var. *gratissima* has a wide distribution, being present outside South America (southern USA and northern Mexico). We fail to understand this gap as a disjunctive distribution as the lack of records in central and northern South America may be due to the absence of collections there.

2a. *ALOYSIA GRATISSIMA* VAR. *GRATISSIMA* (FIG. 6A–E)

Aloysia lycioides Cham., Linnaea 7: 237. 1832. *Lippia lycioides* (Cham.) Steud., Nomencl. Bot. 2: 62. 1841. *Type:* Brazil meridian, *F. Sellow s.n.* (lectotype designated by Múlgura *et al*. [2012:17] K 487005!; duplicate BR 5720156! HAL 107062!, HAL 107063! K 487006!, M 0111831!, NY 1163417!, US 1049793!, SI 3390!, SI 3391!!).

Aloysia floribunda M.Martens & Galeotti, Bull. Acad. Brux. xi. II: 320. 1844. *Type:* México. Veracruz: Jun.–Oct. 1840, *H. Galeotti 774* (holotype BR 5187607!; isotype BR 5187300!).

Lippia ligustrina var. *lasiodonta* Briq., Annuaire Conserv. Jard. Bot. Genéve 7–8: 305. 1904. *Type:*

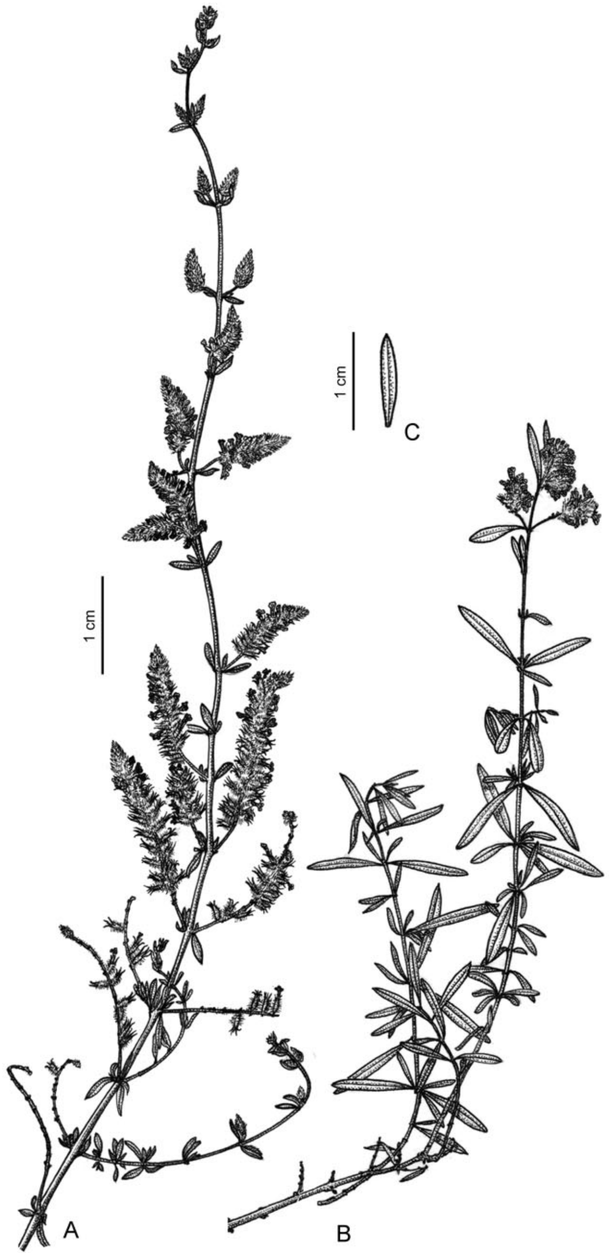

Figure 5. *Aloysia decipiens*. A, fruiting branch; B, flowering branch; C, detail of a leaf. A, from *Burkart 23805* (SI); B and C, from *Burkart 6695* (type SI).

Paraguay. Paraguarí, 15.iii.1881, *B. Balansa 3117* (holotype G 166265!; isotypes F 92411F!, GH 299001!, K 487007).

Lippia ligustrina (Lag.) Britton var. *paraguariensis* Briq., Annuaire Conserv. Jard. Bot. Genève 7–8: 305. 1904. *Aloysia ligustrina* var. *paraguariensis* (Briq.) Moldenke, Phytologia 1: 167. 1935. *Aloysia lycioides* var. *paraguariensis* (Briq.) Moldenke, Phytologia 2: 464. 1948. *Aloysia gratissima* var. *paraguariensis* (Briq.) Moldenke, Phytologia 9: 500. 1964. *Type*: Paraguay. Paraguarí: i.1875, *B. Balansa 1015* (holotype G 166413!; isotypes BR 5213337!, G 166414!, SI 3546!).

Lippia ligustrina var. *schulziae* Standl., Field Museum Pub. Bot. 4: 256. 1929. *Aloysia lycioides* var. *schulziae* (Stand.) Moldenke, Phytologia 1: 95. 1934. *Aloysia gratissima* var. *schulziae* (Stand.) Moldenke, Phytologia 9: 500. 1964, **syn. nov.** *Type:* United States of America, Texas: Jeff Davis Co., Fort Davis, 5 Aug. 1928, *E. D. Schulz 2020* (holotype F 92408F!).

Aloysia chacoensis Moldenke, Lilloa 5: 373. 1940. *Aloysia gratissima* var. *chacoensis* (Moldenke) Botta, Darwiniana 22: 89. 1979, **syn. nov.** *Type:* Argentina. Chaco: Tirol, on side of mountain, ii.1934, *A. G. Schulz 1494* (holotype NY 103873; isotype CTES 13810!).

Aloysia lycioides var. *revoluta* Moldenke, Phytologia 3: 108. 1949. *Aloysia gratissima* var. *revoluta* (Moldenke) Moldenke, Phytologia 9: 500. 1964, **syn. nov.** *Type:* Uruguay. no label – probably collected by *J. Arachavaleta* s.n. (holotype MVM!; isotype NY 103880!).

Aloysia gratissima fo. *macrophylla* Moldenke, Phytologia 29: 75. 1974. *Type:* United States of America, Texas. Presidio Co., in mountain tracks, 4.viii.1852, *C. Parry* s.n. (holotype NY 103863!).

Aloysia famatinensis Ravenna, Onira 11(4): 15. 2007. *Type:* Argentina. La Rioja: Sierra de Famatina, Guanchín Viejo, 25.i.1928, *A. Castellanos* s.n. (holotype BA 28328!).

This variety is distinguished by the smaller leaf blades, (5.3–) 9.9–19 (–28.3) × (2.0–) 3.6–7.3 (–11.0) mm.

Synonymy: The type material of the new synonyms was included in the data matrix and all were nested in this group in the CA.

Distribution: Aloysia gratissima var. *gratissima* is found from 37°S in South America as its southern limit of distribution to southern North America, in Texas (Fig. 9B). However, collections have not been reported in northern South America or Central America.

Representative specimens examined: ARGENTINA. Buenos Aires. San Nicolás, barrancas, 12.x.1941, *Cabrera 7209* (SI). Catamarca. La Paz, Puesto del Lobo, 13.xii.1946, *Brizuela 437* (SI). Chaco. Campo del Cielo, Santa Sylvina, 25.i.1947, *Schulz 1128* (NY). Córdoba. Tulumba, orillas del río, 24.xii.1947, *Balegno 1584* (NY). Corrientes. Sto. Tomé, Ao. Chimiray y Río Uruguay, 12.xi.1974, *Schinini 10366* (CTES; SI). Entre Ríos. Concordia. Pto. Yeruá, 30.i.1973, *Burkart 29465* (SI). Formosa. Formosa, ii.1918, *Joergensen 2473* (NY). Jujuy. El Carmen, Dique La Ciénaga, 10.xi.1974, *Burkart 30577* (SI). La

Figure 6. *Aloysia gratissima* var. *gratissima*. A and B, basal branches; C, fruiting branch; D, flower; E, extended calyx, inner surface. *Aloysia gratissima* var. *sellowii*. F, branch general aspect. A–E, from *Schinini 10366* (SI); F, from *Schwartz 3803* (SI).

Pampa. Guatraché, Ea. Remecó, 18.x.1990, *Rúgolo 1275* (SI). La Rioja. Arauco. Qda. de la Cébila, 26.ii.1977, *Troncoso 1930* (SI). Mendoza. Lujan de Cuyo. Cuchilla del Carrizal, 12.xi.1944, *Ruiz Leal 9848* (NY). Misiones. Capital, entre San José y Sta. Inés, 25.iii.1977, *Cabrera 28331* (NY). Salta. Santa Victoria Oeste. Izquierda Rio santa Victoria, 9.xii.2001, *Forzza 1977* (SI). San Juan. Tumbaya, Laguna Volcán, 23.i.1988, *Zuloaga 3738* (SI). Santiago Del Estero. Pellegrini. Cerro Del Benicate, 23.xii.1927, *Venturi 5747* (SI). Tucumán. Burruyacú, Cerro del Campo, 3.xii.1928, *Venturi 7656* (SI).

BOLIVIA. Chuquisaca. Sucre, Cerro Macho, iv.1933, Herb. Cardenasianum 637 (NY). Cochabamba, hillsides, 14.ii.1936, *Wolstenholme 24* (NY). Potosí. Charcas, San Pedro de Buena Vista, 16.iii.1993, *Torrico 163* (SI). Santa Cruz. Samaipata, 14.x.1928, *Steinbach 8248* (NY). Tarija. Arce, Padcaya, 28.i.1988, *Liberman 1823* (SI).

BRAZIL. Rio Grande do Sul. Camino de Uruguayana a Alegrete, 25.iii.1947, *Nicora 4726* (SI). São Paulo. Campinas, Terra Preta, *Campos Novaes 1950* (SI).

CHILE. Valparaiso. Limache, *Garaventa 7092* (SI).

PARAGUAY. Boquerón. Colônia Menno, Paratodo, 13.iii.1974, *Arenas 455* (SI). Central. Asunción, 20.vi.1975, *Krapovickas 28539* (SI). Guairá. Villa Rica, 16.xii.1936, *Archer 4678* (NY). Itapua. Yacyretá, 8.xii.2002, *Zardini 59637* (SI). Paraguarí. Entre Paraguarí y Carapeguá, 12.ix.1980, *Fernandez Casas 3500* (NY). Pte. Hayes. NO Rio Paraguay, 14.i.1976, *Cordo 149* (SI). San Pedro. Villa Primavera, 28.i.1957, *Woolston 794* (SI).

URUGUAY. Artigas. s./loc. 9.i.1971, *Marchesi 10078* (SI). Lavalleja. Abra de Zabaleta, 11.x.1970, *Krapovickas 16138* (SI). Minas. Ao. Águas Blancas, 11.x.1970, *Crespo 26457* (SI). Paysandú. Ao. Negro, 12.xi.1937, *Rosengurt 2245* (SI). Rivera. Ruta 29, pasando Minas de Corrales, 16.iv.2010, *Denham 333* (SI). San José. Ao. Sta. Lucía, 1.xii.2001, *Seijo 2655* (SI). Tacuarembó. Rd. 26, entre Tacuarembó y Melo, 19.i.1994, *Pedersen 15899* (NY).

USA. Texas. Medina Co., 24.vi.1975, *DeLoach* s.n. (SI). MEXICO. Chihuahua. Bachimba Canyon, 23.iii.1885, *Pringle 100* (SI). Nuevo León. Valle Monterey, 31.viii.1903, *Pringle 11666* (SI).

2b. *Aloysia gratissima* var. *sellowii* (Briq.) Botta (Fig. 6F)

Darwiniana 22: 85. 1979. *Lippia sellowii* Briq., Annuaire Conserv. Jard. Bot. Genève 4: 21. 1900. *Aloysia sellowii* (Briq.) Moldenke, Revista Sudamer. Bot. 4: 15. 1937. *Type*: Uruguay. Montevideo, *F. Sellow 1744* (holotype B†, photograph F neg. 24670!; isotype F 0092930F!, SI 66318!).

Lippia pulchra Briq., Ark. Bot. 2(10): 18. 1904. *Aloysia pulchra* (Briq.) Moldenke, Phytologia 1: 95. 1934. *Type*: Brazil. Rio Grande do Sul, Porto Alegre, 1892, *C. A. M. Lindman 579* (holotype G 0386457!; isotypes F 0092929F!, G 0386456!, GH 299004!, NY 137807!, NY 137809!, RB not seen, S 11–10470!, S 11–10959!, SI 3403!, US 01049794!).

This variety is distinguished by its smaller leaf blades, (17.7–) 20.7–32.4 (–38.7) × (5.7–) 7.7–13.0 (–16.0) mm.

Distribution and habitat: This variety is distributed only in South America, being frequent in northern Argentina, in the provinces of Misiones, Corrientes and Salta, and southern Brazil (Fig. 9C). It has a more restricted distribution than *A. gratissima* var. *gratissima*. It is found in rocky soils or along woodland paths.

Representative specimens examined: ARGENTINA. Catamarca. Belén, ruta 40, a 3 km S Londres, 15.ii.2011, *Zuloaga 12912* (SI). Corrientes. Ituzaingó, E de ruta 12 camino san Carlos, ii.1971, *Krapovickas 17970* (SI). Formosa. Pilcomayo, Clorina, 11.ix.1946, *Morel 1257* (SI). Jujuy. San Pedro, 21.iv.1981, *Ahumada 4216* (SI). La Rioja. Sierra de Ambato, 22.ii.1977, *Troncoso 1860* (SI). Misiones. La Viña, Puerta de Díaz, 6.xii.1987, *Novara 7260* (SI). San Javier, *Schwartz 3803* (NY, SI). Salta. Orán, camino de General Ballivián a Rio Seco, 10.xii.1986, *Zuloaga 2687* (NY). Tucumán. Capital, Rio Sali, vi.1920, *Venturi 834* (NY).

BOLIVA. Chuquisaca. Luis Calvo, El Salvador, 23.i.1992, *Saravia Toledo 10335* (SI). Santa Cruz. Caballero, 2.5 km Tambo, 4.ii.1984, *Schmitt 10* (SI). Tarija. Ruta Tarija-Villa Montres, Entre Ríos, 21.v.1971, *Krapovickas 19035* (SI).

BRAZIL. Paraná. Paula Freitas, 29.ii.1975, *Cordo 75375.2* (SI). Rio Grande do Sul: Granja Sodo, Girua, *Hagelund 132-59* (SI). Santa Catarina. Serra do Matador, Rio do Sul, 26.i.1959, *Reitz 8340* (SI). São Paulo. Moema, 20.iv.1955, *Scavone 15200* (SI).

PARAGUAY. Alto Paraná. S.loc. 1909, *Fiebrig 5904* (SI). Itapuá. Villa Encarnación, 14.xi.1902, *Schrottky 66* (SI). Paraguarí. Carapegua, ii.1919, *Rojas 3278* (SI).

URUGUAY. Rivera. Bajada de Pena, 7.ii.1966, *Marchesi 6053* (SI). Tacuarembó. 10 km SW Tacuarembó, 7.ii.1981, *Cabrera 32345* (SI).

3. *Aloysia oblanceolata* Moldenke Figure 7

Phytologia 3: 108. 1949. *Type*: Paraguay. San Bernardino, 1915, *T. Rojas 53a* (holotype NY 103883!; isotype MVM, SI 3407!).

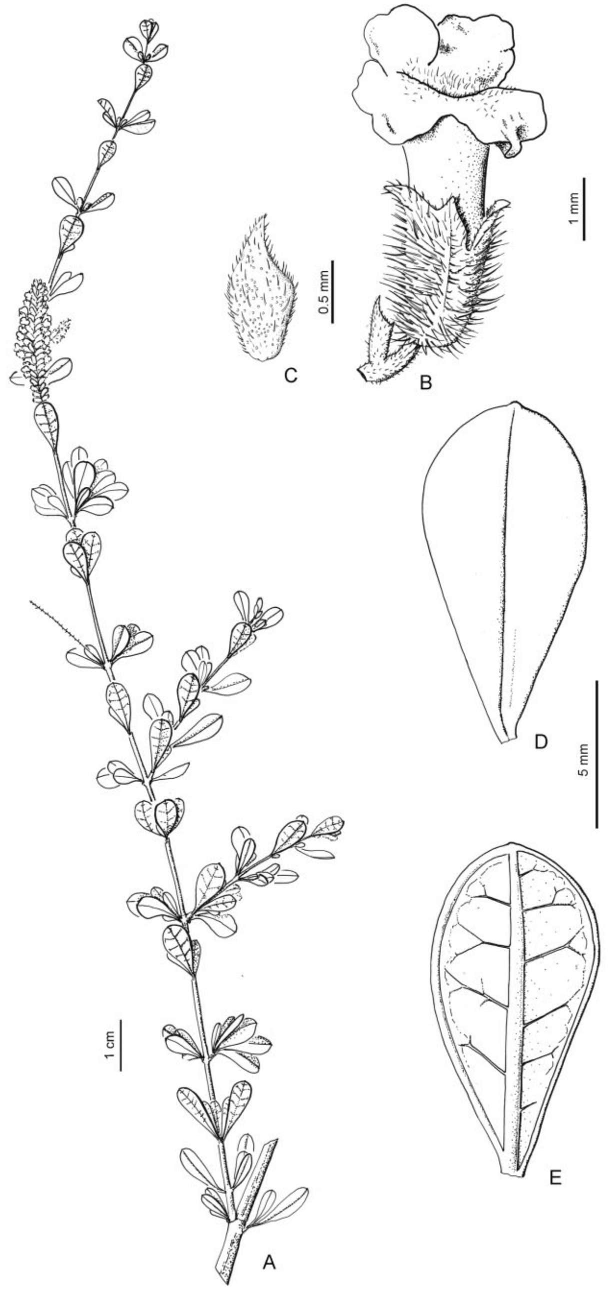

Figure 7. *Aloysia oblanceolata*. A, flowering branch; B, flower; C, floral bract; D, leaf, adaxial surface; E, leaf, abaxial surface. From *Arenas 148* (SI).

Aloysia gratissima var. *oblanceolata* Moldenke, Phytologia 15: 462. 1968. *Type*: Brazil. Rio Grande do Sul: Gloria, south-east of Porto Alegre, 2.x.1948, *A. L. Moldenke 19684* (holotype NY 103875!).

Aloysia beckii Moldenke, Phytologia 52(1): 18. 1982, **syn. nov.** *Type*: Bolivia. Cochabamba: Carrasco, Cochabamba hacia Santa Cruz, 27.ix.1981, *S. Beck 7036* (holotype TEX 374937!; isotype LPB not seen, M 112713!, SI 3374!).

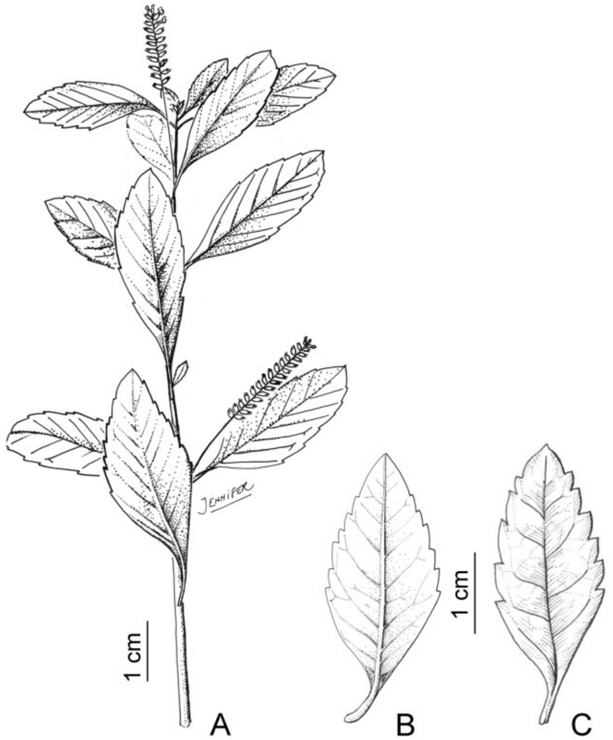

Figure 8. *Aloysia schulziana*. A, flowering branch; B, leaf, abaxial surface; C, leaf, adaxial surface. From *Cabrera 14633* (SI).

SHRUBS or suffruticose plants, 1.0–2.5 m high, stems glabrous. Leaves more than two per node, sessile, blade obovate, (4.7–) 8.9 (–11.0) × (1.5–) 3.8 (–4.7) mm, obtuse mucronate apex, acute base, central vein conspicuous, brochidodromous venation, adaxial surface strigose, abaxial surface sclerous–puberulous, entire margin, revolute border. Axillary inflorescences, two or more per node, (1.2–) 2.4 (–4.1) cm long, peduncle (0.4–) 1.0 (–2.1) cm long. Flowers white or yellowish, perfumed, slightly pedicellate, floral bracts ovate–elliptic, glabrous, strigose or hispid, (0.6–) 1.1 (–1.6) mm. Calyx (1.8–) 2.5 (–3.2) mm long, hispid, with four acute or triangular teeth, (0.5–) 0.8 (–1.2) mm long, corolla tube (3.2–) 3.7 (–5.2) mm long, externally subglabrous to pilose. Nutlets two, 1.5–1.8 mm long, glabrous.

Synonymy: The type material of the new synonym *A. beckii* was included in the data matrix, and it appeared nested in this group in the CA.

Distribution and habitat: Aloysia oblanceolata grows in southern Paraguay, southern Bolivia and Brazil in Paraná and Rio Grande do Sul (Fig. 9D). It is found in sandy or rocky soil in dry forests.

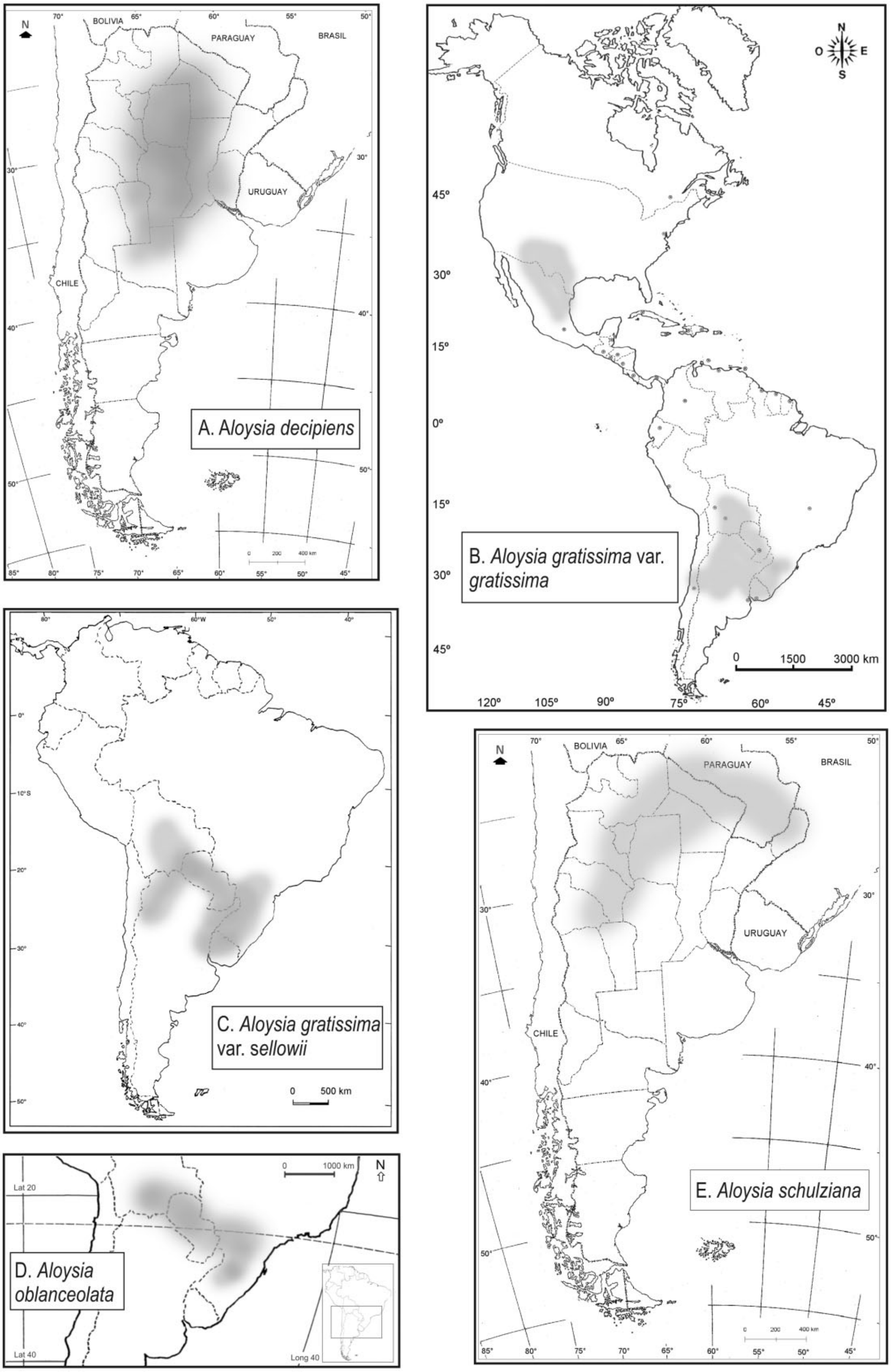

Figure 9. Geographical distribution of the species and varieties accepted in this work.

Representative specimens examined: BRAZIL. Paraná. Guarapuava, Rio Cavernoso, 12.xii.1962, *Hatschbach 9339* (SI). PARAGUAY. Central. In regione Ypacaray, i.1913, *Hassler 11497* (NY). Cordillera. Itaugua, Cantera, 12.xi.1973, *Arenas 148* (SI).

4. *ALOYSIA SCHULZIANA* MOLDENKE FIGURE 8

Lilloa 5: 381. 1940. *Aloysia gratissima* var. *schulziana* (Moldenke) Botta, Darwiniana 22: 87. 1979. *Type*: Argentina. Salta: Colonia San Bernardo, ii.1936, *A. G. Schulz 1447* (holotype NY 103887!; isotypes CTES 13830!, SI 3394!).

Aloysia looseri Moldenke, Lilloa 5: 377. 1940. *Lippia looseri* (Moldenke) Looser, Revista Univ. (Santiago) 26, no. 2: 141. 1941. *Type*: Chile. Santiago, 15.xii.1925, *G. Looser 4008* -cult.- (holotype SGO 4202!; isotypes NY 103879!, SI!).

Aloysia meyeri Moldenke, Lilloa 5: 378. 1940. *Type*: Argentina. Tucumán: Trancas, San Pedro de Colalao, 4.i.1940, *T. Meyer 3092* (holotype NY 103881!; isotypes LIL 1365!, SI 3389!).

Aloysia casadensis Hassler ex Moldenke, Phytologia 3: 107. 1949, **syn. nov.** *Type*: Paraguay. Puerto Casado, ii.1917, *T. Rojas 2529* (holotype MVM; isotypes NY 103868!, NY 103869!, SI 3389!).

Aloysia lomaplatae Ravenna, Onira 10(19): 60. 2006. *Type*: Paraguay. Boquerón: In scopulosis ad Loma Plata, civit., 2001-9-13, *P. Ravenna 5030* (holotype FCQ).

SHRUBS or suffruticose plants, 1.5–2.4 (–3.0) m high, stems subglabrous. Leaves two per node, exceptionally verticillate, petiole (1.5–) 3.2 (–5.3) mm, blade elliptic, (19.3–) 26.4 (–40.0) × (0.7–) 11.5 (–17.3) mm, acute apex, acute base, craspedodromous venation, adaxial surface strigose, abaxial surface strigose–puberulous, exceptionally sclerous, completely serrate–dentate margin, non-revolute border. Axillary inflorescences two per node, sometimes one, (2.7–) 5.5 (–8.8) cm long, peduncle (1.1–) 1.6 (–1.8) cm long. Flowers white, perfumed, slightly pedicellate, floral bracts ovate–elliptic, strigose or hispid, (0.8–) 1 (–1.2) mm. Calyx (1.8–) 2.2 (–2.8) mm long, hispid, with four acute or triangular teeth, (0.6–) 0.9 (–1.1) mm long, corolla tube (2.8–) 3.3 (–3.8) mm long, externally subglabrous to pilose. Nutlets two, 1.5 mm long, glabrous.

Synonymy: The type material of the new synonym *A. casadensis* was included in the data matrix, and it appeared nested in this group in the CA.

Distribution and habitat: Aloysia schulziana grows in north-western Argentina and Paraguay (Fig. 9E). It lives in thickets and roadsides, on rocky soils. The type specimen of *A. looseri* is from Chile, but it is from cultivated material.

Representative specimens examined: ARGENTINA. Chaco. Colonia Benítez, 12.ii.1954, *Schulz 8699* (SI). Jujuy. El Carmen, Dique La Ciénaga, 6.i.1971, *Krapovickas 17543* (SI); Entre San Pedro y Santa Clara: 18.v.1962, *Cabrera 14633* (SI). La Rioja. Sierra de Ambato, Qda. de la Sébila, 22.ii.1977, *Troncoso 1856* (SI). Misiones. Posadas, 2.iii.1945, *Bertoni 761* (NY). Salta. Anta. Entre Los Puestos y El Rey, vii.1934, *Ragonese* s.n. BA 23993 (NY); San Martín: Río Seco, 4.iv.1977, *Krapovickas 30899* (SI). San Juan. Valle Fértil, ruta 142, entre San Agustín y Las Tumanas, 6.iii.1995, *Biurrum 4002* (SI). Santiago del Estero. Guasayán, sierras, falda oriental, 20.iii.1984, *Ulibarri 1644* (SI). Tucumán. Trancas, ruta 311, E Colalao, 3.iii.1978, *Cordo 78-A-46* (SI).

PARAGUAY. Boquerón. Puerto Casado, 25.x.1956, *Pedersen 4186* (SI). Pte. Hayes. Colonia Menno, ii.1976, *Arenas 1493* (SI).

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REFERENCES

Botta SM. 1979. Las especies argentinas del género *Aloysia* (Verbenaceae). *Darwiniana* **22:** 67–108.

Davis JI, Nixon KC. 1992. Populations, genetic variation, and the delimitation of phylogenetic species. *Systematic Botany* **41:** 421–435.

Di Rienzo JA, Casanoves F, Balzarini MG, Gonzalez L, Tablada M, Robledo CW. 2013. InfoStat versión 2013. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. Available at: http://www.infostat.com.ar

Henderson AJ. 2004. A multivariate analysis of *Hyospathe* (Palmae). *American Journal of Botany* **91:** 953–965.

Henderson AJ. 2005a. A multivariate study of *Calyptrogyne* (Palmae). *Systematic Botany* **30:** 60–83.

Henderson AJ. 2005b. The methods of herbarium taxonomy. *Systematic Botany* **30:** 456–459.

Lu-Irving P, Olmstead RG. 2013. Investigating the evolution of Lantaneae (Verbenaceae) using multiple loci. *Botanical Journal of the Linnean Society* **171:** 103–119.

Lu-Irving P, O'Leary N, O'Brien A, Olmstead RG. 2014. Resolving the genera *Aloysia* and *Acantholippia* within tribe Lantaneae (Verbenaceae), using chloroplast and nuclear sequence data. *Systematic Botany* **39:** 644–655.

Luckow M. 1995. Species concepts: assumptions, methods, and applications. *Systematic Botany* **20:** 589–605.

Martens M, Galeotti H. 1844. Enumeratio synoptica plantarum phanerogamicarum in regionibus Mexicanis ab Henrico Galeotti collectarum (continuatio). *Bulletin de l'Académie Royale des Sciences et Belles-Lettres de Bruxelles* **11:** 227–243.

Marx H, O'Leary N, Yuan Y, Lu-Irving P, Tank D, Múlgura ME, Olmstead RG. 2010. A molecular phylogeny and classification of Verbenaceae. *American Journal of Botany* **97:** 1647–1663.

McNeill J, Barrie FR, Buck WR, Demoulin V, Greuter W, Hawksworth DL, Herendeen PS, Knapp S, Marhhold K, Prado J, Prud'homme van Reine WF, Smith GF, Wiersema JH, Turland NJ. 2012. *International Code of Nomenclature for algae, fungi and plants (Melbourne Code): adopted by the Eighteenth International Botanical Congress Melbourne, Australia, July 2011*. Regnum Vegetabile 154. Königstein: Koeltz Scientific Books.

Moldenke HN. 1934. Studies of new and noteworthy tropical American plants – II. *Phytologia* **1:** 95–105.

Moldenke HN. 1940. The flora of extra-tropical South America. *Lilloa* **5:** 353–440.

Moldenke HN. 1949. Notes on new and noteworthy plants VIII. *Phytologia* **3:** 106–122.

Moldenke HN. 1968. Notes on new and noteworthy plants. XLIX. *Phytologia* **15:** 462–463.

Moldenke HN. 1974. Notes on new and noteworthy plants LXXI. *Phytologia* **29:** 75–78.

Moldenke HN. 1982. Notes on new and noteworthy plants CLX. *Phytologia* **52:** 17–18.

Múlgura ME, O'Leary N, Rotman AD. 2012. Verbenaceae En F. Zuloaga, & A. Anton, eds. *Flora Argentina* **14:** i–v, 1–220.

Nelder J, Wedderburn R. 1972. Generalized linear models. *Journal of the Royal Statistical Society. Series A (General)* **135:** 370–384.

Nixon KC, Wheeler QD. 1990. An amplification of the phylogenetic species concept. *Cladistics* **6:** 211–223.

O'Leary N, Calviño C, Martínez S, Olmstead RG, Múlgura ME. 2012. Evolution of morphological traits in Verbenaceae. *American Journal of Botany* **99:** 1778–1792.

O'Leary N, Lu-Irving P, Moroni P, Siedo S. 2016. Recircumscription and taxonomic revision of *Aloysia* in South America. *Annals of the Missouri Botanical Garden* (in press).

Ravenna P. 2006. Studies in Verbenaceae II. A survey of the *Aloysia* species previously, merged in *Aloysia gratissima* including new species from Argentina, Bolivia and Paraguay. *Onira* **10:** 59–64.

Ravenna P. 2007. Studies in Verbenaceae IV. New species of *Aloysia* from Argentina, Bolivia and Paraguay, and notes on *A. lomaplatae* and *A. decorticans*. *Onira* **11:** 14–17.

Sanders RW. 2001. The genera of Verbenaceae in the southeastern United States. *Harvard Papers in Botany* **5:** 303–358.

Siedo SJ. 2006. *Systematics of* Aloysia *(Verbenaceae)*. PhD dissertation, The University of Texas, Austin.

Thiers B. 2014. [continuously updated]. *Index herbariorum: a global directory of public herbaria and associated staff*. New York Botanical Garden's Virtual Herbarium. Available at: http://sweetgum.nybg.org/ih/

Troncoso NS. 1964. Plantas vasculares nuevas o interesantes de la Floras de Entre Ríos I. *Darwiniana* **13:** 625–631.

APPENDIX 1

List of specimens (collector and number) employed in the analysis

A. decipiens (1); *A. gratissima* var. *gratissima* (2a); *A. gratissima* var. *sellowii* (2b); *A. oblanceolata* (3); *A. schulziana* (4).

Aguilar 1027 (1). **Ahumada** 8 (2a), 4216 (2b). **Alboff** sn1896 (2a). **Archer** 4678 (2a). **Arenas** 148 (3), 370 (3), 455 (2a), 1493 (4). **Argañarás** 61 (1). **Balegno** 1068 (1), 1410 (1), 1584 (2a). **Barkley** sn111249 (2a), 20–214 (2a). **Bartlett** 19543 (2a). **Bastián** 167 (2a). **Basualdo** 1354 (2a). **Bertoni** 761 (4), 3617 (2a), 98421 (2a). **Biurrum** 4002 (4). **Boelcke** 16005 (2a). **Brizuela** 386 (1), 437 (2a), 550 (2a), 882 (1) 1180 (2a). **Bruch** sn1908 (2a). **Buratovich** 701 (2a), 710 (2a), 941 (2a). **Burkart** 8078 (2a), 14209 (2b), 18002 (2a), 23800 (1), 27876 (2a), 29465 (2a), 30577 (2a). **Cabrera** 3046 (2a), 5461 (2a), 7209 (2a), 28331 (2a), 28634 (2a), 29751 (2a), 29845 (2a), 32345 (2b), 32798 (1). **Campos Novaes** 1950 (2a). **Cardenasianum** 637 (2a). **Carette** 3877 (2a). **Castellanos** 585 (1), 19615 (1), 33892 (1). **Cervi** 3871 (3). **Cordo** 149 (2a), 28452 (2a), 75375.2 (2b), 78-A-46 (4). **Correa** 37059 (2a). **Crespo** 26457 (2a). **Rodriguez, D.** 1175 (1). **De la Sota** 198 (1), 261 (2a), 447 (2a), 458 (2a), 580 (2a). DeLoach s.n. (2a). **Denham** 333 (2a). **Ekman** Sn 1907 (2a). **Eukontes** 534 (2b). **Fasanella** 9063 (1). **Felippone** 2867 (2b). **Fernandez Casas** 3500 (2a). **Fiebrig** 5904 (2b). **Fortunato** 5040 (2a), 5950 (2a). **Forzza** 1977 (2a). **Frenguelli** 19 (2a). **Garaventa** 7092 (2a). **García** 814 (1). **Golbach** 9 (2a). **Hagelund** 132-59 (2b). **Hassler** 1904 (2a), 1904B (2a), 6488 (2a), 11497 (3). **Hatschbach** 9339 (3), 16420 (2a), 35189 (3). **Hicken** 3537H (2a). **Huidobro** 3134 (1), 4565 (2a), 4886 (2a), 5284 (2a), 5457 (2a), 5551 (2a). **Hunziker** 1042 (2a), 8529 (2a). **Job** 573 (1), 835 (1), 1072 (1). **Joergensen** 2473 (2a), 3781 (2a). **Kiesling** 6670 (2a). **Krapovickas** 2597 (2a), 781 (1), 11743 (2a), 16138 (2a), 17543 (4), 17970 (2b), 19035 (2b), 19655 (3), 28539 (2a). **Kuntze** sn1892 (2a). **Lanfranchi** 1206 (1). **Legname** 6887 (2b), 9108 (2b). **Liberman** 1823 (2a). **Lichtenstein** 17475 (1). **Lillo** 3278 (2b), 7183 (2a), 32305 (1). **Lindman** 3637 (2a). **Lossen** 227 (1). **Lourteig** 874 (2a), 1037 (2a). **Maldonado** 206 (1). **Malvárez** 286 (2a), 1402 (2a),1431 (2a). **Marchesi** 6053 (2b), 10078 (2a). **Martinez Crovetto** 8956 (2b), 10638 (2a). **Medina** 240 (2b). **Meyer** 277 (1), 2671 (2a), 2944 (2a), 4289 (2a), 4867 (2a), 4868 (2b), 5066 (2a), 8590 (1), 9994 (2a), 10639 (2a), 10954 (2a), 11090 (2a), 11742 (2a), 11965 (2b), 12156 (2a), 12156 (2a), 23456 (2b). **Monetti** 1035 (2a). **Montes** 12 (2a), 599 (2b), 1410 (2b), 1864 (2b), 14841 (2b), 14911 (2b), 27627 (2a). **Morel** sn1948 (2a), 146 (2a), 379 (2a), 913 (1), 1257 (2b), 2049 (2a). **Morrone** 635 (2a). Nicora 4726 (2a). **Novara** 7260 (2b). **O'Donell** 3159 (2b), 4156 (2a), 4366 (2a), 5377 (2b). **Olea** 99 (2a). **Pedersen** 3703 (2a), 4089 (2a), 4186 (4), 15899 (2a). **Peirano** 32847 (2a), 32868 (2a). **Perez Moreau** 13577 (1). **Pierotti** 16 (1). **Pozzo** sn101946 (2a). **Pringle** 100 (2a)**,** 11666 (2a)**. Ragonese** s.n. BA 23993 (4), 9719 (2a), 23993 (4). **Reales** 625 (1). **Reitz** 8340 (2b). **Renvoize** 30 (2b), 2898 (2a), 3156 (2a), 3389 (2a). **Rodriguez** 66 (2a), 661 (2a), 891 (2a), 2059 (2a), 23848 (2a), 32320 (2a). **Rojas** 3278 (2b). **Rosengurt** 2245 (2a)**. Rotman** 296 (1). **Rúgolo** 1275 (2a)**. Ruiz** 422 (1) 1505 (2a) 25/2192 (2a). **Ruiz Leal** 1102 (2a), 1220 (2a), 1505 (2a), 9848 (2a), 10439 (1). **Saldias** 4404 (2a). **Saravia Toledo** 1752 (1), 10335 (2b). **Scavone** 15200 (2b). **Schinini** 9552 (3). **Schmitt** 10 (2b). **Schrottky** 66 (2b). **Schulz** 933 (1), 1128 (2a), 1492 (2a), 1493 (1), 6875 (2a), 8699 (4), 9001 (2a). **Schwarz** 763 (2b), 1687 (2b), 1924 (2a), 1952 (2b), 2312 (2b), 3233 (2b), 3334 (2b), 3742 (2a), 3803 (2b), 4444 (2b), 4561 (2a), 4610 (2a), 5536 (2b), 6398 (2a), 35439 (2a). **Schwindt** 110 (2b). **Seijo** 2655 (2a)**. Semper** 116 (2a), 339 (2a), 9848 (2a). **Serrano** 3529 (1). **Sesmero** 304 (2a). **Soria** 2098 (3). **Steinbach** 8248 (2a). **Toledo** 1752 (1). **Torrico** 163 (2a). **Troncoso** 1856 (4), 1860 (2b), 1930 (2a). **Ulibarri** 900 (2b), 1644 (4). **Varela** 675 (1). **Vargas** 35 (2a). **Vega** 847 (1). **Venturi** 834 (2b)**,** 5747 (2a), 7656 (2a). **Vervoorst** 498 (2b), 8642 (2a). **Villafañe** 342 (2a), 464 (2a), 499 (2a), 558 (2a), 693 (2a), 751 (2a). **Wall** sn28/1946 (2a). **Wolstenholme** 24 (2a)**. Woolston** 794 (2a). **Zardini** 59637 (2a)**. Zuloaga** 2687 (2b), 3738 (2a), 12912 (2b).