

## **Biscutella pseudolyrata (Brassicaceae, Biscutelleae), a new species endemic to NW Morocco based on morphological and molecular evidence**

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## *Biscutella pseudolyrata* (Brassicaceae, Biscutelleae), a new species endemic to NW Morocco based on morphological and molecular evidence

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**Abstract:** *Biscutella pseudolyrata* is described from the Atlantic coastal areas of NW Morocco, where several populations are known to occur in deep sandy soils at low elevation. It belongs to *B.* ser. *Biscutella* (*B.* ser. *Lyratae*, Brassicaceae) and is morphologically close to the Spanish endemic *B. lyrata* and the C Mediterranean *B. maritima*, two species to which it has sometimes been considered related and with which it shares a diploid chromosome number  $2n = 16$ . Re-evaluation of morphological characters in the light of phylogenetic trees from plastid (*rpl32-trnL* and *trnV*) and nuclear (ITS region) DNA sequence data support description of those Moroccan populations as a new species, which is phylogenetically closer to the W Mediterranean *B. boetica* but morphologically quite easy to distinguish from it. Data on morphology, ecology and distribution are reported, and similarities and differences with regard to other members of the series are discussed.

**Key words:** *Biscutella*, *Biscutella* ser. *Biscutella*, *Biscutelleae*, Brassicaceae, Cruciferae, molecular phylogeny, Morocco, new species, North African flora, plant morphology, taxonomy

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### Introduction

*Biscutella* L. (Brassicaceae) includes annual herbs or dwarf shrubs distributed throughout Europe, N Africa and SW Asia, with a centre of high diversity in the Mediterranean basin. The number of species attributed to that genus has varied notably, depending on different sources (cf. Candolle 1811; Jordan 1864; Malinowski 1911; Machatschki-Laurich 1926; Guinea 1964; Appel & Al-Shehbaz 2003; The Plant List 2010), but recent studies accept some 45–53 species (Warwick & Al-Shehbaz 2006; Marhold 2011+; Al-Shehbaz 2012), many of which are endemics with narrow distribution areas (Greuter & al. 1986; Marhold 2011+). *Biscutella* is the type of tribe *Biscutelleae* where other genera morphologically divergent such as *Heldreichia* Boiss., *Lunaria* L., *Megadenia* Maxim. and *Ricotia* L. are also placed (Özüdoğru & al. 2015, 2017).

Mostly annual species of *Biscutella* with petals gradually tapering at the base and lateral intrastaminal nectaries have been placed in *B.* ser. *Biscutella* (Malinowski 1911; Olowokudejo 1986a; Guinea & Heywood 1993), a group widely distributed in the Mediterranean basin and SW Asia (Machatschki-Laurich 1926; Maire 1967; Hedge 1968).

The relative uniformity of flower and fruit characters together with the wide variation in most of the vegetative features in *Biscutella* make the taxonomy of the genus highly complex (Olowokudejo 1985; Guinea & Heywood 1993), with contrasting taxonomic treatments available. This morphological plasticity is unequally distributed within *B.* ser. *Biscutella*, where a great range of morphological variation is found in N Africa, whereas a relative homogeneity can be found in Europe and SW Asia (cf. Vicente & al. 2016a). In particular, the variation observed in the NW African taxa of *Biscutella* with lyrate leaves is especially remarkable and their taxonomy is

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consequently complicated (Vicente & al. 2016a, 2017). In general terms, annual plants with lyrate to sublyrate leaves have been traditionally considered related to *B. lyrata* L. (*B. microcarpa* DC.) through a broad number of infraspecific taxa (Battandier & Trabut 1905; Maire 1967; Pottier-Alapetite 1979). More recently, Vicente & al. (2016a, 2017) have analysed such variation and suggested that part of these N African populations (mostly those from E Algeria and Tunisia) belong to either *B. raphanifolia* Poir. (annuals or short-lived perennials with well-developed, amplexicaul to auriculate cauline leaves and profusely paniculate inflorescences) or *B. maritima* Ten. (*B. lyrata* auct. non L.; annuals or short-lived perennials lacking well-developed cauline leaves, and with inflorescences of several rather dense racemes). In the NW areas of Morocco, remarkable populations can be found with pinnatifid to pinnatisect (apparently sublyrate) leaves but mostly lacking paniculate inflorescences or only presenting up to eight terminal racemes. Some authors (Grau 1999, 2002) have assigned them to the range of variation of *B. boetica* Boiss. & Reut. (for the correct spelling of this name see Vicente & al. 2016b: 294), a treatment that can be provisionally accepted until an ongoing deeper study on the whole *B. ser. Biscutella* is accomplished. As part of that research, and during field work in NW Morocco to clarify the identity of some conflicting members of the *B. boetica* aggregate, some populations of a plant with all leaves rosulate and mostly lyrate were found on deep sandy soils in oak forests near Rabat and Larache (El Araich), which at first glance showed some resemblance to both the S Iberian *B. lyrata* and the C Mediterranean *B. maritima*, and clearly differed from the neighbouring populations of *B. boetica*. A close examination of many individuals both in the field and in the laboratory revealed the existence of a unique combination of vegetative and reproductive characters not present in any of the described taxa of *B. ser. Biscutella*, which were later supported by molecular phylogenetic analyses based on DNA sequence data from two plastid (*rpl32-trnL* and *trnV*) and one nuclear (ITS) regions. Consequently, in the present contribution, based on morphological, molecular and ecological evidence the NW Moroccan plant is described as a new species, *B. pseudolyrata*.

## Material and methods

### Morphological study

Fresh material collected during field work in Morocco as well as herbarium specimens from ABH, B, BC, K, MA, MPU, P and SEV (herbarium codes according to Thiers 2019+) were used for morphological comparison. Over 200 herbarium specimens were visually examined, and both qualitative and quantitative analyses were conducted on 26 herbarium specimens (see below), mostly on well-developed, flowering and fruiting plants. The characters observed or measured were selected from those

typically used in the literature on the genus (Malinowski 1911; Guinea 1964; Maire 1967; Raffaelli 1991; Vicente & al. 2016a), together with those considered relevant according to our own experience. Selected characters and taxa studied from *Biscutella ser. Biscutella* with lyrate to pinnatisect leaves are shown in Table 1. Fruit measurements were taken only from mature silicles. Pedicel mean was calculated by measuring the first six basal fruits of the terminal panicle branches. Panicle density was obtained by calculating the number of fruits on the first 3–4 cm of the terminal branches, depending on the panicle length. ImageJ (Rasband 1997–2015) was used to measure these three characters in some specimens from P. For taxonomic identification and synonymy the main literature on the genus and the principal N African Floras were consulted (Cosson 1887; Battandier 1888; Jahandiez & Maire 1932; Quézel & Santa 1963; Maire 1967; Pottier-Alapetite 1979; Fennane & al. 1999; Le Floch & al. 2010). Nomenclatural aspects conform with the *Shenzhen Code* (Turland & al. 2018).

### Molecular analyses

The molecular analyses shown here are part of a broader study on *Biscutella ser. Biscutella*, currently underway (cf. Vicente & al. 2016a). Ten samples belonging to five species of *Biscutella* with lyrate to pinnatisect leaves (apparently sublyrate leaves, but with the terminal segment clearly lobate), were used for phylogenetic reconstruction, including those of the new species and using *Lepidium draba* L. (*Cardaria draba* (L.) Desv.) and *Megadenia speluncarum* Vorob. & al. (sensu Artyukova & al. 2014) as the outgroup. Plant source information and GenBank accession numbers are shown in Table 2.

The DNA extraction was made according to a modification of the 2× CTAB protocol (Doyle & Doyle 1987), from silica-gel-dried leaf material (Chase & Hill 1991) or voucher material. Total DNA was purified using MOBIO minicolumns and kept in 0.1× TE buffer. The study is based on one nrDNA internal transcribed spacer region (ITS) and two cpDNA regions, namely the *trp32-trnL* intergenic spacer and the *trnV* intron. The PCR amplifications of ITS were obtained using the primers ITS5/ITS4 (White & al. 1990); meanwhile the *rpl32-trnL* and *trnV* intron sequences were obtained using the primer pairs *rpl32F/trnL* (Shaw & al. 2007) and *trnV\_F/R* (Wang & al. 2003), respectively. The amplifications were performed on a reaction volume of 25 µl containing 22.5× ABGene 1.1× Master Mix 2.5 mM MgCl<sub>2</sub> (Thermo Scientific Waltham, MA, U.S.A.), 0.5 µl of 0.4% bovine serum albumin (BSA), 0.5 µl of each primer (10 pmol/µl) and 1 µl of template DNA, on a 9700 GeneAmp thermocycler (Applied Biosystems). The PCR programmes were, for ITS: 2 min at 95°, followed by 30 cycles of 95° for 1 min, 53° for 1 min, 72° for 2 min and a final extension of 72° for 5 min; for *rpl32-trnL*: 2 min at 94°, followed by 30 cycles of 94° for 1 min, 56° for 1.5 min,

Table 1. Morphological characters and *Biscutella* taxa studied.

Character	<i>B. raphanifolia</i> Poir. var. <i>raphanifolia</i>	<i>B. raphanifolia</i> var. <i>algeriensis</i> (Jord.) A. Vicente & al.	<i>B. maritima</i> Ten.	<i>B. pseudolyrata</i> A. Vicente, M. Á. Alonso & M. B. Crespo	<i>B. lyrata</i> L.	<i>B. boetica</i> Boiss. & Reut.
Raceme density (fruits/cm)	(1.5)–2–5	(1.8)–2–4.5	1.5–4.5(–6)	1.3–2.8	1.5–3.5	1.5–3(–3.5)
Pedice length (mean of first 5 fruits)	5.3–11	5.4–10.4	(5.5)–7–11	6.5–11	5–9	6.5–9 (–10)
Sepals length (mm)	1.8–3	1.4–2.6	(1.5)–2–3	1.8–3	1.3–2	1.5–2.5
Petals length (mm)	3–6	2.9–5(–6)	(3.5)–4–6.5	2.8–5(–6)	2–4	2.5–5
Median nectary length (mm)	inconspicuous or to 0.4	inconspicuous or to 0.4	0.5–0.7(–0.8)	0.4–0.5	inconspicuous or to 0.4	0.2–0.4
Stamen filament	not winged	not winged	not winged	not winged	broadly winged	not winged
Style length (mm)	2.6–4.5(–5)	2–3	2–3.5	(1.6)–1.9–2.9	1–2(–2.5)	1.8–3(–3.8)
Fruit width (mm)	(8.2)–9–15	4.5–9(–11)	(6)–7–13	(6.5)–7–11	4–6	(4.5)–5–10
Style length/fruit width ratio	0.27–0.4	0.28–0.45	(0.23)–0.25–0.37	0.22–0.33	0.3–0.4	(0.28)–0.3–0.45

Table 2. Plant material used in the molecular analyses.

Taxon	Label on Fig. 3	Locality	Voucher	GenBank accession no. <i>rpl32-trnL</i>	GenBank accession no. <i>trnY</i>	GenBank accession no. ITS
<i>Biscutella boetica</i> Boiss. & Reut.	<i>B. boetica</i> MO4	Morocco: Meknès, Azrou-Meknès road	ABH68336	MF521264	MF521299	MF521229
<i>Biscutella boetica</i>	<i>B. boetica</i> MO5	Morocco: Oujda, Beni Snassen	ABH68371	KU570219	KU574028	KU570209
<i>Biscutella lyrata</i> L.	<i>B. lyrata</i> ES	Spain: Cádiz: Alcalá de los Gazules	ABH70649	KU570211	KU574020	KU570201
<i>Biscutella maritima</i> Ten.	<i>B. maritima</i> IT2	Italy: Sicily, Cerami	ABH70575	MF521249	MF521284	MF521214
<i>Biscutella maritima</i>	<i>B. maritima</i> TN1	Tunisia: Nabeul, Takelsa, Cap Bon	MA797732	KU570214	KU574023	KU570204
<i>Biscutella pseudolyrata</i> A. Vicente, M. Á. Alonso & M. B. Crespo	<i>B. pseudolyrata</i> MO1	Morocco: Salé to Sidi Allal el Bahraoui (isotype)	ABH74994	MF521261	MF521296	MF521226
<i>Biscutella pseudolyrata</i>	<i>B. pseudolyrata</i> MO2	Morocco: Sidi Allal el Bahraoui to Kenitra	ABH75002	MF521262	MF521297	MF521227
<i>Biscutella pseudolyrata</i>	<i>B. pseudolyrata</i> MO3	Morocco: Larache to Ksar-el-Kebir	ABH74993	MF521263	MF521298	MF521228
<i>Biscutella raphanifolia</i> var. <i>algeriensis</i> (Jord.) A. Vicente & al.	<i>B. raph.</i> var. <i>algeriensis</i> DZ35	Algeria: Blida, Chrea National Park	ABH72641	KU570217	KU574026	KU570207
<i>Biscutella raphanifolia</i> Poir. var. <i>raphanifolia</i>	<i>B. raph.</i> var. <i>raphanifolia</i> DZ1	Algeria: Wilaya de Tizi-Ouzou	VAL33967	KU570218	KU574027	KU570208
<i>Lepidium draba</i> L.	<i>L. draba</i> ES	Spain: Alicante, San Vicente del Raspeig	ABH71952	KU746330	KU746332	KU746329
<i>Megadenia speluncarum</i> Vorob. & al.	<i>M. speluncarum</i> RU	Russia: Primorskii Krai, Lozovy	VLA10454	KX943557	KX943556	KX943555

72° for 10 min and a final extension of 72° for 10 min; for *trnV*: an initialization step of 3 min at 94°, followed by 42 cycles of 94° for 1 min, 62° for 1 min, 72° for 1.5 min and a final extension of 72° for 10 min.

Sequencher 4.1 (Gene Codes Corp., Ann Arbor, MI, U.S.A.) was used to assemble complementary strands. The three regions were aligned using Clustal W, conducted in MEGA X (v. 10.0.5) (Kumar & al. 2018) with minor manual adjustments to get the final aligned matrix. Three different data sets were produced, corresponding respectively to: (1) the combined plastid (two regions) data matrix; (2) the nuclear (ITS) data matrix; and (3) the combined molecular (plastid + nuclear) data matrix. When appropriate, the gaps were codified with FastGap 1.2 (Borchsenius 2009) according to the method of Simmons & Ochoterena (2000), and added to the DNA data matrix as separate partitions. The incongruence length difference (ILD) test (Farris & al. 1994) was implemented in PAUP v.4.0.b10 (Swofford 2002).

Maximum parsimony analyses (MP) were conducted in PAUP, using Branch and Bound search options with 10 000 replicates and MP support was assessed by 10 000 bootstrap replicates. Maximum Likelihood (ML) (Felsenstein 1981) and Neighbour-Joining (NJ) (Saitou & Nei 1987) analyses were performed in MEGA. Models with the lowest BIC (Bayesian Information Criterion) scores were considered to best describe the substitution pattern for the ML and NJ analyses. Furthermore, a Bayesian inference (BI) analysis was conducted with MrBayes 3.2 (Ronquist & al. 2012). Evolutionary distances for NJ and phylogenetic reconstructions for ML were estimated using the 3-parameter method of Tamura (1992) for all matrices excepting the ITS one, for which the 2-parameter method of Kimura (1980) was applied; the rate variation among sites was modelled with a Gamma distribution ( $G = 0.2009$ ), and partial deletion of gaps was applied in all cases (positions with less than 95% site coverage were eliminated). ML was conducted with the tree-searching strategy based on Nearest Neighbour Interchange (NNI). For BI analyses, the Markov chain Monte Carlo runs were performed for 10 million generations and sampled every 1000 generations. Two runs were executed. The general time reversible (GTR) + proportion of invariant sites (I) + gamma distribution (G) model was used in the analyses (set  $nst = 6$  rates =  $invgamma$ ). The first 25% generations ( $burnfrac = 0.25$ ) were excluded and the remaining trees were used to compile a posterior probability (PP) distribution using a 50% majority-rule consensus. For all methods, support was assessed by bootstrap (Felsenstein 1985) with 1000 replicates, but holding only 10 trees per replicate. Clades showing bootstrap (BS) values of 50%–74% were considered as weakly supported, 75%–89% moderately supported and 90%–100% strongly supported. Because our preliminary analyses of each matrix revealed that the resulting trees prior to and after gap inclusion were identical, gaps were therefore considered only in the MP analyses performed in PAUP, but they were disre-

garded in the ML and NJ analyses performed in MEGA due to their computing features.

## Results and Discussion

*Biscutella pseudolyrata* A. Vicente, M. Á. Alonso & M. B. Crespo, **sp. nov.**

Holotype: Morocco, Rabat-Salé-Zemmour-Zaër province, ctra. de Salé a Sidi Allal el Bahraoui, 29SQT239668 [34°01'04.7"N, 06°34'30.8"W], 178 m, bosque de *Quercus suber*, 6 May 2015, A. Vicente & M. Á. Alonso (ABH72445! [Fig. 1]; isotypes: ABH74994!, ABH75001!, MA01–00931693!).

*Diagnosis* — Planta speciosa *Biscutellae* seriei *Biscutellae* (= *B. seriei Lyratae*), a *B. lyrata* et *B. maritima* primo intuito aemulans sed ab eis singulari characterum combinatione bene diversa et facile distinguenda. A *B. lyrata* differt imprimis floribus siliculisque valde majoribus et staminum filamentis non membranaceo-dilatatis. A *B. maritima* tamen discrepat praecipue caulibus generaliter humilioribus; racemis fructiferis paniculae saepissimae laxioribus; petalis plerumque brevioribus; extrastaminalibus mediis nectariis brevioribus, non cylindricis.

*Description* — Annual plant, 25–45 cm tall. Stems 1–10 glabrescent to hirsute below. Basal leaves 4–25, in a rosette, to 12 × 3 cm, hirsute, lyrate with terminal lobe relatively entire and ovate, occasionally with very few or minute lateral lobes (acquiring spatulate to oblanceolate appearance); cauline leaves often absent (exceptionally 1, ± well developed, attenuate at base). Inflorescence a raceme or simple panicle (sometimes sparingly branched at base), usually with up to 8 terminal racemes, elongated and often loose in fruit, bearing 1.3–2.8 fruits/cm at base; pedicels patent to erect, first 5 basal ones 6–12 mm long. Sepals 1.8–3 mm long; petals 2.8–5(–6) mm long, gradually attenuate at base; stamen filaments filiform, not winged; extrastaminal median nectaries elongated, 0.4–0.6 mm, usually clavate. Silicles 3–6 mm long × 6.5–11 mm wide, flat to conspicuously swollen at margin, glabrous to hirsute, variously covered with clavate and/or tiny conical papillae; style (1.6–)1.9–2.9 mm long, with a style length/fruit width ratio of 0.22–0.33.

*Chromosome number* —  $2n = 16$  (cf. Vogt & Oberprieler 2009 as “*B. boetica*”; Morocco, Rharb, road S 216 between Arbaoua and Moulay Bousselham, B 10-1013203!).

*Distribution and ecology* — *Biscutella pseudolyrata* is endemic to the Atlantic coastal areas of NW Morocco, between Larache (El Araich) and Rabat (Fig. 2). It grows in ephemeral grasslands, disturbed ground and open *Quercus suber* L. woodlands, on deep siliceous sandy soils of Neogenic-Quaternary origin from the Gharb region, at 1–300 m elevation.





Fig. 1. Holotype of *Biscutella pseudolyrata* (ABH72445).



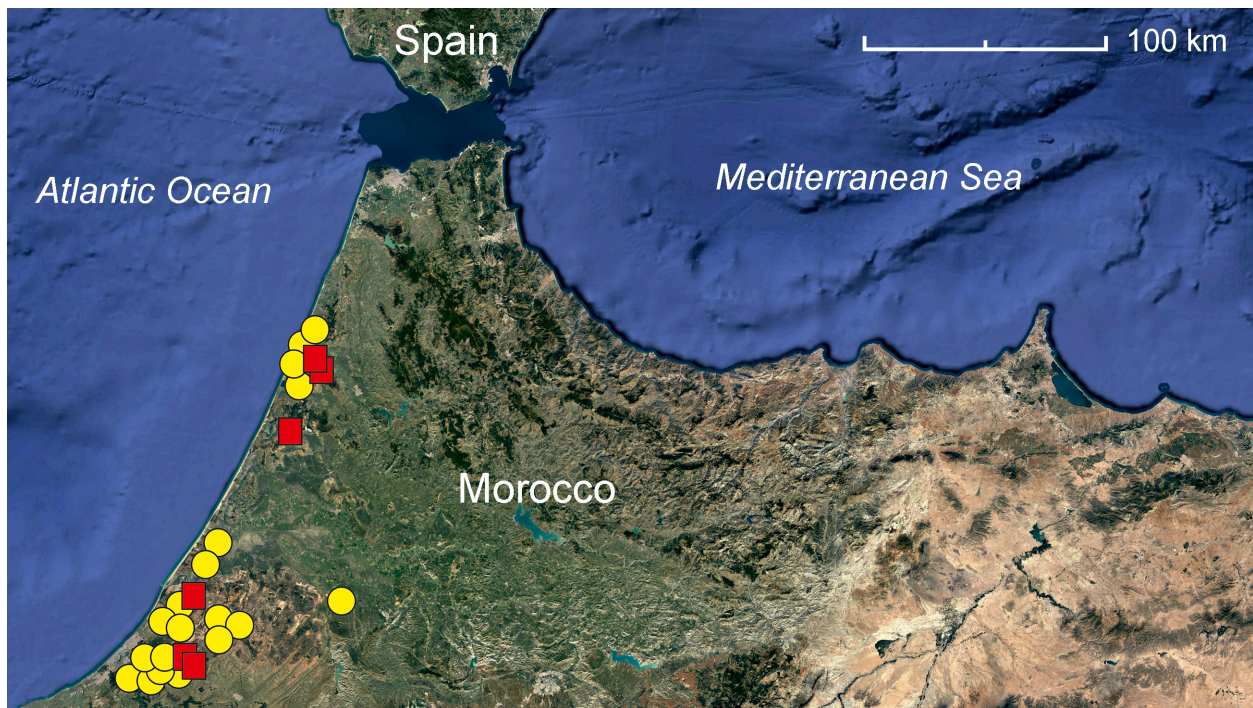


Fig. 2. Known distribution of *Biscutella pseudolyrata* (NW Morocco). Red squares indicate georeferenced collections; yellow circles are tentative locations from data on labels. Satellite image from Google Earth Pro (<https://www.google.com/earth/>).

*Additional specimens examined (paratypes)* — MOROCCO: Tanger-Tetouan-Al Hoceima region, Larache, Feb 1886, *M. Mellerio* (P05438254, P05438255); Larache, 1914, *Pérez Camarero* (BC05077); El Araix [Larache], 20 m, in arenosis, 16 Mar 1930, *Font Quer* 233 (MA44485); ibidem, *Font Quer* 234 (MA44486; MPU006773); Larache, ctra. de Larache a Ksar-el-Kebir, 29SQU615893, 19 m, 6 May 2015, *A. Vicente & M. Á. Alonso* (ABH74993, ABH74995); Rabat-Salé-Kénitra region, Gharb-Chrarda-Béni Hssen province, Ain Felfe, ctra. 4214, 29SQU534586, 25 m, sobre sustrato arenoso, 6 May 2015, *A. Vicente & M. Á. Alonso* (ABH74997); Rharb [Gharb], road S 216 between Arbaoua and Moulay Bousselham, c. 3.4 km W of junction with road to Lalla-Rhano and Ksar-el-Kebir, 10 m, ungrazed field margin, 34°51'N, 06°10'W, 24 Apr 1993, *Vogt 10190 & Oberprieler 4638* (B 10 1013203 [digital image!]); Mamora forest, May 1888 (K); Forêt de Mamora, 4 Apr 1888, *Grant* (P05438793); in planitie Gharb, in silva Mamora, 22 Apr 1925 (P05438224); Mamora, [Camp] Monod, 10 Feb 1939, *G. L'Hermite* (P00898612); Kenitra, Rabat, Mamora forest, Feb 1930, *A. W. Trethewy* (K); Kenitra, Ma'mora, 12 km from Rabat on road to Meknès, Forêt de la Mamora, 34°02'N, 06°42'W, 80 m, *Quercus suber* forest, 9 Jun 1992, *B. Valdés & al.* 01-0032 (B 10 0298334); Kenitra, Mar 1931, *A. W. Trethewy* (K); Sidi Sliman, 60 km from Meknes, 1936, *A. W. Trethewy* (K); Kenitra, plantation d'agrumes au N de la ville, sol sableux, 14 Feb 1974, *J. Lewalle* 7438 (MA268268, P04657216, P04743559); région de Rabat, bois du Souissi, aux environs immédiats de Rabat, sur sol sableux, 15 Dec 1966, *J.*

*Veilex* (MA802415, P04657219, P04718125, P04745864, P05432970); Rabat, Forêt des Sers [Zaërs?], *G. L'Hermite* (P00898557); ctra. de Sidi Allal el Bahraoui a Kenitra, 29SQT235881, 78 m, *Quercus suber* forest, 6 May 2015, *A. Vicente & M. Á. Alonso* (ABH75002).

#### *Phylogenetic and taxonomic discussion*

Preliminary analyses of the three individual matrices (namely the plastid data set, ITS data set, and combined plastid+ITS data set) yielded trees (not shown) with similar topologies, both the plastid and combined molecular ones exhibiting the same topology and almost identical support in most branches. Conversely, the ITS tree (not shown) slightly differed by a lower support in most branches and the unresolved position or very weak support of some others (see below). Bayesian PP and parsimony BS values were well correlated in all three cases (data not shown). Application of the Incongruence Length Difference test (ILD) suggested the existence of slight incongruence between data sets ( $P = 0.01$ ). Nevertheless, as all obtained phylogenies did not show at first sight strong differences in their topologies, and also because some authors (Barker & Lutzoni 2002) argued that combining heterogeneous data can also increase accuracy even if ILD analyses do not explicitly incorporate that heterogeneity, we accept the combined phylogeny as a good reconstruction of the evolutionary history of the studied group, according to our previous results (Vicente & al. 2016a).

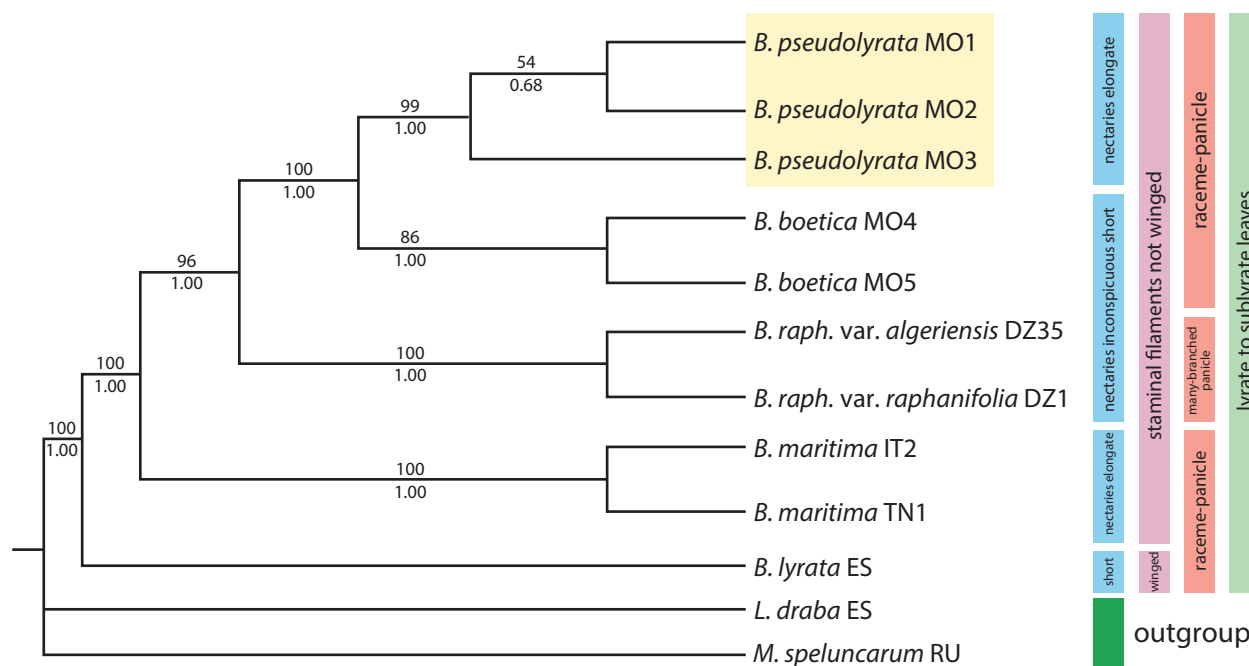


Fig. 3. Phylogenetic tree estimated using a combination of cpDNA (*rpl32-trnL* and *trnV*) and nrDNA (ITS) sequences, showing the position of *Biscutella pseudolyrata*. Bootstrap values (BS) and Bayesian posterior probability (PP) are shown respectively above and below branches. Taxon names and codes refer to the material listed in Table 2.

Analyses using MP, ML, NJ and BI methods yielded trees with identical topologies and similar bootstrap and branch-length values. Combination of all plastid and nuclear regions (combined molecular matrix), together with gaps codification, generated a matrix of 2679 characters, of which 2149 were constant, 315 parsimony-uninformative and 215 were parsimony-informative. In the MP analysis, two most parsimonious trees were obtained with a tree length (TL) of 681, a consistency index (CI) of 0.847 and a retention index (RI) of 0.757. In Fig. 3, the phylogenetic relationships of taxa of *Biscutella* are shown as recovered in our BI consensus tree of the combined molecular matrix, in which PP values are placed below branches and BS percentages above branches (respectively, from the BI and MP analyses).

The topology of the tree in Fig. 3, like in the trees resulting from our all combined analyses, is identical to that reported by Vicente & al. (2016a) for a similar set of taxa, but shows even higher support for most branches. All three accessions of *Biscutella pseudolyrata* form a strongly supported group (99 BS, 1.0 PP), not well resolved internally, which is sister to *B. boetica* (100 BS, 1.0 PP), this clade being successively sister to *B. raphanifolia* (96 BS, 1.0 PP), *B. maritima* (100 BS, 1.0 PP) and *B. lyrata* (100 BS, 1.0 PP) with a strong support in all cases. Among the rest of the analyses performed, only the ITS tree differed in the position of the *B. raphanifolia* clade, which was sister to the *B. maritima* one with moderate support (79 BS, 0.95 PP), and they both moderately sister to the *B. boetica*–*B. pseudolyrata* clade (79 BS, 0.92 PP). Furthermore, in this latter clade the *B. pseudolyrata* accessions did not form a well-supported

subclade (– BS, 0.59 PP) in the ITS tree, and their relationships remained unresolved.

The obtained phylogenetic connections are apparently contradictory with morphological characteristics of the studied taxa, but can be understood in the light of biogeographical and ecological data. Populations of *Biscutella pseudolyrata* are well characterized by a unique combination of morphological features not present in any described taxon of *B. ser. Biscutella* (Table 1). All studied specimens showed stems medium-sized with regard to the remaining taxa of the series; basal leaves in a rosette, lyrate or occasionally spatulate to oblanceolate after reduction or lack of lateral lobes (Fig. 4A), and cauline leaves mostly absent or rarely only one well developed at the basal part of the stems; inflorescence a raceme or simple panicle (sometimes sparingly branched at base), usually with up to 8 terminal racemes, elongated and loose in fruit; flowers relatively large; stamen filaments filiform; median nectaries elongated and usually clavate (Fig. 4B); silicles medium-sized, with various indumentum and a long style. Leaf size, however, was very variable even within a single population, like in other related species (cf. Raffaelli 1991; Vicente & al. 2016a), and no special indumentum types were observed with regard to other members of the series, which makes those characters have little value for taxonomic purposes (cf. Olowokudejo 1992).

At first glance, plants of the newly described species resemble morphologically both *Biscutella lyrata* and *B. maritima* on account of the general habit with leaves in a dense rosette, lacking well-developed cauline leaves, and the long, loose inflorescences. However, *B. lyrata* is nar-





Fig. 4. *Biscutella pseudolyrata* – A: leaf variation (1: MA44485; 2: ABH74993; 3: ABH74995; 4: ABH75002; 5: ABH72445; 6: ABH74995; 7: P04657219); scale bars = 2 cm. – B: morphology of extrastaminal nectaries, with arrows pointing at nectaries; B1: general view of flower base, from ABH75002; B2: detail of the nectaries, from ABH74993.

rowly endemic to S Spain (Cádiz province, not confirmed in Huelva and Málaga; cf. Grau & Klingenberg 1993), with  $2n = 12$  chromosomes (cf. Olowokudejo & Heywood 1984 as “*B. microcarpa* DC.”), and is easy to recognize on the basis of its small flowers with short petals (2–4 mm), outer stamens with broadly winged filaments, and very small silicles (2–3 × 4–6 mm) with short style (1–2.5 mm) arranged in long and very loose racemes, features that are unique in *B. ser. Biscutella* (Vicente & al. 2016a). Similarly, *B. pseudolyrata* is also morphologically close to *B. maritima*, a C Mediterranean species (W and S Italy, Sicily, N Tunisia and NE Algeria; cf. Vicente & al. 2017) with which it shares the same chromosome number ( $2n = 16$ ; cf. Olowokudejo & Heywood 1984 as “*B. lyrata* L.”), but the latter clearly differs by its taller stems (up to 120 cm), and broad inflorescences paniculately branched with denser racemes, bearing flowers with cylindrical, longer (to 0.8 mm) nectaries, among other characters (Table 1).

However, the described morphological affinities with both cited taxa are not confirmed in our molecular analyses, in which *Biscutella pseudolyrata* is sister to *B. boetica*. As suggested by Grau (1999), *B. boetica* (sensu lato) seems to have its centre of diversity in N Africa on account of the broad morphological variation it displays in its whole distribution area. Although that species was described as producing regularly dentate leaves (Boissier 1854), with stems bearing up to 5 well-developed ones in

the basal part, in the natural populations near the type locality (Málaga province, S Spain), as well as more generally in S Spain and N Africa, a wide range of morphological variation can be observed even in a single population, with plants bearing dentate leaves occurring together with others producing pinnatifid to pinnatisect or sublyrate leaves (as, for instance, in ABH70653, ABH70655, ABH70821, ABH70822 and ABH70948). Infructescence racemes typically are elongated (often covering up to half the total length of stems) and loose, bearing 1.5–3(–3.5) fruits/cm at the base, and the flowers show short median nectaries (0.2–0.4 mm). Although some characteristics are similar in both plants (e.g. sepal and petal size, infructescence density or fruit features and indumentum; Table 1), the broad leaf variation in *B. boetica* does not include in any case the characteristic lyrate leaves found in *B. pseudolyrata*, even though in the latter species they can occasionally be spatulate to oblanceolate in a single population or individual after reduction or lack of lateral lobes (see, for instance, P04657216 and P04743559). A similar morphological variation pattern of leaves had also been described previously for other close relatives such as *B. maritima* (Raffaelli 1991) and *B. raphanifolia* (Vicente & al. 2016a). Furthermore, *B. pseudolyrata* often lacks cauline leaves, and median nectaries are constantly longer (up to 0.6 mm) than in *B. boetica*. It is worth mentioning that the size and shape of nectaries are revealed as taxonomically diagnostic in some taxa of the series

(Olowokudejo 1986b), mostly when combined with leaf morphology, infructescence structure and fruit features (cf. Vicente & al. 2016a). It should be noted that *B. pseudolyrata* was sometimes identified as “*B. didyma* f. *scabrida* Pau & Font Quer” (see collections *Font Quer* 233, MA44485; *Font Quer* 234, MA44486), a name sometimes applied to *B. boetica* (cf. Machatschki-Laurich 1926; Maire 1967). Similarly, Grau (1999) commented that some populations from the Rif area that he identified as *B. boetica* closely resembled *B. lyrata*, although they always produced stamens with filiform filaments. Most probably these populations indeed belonged to the newly described species. According to the above mentioned data, *B. pseudolyrata* and *B. boetica* are treated here as different at specific rank, despite their also sharing the same chromosome number ( $2n = 16$ ; Olowokudejo & Heywood 1984) and occupying a similar geographic range in the Atlantic coastal areas of NW Morocco. Both species were previously treated by North African authors in diverse ways. On the one hand and following Maire’s (1967) treatment, plants belonging to *B. pseudolyrata* would fit with the concept of “*B. didyma* subsp. *lyrata* (L.) Nyman”, a name that Maire used in the sense of the current *B. maritima*. Similarly, plants belonging to the morphologically variable *B. boetica* would match several varieties and forms within “*B. didyma* subsp. *apula* Nyman”. On the other hand, according to the treatment of Fennane & al. (1999), both *B. pseudolyrata* and *B. boetica* would fit with the concept of “*B. didyma*”, a species regarded as quite polymorphic by those latter authors. Indeed, it is worth mentioning that Maire’s (1967) analytic arrangement of *B. didyma*, including 30 infraspecific taxa (3 subspecies, 20 varieties and 7 forms), was based on characters such as the indumentum and size of silicles, the colour and scent of flowers, and the density of racemes, which are extremely variable even within a single population. This resulted in a confusing arrangement in which most taxa are poorly defined and difficult to delimit in both fresh or dried specimens. Therefore, according to Fennane & al. (1999), that unrealistic arrangement is not followed here.

Diversification of the “*Biscutella boetica*–*B. pseudolyrata* clade” should probably respond to ecological specialization in distinct types of substrates. All studied populations of *B. pseudolyrata* occur in the Gharb (Rharb) region, a Neogenic-Quaternary lowland basin placed between Larache and Rabat (Fig. 2), which corresponds to the external plains of the Western Rif mountains, also called the Pre-Rif area (Piqué 1994). In that basin, the new species is constantly found on red substrates in which sand is an important constituent of the soil matrix (Laouina 2013). Those peculiar red sandy soils are variable in depth, being up to 20 m deep (Bagaram 2014), and come from oblique lixiviation of red clayish substrates (De Beaucorps 1956). It is important to note that a number of narrow Moroccan endemics are also found mostly in this area, such as *Anthemis*

*gharbensis* Oberpr., *Asphodelus gracilis* Braun-Blanq. & Maire, *Centaureum erythraea* subsp. *bernardii* (Maire & Sauvage) Greuter, *Elizaldia heterostemon* (Murb.) I. M. Johnst., *Gaudinia valdesii* Romero Zarco, *Linaria arenicola* Pau & Font Quer, *Micropyrum mamoraem* (Maire) Stace (*Catapodium mamoraem* (Maire) Maire & Weiller) and *Pyrus communis* subsp. *mamorensis* (Trab.) Maire among others (cf. Valdés & al. 2002), to which *B. pseudolyrata* should be added. Gharb, including the Mamora plains, is therefore considered as an important area for plant conservation (Radford & al. 2011). Conversely, populations of *B. boetica* usually are found in a variety of substrates, often shallow or stony schistose sandy soils, geologically quite different from the above-described Neogenic-Quaternary soils from the Gharb region.

Morphological similarities of *Biscutella pseudolyrata* to other lyrate-leaved members of *B. ser. Biscutella* are to be regarded as the result of convergence from different lineages. The clade formed by *B. raphanifolia* (incl. *B. algeriensis* Jord.) is easy to recognize by the stems sometimes being perennial, thickened, with 1–4, well-developed, broad leaves, amplexicaul to auriculate at base; infructescence profusely paniculate with denser racemes bearing (1.5–)2–5 fruits/cm at base, which tend to be larger, and nectaries inconspicuous or up to 0.4 mm long (cf. Vicente & al. 2016a). However, some resemblances exist to *B. pseudolyrata* regarding the leaf morphology, flower size and infructescence features (Table 1). These and other morphological connections to the remaining sister lineages, such as *B. lyrata* and *B. maritima* (as already discussed above), are more likely due to convergent evolution in every case.

The description of the new species, *Biscutella pseudolyrata*, once more supports discarding any close phylogenetic relationship between the true *B. lyrata* (which is a narrow endemic of S Spain) and the other N African members with lyrate or pinnatisect leaves (cf. Vicente & al. 2016a), which was historically assumed to be probable by many authors (cf. Maire 1967, as *B. scutulata* Boiss.; Fennane & al. 1999, as *B. microcarpa* DC.). It also helps to disentangle the traditionally complex taxonomy of *B. ser. Biscutella* in the W Mediterranean basin.

#### Key to the species of *Biscutella ser. Biscutella*

1. Staminal filaments with wide membranous wing; silicles 2–3.2 × 4–6 mm . . . . . ***B. lyrata***  
– Staminal filaments filiform, unwinged; silicles usually larger . . . . . **2**
2. Rosette leaves mostly oblanceolate, dentate or occasionally pinnatisect . . . . . **3**  
– Rosette leaves lyrate (occasionally lowermost dentate, and then intrastaminal nectaries ≥ 0.4 mm long) . . . . . **4**

3. Inflorescence dense, with 2.5–9 flowers per cm; intrastaminal median nectaries to 0.2 mm long; fruit 7–13.5 mm wide; style length/fruit width ratio < 0.25 ..... *B. didyma*  
 – Inflorescence usually loose, with 1.5–4 (rarely up to 8) flowers per cm; intrastaminal median nectaries 0.2–0.4 mm long; fruit 4.5–10 mm wide; style length/fruit width ratio  $\geq$  0.25 ..... *B. boetica*
4. Cauline leaves well developed (occasionally uppermost ones bract-like); infructescence a profusely branching panicle, with 8–30 terminal racemes per branch; median nectaries inconspicuous to 0.4 mm long ..... *B. raphanifolia*  
 – Cauline leaves absent or bract-like (occasionally lowermost ones well developed); infructescence a raceme to simple panicle, usually with 2–8 terminal racemes per branch; median nectaries 0.4–0.8 mm long ... **5**
5. Stem to 120 cm tall; median nectaries 0.5–0.8 mm long, usually cylindrical; racemes bearing 1.5–4.5(–6) fruits per cm at base ..... *B. maritima*  
 – Stem to 45 cm tall; median nectaries 0.4–0.5 mm long, usually clavate; racemes bearing 1.3–2.8 fruits per cm at base ..... *B. pseudolyrata*

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