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

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; Guine & Heywood 1993; Al-Shehbaz 2012). 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 (Al-Shehbaz 2012; Guine & 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 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 (Algeria) 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* Cos. & Durieu, and on the other hand the annual plants, which he first named *B. didyma* var. *lyrata* (L.) Cos. (including *B. raphanifolia*) and later (Cosson 1887) *B. didyma* var. *raphanifolia* (Poir.) Cos. 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 Floch & 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</td>
</tr>
<tr>
<td>Silicle size</td>
<td>(4.5–)–5×8 × (8.2–9)–15 mm</td>
<td>2.5–6 × 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</td>
<td>clavate and conical trichomes,</td>
</tr>
<tr>
<td></td>
<td>tiny, conical trichomes</td>
<td>occasionally 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: Alicante</td>
<td>ABH71952</td>
<td>KU746330</td>
<td>KU746332</td>
<td>KU746329</td>
</tr>
<tr>
<td>M. speluncarum RU</td>
<td>Megadenia speluncarum Vorob. &amp; al.</td>
<td>Russia: Primorski Krai, Lozovy</td>
<td>VLA10454</td>
<td>KX943557</td>
<td>KX943556</td>
<td>KX943555</td>
</tr>
<tr>
<td>B. lyrae ES</td>
<td>Biscutella lyrae L.</td>
<td>Spain: Cádiz</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: Soumion, temple of Poseidon</td>
<td>ABH58628</td>
<td>KU570212</td>
<td>KU574021</td>
<td>KU570202</td>
</tr>
<tr>
<td>B. didyma IT</td>
<td>B. didyma</td>
<td>Italy: Martina Franca</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: Nabeul</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: Rohnia</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>Algerie: Berrouaghia</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>Algerie: Blida, Chrea National Park</td>
<td>ABH72641</td>
<td>KU570217</td>
<td>KU574026</td>
<td>KU570207</td>
</tr>
<tr>
<td>B. raph. var. rifanifolia DZ12</td>
<td>B. raphanifolia Poir. var. raphanifolia</td>
<td>Algerie: Wilaya de Tizi-Ouzou</td>
<td>VAL33697</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: Oujda, Beni Snassen</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>Algerie: Tlemcem</td>
<td>ABH69561</td>
<td>KU570220</td>
<td>KU574029</td>
<td>KU570210</td>
</tr>
</tbody>
</table>

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 minicolumns 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 intragenic 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 rpl32F/trnL (Shaw & al. 2007) and trnV/F/R (Wang & al. 2003), respectively. The ampli-
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 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.

Sequencer 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; Akaile 1974). For the three data sets, the Markov and Monte Carlo chains were run for 1.0 × 10⁶ generations and corrections were made to get the final alignment. The in-

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 pinni-

The N African endemic Biscutella raphanifolia

 republic 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, 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.

Fig. 1. Map of localities and morphotypes found.

Three results were 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. 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. (MPS007633, MPS007634, P00166951, P00364814) and B. confusa Pomel (MPS005072) 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 $\times $ 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...
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. B. tereocra incana (L.) DC., Cardamine glauca Spreng. ex DC., C. monensis (L.) Greuter & Burdet, Diplotaxis harra (Forsk.) Boiss., D. maralis (L.) DC., Erucastrum nasturtiifolium (Poir.) O. E. Schulz, Marcuskochia triloba (L.) Al-Shehbaz (Malcolmia lacerca (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).


de Bône, May 1864, V. Rebound [Fragmenta flrorae algeriensis exsiccata no. 503] (P05438661!); isolec-
totyptypes: B10015479!, MPU008649!, MPU023098!, MPU023099!, MPU023100!, P04632019!, P05438730!).

– Lectotype (designated here): In pascuis supra Ben Chicao, solo arenaceo, 24 May 1936, R. Maire  
(MPU003738!).


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-pinnatifidipartite, to 20 × 6 cm, with broad, entire terminal lobe; cauline leaves (absent or) 1–4, well developed, broad, amplexi-
caul to auriculate at base. Inflorescence a profusely 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 fil-
ants filiform; median nectaries inconspicuous or elong-
gated 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 lack-
ing conical trichomes, showing only clavate indumentum on margin and/or centre.

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Appendix: Specimens examined

*Biscutella raphanifolia* var. *raphanifolia*

**ALGERIA**: Massif de l’Akaïdou (Algérie, wilaya de Tizi-Ouzou), route forestière de l’Afakdou, près de la maison forestière de Tala Kitane, à env. 20 km au SE d’Azaiga, 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’Akaïdou (Algérie, wilaya de Tizi-Ouzou), route forestière de l’Akaïdou, près de la maison forestière de Chelia, Massif de l’Aïr, 25 May 1935 (P04745853); Route du fort Gerion près Bône, prov. de Constantine, May 1863, V. Reboud (P05438666); Djebel Edough, 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, prov. de Constantine, Algérie, 26 Jun 1861, E. Cosson (P05438715); Collins de Djebel-Edough, subdivision de Bône, May 1864, V. Reboud (lectotype of *B. radicata*: P05438661); isolectotypes: B100154798, MPU008649, MPU023098, MPU023099, MPU023100, P04632019, P05438718); Plaine de Tamedjadjout, entre les monts Babor et Tababar, prov. de Constantine, 21–22 Juin 1880, E. Cosson (K); Ad acumen 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 Chicaou, 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 Maboua, 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-Fejda (Ouchteta), oued El Hadjar, 21 May 1886. A. Letourneux (P05438658); El Fedja, 11 May 1888, E. Cosson (P05438689).

*Biscutella raphanifolia* var. *algeriensis*


1975 & 25 Jun 1976, A. Dubuis (BC530757, BC630756, MA268267, SEV43939); Bouchata Mahmouda, 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); Azzefoun (Algérie, Kabylie), pentes NW du Djebel Tangout, à 10 km NNE d’Azazga, terrains rocaillieux 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 Kadous, 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; isoelectotypes: 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 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).