BioOne DIGITAL LIBRARY

Taxonomic circumscription of the N African endemic Biscutella raphanifolia (Brassicaceae) based on morphological and molecular characters

Authors: Vicente, Alicia, Alonso, Ma Ángeles, and Crespo, Manuel B.

Source: Willdenowia, 46(3) : 411-422

Published By: Botanic Garden and Botanical Museum Berlin (BGBM)

URL: https://doi.org/10.3372/wi.46.46309

The BioOne Digital Library (https://bioone.org/) provides worldwide distribution for more than 580 journals and eBooks from BioOne's community of over 150 nonprofit societies, research institutions, and university presses in the biological, ecological, and environmental sciences. The BioOne Digital Library encompasses the flagship aggregation BioOne Complete (https://bioone.org/subscribe), the BioOne Complete Archive (https://bioone.org/archive), and the BioOne eBooks program offerings ESA eBook Collection (https://bioone.org/esa-ebooks) and CSIRO Publishing BioSelect Collection (https://bioone.org/csiro-ebooks).

Your use of this PDF, the BioOne Digital Library, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Digital Library content is strictly limited to personal, educational, and non-commmercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne is an innovative nonprofit that sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Downloaded From: https://complete.bioone.org/journals/Willdenowia on 19 May 2025
Terms of Use: https://complete.bioone.org/terms-of-use

Willdenowia
Annals of the Botanic Garden and Botanical Museum Berlin-Dahlem

ALICIA VICENTE[1*], Mª ÁNGELES ALONSO[1] & MANUEL B. CRESPO[1]

Taxonomic circumscription of the N African endemic *Biscutella raphanifolia* (*Brassicaceae*) based on morphological and molecular characters

Version of record first published online on 21 November 2016 ahead of inclusion in December 2016 issue.

Abstract: *Biscutella raphanifolia* is a species distributed in N Algeria and Tunisia, characterized by the presence of large, lyrate basal leaves, amplexicaul cauline leaves, and paniculate-branched inflorescences. It has been traditionally considered the only perennial taxon from *B.* sect. *Biscutella*, lifespan being the strongest argument used to differentiate *B. raphanifolia* from other annual taxa such as *B. algeriensis*, *B. confusa* or *B. didyma* var. *coriophora*, with which it shares both morphological characters and distribution. Reevaluation of morphological characters in the light of phylogenetic trees from plastid (*rpl32-trnL* and *trnV*) and nuclear (ITS region) sequence data support a new circumscription of *B. raphanifolia* to include all those annual taxa. As a result, the new combination *B. raphanifolia* var. *algeriensis* is established, descriptions are provided for both varieties, and synonyms and lectotypes are indicated or designated for the names concerned.

Key words: Algeria, *Biscutella*, *Brassicaceae*, *Cruciferae*, molecular phylogeny, North Africa, plant morphology, taxonomy, Tunisia, new combination, lectotype

Article history: Received 20 April 2016; peer-review completed 26 September 2016; received in revised form 11 October 2016; accepted for publication 17 October 2016.

Citation: Vicente A., Alonso M. A. & Crespo M. B. 2016: Taxonomic circumscription of the N African endemic *Biscutella raphanifolia* (*Brassicaceae*) based on morphological and molecular characters. – Willdenowia 46: 411–422. doi: http://dx.doi.org/10.3372/wi.46.46309

Introduction

The genus *Biscutella* L. includes annual herbs or dwarf shrubs distributed throughout Europe, N Africa and SW Asia, with a centre of high diversity in the Mediterranean basin. It is placed in tribe *Biscutelleae* together with *Lunaria* L., *Megadenia* Maxim. and *Ricotia* L. (Özüdoğru & al. 2015). The number of species attributed to *Biscutella* 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 more 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 (Marhold 2011+).

Two main groups, morphologically well differentiated, are recognized in the genus, to which sectional rank is usually applied (cf. Raffaelli 1992; Guinea & Heywood 1993): *Biscutella* sect. *Biscutella* and *B.* sect. *Jondraba* (Medik.) Rchb. (≡ *Jondraba* Medik.). In the former, *Biscutella* ser. *Biscutella* (= *B.* ser. *Lyratae* Malin.) comprises annual or rarely perennial species with petals gradually tapering at the base and lateral intrastaminal nectaries (Malinowski 1911; Olowokudejo 1986a; Guinea & Heywood 1993); they are distributed in the Mediterranean basin and SW Asia (Machatschki-Laurich 1926; Maire 1967; Hedge 1968).

The wide variation in most of the vegetative features and the relative uniformity of flower and fruit characters in *Biscutella* make the taxonomy of the genus highly complex (Olowokudejo 1985; Guinea & Heywood 1993),

---

[1] Depto. de Ciencias Ambientales y Recursos Naturales (dCARN) & CIBIO (Instituto de la Biodiversidad), Apartado 99, ES-03080 Alicante, Spain; *e-mail: alicia.vicente@ua.es (author for correspondence).

Downloaded From: https://complete.bioone.org/journals/Willdenowia on 19 May 2025
Terms of Use: https://complete.bioone.org/terms-of-use

with contrasting taxonomic treatments available. This morphological plasticity is unequally distributed within *B.* sect. *Biscutella*, where a relative homogeneity can be found in Europe and SW Asia, whereas a great range of morphological variation is found in N Africa.

The morphological variation of the N African taxa of *Biscutella* with lyrate leaves is especially remarkable and their taxonomy is consequently complicated. In general terms, *B. raphanifolia* Poir. (Poiret 1789: 198) is considered the only perennial taxon in the section, whereas the annual N African plants have been traditionally related to *B. lyrata* L. due to leaf morphology and life span (Battandier & Trabut 1905; Maire 1967; Pottier-Alapetite 1979). Among these annual taxa, one shares many characters and its distribution area with *B. raphanifolia*, the paniculate inflorescence being the most outstanding diagnostic feature. The name better applying to this annual taxon is *B. algeriensis* Jord. (Jordan 1864: 318), which is known to occur from the surroundings of Alger (Algiers) to the border with Tunisia. Beyond the W limit of the distribution of *B. algeriensis*, populations can be found with pinnatifid to pinnatisect leaves, but mostly lacking paniculate inflorescences or only presenting up to eight terminal racemes. They have been recently assigned by some authors (Grau 1999) to the range of variation of *B. boetica* Boiss. & Reut. By now, this seems to be a suitable treatment for these populations, even though a deeper study of that taxonomic complex is currently underway.

The protologue of *Biscutella raphanifolia* describes a large plant over 75 cm tall, showing amplexicaul cauline leaves, paniculate inflorescence and glabrous silicles (Poiret 1789). Distinction of *B. raphanifolia* from *B. lyrata* was established by Poiret based on the smaller size, the non-branched stem and the pilose silicles of the latter, but no specific mention was made to life span. However, Poiret included "*Thlaspi bifurcatum raphani aut irionis folio* (Bocc. fic. 45, t. 23)" in synonymy, a polynomial that, according to Boccone's publication (Boccone 1674), applies to an annual plant from Sicily that corresponds to *B. maritima* Ten. (cf. Raffaelli 1991).

Later, Cosson (1873) noted the existence of these two different entities in Poiret's protologue: on the one hand the perennial plants described in the protologue, which he named *Biscutella radicata* Coss. & Durieu, and on the other hand the annual plants, which he first named *B. didyma* var. *lyrata* (L.) Coss. (including *B. raphanifolia*) and later (Cosson 1887) *B. didyma* var. *raphanifolia* (Poir.) Coss. However, as Raffaelli (1985) stated, *B. radicata* must be synonymized under *B. raphanifolia*. From then onwards, *B. raphanifolia* has been generally circumscribed to include perennial individuals completely fitting Poiret's protologue, whereas the smaller plants with lyrate leaves, distributed in the same geographical areas, have received quite different treatments in the principal local floras (Battandier 1888; Maire 1967; Pottier-Alapetite 1979).

Biscutella algeriensis was described by Jordan (1864) on the basis of one specimen received from Revelière, which was collected in the surroundings of Alger. According to Jordan's description, *B algeriensis* is a plant 30–40 cm tall; lower leaves sublyrate, obovate upwards; upper leaves attenuate to semiamplexicaul; stem many times branched in the inflorescence; and silicles 4–5 × 8–9 mm, ciliate in the central part and on the margin, and totally covered with very thin pubescence. Despite the traditionally suspected relationship of this taxon with *B. lyrata*, some remarkable morphological features point to a possible connection with *B. raphanifolia*. The aim of the present work is to study the taxonomic circumscription of *B. raphanifolia* and to explore the eventual relationships with *B. algeriensis*, by combining both morphological and molecular phylogenetic analyses based on sequence data from two plastid (*rpl*32-*trnL* and *trnV*) and one nuclear (ITS) regions.

Material and methods

Morphological study

Fresh material collected during field work in Algeria as well as herbarium specimens from ABH, B, BC, K, MA, P, SEV and VAL (acronyms according to Thiers 2016+) were used for morphological examination. Over 200 herbarium specimens were visually examined, and both qualitative and quantitative analyses were conducted on over 120 specimens (listed in the Appendix), 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 (Poiret 1789; Jordan 1864; Malinowski 1911; Guinea 1964; Maire 1967; Raffaelli 1985), together with those considered relevant according to our own experience. Selected characters 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 (Battandier 1888; Quézel & Santa 1963; Maire 1967; Pottier-Alapetite 1979; Guinea & Heywood 1993; Le Floc'h & al. 2010).

Molecular analyses

The molecular analyses shown here belong to a broader study on *Biscutella* sect. *Biscutella*, currently underway. Ten samples belonging to five species of *Biscutella* were used for phylogenetic reconstruction; the selection was made among taxa of *B.* ser. *Biscutella* that share leaf mor-

Downloaded From: https://complete.bioone.org/journals/Willdenowia on 19 May 2025
Terms of Use: https://complete.bioone.org/terms-of-use

Table 1. Characters studied. Results are given for both extremes of variation.

Character	***Biscutella raphanifolia*** var. ***raphanifolia***	***Biscutella raphanifolia*** var. ***algeriensis***
Life span	perennial (or biennial)	annual (or biennial)
Stem length	30–100 cm	23–40 cm
Stem indumentum	hirsute to lanate at base	hirsute at base
Leaf indumentum	hirsute to lanate	hirsute
Basal leaf shape	lyrate-pinnatipartite	lyrate-pinnatipartite
Basal leaf size	to 20 × 6 cm	to 8.5 × 3.5 cm
Insertion of cauline leaves	amplexicaul to auriculate	amplexicaul to attenuate
Inflorescence branching pattern	profusely branched panicle	profusely branched panicle
Pedicel insertion	erecto-patent	erecto-patent
Sepal length	1.8–3 mm	1.4–2.6 mm
Petal length	3–6 mm	2.9–4.6 mm
Nectary length	to 0.4 mm	to 0.4 mm
Median nectary morphology	inconspicuous or elongated	inconspicuous or elongated
Panicle density (fruits/cm)	(1.5–)2–5	(1.8–)2–4.5
Silicle size	(4.5–)5–8 × (8.2–)9–15 mm	2.5–6 × 4.5–9(–11) mm
Silicle indumentum	mostly glabrous, occasionally covered with clavate and/or tiny, conical trichomes	mostly covered with both clavate and conical trichomes, occasionally glabrous

phology and distribution with *B. raphanifolia* (namely *B. boetica* and *B. maritima*) and those to which it has been traditionally related (namely *B. didyma* L. and *B. lyrata*). *Lepidium draba* L. (*Cardaria draba* (L.) Desv.) and *Megadenia speluncarum* Vorob. & al. (sensu Artyukova & al. 2014) were used as the outgroup. The studied specimens of *B. boetica*, which showed pinnatifid to pinnatisect leaves (apparently sublyrate, but with the terminal segment clearly lobate), were collected from the E Moroccan border of their distribution area. 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 & Hills 1991) or herbarium specimens. Total DNA was purified using MOBIO minicolums and kept in 0.1× TE buffer. The study is based on one nrDNA internal transcribed spacer region (ITS) and the cpDNA regions *rpl32-trnL* intergenic spacer and *trnV* intron. The PCR amplifications of ITS were obtained using the primers ITS5/ITS4 (White & al. 1990), while *rpl32-trnL* and *trnV* intron sequences were obtained using the primer pairs *rpl32*F/*trnL* (Shaw & al. 2007) and *trnV*_F/R (Wang & al. 2003), respectively. The ampli-

Table 2. Materials used in the molecular analysis.

Label in Fig. 3–5	Taxon	Locality	Voucher	GenBank acc. no. *rpl32-trnL*	GenBank acc. no. *trnV*	GenBank acc. no. ITS
L. draba ES	*Lepidium draba* L.	Spain: Alicante	ABH71952	KU746330	KU746332	KU746329
M. speluncarum RU	*Megadenia speluncarum* Vorob. & al.	Russia: Primorskii Krai, Lozovy	VLA10454	KX943557	KX943556	KX943555
B. lyrata ES	*Biscutella lyrata* L.	Spain: Cádiz	ABH70649	KU570211	KU574020	KU570201
B. didyma GR	*B. didyma* L.	Greece: Sounion, temple of Poseidon	ABH58628	KU570212	KU574021	KU570202
B. didyma IT	*B. didyma*	Italy: Martina Franca	ABH70559	KU570213	KU574022	KU570203
B. maritima TN1	*B. maritima* Ten.	Tunisia: Nabeul	MA797732	KU570214	KU574023	KU570204
B. maritima TN2	*B. maritima*	Tunisia: Rohnia	MA724301	KU570215	KU574024	KU570205
B. raph. var. *algeriensis* DZ29	*B. raphanifolia* var. *algeriensis* (Jord.) A. Vicente & al.	Algerie: Berrouaghia	ABH72640	KU570216	KU574025	KU570206
B. raph. var. *algeriensis* DZ35	*B. raphanifolia* var. *algeriensis*	Algerie: Blida, Chrea National Park	ABH72641	KU570217	KU574026	KU570207
B. raph. var. *raphanifolia* DZ1	*B. raphanifolia* Poir. var. *raphanifolia*	Algerie: Wilaya de Tizi-Ouzou	VAL33967	KU570218	KU574027	KU570208
B. boetica MO	*B. boetica* Boiss. & Reut.	Morocco: Oujda, Beni Snassen	ABH68371	KU570219	KU574028	KU570209
B. boetica DZ	*B. boetica*	Algerie: Tlemcem	ABH69561	KU570220	KU574029	KU570210

Downloaded From: https://complete.bioone.org/journals/Willdenowia on 19 May 2025
Terms of Use: https://complete.bioone.org/terms-of-use

fications were performed on a reaction volume of 25 µl containing 22.5 µl of ABGene 1.1× Master Mix, 2.5 mM $MgCl_2$ (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 GeneAmpl thermocycler (Applied Biosystems). The PCR programs were, for ITS: 2 min at 95°C, followed by 30 cycles of 95°C for 1 min, 53°C for 1 min, 72°C for 2 min and a final extension of 72°C for 5 min; for *rpl32-trnL*: 2 min at 94°C, followed by 30 cycles of 94°C for 1 min, 56°C for 1.5 min, 72°C for 10 min and a final extension of 72°C for 10 min; for *trnV*: 3 min at 94°C, followed by 42 cycles of 94°C for 1 min, 62°C for 1 min, 72°C for 1.5 min and a final extension of 72°C for 10 min.

Sequencher 4.1 (Gene Codes Corp., Ann Arbor, MI, U.S.A.) was used to assemble complementary strands. The four regions were aligned using Clustal W, conducted in MEGA 5.05 (Tamura & al. 2011), where minor manual corrections were made to get the final alignment. The incongruence length difference (ILD) test (Farris & al. 1994) was implemented in PAUP v.4.0.b10 (Swofford 2002). Maximum parsimony (MP) analyses were conducted in PAUP, using Branch and Bound search options with 5000 replicates, and MP support was assessed by 5000 bootstrap replicates. A Bayesian inference (BI) analysis was conducted with MrBayes 3.2 (Ronquist & al. 2012). To determine the best model of DNA substitutions for each region, JMODELTEST 2.1.5 (Darriba & al. 2012) was used, using the Akaike Information Criterion (AIC; Akaike 1974). For the three data sets, the Markov and Monte Carlo chains were run for 1.0×10^6 generations and sampled every 1000 generations. For all the analyses, the first 25 % of the trees were excluded ("burn-in") and the remaining trees were used to compile a posterior probability (PP) distribution using a 50 % majority-rule consensus. Clades showing bootstrap (BS) values of 50 %–74 % were considered as weakly supported, 75 %–89 % moderately supported and 90 %–100 % strongly supported.

Results and Discussion

Morphological analyses

A wide range of variation in morphological characters was observed in the analyses, and in many specimens the plants showed intermediate morphological features between the types of *Biscutella raphanifolia* and *B. algeriensis*. Nonetheless, to facilitate interpretation of the results, we will go on using both names to describe the two extremes of variation found, despite the existence of many intermediate states for every character.

Plants fitting the typical *Biscutella raphanifolia* concept (30–105 cm tall) were generally larger in size than plants matching the *B. algeriensis* concept (21–47 cm tall), but the ranges were partially overlapping and many individuals with intermediate characters were found even in a single population.

All the studied specimens showed basal rosettes of lyrate leaves (with a broad and entire terminal lobe), which in some specimens tended to be pinnatipartite to pinnatisect. Leaf length, however, was very variable in both species, being usually larger in *Biscutella raphanifolia* (occasionally reaching up to 20 × 6 cm) than in typical *B. algeriensis* (usually to 8 × 3 cm). For instance, plants from Djebel Ouach, Algeria, with a clear *B. raphanifolia* appearance, had smaller leaves (maximum length 6–9 cm).

As pointed out by Olowokudejo (1992), trichomes in *Biscutella* are variable in structure, size and distribution, both within and between species. This infraspecific variation, absent in only a few species, makes leaf indumentum have little value for taxonomic purposes. Most of the examined specimens presented hirsute indumentum, with short trichomes to 1 mm long, sparsely distributed on the leaf surface and more densely arranged on the veins, where they tended to be more rigid. Some specimens of *B. raphanifolia* presented, together with the typically hirsute indumentum, a villous indumentum of soft, flexuose trichomes often reaching 1.5 mm long, but this character was absent in most of the samples and consequently lacks diagnostic value at specific rank.

Stems generally bore 1–4 well-developed leaves, amplexicaul to auriculate at the base, but a few individuals were also found lacking cauline leaves or they were attenuate at the base (not auriculate).

In all analysed material, inflorescences formed a profusely branched panicle consisting of 8–20(–30) terminal racemes. No differences between the two morphotypes were found in the total number of terminal racemes, since 8–30 terminal racemes were counted for both taxa (e.g. P05438710, P04745854). Fruit density varied from 2–5.5 fruits/cm, with no difference between morphotypes.

Pedicels were glabrous in all cases and no differences in length (5–11 mm) were found between samples of both taxa. The same pattern occurred regarding length of sepals (1.4–3 mm) and petals (2.4–6.2 mm), even though longer petals were more frequent in the typical *Biscutella raphanifolia* specimens.

Floral nectaries displayed the characteristic features of *Biscutella* sect. *Biscutella* (cf. Olowokudejo 1986b), with two extrastaminal median nectaries (which are placed between the two pairs of median stamens) plus two intrastaminal lateral nectaries. Interestingly, a broad range of elongation in the pair of median nectaries (0–0.4 mm) was found in all studied material, without detectable trends between both morphotypes.

Regarding the fruit size, a wide range was also found in the studied material. The typical *Biscutella raphanifolia* individuals generally produced larger fruits (4–9 × 7–15 mm), while plants fitting *B. algeriensis* produced smaller fruits (2.4–6 × 4.5–9(–11) mm). The range observed for *B. raphanifolia* matches data in Poiret's protologue (4–11 × 8.5–18 mm), whereas the range for *B. algeriensis* in the original description (4–5 × 8–9 mm) (Jordan 1864) is slightly enlarged here in the light of

Downloaded From: https://complete.bioone.org/journals/Willdenowia on 19 May 2025
Terms of Use: https://complete.bioone.org/terms-of-use

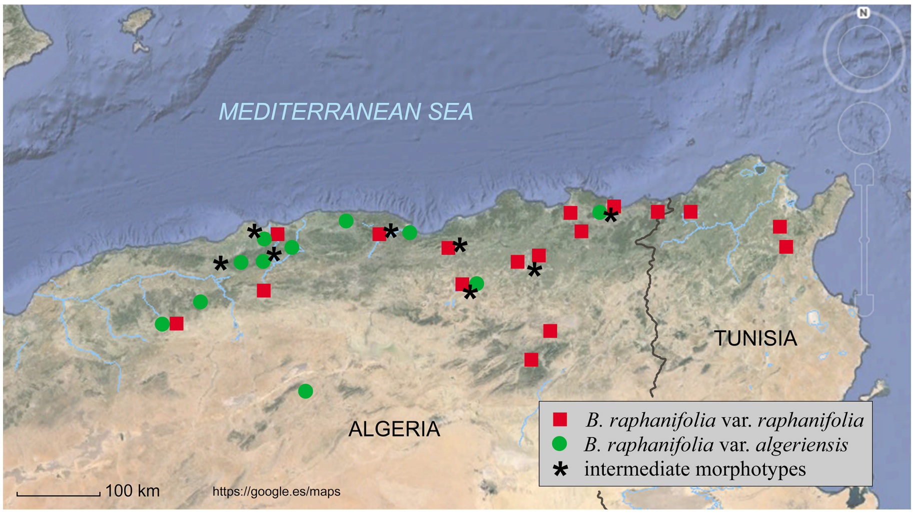

Fig. 1. Map of localities and morphotypes found.

our results. In addition, several individuals were found (e.g. SEV43940, BC630756) that fit the type of *B. algeriensis* but produce larger silicles. Conversely, and more frequently, many typical specimens of *B. raphanifolia* showed mature fruits less than 9 mm wide (e.g. P05438282, P05438730).

A general trend in fruit indumentum can be observed. On the one hand, larger individuals of *Biscutella raphanifolia*, fitting well Poiret's description, mostly produced glabrous silicles, as indicated in the protologue. On the other hand, individuals matching the typical *B. algeriensis* generally showed silicles completely covered with two types of trichomes: (1) longer, clavate trichomes, densely distributed on the margin and more scarcely on the centre; and (2) shorter, unequal, recurved, conical trichomes, generally flattened and spread all over the surface. Nevertheless, a wide variation in fruit indumentum was found, particularly in *B. raphanifolia* specimens, in which all combinations of indumentum types were observed. In fact, some plants produced silicles glabrous on the margin but bearing clavate trichomes on the surface together with short, conical trichomes (e.g. MPU003739, MPU003738), whereas some others showed these same indumentum types but with additional clavate trichomes on the margin (MPU003737; Plaine de Tamedjadjout, K). These results strengthen the observations on variation of fruit indumentum discussed in Cosson (1873: 225), and later highlighted by Maire (1937: 337), who described three varieties of *B. raphanifolia* (var. *genuina* Maire, var. *ditrichocarpa* Maire and var. *orivillosa* Maire) based on the different silicle indumentum. However, in our opinion those variations do not deserve taxonomic recognition, since differences respond mostly to individual variation and hence several of those taxa occur together in a single population.

Perennial growth habit is perhaps the main feature used to distinguish *Biscutella raphanifolia* from some other, related annual taxa with which it shares its distribution area, such as *B. algeriensis* (in Algeria and W Tunisia) or *B. maritima* (in NE Tunisia). This character, nevertheless, does not seem consistent enough for specific separation, since many specimens with intermediate characters were observed during our morphological study. On the one hand, some specimens were found with typical *B. algeriensis* features, but presenting thickened roots or biennial (short-lived perennial) appearance (P04657205, P05326056, P05438160). On the other hand, plants with larger, glabrous fruits but thinner roots connected to an annual life span (P04745850, P05438688) were also found without any clear geographical or ecological pattern. In general terms, specimens comprising plants with all kinds of intermediate life span were commonly found (e.g. P04745963, P05438159) in the distribution range of both *B. raphanifolia* and *B. algeriensis*.

The distribution of the two morphotypes is shown in Fig. 1. Some of the studied specimens (listed in the Appendix) were difficult to classify, since they presented characters attributable to both morphotypes, the most common situation being a combination of perennial life span and small fruits with dense indumentum. Populations from Djebel Magris, Algeria, are a good example of the high variation found, since in this location individuals perfectly matching the protologue of *Biscutella raphanifolia* (Fig. 2C, D), typical *B. algeriensis* (Fig. 2A) and other individuals sharing characters with both extremes of variation (Fig. 2B) live together in the same habitats.

Furthermore, the type material of both *Biscutella didyma* var. *coriophora* Batt. (MPU007633, MPU007634, P00166951, P00364814) and *B. confusa* Pomel (MPU005072) fit perfectly with the original description of *B. algeriensis* and are all therefore treated as synonyms.

Molecular analyses

Combination of both cpDNA regions generated a matrix of 1892 characters, of which 1636 were constant, 153 parsimony-uninformative and 103 parsimony-informative. The MP analysis yielded one unique parsimonious tree, with a tree length (TL) of 297, a consistency index (CI) of 0.912 and a retention index (RI) of 0.863. The phylogenetic trees estimated using MP and BI analyses showed the same topology (Fig. 3). The ITS matrix had a total of 644 characters, of which 498 were constant, 82 parsimony-uninformative and 64 parsimony-informative. The MP analysis yielded two most parsimonious trees, with TL of 195 (CI of 0.897 and RI of 0.828), of which the one sharing topology with the Bayesian tree is shown in Fig. 4.

Downloaded From: https://complete.bioone.org/journals/Willdenowia on 19 May 2025
Terms of Use: https://complete.bioone.org/terms-of-use

Fig. 2. Morphotype diversity in Djebel Magris, Algeria. – A: typical morphotype of *B. algeriensis* (P05438752); B: specimen sharing characters of both morphotypes (P05438157); C, D: typical morphotypes of *B. raphanifolia* (P05438268, P05438663). – Images from Herbier National – MNHN Paris.

Downloaded From: https://complete.bioone.org/journals/Willdenowia on 19 May 2025
Terms of Use: https://complete.bioone.org/terms-of-use

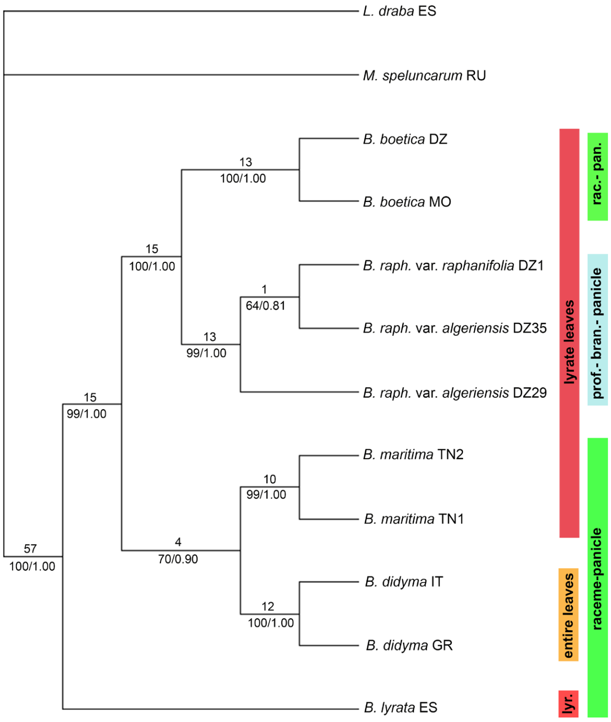

Fig. 3. Phylogenetic tree estimated using cpDNA sequences. Bootstrap values and Bayesian posterior probability are shown below branches (BS/PP). Raceme-panicle: up to 8 terminal racemes per inflorescence. Profusely branched panicle ("prof.-bran.-panicle"): 8–20(–30) terminal racemes per inflorescence.

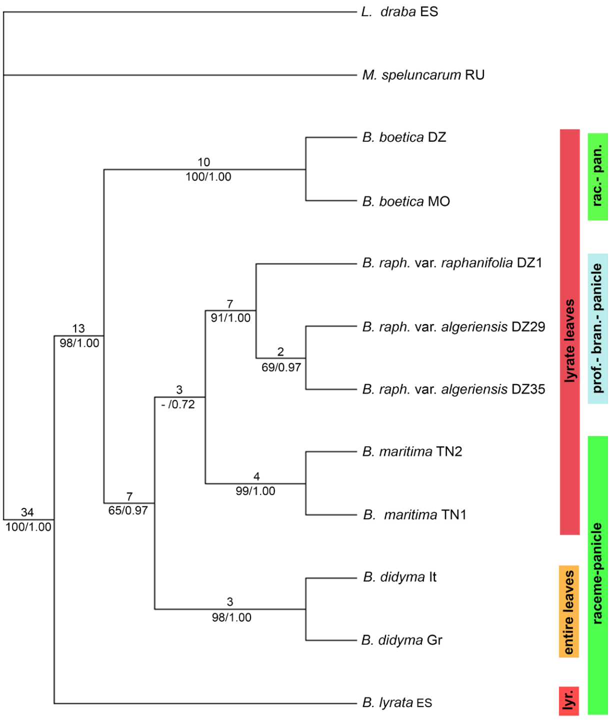

Fig. 4. One of the two most parsimonious trees obtained from ITS sequences. Bootstrap values and Bayesian posterior probability are shown below branches (BS/PP). Raceme-panicle: up to 8 terminal racemes per inflorescence. Profusely branched panicle ("prof.-bran.-panicle"): 8–20(–30) terminal racemes per inflorescence.

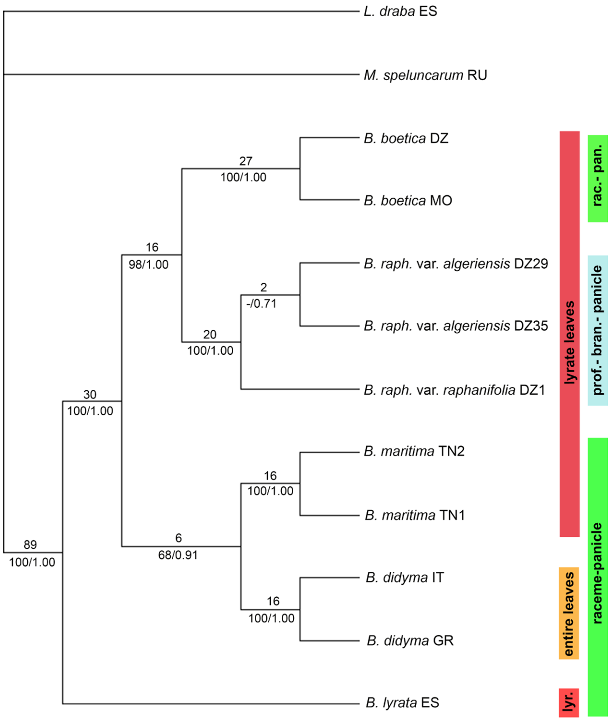

Fig. 5. Phylogenetic tree estimated using a combination of cpDNA and nrDNA sequences. Bootstrap values and Bayesian posterior probability are shown below branches (BS/PP). Raceme-panicle: up to 8 terminal racemes per inflorescence. Profusely branched panicle ("prof.-bran.-panicle"): 8–20(–30) terminal racemes per inflorescence.

Application of the Incongruence Length Difference (ILD) test suggested the existence of slight incongruence between data sets (P = 0.01). Nevertheless, as both 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 merged both plastid and nuclear data to generate a combined phylogeny. The combination of plastid and nuclear regions generated a matrix of 2536 characters, from which 235 were parsimony-uninformative and 167 parsimony-informative. The MP analysis generated two most parsimonious trees, with TL 496, CI 0.899 and RI 0.837. One of the obtained trees, sharing the same topology with the Bayesian tree, is shown in Fig. 5, which is coincident with the plastid tree and yields almost identical support in most branches. Bayesian PP and parsimony BT values are well correlated in all three cases (Fig. 3–5).

The Spanish sample of *Biscutella lyrata* presented a strongly supported basal and isolated position (100 BS, 1.00 PP in all three trees). Plants in this branch are easy to recognize on the basis of their inner stamens with widely dilated filaments, and the very small silicles (2–3 × 4–6 mm) arranged in long and very loose racemes, characteristics not found in any other member of *B.* ser. *Biscutella*. This result supports discarding any phylogenetic relationship between the true *B. lyrata* (which is endemic

Downloaded From: https://complete.bioone.org/journals/Willdenowia on 19 May 2025
Terms of Use: https://complete.bioone.org/terms-of-use

to S Spain) and the other N African members with lyrate or pinnatisect leaves, which was historically assumed by many authors (cf. Maire 1967).

In all the obtained trees, samples of *Biscutella didyma* form a strongly supported clade (98–100 % BS, 1.00 PP). Plants in this group constantly show dentate to subentire leaves usually arranged in a dense basal rosette (sometimes together with several well-developed, sessile cauline leaves), flowers with short nectaries (to 0.2 mm long), and long, densely arranged, racemose infructescences. Similarly, samples of *B. maritima* also form a strongly supported clade in all trees (99–100 % BS, 1.00 PP). Morphologically they show a dense basal rosette of lyrate leaves (lacking well-developed cauline ones), flowers with long nectaries (0.5–0.8 mm), and dense, racemose inflorescences, usually elongated in fruit. Relationships of both clades are not constant in all three trees. They are sister groups, weakly supported in both the plastid (70 % BS, 0.90 PP) and combined trees (68 % BS, 0.91 PP), while in the ITS tree *B. maritima* is sister to the *B. raphanifolia* + *B. algeriensis* clade (see below), and they both are sister to *B. didyma*, though the internal relationships in this three-clade aggregate are not resolved. Nevertheless, the morphological and phylogenetic data obtained are congruent with considering all those clades as separate species, as usually accepted in recent times (cf. Raffaelli 1985, 1991).

Interestingly, the clade composed of *Biscutella raphanifolia* and *B. algeriensis* formed a group strongly supported (99–100 % BS, 1.00 PP in the plastid and combined matrices; 91 % BS, 1.00 PP in the nuclear one). On the one hand, it is sister to a clade including the N African populations of *B. boetica* with pinnatifid to pinnatisect leaves, but showing racemes or slightly paniculate inflorescences, in the plastid and combined matrices (100 % BS, 1.00 PP; 98 % BS, 1.00 PP, respectively). However, each of these two groups is strongly supported and morphologically consistent enough to allow the treatment of *B. algeriensis* and the pinnatifid-leaved *B. boetica* as different species. On the other hand, in the ITS tree the *B. raphanifolia* lineage forms a weakly supported clade (65 % BP, 0.97 PP) with those of *B. didyma* and *B. maritima*, whose internal relationships are however not well resolved.

Internal relationships within the *Biscutella raphanifolia* clade are unresolved, since the position of the three studied specimens is not constant in all trees and it is not strongly supported. The two annual individuals constitute a clade in the ITS tree, while closer connections exist between the perennial individual and one of the annual plants (labelled DZ35) in the case of the plastid matrix (Fig. 3). Provided that those annual individuals were collected in two close localities about 150 km W from where the perennial one grew, our results might be influenced by the geographical origin of samples and they would probably not reflect their true phylogenetic relationships. Further studies are needed to clarify this point.

Conclusion

Even though life span has traditionally been used as the main argument to differentiate *Biscutella raphanifolia* from the other species of *B.* ser. *Biscutella*, many examples of species of *Brassicaceae* can be found that include both annual and perennial individuals, e.g. *Berteroa incana* (L.) DC., *Cardamine glauca* Spreng. ex DC., *Coincya monensis* (L.) Greuter & Burdet, *Diplotaxis harra* (Forssk.) Boiss., *D. muralis* (L.) DC., *Erucastrum nasturtiifolium* (Poir.) O. E. Schulz, *Marcuskochia triloba* (L.) Al-Shehbaz (*Malcolmia lacera* (L.) DC.) or *Moricandia arvensis* (L.) DC. In some species, such as *Brassica fruticulosa* Cirillo, the annual or perennial habit, in combination with other flower and fruit characters, has been used to differentiate infraspecific taxa (subsp. *fruticulosa*, usually biennial to perennial, and subsp. *cossoniana* (Boiss. & Reut.) Maire, annual). In addition to this, our fieldwork observations on *Biscutella maritima* revealed the presence of both annual and short-lived perennial individuals growing together in the same locality, probably related to slightly different microecological soil conditions. Therefore, contrary to the general assumption of many taxonomists, the perennial versus annual habit lacks any diagnostic value for species differentiation in the *B. raphanifolia* clade, as well as in the entire *B.* ser. *Biscutella*.

New approach to the taxonomy and circumscription of *Biscutella raphanifolia*

The broad morphological variation, including life span, observed in the studied group, together with the obtained molecular results, point out the difficulty in separating *Biscutella raphanifolia* and *B. algeriensis* at specific rank. The morphotypes representing both extremes of variation are distributed throughout NE Algeria and NW Tunisia from Theriet el Had to Oued el Hadjar (Fig. 1), and perennial and annual plants can be found living together in many populations in the same habitats. This pattern is common to other species of *B.* ser. *Biscutella*. Accordingly, we consider varietal rank to be the most suitable option, since geographical or ecological isolation diminishing genetic flow is widely assumed to justify application of higher taxonomic ranks, such as subspecies (Avise & Ball 1990; Hamilton & Reichard 1992; Crespo & al. 1998; Ellison & al. 2014).

Biscutella raphanifolia Poir., Voy. Barbarie 2: 198. 1789 var. ***raphanifolia*** ≡ *Biscutella raphanifolia* var. *genuina* Maire in Bull. Soc. Hist. Nat. Afrique N. 28: 337. 1937, nom. inval. (McNeill & al. 2012: Art. 24.3). – Lectotype (designated by Raffaelli 1985: 114): Numidia (P00166955 specimen on right side of sheet!).

= *Biscutella radicata* Coss. & Durieu in Bull. Soc. Bot. France 19: 224. 1873 [“1872”]. – **Lectotype (designated here):** Collines de Djebel-Edough, subdivision

Downloaded From: https://complete.bioone.org/journals/Willdenowia on 19 May 2025
Terms of Use: https://complete.bioone.org/terms-of-use

de Bône, May 1864, *V. Reboud* [Fragmenta florae algeriensis exsiccata no. 503] (P05438661!; isolectotypes: B100154798!, MPU008649!, MPU023098!, MPU023099!, MPU023100!, P04632019!, P05438730!).

= *Biscutella raphanifolia* var. *ditrichocarpa* Maire in Bull. Soc. Hist. Nat. Afrique N. 28: 337. 1937. – **Lectotype (designated here):** In pascuis supra Ben Chicao, solo arenaceo, 24 May 1936, *R. Maire* (MPU003738!).

= *Biscutella raphanifolia* var. *orivillosa* Maire in Bull. Soc. Hist. Nat. Afrique N. 28: 337. 1937. – **Lectotype (designated here):** Djebel-Ouach, près de Constantine (Algérie), pentes arides, (end of) May 1880, *J. Reboud* [Société Dauphinoise 1881 no. 2762] (MPU003737!; isolectotypes: K!, P05325975!, P05325976!, P05438671!, P05438709!, P05438720!).

Herbs perennial, usually with a thickened caudex, 30–100 cm tall. *Stems* 1 or 2, hirsute to lanate at base. *Basal leaves* 4–10, in a rosette, lyrate-pinnatipartite, to 20 × 6 cm, with broad, entire terminal lobe; *cauline leaves* (absent or) 1–4, well developed, broad, amplexicaul to auriculate at base. *Inflorescence* a profusely branched panicle, with short racemes bearing (1.5–)2–5 fruits/cm at base; *pedicels* erecto-patent, 5–8(–8.8) mm long. *Sepals* 1.8–3 mm long; *petals* 3–6 mm long, gradually attenuate at base; *stamen filaments* filiform; *median nectaries* inconspicuous or elongated to 0.4 mm. *Silicles* (4.5–)5–8 × (8.2–)9–15 mm, generally flat and glabrous, sometimes swollen at margin and hirsute, with clavate trichomes and/or tiny conical indumentum.

Biscutella raphanifolia var. ***algeriensis*** (Jord.) A. Vicente, M. A. Alonso & M. B. Crespo, **comb. nov.** ≡ *Biscutella algeriensis* Jord., Diagn. Esp. Nouv.: 318. 1864 ≡ *Biscutella didyma* var. *algeriensis* (Jord.) Batt., Fl. Algérie [Dicot.]: 38. 1888. – Lectotype (designated by Vicente & al. 2015: 237): Env. d'Alger, Birmandreis, 16 Apr 1862, *E. Revelière* (MPU024556!).

= *Biscutella confusa* Pomel in Bull. Soc. Sci. Phys. Algérie 11: 231. 1874 [Nouv. Mat. Fl. Atl.] ≡ *Biscutella didyma* var. *confusa* (Pomel) Batt., Fl. Algérie [Dicot.]: 38. 1888. – **Lectotype (designated here)**: Teniet-el-Had, *Pomel* (MPU005072!).

= *Biscutella didyma* var. *coriophora* Batt., Fl. Algérie [Dicot.]: 37. 1888. – **Lectotype (designated here)**: Duperré, Apr 1882, *J. A. Battandier* (P00166951!; isolectotypes: MPU007633!, MPU007634!, P00364814!).

Herbs annual, 23–40 cm tall. *Stems* 1–4, hirsute below. *Basal leaves* 4–10, in a rosette, lyrate-pinnatipartite, to 8.5 × 3.5 cm, with broad, entire terminal lobe, sometimes with very few lateral lobes (acquiring spatulate appearance); *cauline leaves* 1–3, well developed in most individuals, amplexicaul to attenuate. *Inflorescence* a profusely branched panicle, with short racemes bearing (1.8–)2–4.5 fruits/cm at base; *pedicels* erecto-patent, 6–8(–10) mm long. *Sepals* 1.4–2.6 mm long; *petals* 2.9–4.6 mm long, gradually attenuate at base; *stamen filaments* filiform; *median nectaries* inconspicuous or elongated to 0.4 mm. *Silicles* 2.5–6 × 4.5–9(–11) mm, with a wide range of indumentum types, mostly covered with tiny, conical trichomes together with clavate trichomes distributed on central part and margin; sometimes lacking conical trichomes, showing only clavate indumentum on margin and/or centre.

Acknowledgements

The curators and personnel of the herbaria BC, K, MA, P, SEV and V are kindly thanked for their help with the studied material, as well as Elena Artyukova, who provided us *Megadenia* samples. Reviewers Ihsan A. Al-Shehbaz and Dmitry German made interesting suggestions that very much improved the text. We are also grateful to Nicholas Turland for his suggestions and accurate editing work. The FPU grant programme (Mº de Educación, Cultura y Deporte, Spanish Government) is kindly thanked for supporting Alicia Vicente. This research was partly supported by the I+D+i research project CGL2011-30140 from Dirección Gral. de Investigación, MICINN (Ministerio de Economía y Competitividad, Spanish Government), and the grants ACIE 13-08, ACIE 14-01 and PPI-2015 from the University of Alicante.

References

Akaike H. 1974: A new look at the statistical model identification. – IEEE Trans. Automatic Control **19:** 16–723.

Al-Shehbaz I. A. 2012: A generic and tribal synopsis of the *Brassicaceae* (*Cruciferae*). – Taxon **61:** 931–954.

Appel O. & Al-Shehbaz I. A. 2003: *Cruciferae*. – Pp. 75–174 in: Kubitzki K. & Bayer C. (ed.), Families and genera of vascular plants **5.** – Berlin & Heidelberg: Springer.

Artyukova E. V., Kozyrenko M. M., Boltenkov E. V. & Gorovoy P. G. 2014: One or three species in *Megadenia* (*Brassicaceae*): insight from molecular studies. – Genetica **142:** 337–350.

Avise J. C. & Ball R. M. 1990: Principles of genealogical concordance in species concepts and biological taxonomy. – Oxford Surv. Evol. Biol. **7:** 45–67.

Barker F. K. & Lutzoni F. 2002: The utility of the incongruence length difference test. – Syst. Biol. **51:** 625–637.

Battandier J. A. 1888: Flore de l'Algérie. Dicotylédones. 1er Fascicule. Thalamiflores**.** – Alger: Adolphe Jourdan; Paris: F. Savy.

Battandier J. A. & Trabut L. C. 1905 ["1902"]: Flore analytique & synoptique de l'Algérie & de la Tunisie. – Alger: Giralt.

Downloaded From: https://complete.bioone.org/journals/Willdenowia on 19 May 2025
Terms of Use: https://complete.bioone.org/terms-of-use

Boccone P. 1674: Icones & descriptiones rariorum plantarum Siciliae, Melitae, Galliae, & Italiae. – [Oxford]: Theatro Sheldoniano.

Candolle A. P. de 1811: Monographie des Biscutelles ou Lunetières. – Ann. Mus. Hist. Nat. **18:** 292–301.

Chase M. W. & Hills H. H. 1991: Silica gel: an ideal material for field preservation of leaf samples for DNA studies. – Taxon **40:** 215–220.

Cosson E. 1873: Descriptio *Biscutellae* novae algeriensis. – Bull. Soc. Bot. France **19:** 224–225.

Cosson E. 1887: Compendium florae atlanticae ou Flore des États Barbaresques, Algérie, Tunisie et Maroc **2.** – Paris: Imprimerie Nationale.

Crespo M. B., Serra L. & Juan A. 1998: *Solenopsis* (*Lobeliaceae*): a genus endemic in the Mediterranean region. – Pl. Syst. Evol. **210:** 211–229.

Darriba D, Taboada G. L., Doallo R. & Posada D. 2012: jModelTest 2: more models, new heuristics and parallel computing. – Nature Meth. **9:** 772.

Doyle J. J. & Doyle J. L. 1987: A rapid DNA isolation procedure for small quantities of fresh leaf tissue. – Phytochem. Bull. Bot. Soc. Amer. **19:** 11–15.

Ellison A. M., Davis C. C., Calie P. J. & Naczi R. F. 2014: Pitcher plants (*Sarracenia*) provide a 21st-century perspective on infraspecific ranks and interspecific hybrids: a modest proposal for appropriate recognition and usage. – Syst. Bot. **39:** 939–949.

Farris J. S., Källersjö M., Kluge A. G. & Bult C. 1994: Testing significance of congruence. – Cladistics **10:** 315–319.

Grau J. 1999: Nota sobre *Biscutella* en el Norte de Marruecos. – Lagascalia **21:** 244–246.

Guinea E. 1964: El género *Biscutella.* – Anales Inst. Bot. Cavanilles **21:** 387–405.

Guinea E. & Heywood V. H. 1993: *Biscutella* L. – Pp. 393–398 in: Tutin T. G., Burges N. A., Chater A. O., Edmondson J. R., Heywood V. H., Moore D. M., Valentine D. H., Walters S. M. & Webb D. A. (ed.), Flora europaea **1.** – Cambridge: Cambridge University Press.

Hamilton C. W. & Reichard S. H. 1992: Current practice in the use of subspecies, variety, and forma in the classification of wild plants. – Taxon **41:** 485–498.

Hedge I. 1968: *Biscutella* L. – Pp: 99–100 in: Rechinger K. H. (ed.), Flora iranica **57.** – Graz: Akademische Druck- u. Verlagsanstalt.

Jordan A. 1864: Diagnoses d'espèces nouvelles ou méconnues, pour servir de matériaux à une flore réformée de la France et des contrées voisines. – Paris: F. Savy.

Le Floc'h E., Boulos L. & Vela E. 2010: Catalogue synonymique commenté de la Flore de Tunisie. Ed. 2. – Tunis: Banque Nationale de Gènes de la Tunisie.

Machatschki-Laurich B. 1926: Die Arten der Gattung *Biscutella* L. Sect. *Thlaspidium* (Med.) DC. – Bot. Arch. **13:** 1–115.

Maire R. 1937: Contributions à l'étude de la flore de l'Afrique du Nord. – Bull. Soc. Hist. Nat. Afrique N. **28:** 337.

Maire R. 1967: Flore de l'Afrique du Nord **13.** – Paris: Paul Lechevalier.

Malinowski E. 1911: Monographie du genre *Biscutella* L. I. Classification et distribution géographique. – Bull. Int. Acad. Sci. Cracovie, Cl. Sci. Math., Ser. B, Sci. Nat. **1910:** 111–139.

Marhold K. 2011+ [continuously updated]: *Brassicaceae*. – In: Euro+Med PlantBase – the information resource for Euro-Mediterranean plant diversity. – Published at http://ww2.bgbm.org/EuroPlusMed [accessed 1 Feb 2016].

McNeill J., Barrie F. R., Buck W. R., Demoulin V., Greuter W., Hawksworth D. L., Herendeen P. S., Knapp S., Marhold K., Prado J., Prud'homme van Reine W. F., Smith G. F., Wiersema J. H. & Turland N. J. (ed.) 2012: International Code of Nomenclature for algae, fungi, and plants (Melbourne Code) adopted by the Eighteenth International Botanical Congress Melbourne, Australia, July 2011. – Königstein: Koeltz Scientific Books. – [Regnum Veg. **154**].

Olowokudejo J. D. 1985: Scanning electron microscopy of fruits in the genus *Biscutella* (*Cruciferae*). – Phytomorphology **35:** 273–288.

Olowokudejo J. D. 1986a: The infrageneric classification of *Biscutella* (*Cruciferae*). – Britonnia. **38:** 86–88.

Olowokudejo J. D. 1986b: The taxonomic importance of nectary variation in the genus *Biscutella.* – Feddes Repert. **97:** 837–845.

Olowokudejo J. D. 1992: Taxonomic significance of leaf indumentum characteristics of the genus *Biscutella* (*Cruciferae*). – Folia Geobot. Phytotax. **27:** 401–417.

Özüdoğru B., Akaydin G., Erik S., Al-Shehbaz I. A. & Mummenhoff K. 2015: Phylogeny, diversification and biogeographic implications of the eastern Mediterranean endemic genus *Ricotia* (*Brassicaceae*). – Taxon **64:** 727–740.

Poiret J. L. M. 1789: Voyage en Barbarie **2.** – Paris: J. B. F. Née de la Rochelle.

Pomel A. 1874: Nouveaux matériaux pour la flore Atlantique **1.** – Alger & Paris: J. St.-Lager & F. Savy.

Pottier-Alapetite G. 1979: Flore de la Tunisie. Angiospermes–Dicotylédones. Apétales–Dialypétales, **1.** – Tunis: Imprimerie Officielle de la République.

Quézel P. & Santa S. 1963: Nouvelle flore de l'Algérie et des régions désertiques méridionales **2.** – Paris: CNRS.

Raffaelli M. 1985: Note tassonomiche e corologiche su *Biscutella raphanifolia* Poir. e *B. lyrata* L. ssp. *laxiflora* (Presl) Raffaelli, comb. et stat. nov. – Webbia **39:** 111–118.

Raffaelli M. 1991: *Biscutella* L. Ser. *Lyratae* Malin. (*Cruciferae*) in Italia. - Discussione sui caratteri morfologici e tassonomia. – Webbia **45:** 1–30.

Raffaelli M. 1992: *Biscutella* sect. *Iondraba* (*Crucifera*e) in the Mediterranean area. – Willdenowia **22:** 19–36.

Downloaded From: https://complete.bioone.org/journals/Willdenowia on 19 May 2025
Terms of Use: https://complete.bioone.org/terms-of-use

Rasband W. S. 1997–2015: ImageJ. – Bethesda: U. S. National Institutes of Health. – Published at https://imagej.nih.gov/ij/

Ronquist F., Teslenko M., van der Mark P., Ayres D. L., Darling A., Höhna S., Larget B., Liu L., Suchard M. A. & Huelsenbeck J. P. 2012: MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. – Syst. Biol. **61:** 1–4.

Shaw J., Lickey E. B., Schilling E. E. & Small R. L. 2007: Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetics studies in angiosperms: the tortoise and the hare III. – Amer. J. Bot. **94:** 275–288.

Swofford D. L. 2002: PAUP* Phylogenetic Analysis Using Parsimony (* and other methods), version 4.0b10 for Macintosh. – Sunderland: Sinauer Associates.

Tamura K., Peterson D., Peterson N., Stecher G., Nei M. & Kumar S. 2011: MEGA5: Molecular Evolutionary Genetics Analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. – Molec. Biol. Evol. **28:** 2731–2739.

The Plant List 2010: The Plant List. Version 1. – Published at http://www.theplantlist.org/ [accessed 1 Jan 2015].

Thiers B. 2016+ [continuously updated]: Index Herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden's virtual herbarium. – Published at http://sweetgum.nybg.org/science/ih/ [accessed 1 Jan 2016].

Vicente A., Alonso M. A., Thiébaut M. & Crespo M. B. 2015: Lectotype designation for three North African names of *Biscutella* (*Brassicaceae*) published by Claude Thomas Alexis Jordan. – Phytotaxa **212:** 236–242.

Wang W. P., Hwang C. Y., Lin T. P. & Hwang S. Y. 2003: Historical biogeography and phylogenetic relationships of the genus *Chamaecyparis* (*Cupressaceae*) inferred from chloroplast DNA polymorphism. – Pl. Syst. Evol. **241:** 13–28.

Warwick S. I. & Al-Shehbaz I. A. 2006: *Brassicaceae*: chromosome number index and database on CD-Rom. – Pl. Syst. Evol. **259:** 237–248.

White T. J., Bruns T., Lee S. & Taylor J. W. 1990: Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. – Pp. 315–322 in: Innis M. A., Gelfand D. H., Sninsky J. J. & White T. J., (ed.), PCR protocols: a guide to methods and applications. – New York: Academic Press.

Appendix: Specimens examined

Biscutella raphanifolia var. *raphanifolia*

ALGERIA: Massif de l'Akfadou (Algérie, wilaya de Tizi-Ouzou), route forestière de l'Akfadou, près de la maison forestière de Tala Kitane, à env. 20 km au SE d'Azazga, 27 May 1983, *A. Dubuis* (BC829995, MA561765, P04719060, VAL33967); Clairières dans la forêt de Teniet-el-Had, 20 Jul 1930 (P04745851); Lieux humides, Rain de la maison forestière de Chelia, Massif de l'Aurès, 25 May 1935 (P04745853); Route du fort Gerion près Bône, prov. de Constantine, May 1863, *V. Reboud* (P05438666); Djebel Magris, lieux arides, sur le calcaire, 1600 m, Jun 1898, *E. Reverchon* (MA44520, P05438663, P05438718); Monts Babors, lieux arides sur le calcaire, 1800 m, Jun 1897, *Reverchon* (P05438711, P05438713); Djebel Edough près Bône, province de Constantine, Algérie, 26 Jun 1861, *E. Cosson* (P05438715); Collines de Djebel-Edough, subdivision de Bône, May 1864, *V. Reboud* (lectotype of *B. radicata*: P05438661; isolectotypes: B100154798, MPU008649, MPU023098, MPU023099, MPU023100, P04632019, P05438730); Plaine de Tamedjadjout, entre les monts Babor et Tababor, prov. de Constantine, 21–22 Jun 1880, *E. Cosson* (K); Ad cacumen montis Bou-Zegza prov. Algeriensis, solo siliceo, 950–1000 m, 22 May 1932, *R. Maire* (syntype of *B. raphanifolia* var. *ditrichocarpa*: MPU003739); In pascuis supra Ben Chicao, solo arenaceo, 24 May 1936, *R. Maire* (lectotype of *B. raphanifolia* var. *ditrichocarpa*: MPU003738); Teniet-el-Had, cédraies sur grès, 1600 m, 21 Apr 1930, *R. Maire* (syntype of *B. raphanifolia* var. *ditrichocarpa*: MPU003818); Djebel Ouach près de Constantine (Algérie), 27 May 1877, *V. Reboud* (P05438166, P05438678, P05438710); Constantine (Algérie), coteaux autour de la ville, May 1877, *V. Reboud* (P05438282); La Calle, 4 May 1841, *M. Durieu* (P05438724); La Calle, prov. de Constantine, Algérie, Apr 1840, *M. Durieu* (P05438721); Lieux sablonneuse couvert de broussailles à La Calle, 11 Apr 1841, *M. Durieu* (P05438712); Forêt de Teniet-el-Had, 27 May 1875 (P00166955 specimen on left side of sheet); Numidia (lectotype of *B. raphanifolia*: P00166955 specimen on right side of sheet); Constantine, Algérie, 14 May 1853, *E. Cosson* (P05438798); Djebel-Ouach, près de Constantine (Algérie), pentes arides, (end of) May 1880, *J. Reboud* (lectotype of *B. raphanifolia* var. *orivillosa*: MPU003737; isolectotypes: K, P05325975, P05325976, P05438671, P05438709, P05438720); Sidi Reghis, Jun 1883, *J. Reboud* (P05438662); Djebel Mabouna, près Guelina, prov. de Constantine, 4 Jun 1880, *E. Cosson* (P05438674); Montagnes de Magris, lieux arides, sur la calcaire, 1000 m, Jun 1898, *Reverchon* (P05438268). — TUNISIA: In quercetis valle El-Fedja (Ouchteta), oued El Hadjar, 21 May 1886, *A. Letourneux* (P05438658); El Fedja, 11 May 1888, *E. Cosson* (P05438689).

Biscutella raphanifolia var. *algeriensis*

ALGERIA: Env. d'Alger, Birmandreis, 16 Apr 1862, *E. Revelière* (lectotype of *B. algeriensis*: MPU024556); Bougara (ex Rovigo) (Algérie, Blida), pentes N du Djebel Beni-Kichnit, entre Bougara et l'arboretum de Meurdja, 1 May

Downloaded From: https://complete.bioone.org/journals/Willdenowia on 19 May 2025
Terms of Use: https://complete.bioone.org/terms-of-use

1975 & 25 Jun 1976, *A. Dubuis* (BC530757, BC630756, MA268267, SEV43939); Bouchata Mahmoua, granitic hills, 36°40'N, 06°40'E, 300 m, 28 Apr 1976, *D. A. Sutton & S. J. Sutton* (MA764865); Bougara, (ex Rovigo) (Algérie, Blida), pentes N du Djebel Beni-Kichnit, entre Bougara et l'arboretum de Meurdja, 400 m, 1 May 1975 & 25 Jun 1976, *A. Dubuis* (SEV43940); Azzefoun (Algérie, Kabylie), pentes NW du Djebel Tambout, à 10 km d'Azazga, 900 m, 27 Jun 1976, *A. Dubuis* (SEV43938); Azzeffoun (Algérie, Kabylie), pentes NW du Djebel Tamgout, à 10 km NNE d'Azazga, terrains rocailleux dénudés, 900 m, 27 Jun 1976, *A. Dubuis* (SEV43937); Vallée des Consuls, in collibus herbosis, 9 Feb 1865 (P05438286); In declivibus Bou Zareu, Feb 1869 (P05438287); Le Gouraya de Bougie, lieux incultes, sur le calcaire, 600 m, May 1896, *Reverchon* (P04745963); Environs d'Alger, coteaux, 1853, *G.-L. Durando* (P05325979); Blida, 1839 (P04745854); Algier, in aridis, Mar 1832, *W. Schimper* (K); Algiers, surroundings of town, 8 Mar 1873 (K); Champs à El Kaddous, à deux lieues d'Alger, 1850, *P. Jamin* (K); Alger, *Trabut* (K); Alger, 5 May, *Battandier* (P05438664); Duperré, Apr 1882, *J. A. Battandier* (lectotype of *B. didyma* var. *coriophora*: P00166951; isolectotypes: MPU007633, MPU007634, P00364814); Teniet-el-Had, *Pomel* (lectotype of *B. confusa*: MPU005072); Montagnes de Magris, lieux arides, sur la calcaire, 1000 m, Jun 1898, *Reverchon* (P05438752); Blida, Parque Nacional de Chrea, 31SDA893327, 1240 m, 1 May 2014, *A. Terrones, M. A. Alonso & A. Juan* (ABH72665); ibid., antenas y zona de telesilla, 31SDA898317, 1485 m, 1 May 2014, *A. Terrones, M. A. Alonso & A. Juan* (ABH72641); Médéa, Berrouaghia, ctra. N62 desde Berrouaghia hacia Boualchoune, 31SDV832996, 630 m, 1 May 2014, *A. Terrones, M. A. Alonso & A. Juan* (ABH72640).

Specimens sharing characters of both morphotypes

ALGERIA: Forêt de Taourirt Ighil, dans l'Akfadou (DT de Constantine), 26 May 1912, *L. Ducellier* (P04745849, P04745852); Boisements des dunes littorales, à l'embouchure de la Mafrag, près de Bône, Dt. de Constantine, 1 May 1947 (P04745850); Pentes schisteuses près de Sidi Madayi, Gorges de la Chifla, 30 Jun 1930 (P04745935); Constantine (Algérie), coteaux autour de la ville, May 1877, *V. Reboud* (K, P05325941, P05438167); El Affroun, Apr 1885, *J. A. Battandier* (P05438229); Bône, May 1833 (P05438303); Reghaia, 15 Apr (P05325982); Djebel Tababor, petite Kabylie, prov. de Constantine, Algérie, 21 Jul 1861, *E. Cosson* (P05438688); Birkadem, 2 Apr 1911 (P05326056); Montagnes de Magris, lieux arides, sur la calcaire, 1000 m, Jun 1898, *Reverchon* (P05438157); Algiers, May 1857 (K); Algiers, Feb 1856 (K); Bougara (ex Rovigo) (Algérie, Blida), pentes N du Djebel Beni-Kichnit, entre Bougara et l'arboretum de Meurdja, 400 m, 1 May 1975 & 25 Jun 1976, *A. Dubuis* (P04657205); Algérie, sous le col de Frehu, 9 Jun 1935 (P05438160).

Willdenowia
Open-access online edition www.bioone.org/loi/will
Online ISSN 1868-6397 · Print ISSN 0511-9618 · Impact factor 0.500
Published by the Botanic Garden and Botanical Museum Berlin, Freie Universität Berlin

© 2016 The Authors · This open-access article is distributed under the CC BY 4.0 licence

Downloaded From: https://complete.bioone.org/journals/Willdenowia on 19 May 2025
Terms of Use: https://complete.bioone.org/terms-of-use