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

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

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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

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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; 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 (Malinowski 1911; Olowokudejo 1986a; Guinea & Heywood 1993); they are distributed in the Mediterranean basin and SW Asia (Machatschki-Laurich 1926; Maire 1967; Hedge 1968).

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. *Lyraeae* 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),
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 annuals, 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 section B. lyrata, which is known to occur from the surroundings of Alger (Algiers) to the border with Tunisia. Battandier & Trabut (1905) 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 amplexical 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 Cosson & Durieu, and on the other hand the annual plants, which he first named B. didyma var. lyrata (L.) Cosson. (including B. raphanifolia) and later (Cosson 1887) B. didyma var. raphanifolia (Poir.) Cosson. 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 (rpl32-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-
Table 1. Characters studied. Results are given for both extremes of variation.

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

Table 2. Materials used in the molecular analysis.

<table>
<thead>
<tr>
<th>Label in Fig. 3–5</th>
<th>Taxon</th>
<th>Locality</th>
<th>Voucher</th>
<th>GenBank acc. no. rpl32-trnL</th>
<th>GenBank acc. no. trnV</th>
<th>GenBank acc. no. ITS</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. draba ES</td>
<td>Lepidium draba L.</td>
<td>Spain</td>
<td>ABH71952</td>
<td>KU746330</td>
<td>KU746332</td>
<td>KU746329</td>
</tr>
<tr>
<td>M. speluncarum RU</td>
<td>Megadenia speluncarum Voroib &amp; al.</td>
<td>Russia</td>
<td>VLA10454</td>
<td>KX943557</td>
<td>KX943556</td>
<td>KX943555</td>
</tr>
<tr>
<td>B. lyra ES</td>
<td>Biscutella lyra L.</td>
<td>Spain</td>
<td>ABH70649</td>
<td>KU570211</td>
<td>KU574020</td>
<td>KU570201</td>
</tr>
<tr>
<td>B. didyma GR</td>
<td>B. didyma L.</td>
<td>Greece</td>
<td>ABH58628</td>
<td>KU570212</td>
<td>KU574021</td>
<td>KU570202</td>
</tr>
<tr>
<td>B. didyma IT</td>
<td>B. didyma IT</td>
<td>Italy</td>
<td>ABH70559</td>
<td>KU570213</td>
<td>KU574022</td>
<td>KU570203</td>
</tr>
<tr>
<td>B. maritima TN1</td>
<td>B. maritima Ten.</td>
<td>Tunisia</td>
<td>MA797732</td>
<td>KU570214</td>
<td>KU574023</td>
<td>KU570204</td>
</tr>
<tr>
<td>B. maritima TN2</td>
<td>B. maritima</td>
<td>Tunisia</td>
<td>MA724301</td>
<td>KU570215</td>
<td>KU574024</td>
<td>KU570205</td>
</tr>
<tr>
<td>B. raph. var. algeriensis DZ29</td>
<td>B. raphanifolia var. algeriensis (Jord.) A. Vicente &amp; al.</td>
<td>Algeria</td>
<td>ABH72640</td>
<td>KU570216</td>
<td>KU574025</td>
<td>KU570206</td>
</tr>
<tr>
<td>B. raph. var. algeriensis DZ35</td>
<td>B. raphanifolia var. algeriensis</td>
<td>Algeria</td>
<td>ABH72641</td>
<td>KU570217</td>
<td>KU574026</td>
<td>KU570207</td>
</tr>
<tr>
<td>B. raph. var. raphanifolia DZ1</td>
<td>B. raphanifolia Poir. var. raphanifolia</td>
<td>Algeria</td>
<td>VAL33967</td>
<td>KU570218</td>
<td>KU574027</td>
<td>KU570208</td>
</tr>
<tr>
<td>B. boetica MO</td>
<td>B. boetica Boiss. &amp; Reut.</td>
<td>Morocco</td>
<td>ABH68371</td>
<td>KU570219</td>
<td>KU574028</td>
<td>KU570209</td>
</tr>
<tr>
<td>B. boetica DZ</td>
<td>B. boetica</td>
<td>Algeria</td>
<td>ABH69561</td>
<td>KU570220</td>
<td>KU574029</td>
<td>KU570210</td>
</tr>
</tbody>
</table>
fications were performed on a reaction volume of 25 µl containing 22.5 µl of ABGene 1.1× Master Mix, 2.5 mM MgCl2 (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 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 1.0×106 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 pinnatifid 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, ampelicaul 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 sepal (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
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* discussed in Cosson (1873: 225), and later highlighted strengthening the observations on variation of fruit indumentum (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* discussed in Cosson (1873: 225), and later highlighted strengthening the observations on variation of fruit indumentum (MPU003737; Plaine de Tamedjadjout, K).

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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.
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.
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. set. Biscutella. This result supports discarding any phylogenetic relationship between the true B. lyrata (which is endemic...
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. *Berterea incana* (L.) DC., Cardamine glauca Spreng. ex DC., *Coineya monensis* (L.) Greuter & Burdet, *Diplotaxis harra* (Forssk.) Boiss., *D. maralis* (L.) DC., *Erucastrum nasturtiifolium* (Poir.) O. E. Schulz, *Marcuschochia 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 *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 sub-species (Avise & Ball 1990; Hamilton & Reichard 1992; Crespo & al. 1998; Ellison & al. 2014).


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


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-pinnatifid, to 20 × 6 cm, with broad, entire terminal lobe; Basal leaves 4-branched panicle, with short racemes bearing (1.5–)2–5 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.

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References


Battandier J. A. 1888: Flore de l’Algérie. Dicotylédones. 1er Fascicule. Thalamiflores – Alger: Giralt. – SEV and V are kindly thanked for their help with the studied material, as well as Elena Artyukova, who provided us Megaedina 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 (M 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 General 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


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 Juil 1930 (P04745851); Lieux humides, Rain de la maison forestière de Chelia, Massif de l’Auniet-el-Had, 20 Juil 1930 (P04745851); Lieux humides, Clairières dans la 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); Numidia (right side of sheet): Constantine, Algérie, 14 May 1853, E. Cosson (P05438715); Collins de Djebel-Eough, subdivision de Bône, May 1864, V. Reboud (P05438662); Djebel Edough, près de Constantine, Algérie, 26 Jun 1861, E. Cosson (P05438715); Collines de Djebel-Eough, subdivision de Bône, May 1864, V. Reboud (lectotype of B. radicata: P05438661); isolectotypes: B100154798, MPU008649, MPU023098, MPU023099, MPU023100, MPU04632019, P05438718); Monts Babors, lieux arides sur le calcaire, 1800 m, Jun 1897, Reverchon (P05438711, P05438713); Djebel Edough près Bône, prov. de Constantine, Algérie, 26 Jun 1861, E. Cosson (P05438715); V. Reboud (P05438282); La Calle, 4 May 1841, M. Durieu (P05438724); La Calle, prov. de Constantine, Algérie, 4 Apr 1840, M. Durieu (P05438721); Lieux sablonneux couvert de brossailles 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 (right side of sheet): Constantine, Algérie, 26 Jun 1861, E. Cosson (P05438715); Adacumen montis Bou-Zegza prov. Algeriensis, solo siliceo, 950–1000 m, 22 May 1932, R. MAIRE (synotype of B. raphanifolia var. distichocarpa: MPU003737); In pascuis supra Ben Chicao, solo arenaceo, 24 May 1936, R. MAIRE (lectotype of B. raphanifolia var. distichocarpa: MPU003737); Teniet-el-Had, cédraies sur grès, 1600 m, 21 Apr 1930, R. MAIRE (syntype of B. raphanifolia var. distichocarpa: 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, 4 Apr 1840, M. Durieu (P05438721); Lieux sablonneux couvert de brossailles 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 (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 Mahoune, près Guelina, prov. de Constantine, 4 Jun 1880, E. Cosson (P05438674); Montanges de Magris, lieux arides, sur la calcaire, 1000 m, Jun 1898, Reverchon (P05438268).

TUNISIA: In queretes valley El-Fedja (Ouchteta), oued El Hadjar, 21 May 1886, A. Letourneux (P05438668); El Fedja, 11 May 1888, E. Cosson (P05438689).

Biscutella raphanifolia var. algeriensis

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

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Specimens sharing characters of both morphotypes

**ALGERIA:** Forêt de Taourirt Ighil, dans l’Afkadou (DT de Constantine), 26 May 1912, L. Ducellier (P04745849, P04745850); 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 Tabbabor, 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).