Morphology, phylogeny, and taxonomy of *Microthlaspi* (Brassicaceae: Coluteocarpeae) and related genera

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Abstract The genus *Thlaspi* has been variously subdivided since its description by Linnaeus in 1753, but due to similarities in fruit shape several segregates have still not gained broad recognition, despite the fact that they are not directly related to *Thlaspi*. This applies especially to segregates now considered to belong to the tribe Coluteocarpeae, which includes several well-studied taxa, e.g., *Noccaea caerulescens* (syn. *Thlaspi caerulescens*), and the widespread *Microthlaspi perfoliatum* (syn. *Thlaspi perfoliatum*). The taxonomy of this tribe is still debated, as a series of detailed monographs on Coluteocarpeae was not published in English and a lack of phylogenetic resolution within this tribe was found in previous studies. The current study presents detailed phylogenetic investigations and a critical review of morphological features, with focus on taxa previously placed in *Microthlaspi*. Based on one nuclear (ITS) and two chloroplast (*matK*, *trnL-F*) loci, four strongly supported major groups were recovered among the Coluteocarpeae genera included, corresponding to *Ihsanalshehbazia* gen. nov., *Friedrichkarlmeyeria* gen. nov., *Microthlaspi* s.str., and *Noccaea* s.l. In addition, two new species of *Microthlaspi*, *M. sylvarum-cedri* sp. nov. and *M. mediterraneo-orientale* sp. nov., were discovered, which are well supported by both morphological and molecular data. Furthermore, *M. erraticum* comb. nov. (diploid) and *M. perfoliatum* s.str. (polyploid) were shown to be distinct species, phylogenetically widely separate, but with some overlap in several morphological characters. Detailed descriptions, notes on taxonomy, geographical distribution, and line drawings for the new species and each species previously included in *Microthlaspi* are provided. In addition, the current taxonomic state of the tribe Coluteocarpeae is briefly discussed and it is concluded that while several annual taxa are clearly distinct from *Noccaea*, many perennial taxa, after thorough phylogenetic and morphological investigations, may have to be merged with this genus.

Keywords biogeography; Brassicaceae; chloroplast capture; Coluteocarpeae; evolution; flow cytometry; *Microthlaspi*; molecular phylogenetics; morphology; *Noccaea*; polyploidy; systematics; taxonomy; *Thlaspi*

Supplementary Material The Electronic Supplement (Tables S1–S2; Figs. S1–S3; Appendix S1) is available in the Supplementary Data section of the online version of this article at http://ingentaconnect.com/content/iapt/tax; alignments are available from TreeBase: http://purl.org/phylo/treebase/phylows/study/TB2:S18849

■ INTRODUCTION

Brassicaceae is a large monophyletic angiosperm family with a predominantly holarctic distribution (Beilstein & al., 2006; APG III, 2009; Soltis & al., 2011). Even though most members of the family are readily recognised because of the conservation of the number and arrangement of floral organs, its infrafamiliar classification has been subject to much debate (Al-Shehbaz & Warwick, 2007; Warwick & al., 2008). Until recently, tribal classification was based mainly on morphological traits, such as the shape of the fruits, which has been shown to be of little phylogenetic relevance for most groups

(Mummenhoff & al., 1997b; Koch & Al-Shehbaz, 2009; Al-Shehbaz, 2012). While some genera that were based on the morphology of fruits have proved to be monophyletic, like *Capsella* Medik. (Medikus, 1792; Slotte & al., 2006), *Cardamine* L. (Linnaeus, 1753; Franzke & Mummenhoff, 1999), and *Lunaria* L. (Linnaeus, 1753; Al-Shehbaz, 1987; Beilstein & al., 2006; Al-Shehbaz, 2012), other genera were shown to be derived from within large genera, such as *Cardaria* Desv. (Desvaux, 1814; Mummenhoff & al., 2001a) and *Coronopus* Zinn (Zinn, 1757; Al-Shehbaz & al., 2002), and some genera were demonstrated to be polyphyletic, such as *Aethionema* R.Br. (Brown, 1812; Hall & al., 2002; Khosravi & al., 2009) and *Thlaspi* L. (Linnaeus, 1753; Koch & al., 1993; Mummenhoff & Koch, 1994; Mummenhoff & al., 1997a).

Thlaspi L. provides one of the most striking examples for fruit-shape convergence in Brassicaceae. Species of *Thlaspi* as originally delineated are characterised by bipartite winged fruits containing several small yellowish to dark brown seeds (Meyer, 1973, 1979; Mummenhoff & al., 1997b). This morphological character is so convenient for the identification of the genus that even more than 15 years after molecular phylogenetic evidence revealed the polyphyly of the genus, many publications still use the genus name in its old circumscription (Koch & al., 2012; Koch & German, 2013). However, there is insurmountable evidence that *Thlaspi* s.str. is a rather small genus, closely related to other species of the Thlaspideae smelling garlic-like when rubbed, such as *Alliaria petiolata* (M.Bieb.) Cavara & Grande with its long, unwinged fruits (Mummenhoff & Zunk, 1991; Mummenhoff & al., 1997a, b; Koch & Mummenhoff, 2001; Meekins & al., 2001).

Seed coat morphology has been identified as a major characteristic distinguishing *Thlaspi* s.str. from other species now placed in tribe Coluteocarpeae (Meyer, 1973, 1979; Dorofeyev, 2004; Warwick & al., 2010). Probably because seed coat morphology was a less straightforward characteristic compared with fruit shape, the *Thlaspi* segregates advocated by Meyer (1973, 1979, 1991, 2001a, b, 2003a–d, 2006a–e, 2010), including *Noccaea* Moench (Moench, 1802), did not gain broad recognition. However, molecular phylogenetic investigations have shown that *Thlaspi* s.str. and the segregate genera now placed in Coluteocarpeae are not closely related, thus necessitating a narrow delimitation of *Thlaspi* (Al-Shehbaz & al., 2006; Couvreur & al., 2010; Al-Shehbaz, 2014).

Among the species formerly placed in *Thlaspi*, only *Noccaea* (German, 2008; Koch & German, 2013) and *Microthlaspi* F.K.Mey. species (Koch, 1997; Mummenhoff & al., 1997a; Koch & al., 1998; Koch & Bernhardt, 2004) have received broader attention in phylogenetic studies. While *Noccaea* contains biennial to perennial species, often with non-flowering side branches upon fruiting, with large showy flowers condensed into corymbose or densely racemose inflorescences with stout axes, fruits with a style mostly extending from the apical notch and a seed coat which is often minutely reticulate, *Microthlaspi* species are annual, without non-flowering side branches, often with inconspicuous flowers in less dense inflorescences with slender axes, a style mostly embedded in the apical notch of fruit, and smooth seeds. Thus, there are several

morphological characters clearly separating *Microthlaspi* from *Noccaea*, in line with phylogenetic evidence supporting their independence (Mummenhoff & al., 1997a; Koch & al., 1998; Koch & Mummenhoff, 2001).

Microthlaspi has been described by Meyer (1973) to include four annual species formerly classified in *Thlaspi*: *M. perfoliatum* (L.) F.K.Mey. (the type), *M. granatense* (Boiss. & Reut.) F.K.Mey., *M. natolicum* (Boiss.) F.K.Mey., and *M. umbellatum* (Steven ex DC.) F.K.Mey., based on overall morphology and seed coat structure. Of these four species only *M. natolicum* and *M. perfoliatum* have been revealed to be closely related in later studies (Mummenhoff & al., 1997a; Koch & Mummenhoff, 2001; Koch & Al-Shehbaz, 2004), while *M. granatense* and *M. umbellatum* were mostly placed outside *Microthlaspi*, rendering the genus paraphyletic with respect to *Noccaea* (Koch & Mummenhoff, 2001; Koch & German, 2013). Within *M. perfoliatum* and *M. natolicum*, which can be considered the core species of *Microthlaspi*, significant variation is present. This is reflected by the fact that Meyer (1973, 1979, 2003a) recognised a multitude of subspecies within *M. natolicum*, and the finding that at least two distinct lineages exist in *M. perfoliatum*, corresponding to diploid and polyploid types (Koch & al., 1998; Koch & Mummenhoff, 2001; Koch & Al-Shehbaz, 2004; Koch & Bernhardt, 2004).

However, having been carried out more than a decade ago, previous phylogenetic studies on *Microthlaspi* did not result in phylogenetic resolution high enough to fully resolve relationships of *Microthlaspi* spp. and also included only few samples from the Balkans and Turkey, the assumed centre of *Microthlaspi* diversity (Koch & al., 1998; Koch & Bernhardt, 2004). Also a critical morphological assessment of the species placed in *Microthlaspi* by Meyer (1973, 1979) is lacking so far.

It was the aim of this study to investigate the morphology and phylogenetic relationships of *Microthlaspi* species with recent collections from representative locations in the native distribution range of the genus to provide an overview of this small yet widespread genus and to discuss the findings in relation to the taxonomy of the tribe Coluteocarpeae.

■ MATERIALS AND METHODS

Plant material. — For a detailed phylogenetic and morphological re-evaluation of *Microthlaspi*, plants were collected throughout the major part of its European and west Asian distribution area in order to obtain a representative sampling of its diversity. In addition, specimens from herbaria were used to obtain additional sequence data for species of *Noccaea* to broaden taxon sampling of this related genus. Details for newly collected plant material and herbarium specimens used in this study are given in Appendix 1. All freshly collected material was air-dried and stored in paper bags until further use. Whenever possible, plants were collected that already had ripe seeds. Some immature plants were later grown in confined climate chambers until mature seeds had developed.

Chromosome number determination. — Root-tips from germinating seeds of 13 accessions of *Microthlaspi* were used

to determine the numbers of chromocentres in DAPI (4′,6-di-amidino-2-phenylindole)-stained cells at somatic interphase. Preparation of root-tips followed Ørgaard & al. (1995). Slides were frozen in liquid nitrogen, coverslips lifted off using a razor blade, slides dried and DAPI-stained (1 μg/μl). Chromosome numbers were inferred based on the number of chromocentres counted.

DNA ploidy levels. — DNA ploidy levels of plant specimens of *Microthlaspi* s.l. and *Noccaea* were estimated by flow cytometry calibrated with chromosome counts for *Microthlaspi perfoliatum* s.l. (diploid and polyploid cytotypes).

Flow cytometric analyses (FCM) of fresh leaf material were carried out using a Partec CyFlow instrument (Partec, Münster, Germany). The samples were prepared using a standard two-step Otto protocol as summarized by Doležel & al. (2007) with *Glycine max* cv. 'Polanka' as an internal size standard (Doležel & al., 1994). DAPI served as DNA-selective stain. Sample to standard fluorescence ratios were calculated from the means of fluorescence histograms based on at least 3000 scored particles. Only histograms with coefficients of variation (CVs) less than 5% for the G0/G1 peak of the analysed sample were considered.

Chromosome-counted individuals served as reference for the DNA ploidy estimation. DNA ploidy has been assigned based on the regression of sample to standard fluorescence ratios against the ratios of the counted individuals. The specimens used in this study are listed in Appendix 1.

DNA extraction, amplification and sequencing. — DNA was extracted from freshly collected specimens using a Biosprint 96 DNA plant kit (QIAgen, Hilden, Germany) on the KingFisher Flex 96 robotic workstation (Thermo Fisher Scientific, Waltham, Massachusetts, U.S.A.) according to the manufacturer's protocol. For herbarium specimens a PTB (N-phenacylthiazolium bromide) protocol as outlined in Telle & Thines (2008) was used.

PCR amplifications of the nuclear ribosomal internal transcribed spacers (ITS), chloroplast *matK*, and *trnL-F* regions were carried out on a Mastercycler pro vapo protect (Eppendorf, Hamburg, Germany) with the following conditions for all loci. Initial denaturation for 240 s at 95°C, followed by 36 cycles of denaturation (40 s at 95°C), primer annealing (40 s at 56°C), and primer extension (60 s at 72°C), and a final elongation of 240 s at 72°C. PCR products were electrophoresed using TBE-buffered agarose gels containing 1% agarose and 0.67 μg/ml ethidium bromide. For amplification of the ITS regions the primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (White & al., 1990) were used, the *matK* region was amplified using the primers 3F KIM (5′-CGTACAGTACTTTTGTGTTTACGAG-3′) and 1R KIM (ACCCAGTCCATCTGGAAATCTTGGTTC) (Ki-Joong Kim, unpub.), and the *trnL-F* regions were amplified using the primers trnL_c (5′-CGAAATCGGTAGACGCTACG-3′) and trnF_f (5′-ATTTGAACTGGTGACACGAG-3′) (Taberlet & al., 1991).

PCR products were bidirectionally sequenced at the sequencing laboratory of the Biodiversity and Climate Research Centre (BiK-F) using the primers used for PCR and the BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems,

Waltham, Massachusetts, U.S.A. on an ABI 3730 xl capillary sequencer according to the manufacturer's instructions.

Phylogenetic analyses. — The obtained chromatograms were viewed and edited in Chromas Lite v.2.1.1 (Technelysium, South Brisbane, Queensland, Australia) and Geneious Basic v.5.6.6 (Biomatters, Auckland, New Zealand). Contig formation and assemblies were carried out using Geneious v.5.6.6. Sequences from the individual loci were aligned using the MAFFT v.7 (Katoh & Standley, 2013) webserver (http://mafft.cbrc.jp/alignment/server/), using the Q-INS-I algorithm (Katoh & Toh, 2008). All following phylogenetic analyses were carried out for individual loci as well as the concatenated dataset. Minimum Evolution Analysis was carried out using MEGA5 (Tamura & al., 2011) with default settings, except for using the TN substitution model, with 10,000 bootstrap replicates (Felsenstein, 1985). Maximum likelihood and Bayesian analyses were conducted using RAxML v.8 (Stamatakis, 2014) and siMBa (Mishra & Thines, 2014), a graphical user interface for MrBayes (Ronquist & Huelsenbeck, 2003), respectively. RAxML was run using the GTRGAMMA substitution model with 1000 fast bootstrap replicates (Pattengale & al., 2010). MrBayes was run using four incrementally heated chains for 5 million generations, sampling every 1000th tree. The first 30% of the trees were discarded before inferring posterior probabilities and a consensus tree. *Ionopsidium acaule* (Def.) Rchb. was used as an outgroup as it occupies a rather basal position outside the monophyletic Coluteocarpeae (Beilstein & al., 2006 and references therin). As it was expected that ITS, for which a broad taxon sampling could be achieved with newly obtained sequence data and sequences deposited in GenBank, would not result in high resolution of the backbone of the phylogenetic tree, a multigene phylogeny based on nuclear ribosomal ITS and two chloroplast loci with focus on *Noccaea* s.str. and *Microthlaspi* s.l. was carried out. Alignments have been deposited in TreeBase, study accession number S18849.

Morphological investigations. — Representative plants for each of the major genotypes found in the phylogenetic reconstructions (Appendix 1) were grown from seeds under controlled climate conditions in confined climate chambers (15 h light, 9 h dark, at 18°C and 14°C respectively) in order to reduce morphological divergence due to different growth conditions. To document morphological traits, plants were photographed regularly, from the seedling stage to seed set. Care was taken to take pictures from angles that would minimise parallax influence on later measurements, even though we are aware that such effects cannot be fully excluded. However, as all plants were photographed in the same manner, any such effects will affect all plants in the same way, rendering the results comparable. Measurements for rosette leaves and fruits were made from these photographs using AxioVision v.4.8.2 (Carl Zeiss, Oberkochen, Germany). Measurements for petals were made on photos taken of detached petals of individual flowers, again using AxioVision.

Statistical analyses. — Statistical analyses were carried out using R (R Core Team, 2011) v.2.15.3 (64-bit application with Rstudio), and corresponding graphs were plotted using the R package ggplot2 (Wickham, 2009).

■ RESULTS

Chromosome counts and DNA ploidy levels. — Chromosome numbers were counted for seven individuals of three taxa. A set of 46 individuals from 43 populations of the four genus-level clades found were investigated by means of flow cytometry (Electr. Suppl.: Table S1). Chromosome count calibrated sample/standard fluorescence ratios revealed one DNA ploidy level for *Microthlaspi erraticum* (0.147 ± 0.0035, $2n = 2x = 14$), *M. natolicum* subsp. *gaillardotii* F.K.Mey. (0.292 ± 0.0009, $2n = 2x = 14$) and the samples of *M. perfoliatum* s.str. investigated (0.438 ± 0.0116, $2n = 6x = 42$). The ratio of the diploid *Microthlaspi erraticum* was almost exactly one-third of the ratio of the predominantly hexaploid *M. perfoliatum*. Assuming that genome size among closely related taxa is conserved, diploid DNA ploidy ($2n = 2x$) can be assumed for *M. mediterraneo-orientale* (0.223 ± 0.0033) and *M. natolicum* subsp. *sporadium* (0.296 ± 0.0035), which are closely related to *M. natolicum* subsp. *gaillardotii*. Additionally, diploidy is also assumed for *Ihsanalshehbazia granatensis* (0.121 ± 0.0005) and *Friedrichkarlmeyeria umbellata* (0.146 ± 0.0023), as exclusively diploid chromosome counts have been previously reported (Koch & al., 2012). Likewise the fluorescence ratio of 0.188 ± 0.0076 in *Noccaea* spp. is associated with $2n = 2x$ as $2n = 14$ is the only reported chromosome number for members of this genus (Koch & al., 2012). The intermediate ratio 0.362 ± 0.0014 of *M. sylvarum-cedri* suggests tetraploidy, but unfortunately no chromosome counts could be obtained for this species.

Molecular phylogeny. — An ITS-based molecular phylogeny of the tribe Coluteocarpeae including all available sequences of *Thlaspi* segregates with affinities to Coluteocarpeae is presented in Fig. 1, together with some traits of the different genera. In this tree, seven highly distinct clades were resolved by all phylogenetic algorithms used, with monophyly strongly supported in at least one of them (i.e., bootstrap support above or equal to 90 % or posterior probabilitiy above or equal to 0.95). These clades correspond to the genera *Neurotropis* F.K.Mey., *Friedrichkarlmeyeria*, *Vania* F.K.Mey., *Ihsanalshehbazia*, *Microthlaspi*, *Callothlaspi* F.K.Mey. (represented by only one sequence which was resolved as the sister clade to *Noccaea* s.l. with maximum support in the Bayesian analysis), and a clade representing *Noccaea* s.l. The latter clade includes *Noccaea* s.str., several perennial *Thlaspi* segregates, and *Coluteocarpus vesicaria* (L.) Holmboe. These taxa, as discussed later, share several morphological traits with *Noccaea* s.str.

As expected, the phylogenetic tree based on ITS did not resolve relationships among the genus-level clades. A multigene phylogeny with focus on *Noccaea* s.str. and the highly distinct clades previously placed in *Microthlaspi* (i.e., *Friedrichkarlmeyeria* and *Ihsanalshehbazia*) resulted in high resolution of the backbone of the phylogenetic tree. The phylogenetic trees of the individual loci were largely congruent, with the exception of *M. sylvarum-cedri*, which was placed as a distinct lineage sister to *M. natolicum* and *M. mediterraneo-orientale* in ITS-based phylogenies (Electr. Suppl.: Fig. S1), but was found embedded within *M. perfoliatum* in the trees based on chloroplast loci (Electr. Suppl.: Figs. S2, S3). However, as no further inconsistencies and supported conflicting topologies were found, the alignments of the three loci were concatenated to produce a multigene phylogeny for *Microthlaspi* and related genera (Fig. 2).

The phylogenetic reconstruction showed four lineages separated by large genetic distances as evident from branch lengths, and strong to maximum support was obtained for the monophyly of each of these four clades with all three phylogenetic methods used. These clades correspond to *Microthlaspi*, which was resolved as the sister clade to *Noccaea*, and two clades representing *Ihsanalshehbazia* and *Friedrichkarlmeyeria*, which were successive sisters to *Noccaea* and *Microthlaspi*.

Within *Microthlaspi* five well-supported clades were observed, which obtained maximum support from at least one of the phylogenetic reconstruction algorithms used. Within these clades variation was generally low, even when specimens from a larger geographical range were included. The five species-level clades correspond to *M. perfoliatum*, the two subspecies of *M. natolicum* included in this study (*M. natolicum* subsp. *gaillardotii* and subsp. *sporadium*), the new species *M. sylvarum-cedri* and *M. mediterraneo-orientale*, as well as *M. erraticum*, a species already proposed by Jordan (Jordan, 1852), but later regarded as a synonym of *M. perfoliatum* by most authors. The rather large-flowered subspecies of *M. natolicum* from Turkey and Greece formed a monophyletic group sister to the small-flowered *M. mediterraneo-orientale* from Greece and Israel. These two species were grouped together with *M. sylvarum-cedri* from Turkey and *M. perfoliatum* with moderate (bootstrap support 75%–89%, posterior probability 0.85–0.94) to strong support. The grouping of *Microthlaspi sylvarum-cedri* and *M. perfoliatum* received moderate to maximum support.

Noccaea s.l. was well-resolved as a monophyletic clade with strong to maximum support. It was independent from *Microthlaspi* s.str., to which it was the sister clade with strong to maximum support. Within *Noccaea*, those species with more than two representatives were monophyletic. However, infrageneric relationships in *Noccaea* were only poorly resolved. Except for the grouping of *N. kovatsii* and *N. tymphaea*, which received strong to maximum support, no other groups with strong support were found. It is noteworthy that genetic distances between the different species in *Microthlaspi* were mostly higher than in *Noccaea*. *Thlaspiceras* F.K.Mey. with horned fruits, but also a long style, represented by *Thlaspiceras oxyceras* (Boiss.) F.K.Mey., occupied a basal position within *Noccaea* s.l. with low support (bootstrap support below 75%, posterior probability below 0.85) in the phylogenetic reconstruction based on ITS (Fig. 1), but was placed within *Noccaea* in the multilocus phylogeny (Fig. 2), as sister to an unsupported clade. *Ihsanalshehbazia* formed a third major lineage which was sister to *Noccaea* and *Microthlaspi* with high to maximum support. A fourth highly distinct lineage was formed by specimens of *Friedrichkarlmeyeria*, and was sister to the above three genera.

Morphological analyses. — Several characters were measured on plants grown under controlled conditions to rule out modification effects by divergent climatic conditions. The
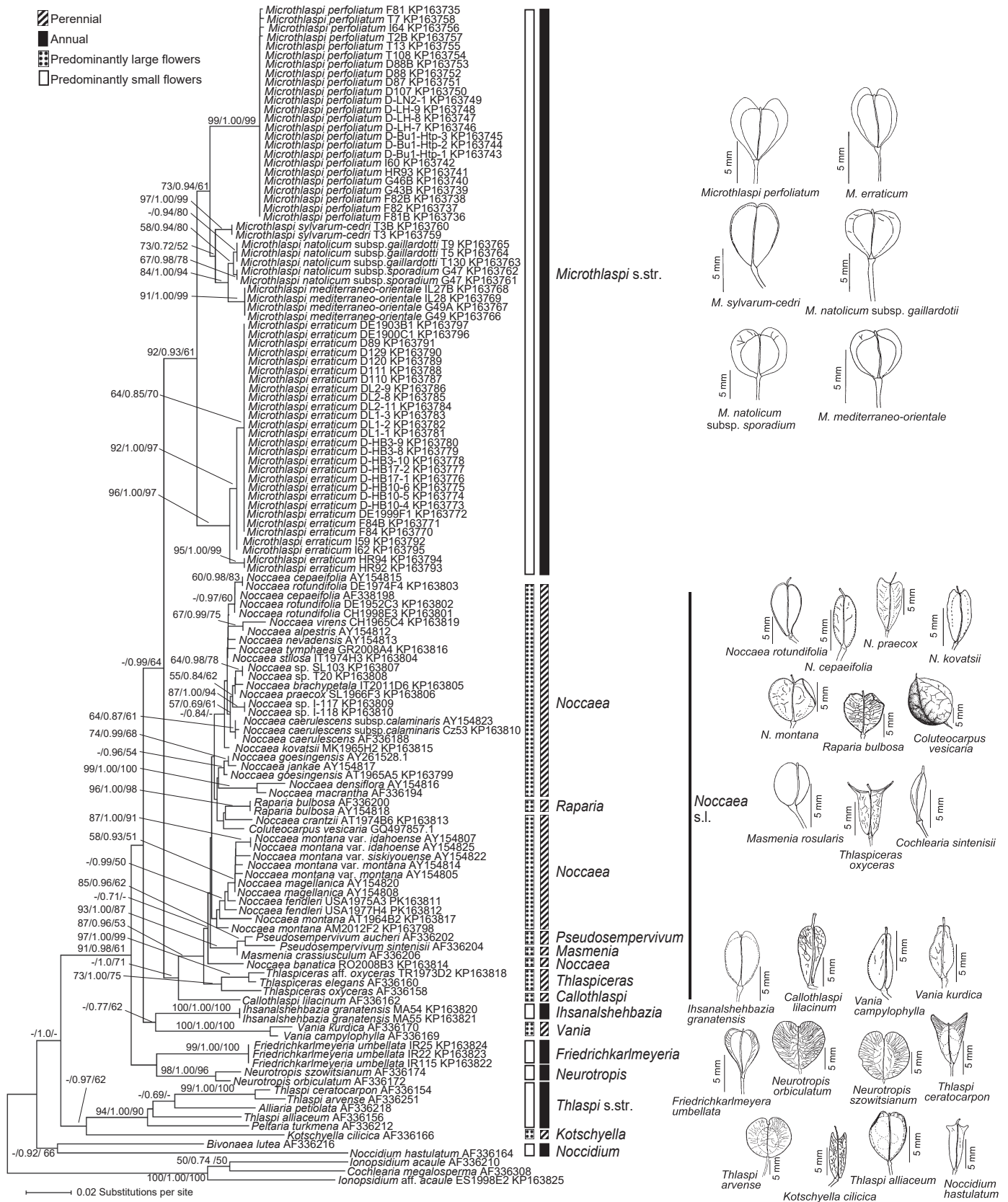
Fig. 1. Overview of fruit shape diversity in Coluteocarpeae and phylogenetic tree (minimum evolution) based on ITS sequences, with support values in minimum evolution (≥50 %), Bayesian inference (≥0.8), and maximum likelihood (≥50 %) at first, second and third position on the branches, respectively. – = no significant support for a conflicting or congruent topology.

Fig. 2. Phylogenetic tree (minimum evolution) based on ITS, *matK*, and *trnL-F* sequences, with support values in minimum evolution (≥50 %), Bayesian inference (≥0.8), and maximum likelihood (≥50 %) at first, second and third position on the branches, respectively. – = no significant support for a conflicting or congruent topology.
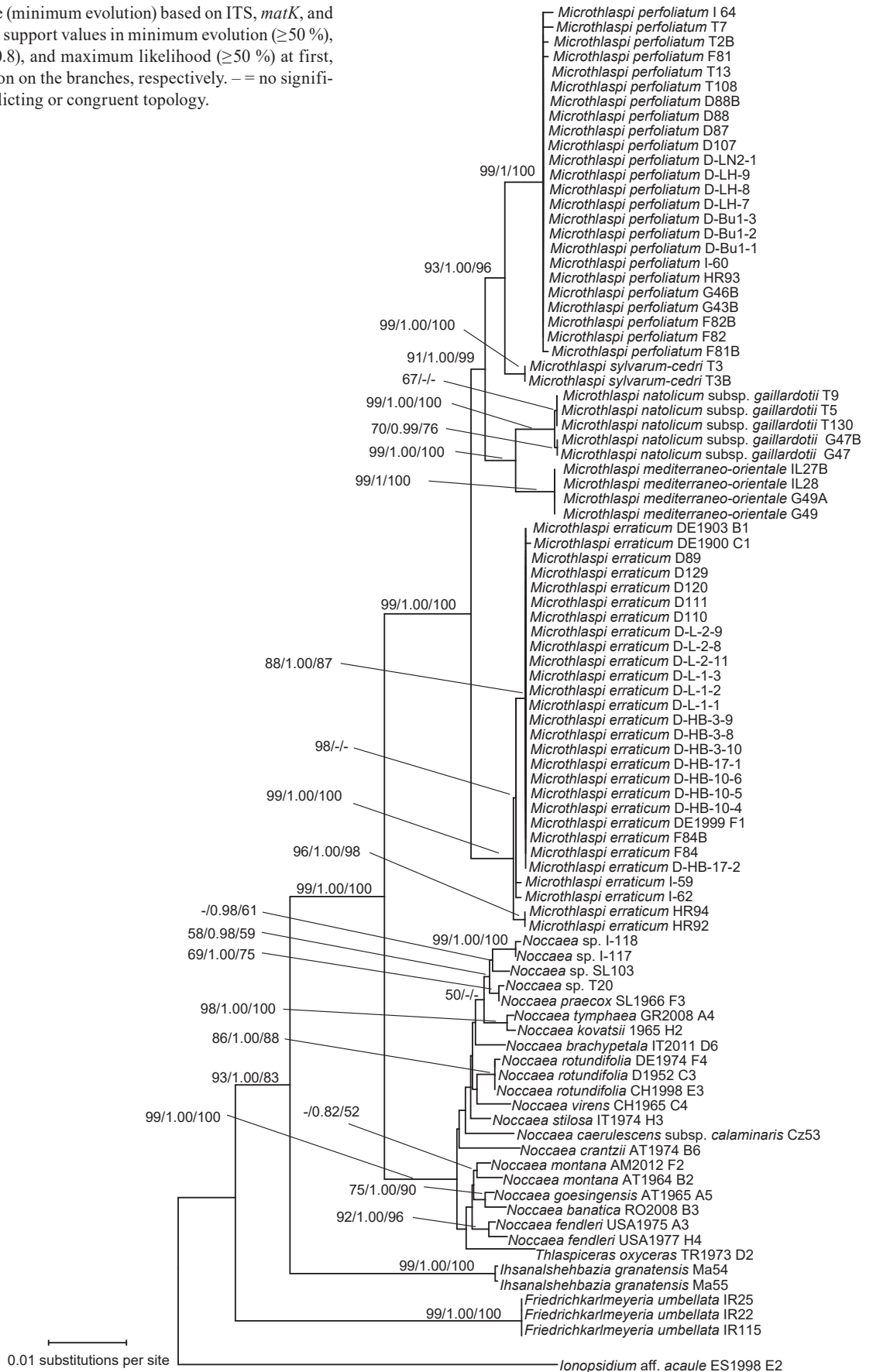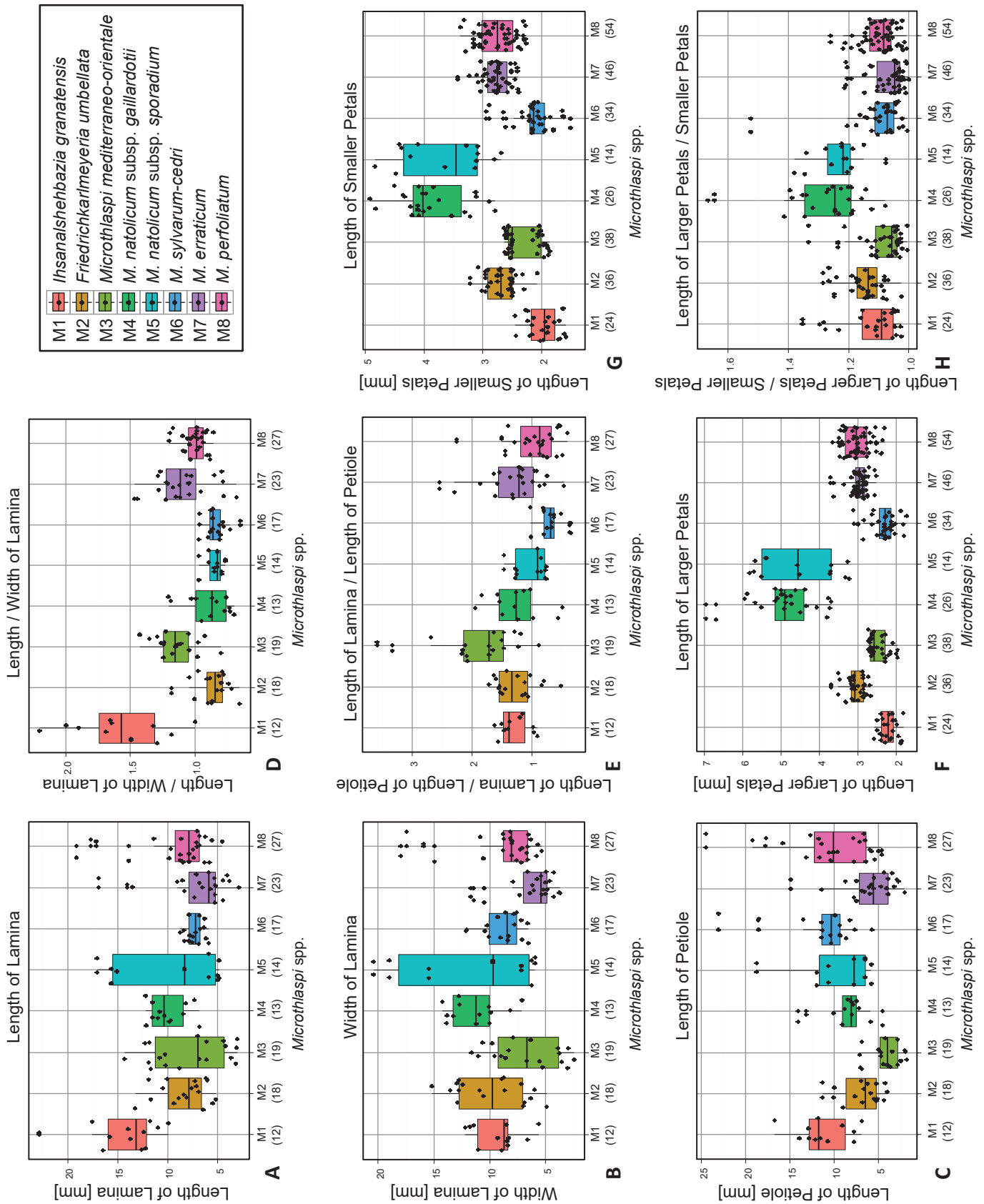
Fig. 3. Scatterplots for various vegetative and floral traits investigated. **A,** Length of the lamina of basal (rosette) leaves; **B,** Width of the lamina of basal (rosette) leaves; **C,** Length of the petiole of basal (rosette) leaves; **D,** Ratio of length to width of basal (rosette) leaves; **E,** Ratio of length of the lamina to length of the petiole of basal (rosette) leaves; **F,** Length of the larger petals; **G,** Length of the smaller petals; **H,** Ratio of length of larger to smaller petals.
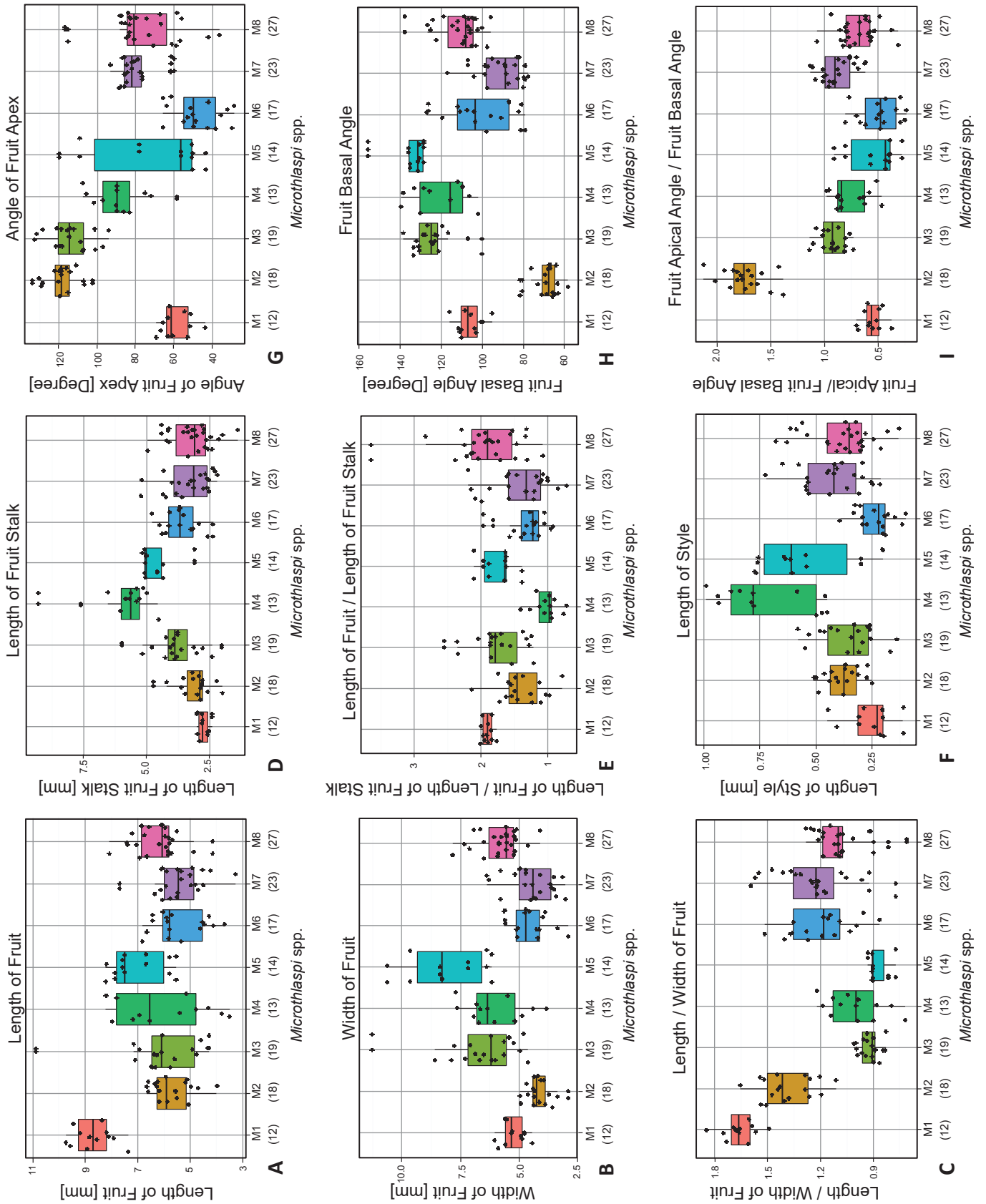
Fig. 4. Scatterplots for various fruit traits investigated. **A,** Length of fruit; **B,** Width of fruit; **C,** Fruit length to width ratio; **D,** Length of fruit stalk; **E,** Length of fruit to length to fruit stalk ratio; **F,** Length of style; **G,** Angle between the wings at the apex of the fruits; **H,** Base angle of the fruit; **I,** Ratio of the fruit apical angle between the wings to fruit basal angle. — For legend, see Fig. 3.

authors are aware that some features might be somewhat different in natural populations. The results of the morphological investigations are presented in Fig. 3 (vegetative traits and flowers) and in Fig. 4 (fruits), as well as in tabular format in Tables 1, 2 and Electr. Suppl.: Table S2.

Rosette leaves were similar in most species investigated, except for *Ihsanalshehbazia*, in which rosette leaves were glossy, with an ovate to lanceolate lamina with entire margins that was tapering into the petiole. In contrast, in *Friedrichkarlmeyeria* and *Microthlaspi* species rosette leaves (Figs. 5B–12B) mostly had a roundish lamina which was irregularly shaped to lobed, often had undulate margins, a base not gradually tapering into the petiole and were comparatively matte. Furthermore, the shape of petals differs among the different lineages. While petals (Figs. 5A–12A) were mostly obtuse at the apex in *Friedrichkarlmeyeria*, they were mostly rounded in *Ihsanalshehbazia* and *Microthlaspi*. However, the most prominent differences between the three genera were observed in their fruits (Figs. 5C–12C). In *Friedrichkarlmeyeria* fruits are heart-shaped, often with convex margins in the lower half, a base tapering narrowly into the stalk, wide wings with prominent parallel venation and delimited by a prominent outer vein, as well as an obtuse to almost straight angle between the wings at the apex (Fig. 6C). In contrast, fruits of *Ihsanalshehbazia* are ovate to elliptic with a rounded base, narrow wings which are broader only at the apex, and an acute angle between the wings at the apex (Fig. 5C), where the wings sometimes even touch above the very short style. Fruits in *Microthlaspi* are heart-shaped to roundish, with an acute to obtuse base, but

are mostly straight to convex in the lower half, and their wings are less wide and have less pronounced venation compared to *Friedrichkarlmeyeria*. However, they are mostly wider than in *Ihsanalshehbazia*, do not touch above the style, and have a less prominent outer vein delimiting the wings compared to *Friedrichkarlmeyeria* (Figs. 7C–12C).

The three genera discussed above are easily distinguished from *Noccaea* s.l. in being annual, by having inflorescences that are rather racemose than corymbose when flowering (except for the early flowering period of *Friedrichkarlmeyeria*), by having inconspicuous white flowers (except for *M. natolicum* with somewhat larger flowers) and a more slender inflorescence
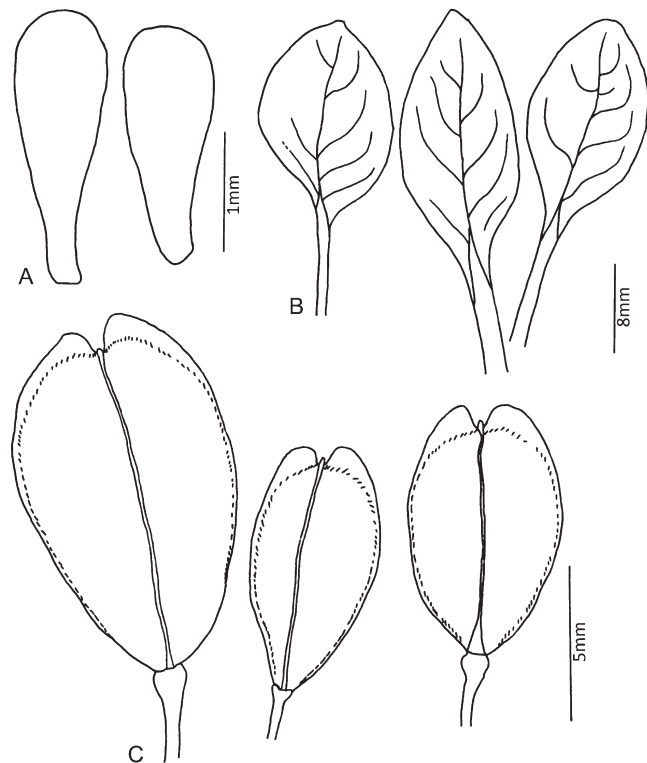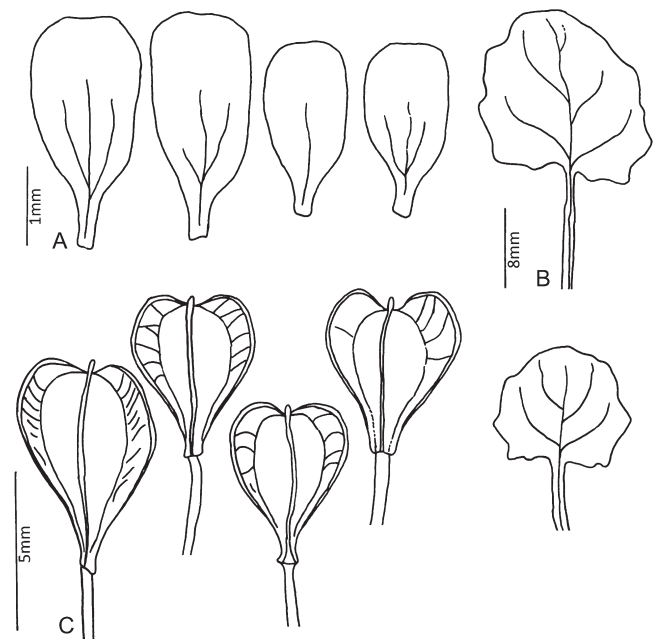
Fig. 6. Line drawings of *Friedrichkarlmeyeria umbellata*. **A,** Petals; **B,** Basal (rosette) leaves; **C,** Fruits.

Fig. 5. Line drawings of *Ihsanalshehbazia granatensis*. **A,** Petals; **B,** Basal (rosette) leaves; **C,** Fruits.
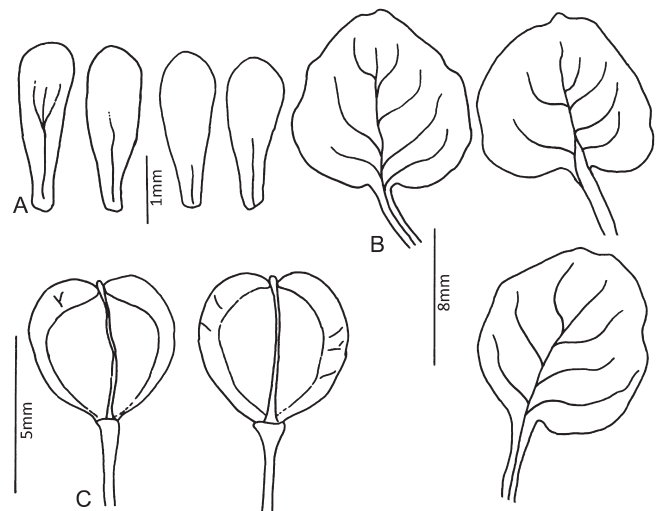
Fig. 7. Line drawings of *Microthlaspi mediterraneo-orientale*. **A,** Petals; **B,** Basal (rosette) leaves; **C,** Fruits.

axis, and by not having non-flowering stems at fruiting. Species of *Noccaea* s.l. are biennial to perennial, with corymbose to densely raceme inflorescences with a stout axis and larger flowers that are often coloured (Table 1).

Within *Microthlaspi* the observed morphological differences corresponded well to the different phylogenetic lineages. The two subspecies of *M. natolicum* included in this study have zygomorphic flowers with larger petals (Figs. 8A, 9A) and

roundish fruits which sometimes are wider than long. Petals were significantly narrowing towards the base in *M. natolicum* subsp. *gaillardotii* as compared to *M. natolicum* subsp. *sporadium*, in which they were slightly wider and more roundish at the apex. Fruits mostly had a rounded base and a short style in *M. natolicum* subsp. *sporadium* from Greece (Fig. 9C). In *M. natolicum* subsp. *gaillardotii* from Turkey (Fig. 8C) a slightly narrower base with an obtuse angle and a comparatively longer style were observed.

Unlike the clearly zygomorphic flowers with large petals in *M. natolicum*, petals in the two new species of *Microthlaspi* described in this study, *M. mediterraneo-orientale* and *M. sylvarum-cedri*, are more similar in length and shorter than 3 mm, separating them from *M. natolicum* (Figs. 3F–H). Fruit shape of the two new species is somewhat similar to that of *M. natolicum*.

Microthlaspi mediterraneo-orientale and *M. sylvarum-cedri* are similar in overall appearance, but the petiole of the rosette leaf is short in *M. mediterraneo-orientale* and long in *M. sylvarum-cedri* (Table 2). The wing of the fruit is wider in *M. mediterraneo-orientale* and the notch angle is narrower in *M. sylvarum-cedri*. Also the average of the style length is different between the two species (Table 2), and the fruits are broader with a more obtuse base in *M. mediterraneo-orientale* than in *M. sylvarum-cedri* (Table 2). *Microthlaspi perfoliatum* and *M. erraticum* differ from *M. sylvarum-cedri* in having wider wings, a mostly less narrow notch angle, and in having usually two more seeds per fruit (Table 2). *Microthlaspi sylvarum-cedri* differs from *M. natolicum* in having more elongated
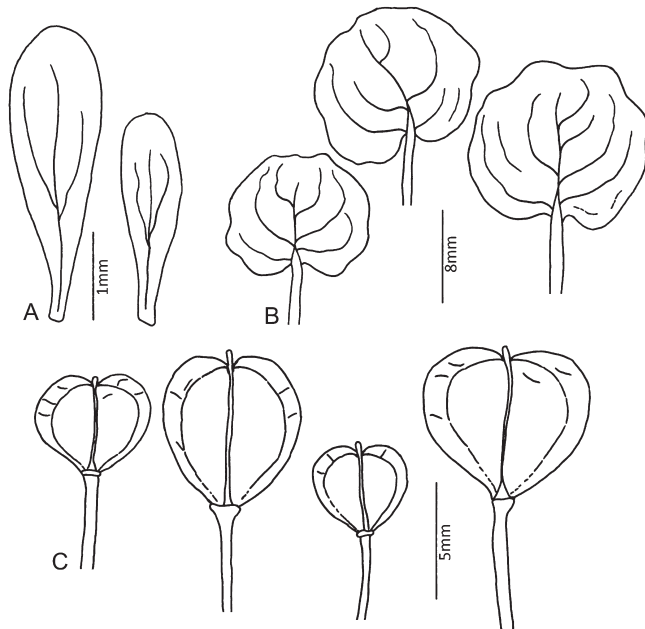

Fig. 8. Line drawings of *Microthlaspi natolicum* subsp. *gaillardotii*. A, Petals; B, Basal (rosette) leaves; C, Fruits.
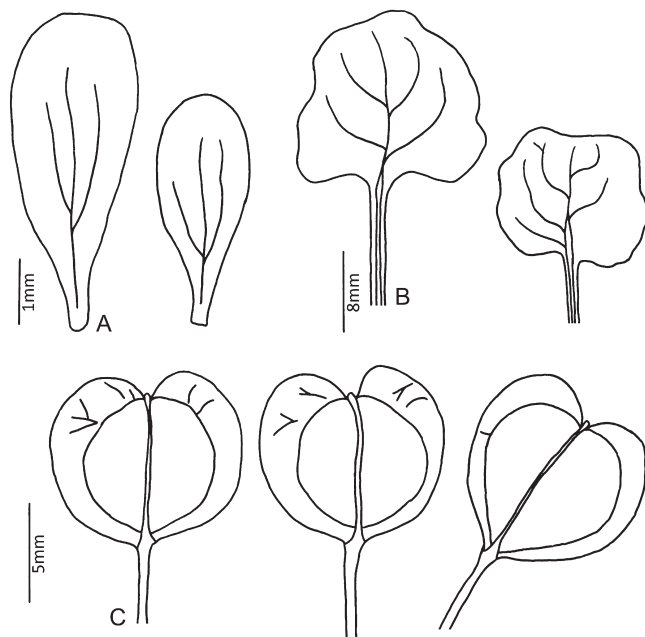

Fig. 9. Line drawings of *Microthlaspi natolicum* subsp. *sporadium*. A, Petals; B, Basal (rosette) leaves; C, Fruits.
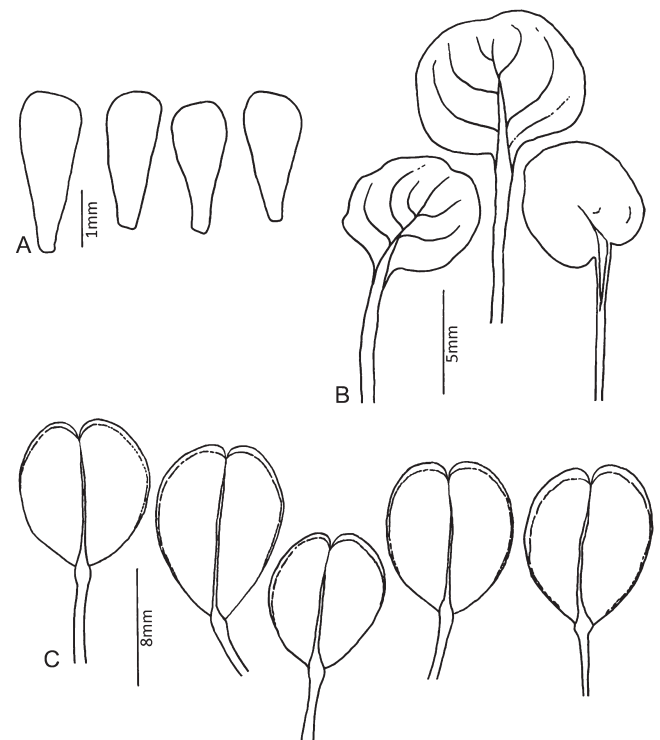

Fig. 10. Line drawings of *Microthlaspi sylvarum-cedri*. A, Petals; B, Basal (rosette) leaves; C, Fruits.

fruits (Table 2). It also differs from this species and *M. mediterraneo-orientale* in having a narrower notch angle.

The morphology of the polyploid *M. perfoliatum* is highly variable and partly overlaps with *M. erraticum*, making it difficult to find clear-cut morphological differences between the two species (Figs. 11, 12). However, there seems to be an overall tendency of *M. erraticum* to have a fruit base with an acute angle, a more acute angle between the wings and generally more elongate fruits. A summary of the morphological features is given in Tables 1, 2, Electr. Suppl.: Table S2.
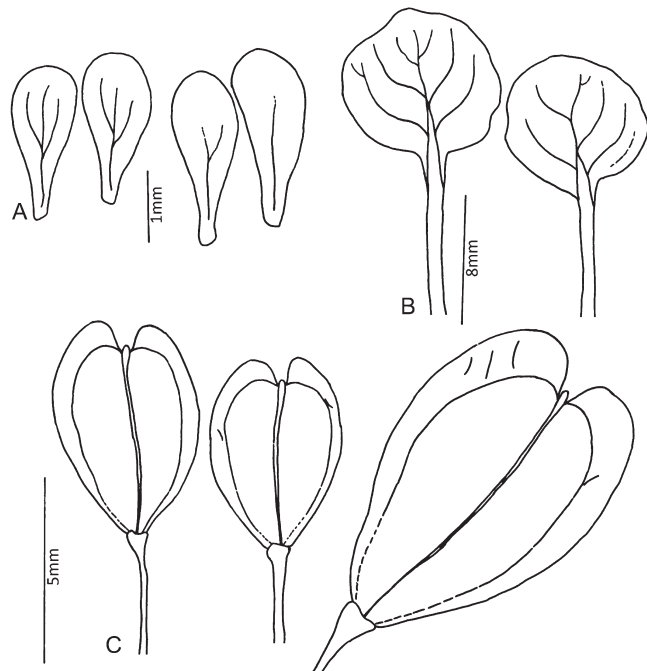

Fig. 11. Line drawings of *Microthlaspi erraticum*. **A,** Petals; **B,** Basal (rosette) leaves; **C,** Fruits.

■ DISCUSSION

Genus concepts of Brassicaceae with winged fruits. — The genus *Thlaspi* was described by Linnaeus (1753) to include a variety of different species with fruits that are broad, bipartite, flattened and often winged. At the same time, Linnaeus (1753) also described additional genera with flattened fruits, either without wings, such as *Clypeola* L. and *Biscutella* L., or even with wings, such as *Iberis* L. Thus, the heterogeneous genus *Thlaspi* was soon divided into several smaller genera, such as *Capsella* (Medikus, 1792; Slotte & al., 2006; Hurka & al., 2012), *Lepidium* (Linnaeus, 1753; Mummenhoff & al., 2001a, 2009), and *Aethionema* (Brown, 1812; Hall & al., 2002). Additional segregates were split from *Thlaspi*, many of which are now widely accepted genus names, such as *Bivonaea* DC.
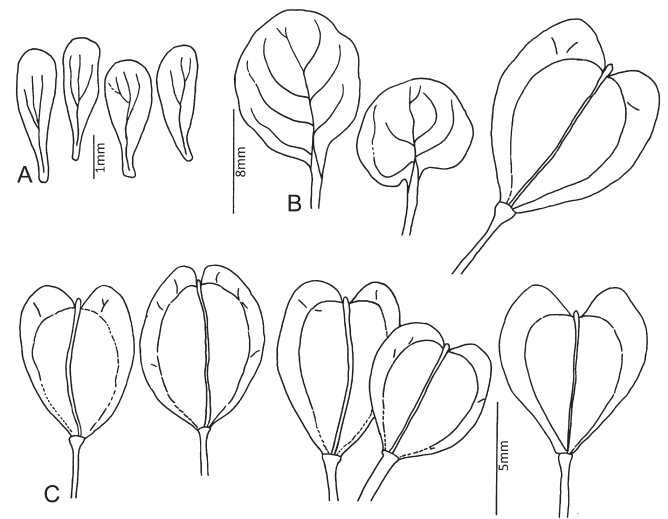

Fig. 12. Line drawings of *Microthlaspi perfoliatum*. **A,** Petals; **B,** Basal (rosette) leaves; **C,** Fruits.

Table 1. Comparison of life cycle and morphological characters of *Noccea* and genera previously placed in *Microthlaspi*.

	Ihsanalshehbazia gen. nov.	*Friedrichkarlmeyeria* gen. nov.	*Microthlaspi* F.K.Mey. s.str.	*Noccaea* Moench
Life cycle	annual	annual	annual	biennial to perennial
Lower leaves				
Margin	entire	undulating, dentate	undulating, dentate	entire
Surface	glossy	matte	matte	matte to slightly glossy
Shape	ovate to lanceolate base tapering into petiole	roundish blunt base	roundish blunt base	mostly roundish to spatulate blunt to tapering base
Inflorescence axis	slender; corymbose at the start of flowering	slender; raceme	slender; raceme	mostly stout; corymbose at (early) flowering
Flower symmetry	radial	radial to slightly zygomorphic	radial to zygomorphic	zygomorphic
Fruits and Seeds				
Fruit shape	ovate	heart-shaped, concave	heart-shaped, convex to roundish	ovate, variously shaped
Style length	highly reduced	short	highly reduced to short	usually long
Mucus production (seeds)	none	limited	pronounced	yes

(Candolle, 1821; Warwick & al., 2010) and *Pachyphragma* (DC.) Rchb. (Reichenbach, 1841; Mummenhof & al., 2001b). However, other segregates from *Thlaspi*, such as *Noccaea* (Koch & German, 2013), were not widely accepted, probably because of the convenience of recognising them as belonging to a single genus of Brassicaceae with flattened and winged fruits. However, also a variety of species without wings on their fruits were retained in *Thlaspi*, such as some species later classified by Meyer (1973) in genera such as *Atropatenia* F.K.Mey.,

Callothlaspi, and *Vania* F.K.Mey. Given the fact that there are several traits clearly separating the *Thlaspi* segregates that are members of the Coluteocarpeae from core *Thlaspi*, such as different chemical composition (Al-Shehbaz & Al-Shammary, 1987; Avestisian & Fursa, 1990; Zunk & al., 1996), flower and fruit anatomy (Schulz, 1936; Koch & al., 1999; Koch & Mummenhof, 2001), leaf shape (Aksoy, 1996; Aksoy & al., 1998), and seed coat morphology (Meyer, 1973, 1979, 2006e), it is at first glance surprising that *Noccaea* and the segregate

Table 2. Comparison of morphological characters of *Microthlaspi* species in culture.

	M. mediterraneo-orientale sp. nov.	M. natolicum subsp. gaillardotii F.K.Mey.	M. natolicum subsp. sporadium F.K.Mey.	M. sylvarum-cedri sp. nov.	M. erraticum comb. nov.	M. perfoliatum (L.) F.K.Mey s.str.
Rosette leaves						
Length of petiole	shorter than lamina	shorter than lamina	shorter than lamina	longer than lamina	slightly shorter to equal to lamina	equal to lamina to longer than lamina
Stem leaves						
Shape	elongated ovate, broadly perfoliate	ovate, broadly perfoliate	ovate, broadly perfoliate	hastate, margins, perfoliate	ovate, broadly perfoliate	roundish to elongated ovate, perfoliate
Margin	entire	slightly serrate	serrate	slightly serrate	slightly serrate to serrate	serrate
Petals						
Flower symmetry	radial, some flowers slightly zygomorphic	zygomorphic	zygomorphic	radial	radial	radial
Petal length	<3 mm	>3 mm	>3 mm	<3 mm	<3 mm	≤3 mm
Petal shape	spatulate	spatulate	spatulate	ovate	spatulate	elongated oval to spatulate
Petal apex	rounded	slightly obtuse	rounded	slightly obtuse	mostly rounded	rounded to slightly obtuse
Fruits						
Fruit shape	broadly ovate to broadly heart-shaped	heart-shaped, roundish	broadly heart-shaped	ovate to oval	narrow heart shaped	heart-shaped, often broadly heart-shaped
Base of fruit	obtuse to rounded	obtuse	slightly obtuse to rounded	slightly roundish	acute	slightly acute to roundish
Fruit length (mean)	6.1 mm	6.5 mm	6.7 mm	5.8 mm	5.5 mm	6.0 mm
Fruit width (mean)	6.2 mm	6.3 mm	6.7 mm	4.7 mm	4.4 mm	5.6 mm
Valve shape	wide wings, widely obtuse angle between the wings at the apex	wide wings, obtuse angle between the wings at the apex	wide wings, obtuse to slightly acute angle between the wings at the apex	very narrow to no wings very acute angle between the wings at the apex	narrow wings, acute angle between the wings at the apex	narrow to wider wings, sometimes acute to usually obtuse angle between the wings at the apex
Valve veins	no prominent veins	few slightly prominent veins	few slightly prominent veins	no prominent veins	few, non-prominent veins	few, non-prominent veins
Style length	short (included in the apical notch)	intermediate	long (protruding from the apical notch)	very short to insignificant	intermediate	short to intermediate
Seed						
Av. number of seeds/fruit	4	6	4	4	6	6

genera described by Meyer (Meyer, 1973, 1979, 2006c, 2010) did not gain broad recognition. Some of the reasons for this have already been discussed by Al-Shehbaz (2014). Apart from the practical ease of determining the genus by some easily accessible characteristics of the fruit, there are probably two main reasons why the segregates never gained wide recognition. One of these might be that Meyer (1973, 1979, 2001a, b, 2003a–d, 2006a–e, 2010) published his most important findings in German only, and in journals not readily available to the scientific community. A second important reason might be that some of the segregates are based on morphological and anatomical peculiarities that are not necessarily criteria generally used for delimiting genera.

The tribe Coluteocarpeae. — Even today the majority of publications use the name *Thlaspi caerulescens* L. for this important heavy metal-tolerant species (for discussion on this, see Koch & German, 2013; Al-Shehbaz, 2014), despite the fact that in terms of relationships this cannot be upheld as no direct relationship with *Thlaspi* exists and the name *Noccaea caerulescens* (L.) F.K.Mey. should instead be applied. Already early phylogenetic investigations (Mummenhoff & Zunk, 1991; Mummenhoff & Koch, 1994) have revealed that the genus *Thlaspi* as traditionally understood is polyphyletic and thus in need of splitting. Later phylogenetic investigations (Zunk & al., 1996; Mummenhoff & al., 1997a, b; Koch & Al-Shehbaz, 2004; Koch & Bernhardt, 2004) provided additional support for the general validity of the splitting concept of Meyer (1973). However, it was also revealed that some of the segregates were closely related to *Noccaea* and probably embedded in this genus (Koch & Mummenhoff, 2001; Koch & Al-Shehbaz, 2004). This is in line with the current study, in which it could be demonstrated that *Thlaspiceras*, represented by its type, *Thlaspiceras oxyceras*, is not clearly distinct from *Noccaea* on the basis of both nuclear ITS and chloroplast DNA sequence variation, and the finding that several other segregates (e.g., *Masmenia* F.Mey., *Raparia* F.K.Mey.) could not be distinguished from *Noccaea* in the ITS-based phylogeny. However, *Noccaea* s.l. containing perennial species with corymbose to densely racemose inflorescences with a stout axis is well-separated from the annual *Microthlaspi* species with elongated racemose inflorescences with a slender axis in the current phylogenetic reconstructions (Figs. 1, 2).

The morphological similarity of some of the perennial genera described by Meyer (1973) and partly unresolved phylogenetic affinities of genera in the Coluteocarpeae led Al-Shehbaz (2014) to propose merging of all genera of the tribe into a single heterogeneous genus, *Noccaea*, based on the claim that family-wide studies by Khosravi & al. (2009) and Warwick & al. (2010) had shown that the ten segregates of Meyer (1973) apart from *Noccidium* F.K.Mey. "grouped together with *Noccaea* to form a rather poorly resolved clade suggesting they form a single genus". Al-Shehbaz (2014) thus came to the conclusion that "molecular data do not support the recognition of more than one entity in the *Noccaea* complex". However, both studies mentioned by Al-Shehbaz (2014) were based solely on ITS sequences, which usually do not offer the possibility to infer relationships among genera in Brassicaceae, even

though genus-level clades can often be identified (Khosravi & al., 2009; Warwick & al., 2010; this study). This is also the reason why Khosravi & al. (2009) and Warwick & al. (2010) aimed at determining the tribal classification of some genera and species of unclear affinity, rather than aiming at reconstructing phylogenetic relationships among genera and tribes. It is noteworthy that the genetic distances in Coluteocarpeae (as Noccaeae) in Warwick & al. (2010) were similar to those in Thelypodieae, Isatideae, and Thlaspideae in the same publication. As an example, the Thlaspideae contain several closely related but well-established genera, such as *Alliaria* Cavarra & Grande and *Thlaspi* (Koch & Mummenhoff, 2001; Koch & Al-Shehbaz, 2004; German & al., 2009). This, in conjunction with the genus-level clades identified in this study and the morphological diversity in Coluteocarpeae, provides evidence that this tribe should not be treated as a single genus.

The two genera *Kotschyella* F.K.Mey. and *Microthlaspi* described by Meyer (1973) were already recognised by Meyer (2003a, d) as being rather heterogeneous. While detailed studies for *Kotschyella* are lacking, the genus *Microthlaspi* was suspected to be polyphyletic or paraphyletic in earlier studies (Koch & Mummenhoff, 2001; Koch & Al-Shehbaz, 2004; Koch & Bernhardt, 2004; Koch & German, 2013) and was shown to contain three genus-level clades in the current study, which are easily distinguished from other genera of the Coluteocarpeae by their annual life cycle and characteristics of the flowers and fruits.

Apart from the genus-level clades supported in the current multigene analysis (*Friedrichkarlmeyeria*, *Ihsanalshehbazia*, *Microthlaspi*, *Noccaea* s.l.), some additional genus-level clades were revealed by previous studies (Koch & Mummenhoff, 2001; Koch & Al-Shehbaz, 2004; Khosravi & al., 2009; Warwick & al., 2010) and the ITS-based phylogenetic analyses in the current study. These clades represent genera that should not be merged with *Noccaea* and include *Vania* (probably including *Kotschyella* s.str. and *Eunomia* DC. p.p.), *Neurotropis* F.K.Mey., and *Callothlaspi*. All of the seven genera recognised in this study show clear-cut morphological synapomorphies (see also the key to the genera of Coluteocarpeae in the taxonomy section) and are phylogenetically distinct.

At the same time, based on previous phylogenetic studies (Koch & Mummenhoff, 2001; Koch & Al-Shehbaz, 2004; Beilstein & al., 2006) and the data presented in the current study, it is apparent that several of the genera described by Meyer (1973, 2006c) could not be separated from *Noccaea*, in line with Al-Shehbaz (2014). Some peculiarities in fruit shape, e.g., horned, inflated, or wingless fruits have probably arisen from within *Noccaea*. A careful revision of the genera with close affinities to *Noccaea*, such as *Callothlaspi*, *Coluteocarpus* Boiss., *Eunomia* p.p., *Masmenia*, *Pseudosempervivum* Pobed., *Raparia*, and *Thlaspiceras*, will have to be conducted to infer whether merging with *Noccaea* might be warranted or whether several genera should be recognised in this complex. However, the proposal by Al-Shehbaz (2014) to merge all Coluteocarpeae species into a single genus, *Noccaea*, is neither supported by morphology nor by molecular data and would result in a highly heterogeneous assembly with few synapomorphic traits. The

uncertainty regarding such an approach is also highlighted by an earlier, non-formal proposal in which Al-Shehbaz (2012) suggested to retain only three genera within Coluteocarpeae, *Coluteocarpus*, *Noccaea*, and *Pseudosempervivum*, even though it had already been shown in an earlier phylogenetic study (Warwick & al., 2010) that this would render *Noccaea* polyphyletic, as some genera included in the synonymy of *Noccaea* (Al-Shehbaz, 2012) are less closely related to *Noccaea* s.str. than *Coluteocarpus* and *Pseudosempervivum*. Also the phylogenetic sketch presented by Koch & German (2013) highlights this situation. Thus, until all sections of *Noccaea* and all other biennial to perennial genera described in the Coluteocarpeae have been included in multigene phylogenies, it seems to be reasonable to abstain from a detailed taxonomic revision of *Noccaea* and other genera probably embedded in the genus (e.g., *Atropatenia* F.K.Mey., *Callothlaspi*, *Coluteocarpus*, *Masmenia*, *Noccaeopsis* F.K.Mey., *Pseudosempervivum*, *Raparia*, *Thlaspiceras*).

Infrageneric classification of *Microthlaspi*. — *Microthlaspi* is well-characterised by comprising annual plants with ovate, perfoliate stem leaves, slender inflorescences, elongate to roundish, often heart-shaped winged fruits, which usually have a straight to convex margin in the lower half, are flattening towards the wings that do not show a prominent venation, and usually leave an opening between the wings that do not touch above the apex, with an angle between 40 and 150 degrees, as well as seeds producing mucus. As revealed in this study, the species *Friedrichkarlmeyeria umbellata* and *Ihsanalshehbazia granatensis*, which were previously placed in *Microthlaspi* (Meyer, 1973, 2003), are distinct from *Microthlaspi* both based on molecular phylogenetic relationships and morphological characteristics. Thus, only two of the species previously accepted in *Microthlaspi* remain in this genus, the type species, *M. perfoliatum*, and *M. natolicum*. These species differ from each other especially in the length of the petals, of which the longer two are on average longer than 3 mm in all subspecies of *M. natolicum* (Meyer, 2003a) and only in rare cases reach 3 mm in *M. perfoliatum* (up to 3.4 mm) according to Meyer (2003a) and in line with own measurements. Both species have been variously subdivided, and especially within *M. natolicum* there seem to be several distinct forms which were treated as subspecies by Meyer (1973, 2003a). *Thlaspi inornatum* Schott (Schott, 1854), collected from the Taurus mountains, was placed in synonymy with *M. natolicum* subsp. *sporadium* by Meyer (2003a), contrary to Boissier (1867), based on the fact that the style was described as relatively short. The phylogenetic reconstruction presented in this study highlights that the morphologically divergent forms representing *M. natolicum*, the *M. natolicum* subsp. *gaillardotii* type, and the *M. natolicum* subsp. *sporadium* type are closely related and only separated by a small genetic distance. Whether all subspecies of *M. natolicum* listed by Meyer (2003a) are similarly closely related needs to be clarified by future studies.

Surprisingly, two new species of *Microthlaspi* were discovered in this study, *M. mediterraneo-orientale* and *M. sylvarum-cedri*, both of which show some similarity to *M. natolicum* in the shape of the fruit, but have petals often of similar length and mostly smaller than 3 mm, clearly separating them from *M. natolicum* and giving the flowers an appearance similar to *M. perfoliatum* and *M. erraticum*. Interestingly, *M. mediterraneo-orientale* seems to have a wider distribution, as it was found both on the Greek Island of Rhodes and in Israel, highlighting the scattered knowledge regarding the diversity and distribution of *Microthlaspi*. It might be possible that the invalid "*Thlaspi micranthum*" (Boissier, 1856) and *Thlaspi perfoliatum* var. *rotundatum* Boiss. (Boissier, 1867), which are, according to Meyer (2003a), based on the same material, are synonyms of *M. mediterraneo-orientale*. Even though it was stated by Boissier (1856) that "*Thlaspi micranthum*" does not differ from *M. perfoliatum* in fruit characteristics, Boissier (1867) described the variety *T. perfoliatum* var. *rotundatum* as having more roundish fruits than *T. perfoliatum* var. *perfoliatum*. However, as apparently no name for this plant on the species level has been validly published previously, we will leave it to future taxonomic studies to reveal if the variety described by Boissier (1867) has to be treated as a synonym of the species described here.

The second new species discovered in the current study, *Microthlaspi sylvarum-cedri*, again has flowers similar to *M. perfoliatum* and *M. erraticum*, fruits with an appearance similar to *M. natolicum* and *M. mediterraneo-orientale*, but less roundish and with a narrower wing than in the former species, rendering it also similar to *M. perfoliatum*. However, the very narrow opening between the wings at the apex of the fruits is even narrower than in *M. erraticum*, from which it can also be distinguished by its more roundish and less elongate fruits. Interestingly, this species is clearly distinct from *M. perfoliatum* in terms of morphology and ITS sequences, but identical in chloroplast sequences. Therefore, *M. sylvarum-cedri* probably represents a case of chloroplast capture through a rare hybridisation event, similar to the situation observed in *Nothofagus* Blume (Acosta & Premoli, 2010; Stegemann & al., 2010; Premoli & al., 2012), *Veratrum* L. (Kikuchi & al., 2010), and some genera of Brassicaceae (Harris & Ingram, 1991; Mummenhoff & al., 1997a; Hansen & al., 2003; Karl & al., 2012).

Microthlaspi perfoliatum is the type of *Microthlaspi* (Meyer, 1973) and has been variously subdivided by Boissier (1856, 1867) and Jordan (1852, 1864). Later authors have usually recognised only two, not easily distinguishable types, the "erraticum-type" and the "improperum-type" (Gandoger, 1884; Schwartz, 1949; Markgraf, 1961; Guterman, 1975), which were usually thought to represent the same species. Koch (1997), Koch & al. (1998), Koch & Hurka (1999), and Koch & Bernhardt (2004) assumed that *Thlaspi erraticum* Jord. would refer to the diploid form and that the diploid form would constitute *M. perfoliatum*, while *Thlaspi improperum* Jord. would refer to the polyploid form. However, Jordan (1852) explicitly stated that *Thlaspi erraticum* differs from the species described by Linnaeus (1753) in having more elongate fruits with a narrower base. An inspection of the type specimen of *Thlaspi perfoliatum* in the Linné Herbarium (available from http://linnean-online.org/7471/) revealed a morphology typical for the polyploid species, *M. perfoliatum*, as the notch angle between the wings is obtuse and the fruits are more roundish than in *M. erraticum*.

Thus, *Thlaspi improperum* apparently refers to a *M. perfoliatum* type with an even more rounded fruit shape, which can often be observed when plants grow in dry habitats (own observations in Spain, Italy and Turkey). The notion that the polyploid species has arisen by early hybridisation of *M. natolicum* and *M. erraticum* (Mummenhoff & al., 1997a; Koch & al., 1998) is not clearly supported. A close inspection of the variable bases shown by Mummenhoff & al. (1997a) revealed that *M. perfoliatum* had 9 private SNPs, while sharing 9 with *M. natolicum* and 9 with *M. erraticum*, *M. natolicum* had 11 private SNPs, while sharing 9 with *M. perfoliatum* and 10 with *M. erraticum*, and *M. erraticum* had 10 private SNPs, while sharing 9 with *M. perfoliatum* and 10 with *M. natolicum*. Thus, the genetic distinctiveness of the three species is similar, suggesting speciation from a common gene pool at about the same time. Thus, the nature and origin of the polyploidy of *M. perfoliatum* remains unclear. As pointed out by various authors and revealed by the detailed investigations by Koch (1995, 1997), it is not easy to separate *M. perfoliatum* and *M. erraticum* based on morphological characters, as the extremes of the variable polyploid species partly overlap with the more conserved morphological features of the diploid species (Koch & Hurka, 1999). However, when the acute angle formed by the base of the fruit together with the acute angle between the wings are considered, there are only few individuals of *M. perfoliatum* that could be easily confused with *M. erraticum*.

■ TAXONOMY

Taxonomic novelties. — The detailed phylogenetic and morphological investigations carried out in the current study revealed the presence of two genus-level clades apart from *Microthlaspi* and *Noccaea* s.l., and the presence of two previously undescribed species in *Microthlaspi*. Therefore, the corresponding taxonomic novelties are introduced here.

Friedrichkarlmeyeria Tahir Ali & Thines, **gen. nov.** – Type: ***Friedrichkarlmeyeria umbellata*** (Steven ex DC.) Tahir Ali & Thines, **comb. nov.** ≡ *Thlaspi umbellatum* Steven ex DC., Syst. Nat. 2: 377. 1821 ≡ *Microthlaspi umbellatum* (Steven ex DC.) F.K.Mey. in Feddes Repert. 84: 453. 1973 ≡ *Noccaea umbellata* (Steven ex DC.) Al-Shehbaz in Harvard Pap. Bot. 19(1): 47. 2014 – Holotype: IRAN, Gilan Province, date unknown, but before 1821, *S.G. Gmelin s.n.*, Herb. Stevens (H barcode H1052113!).

Diagnosis. – Differs from *Ihsanalshehbazia* gen. nov., *Microthlaspi* and *Noccaea* in having heart-shaped fruits with often convex margins in the lower half, with an almost straight angle between the wings at the apex in conjunction with a narrow angle at the base, prominent venation of the wings and a more prominent vein that delimitates the outer margins of the wings (Fig. 6). *Friedrichkarlmeyeria* further differs from these genera in having smaller seeds (1.2 × 0.85 mm). Differs from *Noccaea* in being annual, having inconspicuous petals and a shorter style. Differs from *Microthlaspi* in having seeds that swell but do not produce mucus when wetted.

Etymology. – Dedicated to the memory of Friedrich Karl Meyer (1926–2012), for his important contribution to Brassicaceae systematics by recognising the importance of seed-coat morphology for delimiting monophyletic groups.

Distribution. – Mountains and steppe adjacent to the southern part of the Caspian Sea, from Armenia and Azerbaijan to Iran.

Ploidy. – $2n = 2x = 14$ (Koch & al., 2012; this study)

Note. – The detailed description of *Friedrichkarlmeyeria umbellata* from Meyer (2003a) in a partly simplified translation is given in the Electr. Suppl.: Appendix S1.

Ihsanalshehbazia Tahir Ali & Thines, **gen. nov.** – Type: ***Ihsanalshehbazia granatensis*** (Boiss. & Reut.) Tahir Ali & Thines, **comb. nov.** ≡ *Thlaspi granatense* Boiss. & Reut. in Boissier, Diagn. Pl. Orient., ser. 2, 1: 40. 1854 – Lectotype (designated by Meyer in Haussknechtia 9: 39. 2003): SPAIN, Sierra de Baza, 21 May 1851, *E. Bourgeau 1025* (G barcode G00371933!).
= *Thlaspi rotundifolium* Tineo, Pl. Rar. Sicil. [ed. 2]: 46. 1846, nom. illeg., non Gaudin 1829 ≡ *Thlaspi tinei* Nyman, Syll. Fl. Eur.: 205. 1855 ≡ *Thlaspi tinnoeanum* A.Huet in Battandier & Trabut, Fl. Algérie 1: 40. 1888 – Holotype: ITALY, Sicily, Madonie, Colma Grande before 1846, *Citarda*, *Flora Sicula Exsiccata 385* (PAL No. 5301!).
= *Thlaspi obtusatum* Pomel, Nouv. Mat. Fl. Atl.: 376. 1875 – Holotype: ALGERIA, Oran, Ghar Rouban, before 1875, *A. Pomel s.n.* (MPU barcode MPU005995!).
For further synonymy see Meyer (2003a).

Diagnosis. – Differs from *Friedrichkarlmeyeria* and *Microthlaspi* in having glossy, oval to ovate to lanceolate rosette leaves with a lamina gradually tapering into the petiole and ovate to elliptic fruits with narrow wings, which are only widening towards the apex to form a very narrow opening or even overlap (Fig. 5). Further differs from these genera in having stem leaves that are less elongate and often roundish. Differs from *Noccaea* in being annual, having inconspicuous petals, seeds that produce limited mucus when wetted, and a shorter style.

Etymology. – Dedicated to Ihsan Ali Al-Shehbaz for his contributions towards a natural classification of Brassicaceae.

Distribution. – Montane to subalpine habitats in southeastern Spain, Sicily and the Atlas Mountains, on calcareous soil.

Ploidy. – $2n = 2x = 14$ (Koch & al., 2012; this study)

Note. – The detailed description of *Ihsanalshehbazia granatensis* from Meyer (2003a) in a partly simplified translation is given in the Electr. Suppl.: Appendix S1.

Microthlaspi erraticum (Jord.) Tahir Ali & Thines, **comb. nov.** ≡ *Thlaspi erraticum* Jord., Pugill. Pl. Nov.: 12 [= in Mém. Acad. Natl. Sci. Lyon, Cl. Sci. 1: 223]. 1852 – Holotype: FRANCE, Lyon, Mts. Beugesi, Herb. Jordan, *Jordan s.n.* (LY [not located]; isotypes: LYJB barcode LYJB010364!, P barcode P05122622!).

Further synonyms. – There are several taxa published after Jordan (1852) that might be considered heterotypic synonyms of *Microthlaspi erraticum*. As most descriptions of these taxa lack sufficient detail to determine if they refer to *Microthlaspi*

erraticum, no detailed synonymy is given here. For reference see Meyer (2003a).

Ploidy. – $2n = 2x = 14$ (Koch & al., 1998; this study).

Microthlaspi mediterraneo-orientale Tahir Ali & Thines, **sp. nov.** – Holotype: GREECE, Rhodos, Archangelos, 36°11′N, 28°06′E, elevation 460 m, in cracks of limestone rocks, 20 Mar 2013, *V. Kummer Mp-G-Rh-11-1* (FR barcode FR-0117884).

Diagnosis. – Differs from *Microthlaspi natolicum* in having petals smaller than 3 mm which often are equal in length. Differs from *M. sylvarum-cedri* in having shorter petioles of the rosette leaves, in having more pronounced wings, a longer style, a wider angle between the wings at the apex of the fruits, and a more roundish base of the fruits. Differs from *M. perfoliatum* and *M. erraticum* in having less elongate fruits.

Etymology. – Referring to the East Mediterranean distribution of the species.

Additional specimens examined. – See Appendix 1.

Distribution. – Greece, Israel.

Ploidy. – $2n = 2x = 14$.

Note. – A detailed description of *M. mediterraneo-orientale* is given the Electr. Suppl.: Appendix S1.

Microthlaspi sylvarum-cedri Tahir Ali & Thines, **sp. nov.** – Holotype: TURKEY, Taurus Mountains, open coniferous forest with cedars, 37°11′N, 30°59′E, elevation 330 m, brown soil from limestone, 26 Mar 2012, *F. Runge & M. Thines Mpn_T12_3-1* (FR barcode FR-0177886).

Diagnosis. – Differs from *Microthlaspi natolicum* in having petals smaller than 3 mm which often are equal in length. Differs from *M. mediterraneo-orientale* in having longer petioles of the rosette leaves, less pronounced wings, a shorter style, a narrower angle between the wings at the apex of the fruits, and a less roundish base of the fruits. Differs from *M. perfoliatum* in having narrower wings, and from *M. erraticum* in having more roundish fruits, while differing from both these species in having a petal nail less clearly separated from the petal blade. Differs from *M. mediterraneo-orientale*, *M. natolicum*, and *M. perfoliatum* in having a very narrow opening between the wings at the apex.

Etymology. – Referring to its occurrence in open forests with cedars.

Additional specimens examined. – See Appendix 1.

Distribution. – Taurus Mountains near Antalya, Turkey.

Ploidy. – $2n = 4x? = 28?$

Note. – A detailed description of *M. sylvarum-cedri* is given in the Electr. Suppl.: Appendix S1.

Key to the genera of the tribe Coluteocarpeae. — Because of the absence of detailed phylogenetic studies for some species probably belonging to *Noccaea* s.l., only those genera which are distinct from *Noccaea* s.l. based on both phylogenetic evidence and morphological characteristics are included in the key.

1. Plants annual ……………………………………………… 2
1. Plants perennial ………………………………………… 5

2. Inflorescence corymbose at the beginning of flowering, fruits heart-shaped, fruit base often concave, tapering into the fruit stalk, angle between the wings at the top of the fruit often almost straight ……… ***Friedrichkarlmeyeria***
2. Inflorescence not corymbose, fruits not concave at the base, not tapering into the fruit stalk, angle at the top of the fruit acute to obtuse ………………………………… 3
3. Wings of the fruits more than 2 mm wide, surrounding the central locule completely, wings and fruits with prominent venation……………………………… ***Neurotropis***
3. Wings of the fruits less than 2 mm wide, narrow or absent in the basal part of the fruit, fruits and wings without prominent venation …………………………………… 4
4. Rosette leaves often glossy, tapering into the petiole, stem leaves glaucous, fruit stalk shorter than the fruits, fruits ovate to elliptical, notch angle acute, wings narrow, widest at the top, wings sometimes touching each other above the short style……………………………… ***Ihsanalshehbazia***
4. Rosette leaves matte, not gradually tapering into the petiole, fruit stalk as long or longer than the fruits, fruits broad elliptical to heart-shaped, notch angle acute to obtuse, wings not touching each other above the style…… ***Microthlaspi***
5. Rosette leaves narrowly spatulate to lanceolate, stem leaves spatulate to lanceolate, leaves leathery, rigid; cushion plants…………………………………………… ***Vania***
5. Rosette leaves broadly spatulate to petiolate, not leathery 6
6. At fruiting without non-fruiting creeping shoots, inflorescence racemose, fruits with prominent venation, without prominent wings …………………………… ***Callothlaspi***
6. At fruiting often with non-fruiting creeping shoots, inflorescences often corymbose at the beginning of flowering, sometimes as a short raceme, often elongating when fruits ripen, fruits inflated, or winged, or horned, or without wings or horns, in the latter case mostly without prominent venation on the fruits…………………………***Noccaea*** s.l.

A key to the species of *Microthlaspi*. — For correct species identification several plants of a population should be examined. As the variation of various traits within *M. perfoliatum* is high, special care is needed when identifying individuals of this species, especially to avoid confusion with *M. erraticum*.

1. Flowers zygomorphic, the larger petals longer than 3 mm . ……………………………………………***M. natolicum***
1. Flowers radial, petals shorter than 3 mm………………… 2
2. Fruits without pronounced wings, notch angle acute……… ……………………………………… ***M. sylvarum-cedri***
2. Fruits with prominent wings ……………………………… 3
3. Fruits roundish to broadly elliptical, base rounded or forming an obtuse angle ……………*M. mediterraneo-orientale*
3. Fruits ovate to heart-shaped, base angle obtuse to acute.. 4
4. Petals spatulate with an often wide, indistinct nail; fruits elongate heart-shaped, base angle acute, angle between the wings at the apex mostly acute ………. ***M. erraticum***
4. Petals spatulate mostly with a distinct nail, fruits heart-shaped, base angle obtuse to acute, angle between the wings at the apex mostly obtuse ……….***M. perfoliatum***

■ ACKNOWLEDGEMENTS

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Appendix 1. Details of newly collected plant samples and herbarium material included in this study.

Taxon name, geographic origin (country/locality), coordinates, year of collection, collector, collection code, herbarium code, code used in Fig. 1 (* indicates samples used for flow cytometry/morphometry), (chromosome count [if available]), and GenBank accessions of newly generated (#) sequences (ITS, *trnL-F*, *matK*). Missing information is indicated by a dash (–).

Friedrichkarlmeyeria umbellata (Steven ex DC.) Tahir Ali & Thines: Iran, 36°50′N/54°26′E, 2012, *MR Mirzaee*, *Mp_IR12_Gs_3*, (FR-0119853), IR115*, KP163822#, KP163914#, KP163728#; Iran, 36°41′N/54°09′E, 2012, *MR Mirzaee*, *Mp_IR12_Cd_1*, (FR-0119854), IR22*, KP163823#, KP163915#, KP163729#; Iran, 36°50′N/54°26′E, 2012, *MR Mirzaee*, *Mp_IR12_Gs_3*, (FR-0119855), IR25*, KP163824#, KP163916#, KP163730#; *Ihsanalshehbazia granatensis* (Boiss. & Reut.) Tahir Ali & Thines: Morocco/Wilaya, –, –, –, –, (01-0124-10-00), Ma54*, KP163820#, KP163911#, KP163732#; Morocco/Wilaya, –, –, –, –, (01-0123-10-00), Ma55*, KP163821#, KP163912#, KP163733#; *Ionopsidium* aff. *acaule* Rch.: Spain/Andalusia, –, 1998, *M. Nydegger-Hügli*, *35981*, (FR-0116000), ES1998_E2, KP163825#, KP163913#, KP163734#; *Microthlaspi erraticum* (Jord.) Tahir Ali & Thines: Germany/Hessen, –, 1900, *A.W. Peipers*, *s.n.*, (FR-0111846), DE1900_C1, KP163796#, KP163878#, KP163676#; Germany/Hessen, –, 1903, *A.W. Peipers*, *s.n.*, (FR-0111847), DE1903_B1, KP163797#, KP163854#, KP163677#; Germany/Thüringen, –, 1999, *K. Baumann*, *99/422*, (FR-0111830), DE1999_F1, KP163772#, KP163855#, KP163678#; Germany, 48°36′N/10°10′E, 2011, *Tahir Ali*, *Mp_D_HB_10_4*, (FR-0119856), D_HB_10_4, KP163773#, KP163856#, KP163679#; Germany, 48°36′N/10°10′E, 2011, *Tahir*

Appendix 1. Continued.

Ali, *Mp_D_HB_10_5*, (FR-0119857), D_HB_10_5, KP163774#, KP163857#, KP163680#; Germany, 48°36′N/10°10′E, 2011, *Tahir Ali, Mp_D_HB_10_6*, (FR-0119858), D_HB_10_6, KP163775#, KP163858#, KP163681#; Germany, 48°36′N/10°10′E, 2011, *Tahir Ali, Mp_D_HB_17_1*, (FR-0119859), D_HB_17_1, KP163776#, KP163859#, KP163682#; Germany, 48°36′N/10°10′E, 2011, *Tahir Ali, Mp_D_HB_17_2*, (FR-0119860), D_HB_17_2, KP163777#, KP163860#, KP163683#; Germany, 48°36′N/10°10′E, 2011, *Tahir Ali, Mp_D_HB_3_10*, (FR-0119861), D_HB_3_10, KP163778#, KP163861#, KP163684#; Germany, 48°36′N/10°10′E, 2011, *Tahir Ali, Mp_D_HB_3_8*, (FR-0119862), D_HB_3_8, KP163779#, KP163862#, KP163685#; Germany, 48°36′N/10°10′E, 2011, *Tahir Ali, Mp_D_HB_3_9*, (FR-0119863), D_HB_3_9, KP163780#, KP163863#, KP163686#; Germany, 48°36′N/9°31′E, 2011, *Marco Thines, Mp_D_L_1_1*, (FR-0119864), D_L_1_1, KP163781#, KP163864#, KP163687#; Germany, 48°36′N/9°31′E, 2010, *Marco Thines, Mp_D_L_1_2*, (FR-0119865), D_L_1_2, KP163782#, KP163865#, KP163688#; Germany, 48°36′N/9°31′E, 2010, *Marco Thines, Mp_D_L_1_3*, (FR-0119866), D_L_1_3, KP163783#, KP163866#, KP163689#; Germany, 48°36′N/9°31′E, 2010, *Marco Thines, Mp_D_L_2_11*, (FR-0119867), D_L_2_11* (14), KP163784#, KP163867#, KP163690#; Germany, 48°36′N/9°31′E, 2010, *Marco Thines, Mp_D_L_2_8*, (FR-0119868), D_L_2_8, KP163785#, KP163868#, KP163691#; Germany, 48°36′N/9°31′E, 2010, *Marco Thines, Mp_D_L_2_9*, (FR-0119869), D_L_2_9, KP163786#, KP163869#, KP163692#; Germany, 48°55′N/11°36′E, 2012, *Sabine Telle, Mp_D_Je12_4_10*, (FR-0119870), D89* (14), KP163791#, KP163874#, KP163697#; Germany, –, 2010, *Marco Thines, Mp_D_N2_1*, (FR-0119871), D111* (14), KP163788#, KP163871#, KP163694#; Germany, 48°36′N/9°31′E, 2010, *Marco Thines, Mp_K3*, (FR-0119872), D120* (14), KP163789#, KP163872#, KP163695#; Germany, 48°36′N/9°31′E, 2010, *Marco Thines, Mp_K11*, (FR-0119873), D129*, KP163790#, KP163873#, KP163696#; Germany, 48°36′N/9°31′E, 2010, *Marco Thines, Mp_D_L2_11*, (FR-0119874), D110*, KP163787#, KP163870#, KP163693#; France, 44°50′N/5°47′E, 2013, *Ann-Katrin Buch & Xiaojuan Xia, Mp_F13_24_1*, (FR-0119875), F84*, KP163770#, KP163852#, KP163670#; France, 44°50′N/5°47′E, 2013, *Ann-Katrin Buch & Xiaojuan Xia,* Mp_F13_24_2, (FR-0119876), F84B*, KP163771#, KP163853#, KP163671#; Croatia, 44°19′N/15°51′E, 2013, *Sebastian Ploch & Tahir Ali, Mp_HR13_22_1*, (FR-0119877), HR92*, KP163793#, KP163875#, KP163672#; Croatia, 44°15′N/16°01′E, 2013, *Sebastian Ploch & Tahir Ali, Mp_HR13_20_2*, (FR-0119878), HR94*, KP163794#, KP163876#, KP163673#; Italy, 45°47′N/9°40′E, 2011, *Marco Thines, Mp_I_Ex_45_6*, (FR-0119879), I_59* (14), KP163792#, KP163877#, KP163674#; Italy, 45°50′N/11°18′E, 2011, *Marco Thines & Lisa Nigrelli, Mp_I12_Ex35_2*, (FR-0119880), I_62*, KP163795#, KP163879#, KP163675#; ***M. mediterraneo-orientale*** Tahir Ali & Thines: Greece, 36°11′N/28°06′E, 2013, *Volker Kummer, Mp_G13_Rh_11_6*, (FR-0119881), G49*, KP163766#, KP163885#, KP163703#; Greece, 36°11′N/28°06′E, 2013, *Volker Kummer, Mp_G13_Rh_11*, (FR-0119882), G49A*, KP163767#, KP163886#, KP163704#; Israel, 33°0.81′N/35°21′E, 2011, *Ouria Orren, Mp_IL_HOR_1*, (FR-0119883), IL27B*, KP163768#, KP163887#, KP163705#; Israel, 32°29′N/35°06′E, 2012, *Ouria Orren, Mp_IL12_Ba_2*, (FR-0119884), IL28*, KP163769#, KP163888#, KP163706#; **M. natolicum** subsp. *sporadium* F.K.Mey.: Greece, 36°09′N/27°46′E, 2013, *Volker Kummer, Mp_G13_Rh_7_2*, (FR-0119885), G47*, KP163761#, KP163880#, KP163698#; Greece, 36°09′N/27°46′E, 2013, *Volker Kummer, Mp_G13_Rh_7*, (FR-0119886), G47B*, KP163762#, KP163881#, KP163699#; **M. natolicum** subsp. *gaillardotii* F.K.Mey.: Turkey, 36°45′N/32°0.65′E, 2012, *Marco Thines & Fabian Runge, Mn_T12_37_2*, (FR-0119887), T130* (14), KP163763#, KP163882#, KP163700#; Turkey, 36°44′N/31°57′E, 2012, *Marco Thines & Fabian Runge, Mn_T12_35_1*, (FR-0119888), T5*, KP163764#, KP163884#, KP163701#; Turkey, 36°30′N/30°19′E, 2012, *Marco Thines & Fabian Runge, Mn_T12_22_2*, (FR-0119889), T9*, KP163765#, KP163883#, KP163702#; **M. perfoliatum** (L.) F.K.Mey.: Germany, 49°32′N/9°20′E, 2010, *Fabian Runge, Mp_D_Bu1_Htp_1*, (FR-0119890), D_Bu1_1, KP163743#, KP163832#, KP163652#; Germany, 49°32′N/9°20′E, 2010, *Fabian Runge, Mp_D_Bu1_Htp_2*, (FR-0119891), D_Bu1_2, KP163744#, KP163833#, KP163653#; Germany, 49°32′N/9°20′E, 2010, *Fabian Runge, Mp_D_Bu1_Htp_3*, (FR-0119892), D_Bu1_3, KP163745#, KP163834#, KP163654#; Germany, 49°09′N/7°12′E, 2011, *Marco Thines, Mp_D_LH_7*, (FR-0119893), D_LH_7, KP163746#, KP163835#, KP163655#; Germany, 49°09′N/7°12′E, 2011, *Marco Thines, Mp_D_LH_8*, (FR-0119894), D_LH_8, KP163747#, KP163836#, KP163656#; Germany, 49°09′N/7°12′E, 2011, *Marco Thines, Mp_D_LH_9*, (FR-0119895), D_LH_9, KP163748#, KP163837#, KP163657#; Germany, 49°03′N/9°09′E, 2010, *Fabian Runge, Mp_D_LN2_htp_1*, (FR-0119896), D_LN2_1, KP163749#, KP163838#, KP163658#; Germany, –, 2010, *Marco Thines, Mp_D_N2_1*, (FR-0119897), D107*, KP163750#, KP163839#, KP163659#; Germany, 48°55′N/11°36′E, 2012, *Sabine Telle, Mp_D_Je12_2_5*, (FR-0119898), D87*, KP163751#, KP163840#, KP163660#; Germany, 48°52′N/10°22′E, 2012, *Marco Thines, Mp_D_Bo12_6_11*, (FR-0119899), D88*, KP163752#, KP163841#, KP163661#; Germany, 48°52′N/10°23′E, 2012, *Marco Thines, Mp_D_Bo12_7_3*, (FR-0119900), D88B* (42), KP163753#, KP163842#, KP163662#; France, 44°22′N/4°31′E, 2011, *Fabian Runge, Mp_F4_8*, (FR-0119901), F81*, KP163735#, KP163826#, KP163644#; France, 44°10′N/5°03′E, 2011, *Fabian Runge, Mp_F7_8*, (FR-0119902), F81B*, KP163736#, KP163848#, KP163645#; France, 48°41′N/5°47′E, 2012, *Marco Thines & Sebastian Ploch, Mp_Fr12_El5_5*, (FR-0119903), F82*, KP163737#, KP163827#, KP163646#; France, 48°52′N/5°50′E, 2012, *Marco Thines & Sebastian Ploch,* Mp_Fr12_El7_7, (FR-0119904), F82B*, KP163738#, KP163828#, KP163647#; Greece, 39°45′N/20°45′E, 2011, *Angelika Schmuker, Mp_Gr9_10*, (FR-0119905), G43B*, KP163739#, KP163849#, KP163648#; Greece, 39°52′N/20°38′E, 2011, *Angelika Schmuker, Mp_Gr11_3*, (FR-0119906), G46B*, KP163740#, KP163829#, KP163649#; Croatia, 44°24′N/15°53′E, 2013, *Sebastian Ploch & Tahir Ali, Mp_HR13_23_1*, (FR-0119907), HR93*, KP163741#, KP163830#, KP163650#; Italy, –, 2011, *Marco Thines, Mp_I_Ex_25_9*, (FR-0119908), I_60*, KP163742#, KP163831#, KP163651#; Italy, 45°33′N/11°11′E, 2012, *Marco Thines & Lisa Nigrelli, Mp_I12_Ex_19_3*, (FR-0119909), I_64*, KP163756#, KP163850#, KP163826#; Turkey, 41°07′N/31°23′E, 2013, *Marco Thines & Tahir Ali, Mp_T13_65*, (FR-0119910), T108*, KP163754#, KP163843#, KP163665#; Turkey, 41°36′N/33°14′E, 2013, *Marco Thines & Tahir Ali, Mp_T13_60_8*, (FR-0119911), T13*, KP163755#, KP163844#, KP163666#; Turkey, 36°31′N/30°20′E, 2012, *Marco Thines & Fabian Runge, Mp_T12_21*, (FR-0119912), T2B*, KP163757#, KP163845#, KP163667#; Turkey, 36°39′N/30°24′E, 2012, *Marco Thines & Fabian Runge, Mp_T12_19_3*, (FR-0119913), T7*, KP163758#, KP163846#, KP163668#; ***M. sylvarum-cedri*** Tahir Ali & Thines: Turkey, 37°21′N/30°59′E, 2012, *Marco Thines & Fabian Runge, Mn_T12_2_2*, (FR-0119914), T3*, KP163759#, KP163851#, KP163663#; Turkey, 37°11′N/30°59′E, 2012, *Marco Thines & Fabian Runge, Mn_T12_3_1*, (FR-0117886), T3B*, KP163760#, KP163847#, KP163664#; ***Noccaea banatica*** (R.Uechtr.) F.K.Mey.: Romania/Prahova, 45°30′N/25°57′E, 2008, *T. Gregor & L. Meierott, 4367*, (FR-0115993), RO2008_B3, KP163814#, KP163891#, KP163708#; **N. brachypetala** (Jord.) F.K.Mey.: Italy/Piemonte, 44°08′N/7°21′E, 2011, *T. Gregor, 8016D*, (FR-0115914), IT2011_D6, KP163805#, KP163900#, KP163721#; **N. caerulescens** subsp. *calaminaris* (Lej.) Holub: Czech Republic, –, –, –, –, (96-0032-10-00), Cz53, KP163800#, KP163890#, KP163726#; **N. crantzii** F.K.Mey.: Austria/Niederösterreich, –, 1974, *F. Grims, s.n.*, (FR-0115918), AT1974_B6, KP163813#, KP163899#, KP163725#; **N. fendleri** (A.Gray) Holub: U.S.A./Arizona, –, 1975, *W.J. Schrenk, 1316*, (FR-0115994), USA1975_A3, KP163811#, KP163908#, KP163710#; U.S.A./Wyoming, –, 1977, *W.J. Schrenk, 2371*, (FR-0115958), USA1977_H4, KP163812#, KP163910#, KP163711#; **N. goesingensis** (Halácsy) F.K.Mey.: Austria/Steiermark, –, 1965, *B. Malende, s.n.*, (FR-0115957), AT1965_A5, KP163799#, KP163898#, KP163709#; **N. kovatsii** (Heuff.) F.K.Mey.: Macedonia, –, 1965, *Fr. Cernoch, s.n.*, (FR-0115997), MK1965_H2, KP163815#, KP163906#, KP163715#; **N. montana** (L.) F.K.Mey.: Austria/Niederösterreich, –, 1964, *K.P. Buttler, 64-1332*, (FR-0111821), AT1964_B2, KP163798#, KP163892#, KP163712#; Armenia/Lorri, 40°45′N/44°37′E, 2012, *T. Gregor, 9431*, (FR-0037882), AM2012_F2, KP163798#, KP163889#, KP163707#; **N. praecox** (Wulfen) F.K.Mey.: Slovenia, –, 1966, *K.P. Buttler, 9682*, (FR-0115980), SL1966_F3, KP163806#, KP163902#, KP163727#; **N. rotundifolia** (L.) Moench: Switzerland, –, 1998, *T. Gregor, 875*, (FR-0115982), CH1998_E3, KP163801#, KP163893#, KP163717#; Germany/Bayern, –, 1952, *B. Malende, s.n.*, (FR-0115992), D1952_C3, KP163802#, KP163894#, KP163718#; Germany/Bayern, –, 1974, *K.Schuh, s.n.*, (FR-0115960), DE1974_F4, KP163803#, KP163895#, KP163719#; **N. stilosa** (Ten.) Rchb.: Italy/Abruzzo, –, 1974, *K.P. Buttler & E. 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Erben, 17870*, (FR-0111818), TR1973_D2, KP163818#, KP163897#, KP163720#.