Phylogeny and systematics of the tribe Thlaspideae (Brassicaceae) and the recognition of two new genera

Shokouh Esmailbegi,[1,2] Ihsan A. Al-Shehbaz,[3] Milan Pouch,[2] Terezie Mandáková,[2] Klaus Mummenhoff,[4] Mohammad Reza Rahiminejad,[1] Mansour Mirtadzadini[5] & Martin A. Lysak[2]

1 *Department of Biology, University of Isfahan, Hezarjarib Street, Isfahan 81746-73441, Iran*
2 *Central European Institute of Technology (CEITEC) and Faculty of Science, Masaryk University, Kamenice 5, 62500 Brno, Czech Republic*
3 *Missouri Botanical Garden, P.O. Box 299, St. Louis, Missouri 63166-0299, U.S.A*
4 *Department of Biology, Botany, University of Osnabrück, Barbarastrasse 11, 49076 Osnabrück, Germany*
5 *Shahid Bahonar University of Kerman, 22 Bahman Blvd., Afzalipour Square, P.O. Box 76169-133, Kerman, Iran*
Author for correspondence: *Martin A. Lysak, martin.lysak@ceitec.muni.cz*

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Abstract Thlaspideae is an Old World tribe of Brassicaceae centered in SW Asia. Thirty-seven of 42 species (ca. 88%) in 13 genera of the tribe were analyzed using nuclear ITS and chloroplast *trnL-F* markers in a family-wide context. Both single-marker and concatenated phylogenies corroborated Thlaspideae as a well-supported monophyletic clade. With the exception of polyphyletic *Alliaria* and *Parlatoria* and paraphyletic *Thlaspi* and *Didymophysa*, the remaining genera were monophyletic. *Alliaria petiolata* comprised diploid and hexaploid populations in two well-resolved clades. The non-weedy diploid and hexaploid populations are restricted to SW Asia, and together with diploid *A. taurica* (formerly *P. taurica*), formed a sister clade to well-resolved *Sobolewskia* (3 spp.) and *P. rostrata* (now treated as the new monospecific genus *Lysakia*) clades. By contrast, the European and North American weedy and invasive hexaploid *A. petiolata* populations clustered with the diploid *P. cakiloidea*. Polyphyletic *Thlaspi* formed two distinct clades easily distinguished morphologically, and two of its six species are segregated into the new genus *Mummenhoffia*. *Elburzia* is reduced to synonymy of *Didymophysa*, and the new combinations *D. fenestrata*, *Lysakia rostrata*, *Mummenhoffia alliacea*, and *M. oliveri* are proposed and a diagnostic key for determination of Thlaspideae genera is presented. Age estimations based only on calibration by the controversial fossil *Thlaspi primeavum* resulted in unrealistic old age estimates. Chromosome counts are reported for 16 species.

Keywords *Alliaria*; chromosome counts; Cruciferae; *Didymophysa*; *Elburzia*; *Lysakia*; *Mummenhoffia*; *Parlatoria*; phylogeny; taxonomy; Thlaspideae

Supplementary Material The Electronic Supplement (Tables S1, S2; Figs. S1–S3; Appendix S1) and DNA sequence alignment are available from https://doi.org/10.12705/672.4.S1 and https://doi.org/10.12705/672.4.S2, respectively.

■ INTRODUCTION

The Brassicaceae (Cruciferae, or mustard family) include some 3980 species in 351 genera of 52 tribes (BrassiBase, 2017; Kiefer & al., 2014). It is of a special interest because it includes many vegetable crops (e.g., *Brassica oleracea* L., *B. rapa* L., *Raphanus sativus* L.), ornamentals (e.g., *Arabis* L., *Aubrieta* Adans., *Erysimum* L., *Iberis L.*, *Lunaria* L.), condiments (*Armoracia rusticana* G. Gaertn. & al., *Eutrema japonicum* (Miq.) Koidz., *Sinapis alba* L.), vegetable oils such as canola (*B. napus* L.), numerous weeds, and several model plants, such as *Arabidopsis thaliana* (L.) Heynh. (Franzke & al., 2011).

The family is distributed on all continents except Antarctica, and it is particularly abundant in the temperate and alpine areas of the world, with the major distribution centers in the Irano-Turanian region, Mediterranean region, and western North America (Hedge, 1976; Appel & Al-Shehbaz, 2003; Al-Shehbaz & al., 2006). Turkey has the highest concentration of native crucifers in any country, and some (e.g., Franzke & al., 2011; Mohammadin & al., 2017) have proposed that it might have been the cradle of the family. The age estimate of the family is not fully settled because some (e.g., Beilstein & al., 2010) suggested an Eocene origin (ca. 54 Ma), whereas most other authors suggested a younger origin in the Late Oligocene, with the major diversification ca. 25 Ma in the Miocene (e.g., Franzke & al., 2009, 2011, 2016; Salariato & al., 2016; Guo & al., 2017; Lopez & al., 2017).

The tribal classifications of the Brassicaceae pre-dating the era of molecular systematics (e.g., Candolle, 1821a, b; Prantl, 1891; Hayek, 1911; Schulz, 1936) were artificial because delimitation of tribal boundaries was based on morphological characters that were subject to considerable convergence during the evolutionary history of the family (Franzke & al., 2011). Al-Shehbaz & al. (2006) introduced the first phylogenetic classification of the family primarily based on molecular phylogenetic studies by Beilstein & al. (2006). The original 25 tribes (Al-Shehbaz & al., 2006) went up to 49 (Al-Shehbaz, 2012) and to 52 most recently (Kiefer & al., 2014).

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Some of the Brassicaceae tribes were subjected to more recent molecular studies, and the relationships of their component genera are fairly well understood. These include the tribes (and latest reference) Aethionemeae (Mohammadin & al., 2017), Alysseae (Španiel & al., 2015), Anchonieae and Euclidieae (Warwick & al., 2007; Jaén-Molina & al., 2009), Aphragmeae (Warwick & al., 2006a), Arabideae (Karl & Koch, 2013), Biscutelleae (Özüdoğru & al., 2017), Boechereae (Alexander & al., 2013), Brassiceae (Arias & al., 2014), Chorisporeae (German & al., 2011), Cochlearieae (Koch, 2012), Coluteocarpeae (Firat & al., 2014), Cremolobeae (Salariato & al., 2013), Descurainieae (Goodson & al., 2011), Erysimeae (Moazzeni & al., 2014), Eudemeae (Salariato & al., 2015), Eutremeae (Warwick & al., 2006a), Halimolobeae (Bailey & al., 2007), Heliophileae (Mummenhoff & al., 2005), Lepidieae (Mummenhoff & al., 2009), Microlepidieae (Mandáková & al., 2017), Physarieae (Fuentes-Soriano & Al-Shehbaz, 2013), Schizopetaleae and Thelypodieae (Cacho & al., 2014; Toro-Núñez & al., 2014), Sisymbrieae (Warwick & al., 2006b), and Smelowskieae (Warwick & al., 2004). The Cardamineae and Thlaspideae are among some of the most widespread tribes that have not been subjected to critical molecular phylogenetic and systematics studies.

Although the tribe Thlaspideae was initially proposed by Candolle (1821a), it was not recognized by subsequent authors except for Prantl (1891). Of the initial 87 species recognized by Candolle (1821b), only 3 of 17 species of *Thlaspi* L. (i.e., *T. alliaceum* L., *T. arvense* L., *T. ceratocarpum* Murr.) are currently maintained in the tribe (Meyer, 2001). The other genera of Thlaspideae accepted by Candolle included *Biscutella* L. (Biscutelleae), *Capsella* Medik. (now in tribe Camelineae), *Cremolobus* DC. and *Menonvillea* DC. (both Cremolobeae), illegitimate *Hutchinsia* W.T.Aiton (now *Hornungia* Rchb. of Descurainieae), *Megacarpaea* DC. (Megacarpaeeae), and *Teesdalia* W.T.Aiton and *Iberis* L. (both Iberideae). The genera *Alliaria* Heist. ex Fabr., *Peltaria* Jacq., and *Sobolewskia* M.Bieb., which are currently accepted in Thlaspideae (Al-Shehbaz, 2012), were assigned by Candolle (1821b) to Sisymbrieae, Alysseae, and Isatideae, respectively. Al-Shehbaz & al. (2006) re-established the Thlaspideae based on molecular phylogenetic data to include 26 species in *Alliaria* (2 spp.), *Graellsia* Fenzl (8 spp.), *Pachyphragma* (DC.) Rchb. (1 sp.), *Parlatoria* Boiss. (2 spp.), *Peltaria* (4 spp.), *Pseudocamelina* (Boiss.) N.Busch (3 spp.), and *Thlaspi* (6 spp.). Later, Al-Shehbaz (2012) expanded the tribe to include 12 genera and 34 species by adding *Didymophysa* Boiss. (2 spp.), *Elburzia* Hedge (1 sp.), *Peltariopsis* (Boiss.) N.Busch (2 spp.), *Pseudovesicaria* (Boiss.) Rupr. (1 sp.), and *Sobolewskia* (4 spp.) and excluding *Peltaria emarginata* (Boiss.) Hausskn. to genus *Bornmullera* Hausskn. (Alysseae) and *Graellsia hederifolia* (Coss.) R.D.Hyam & Jury to genus *Draba* L. (Arabideae).

While family-wide molecular surveys of Brassicaceae (e.g., Beilstein & al., 2006, 2008; German & al., 2009; Khosravi & al., 2009; Couvreur & al., 2010; Warwick & al., 2010; Huang & al., 2015) included some species of Thlaspideae, no study focused on the relationships among its genera, especially those of Iran where more than 50% of the species occur. In the present study, we aimed to test the monophyly of Thlaspideae and its genera and determine the relationships among them. Furthermore, we attempted to utilize the molecular data to resolve the taxonomy of problematic genera such as *Alliaria*, *Didymophysa*, *Elburzia*, *Parlatoria*, and *Thlaspi*.

■ MATERIALS AND METHODS

Taxon sampling. — Samples for the molecular studies included silica-gel dried leaves collected during fieldwork mainly in Iran (with a few samples of *Alliaria petiolata* (M.Bieb.) Cavara & Grande from Austria, Czech Republic, and France), fresh leaf material from plants grown from seeds, and tiny fragments taken from herbarium specimens deposited at FUMH, G, HUB, Isfahan (Isfahan University herbarium, Iran), LE, Lysak (Martin A. Lysak private herbarium, Czech Republic), M, MIR, MO, MSB, PR, Sanandaj (Herbarium of Kurdistan, Iran), W, and WU. Vouchers of all samples collected by the first author are deposited in MIR, MO, and W. In total, 91 accessions from 37 of the 42 Thlaspideae species of 13 genera were included in the analyses, representing the full range of morphological variation and the entire geographic distribution of the tribe. *Graellsia chitralensis* O.E.Schulz, *G. hissarica* Junussov, *Pseudocamelina conwayi* (Hemsl.) Al-Shehbaz, and *Thlaspi kochianum* F.K.Mey. are known only from the type collections and, therefore, were not included in the study. Furthermore, *Pseudocamelina campylopoda* was not included due to the insufficient quality of DNA isolated from its three accessions. In addition, sequence data of 13 species in 10 Thlaspideae genera were taken from GenBank. To examine the monophyly of Thlaspideae, 22 species of 16 Brassicaceae tribes in lineage II and expanded lineage II were used, and the outgroup included three species of three tribes of lineage III Appendix 1). For component tribes of all lineages, see Kiefer & al. (2014).

DNA extraction. — Total DNA was extracted using either the modified CTAB method (Doyle & Doyle, 1987) or NucleoSpin Plant II kit (Macherey-Nagel, Düren, Germany).

PCR amplification and sequencing. — ITS and *trnL-F* sequences were amplified by the polymerase chain reaction (PCR) in 50 µl volumes containing 1× Dream Taq buffer, 0.2 mM dNTPs, 0.3 µM of each primer, 1.25 U of Dream Taq DNA polymerase (Thermo Fisher Scientific, Waltham, Massachusetts, U.S.A.), and 1 µl of unquantified DNA template. All the used primers are listed in Table S1 (Electr. Suppl.); ITS sequences were amplified using two different primer pairs (Appendix 1; Electr. Suppl.: Table S1).

For ITS amplification, the reaction conditions were as follows: the first denaturation step was at 95°C for 5 min, followed by 40 cycles of 95°C for 20 s, annealing at 59°C for 20 s, and extension at 72°C for 1 min, with a final extension step at 72°C for 7 min. The *trnL-F* region was amplified as follows: denaturation at 95°C for 5 min, followed by 38 cycles of 95°C for 20 s, 55.5°C for 20 s, and 72°C for 1 min, with a final extension at 72°C for 7 min. Subsequently, PCR products were purified using GenElute PCR Clean-Up Kit (Sigma-Aldrich, St. Louis, Missouri, U.S.A.) following the manufacturer's protocol. Purified PCR fragments were sequenced in both directions (Macrogen, Amsterdam, Netherlands).

Alignments and phylogenetic analyses. — Sequences were edited in Geneious v.8.0.5 (Biomatters, 2012), aligned with MAFFT v.7 (online version: http://mafft.cbrc.jp/alignment/server/), and manually inspected. Best-fit models of evolution were chosen based on Akaike information criterion (Akaike, 1974; Posada & Buckley, 2004) in jModelTest v.0.1.1 (Posada, 2008) by choosing the GTR+I+Γ model for the ITS dataset and the TVM+I+Γ model for the *trnL-F* dataset. Phylogenetic analyses were conducted individually for each DNA region and for concatenated sequences of ITS and *trnL-F* partitions using maximum likelihood (ML) and Bayesian interference. Bayesian analyses were performed using MrBayes v.3.1.2 (Ronquist & Huelsenbeck, 2003). Each analysis was run for 12×10^6 generations with three Markov chains (two heated and one cold), and trees saved every 100 generations. Trees were checked to be stable by plotting log likelihood values vs. generation with MCMC (Markov chain Monte Carlo) using Tracer v.1.6.0 (Drummond & al., 2012), and 30,000 burn-in trees (or 25%) were discarded using MrBayes. The ML analyses were performed using PhyML v.3.0 (Guindon & al., 2010) implemented in Geneious v.8.0.5, selecting the GTR GAMMA nucleotide model, random seed, and 1000 bootstrap replicates. Finally, all support values were presented on Bayesian trees, and all potential topological conflicts between nuclear and chloroplast trees were assessed before combining the respective datasets.

Divergence time estimation. — The concatenated datasets were used for divergence time estimation using BEAST2 (Bouckaert & al., 2014) on CIPRES Science gateway (http://www.phylo.org/; Miller & al., 2010). The analyses were performed twice, based on both fossil calibration and secondary calibration; a uniform distribution was used for all calibrations. BEAST2 was run for 5×10^7 generations, and data were saved every 1000 generations under the Birth-Death model prior and an uncorrelated relaxed clock. In this study, the GTR model was used for the dataset. For fossil calibration, we tested the effect of the controversial fossil *Thlaspi primeavum* and used an average age of 29.8 million years (29.4–30.2 Ma; Beilstein & al., 2010) to calibrate the clade of *Thlaspi* and related taxa (*Didymophysa*, *Mummenhoffia* gen. nov., and *Thlaspi*). For a thorough discussion of this fossil and calibration by fossils in Brassicaceae in general, see Franzke & al. (2016). For secondary calibration, we followed Couvreur & al. (2010), Huang & al. (2015), and Salariato & al. (2016) to set two calibration points, one at 31.5 (30–33) Ma at the split of lineage II s.l. and lineage III, and the second at the origin of lineage II s.str. (Brassiceae, Isatideae, Sisymbrieae, and Thelypodieae) at 21.5 (20–23) Ma.

Chromosome counting. — Young inflorescences were fixed in freshly prepared ethanol : acetic acid (3 : 1) fixative at 4°C for 24 h. The fixative was replaced by 70% ethanol and the material stored at −20°C until further use. Selected flower buds were rinsed in distilled water (twice for 5 min) and citrate buffer (10 mM sodium citrate, pH 4.8; twice for 5 min), and digested in 0.3% cellulase, cytohelicase, and pectolyase in citrate buffer at 37°C for 3 h. After digestion, individual anthers were dissected on a microscope slide in 20 µl of acetic acid and the material spread on the slide placed on a metal hot plate (50°C) for ca. 30 s. Then, the preparation was fixed in freshly prepared ethanol : acetic acid (3 : 1) fixative by dropping the fixative around the drop of acetic acid and into it. The preparation was dried using a hair dryer and staged using a phase contrast microscope for the presence of suitable mitotic metaphase spreads. Chromosomes were counterstained with 2 µg/ml DAPI (4', 6-diamidino-2-phenylindole) in Vectashield (Mandáková & Lysak, 2016). Preparations were analyzed and photographed using a Zeiss Axioimager epifluorescence microscope equipped with a Cool Cube camera. The list of accessions investigated for chromosome counts is given in Table S2 (Electr. Suppl.).

Scanning electron microscopy (SEM). — Pollen samples were taken from plants collected during field trips and also obtained from the W and WU herbaria. Individual samples were fixed onto the electron microscopy stubs using two-sided carbon tape and were coated with 5 nm of Au/Pd (gold/palladium) prior to imaging. SEM imaging was performed in FEI Versa 3D FIB/SEM microscope with an accelerating voltage 5 kV, working distance of 10 mm, and a current of 50 pA at various magnifications. Secondary electron detection was performed with ETD (Everhart Thornley Detector), and the data was exported as tiff images of the size of 3072×2048 pixels. The measurements were taken from SEM images of at least five pollen grains per sample.

■ RESULTS

ITS and *trnL-F* phylogenies. — The ITS phylogeny included 119 accessions: 94 from Thlaspideae, 22 from lineage II and expanded lineage II, and 3 from lineage III (*Chorispora tenella* (Pall.) DC., *Dontostemon integrifolius* (L.) C.A.Mey., *Hesperis sibirica* L.) as outgroup (Electr. Suppl.: Fig. S1). Trees derived from the analyses of non-coding cpDNA sequences (*trnL-F*) were based on 71 of the 94 Thlaspideae taxa included in the ITS analysis (Electr. Suppl.: Fig. S2). The data matrix consisted of 96 sequences, including 22 accessions from lineage II and expanded lineage II, and 3 outgroup species from lineage III.

For both ITS and *trnL-F*, Bayesian 50% majority-rule consensus trees were constructed. Thlaspideae taxa formed a strongly supported clade in both Bayesian and ML analyses of both markers. Since the topologies of both trees (Bayesian and ML) were almost the same (>90% congruency), all support values were shown on the Bayesian trees (Electr. Suppl.: Fig. S1 for ITS and Fig. S2 for *trnL-F*).

Both nrDNA and cpDNA phylogenies supported the monophyly of the tribe, but the backbone of Thlaspideae remained unresolved in both datasets. There were only a few discrepancies between ITS and *trnL-F* trees. The four major Thlaspideae clades in the *trnL-F* cladogram (Electr. Suppl.: Fig. S2) included A (*Didymophysa*, *Peltaria*, *Pseudocamelina*, *Thlaspi*), B (*Alliaria*, *Pachyphragma*, *Parlatoria*, *Pseudovesicaria*, *Sobolewskia*), C (*Graellsia*), and D (*Peltariopsis*). In the ITS tree (Electr. Suppl.: Fig. S1), clades B, C, and D had almost the same topology, but clade A formed a polytomy of four independent clades. The monophyly of *Graellsia*, *Pachyphragma*, *Peltaria*, *Peltariopsis*, *Pseudocamelina*, *Pseudovesicaria*, and *Sobolewskia* was supported by both markers. *Didymophysa*

was paraphyletic because *Elburzia* was nested within, and both genera formed a polytomy with paraphyletic *Thlaspi*. *Alliaria* and *Parlatoria* were polyphyletic, and all of their species fell into well-supported clades.

ITS-*trnL-F* concatenated phylogeny. — Since the comparison of topologies and support for the nuclear ITS and plastid *trnL-F* regions do not show major conflicts (i.e., no well-supported conflicts receiving bootstrap and Bayesian posterior probability values above 70% and 0.95, respectively), these datasets were combined and analyzed. Ninety-two ingroup and three outgroup samples were used to construct the concatenated tree based on ITS and *trnL-F* markers. The backbone was an unresolved polytomy of three branches (Fig. 1). Monophyletic *Graellsia* (clade C) was sister to clade B that included *Alliaria*, *Pachyphragma*, *Parlatoria*, *Pseudovesicaria*, and *Sobolewskia*. The polyphyletic *Parlatoria* had three species, of which the type *P. cakiloidea* was sister to the European and North American *Alliaria* accessions, *P. taurica* was nested within the Asian *Alliaria* clade, and *P. rostrata* fell into a collapsed relationship with *Sobolewskia* and Asian *Alliaria* clade. In clade A, two species groups representing *Thlaspi* and *Didymophysa* formed an unresolved clade sister to a clade of monophyletic *Peltaria* and *Pseudocamelina*. As in the single-gene trees, *Peltariopsis* formed a well-supported lineage (clade D).

Divergence time estimation. — Because the present study focuses on tribe Thlaspideae, the use of the fossil *Thlaspi primaevum* Becker to calibrate the Thlaspideae phylogeny looked promising. However, calibration using the *T. primaevum* fossil resulted in an unrealistic divergence time of ~88 Ma for the separation of lineage II and expanded lineage II from lineage III. By comparing the fossil versus secondary calibration, it becomes apparent that all ages have increased twice or more using the fossil calibration (see Table 1).

The present study is based on a secondary calibration using two divergence time points—one at the split of lineages II+expanded lineage II from lineage III, the other at the split of lineage II from expanded lineage II. Our results show that Thlaspideae originated around 15.56 Ma during the Miocene (Fig. 2; Table 1)

Chromosome numbers. — Chromosome numbers were determined in 20 populations of 16 taxa. The analyzed populations of *Peltariopsis grossheimii*, *P. planisiliqua*, *Pseudocamelina aphragmodes*, *P. campylocarpa*, and *P. kermanica* were diploid ($2n = 2x = 14$); the first reported counts for these species. Also, ten other species (*Didymophysa aucheri*, *Elburzia fenestrata*, *Graellsia saxifragifolia*, *G. stylosa*, *Parlatoria cakiloidea*, *Peltaria angustifolia*, *P. turkmena*, *Pseudocamelina glaucophylla*, *Thlaspi arvense*, *T. ceratocarpum*) were diploid (Fig. 3; Electr. Suppl.: Fig. S2). Four populations of *Alliaria petiolata* from Austria, Czech Republic, France, and Iran were hexaploid ($2n = 6x = 42$). Unfortunately, we did not have any materials of diploid *A. petiolata* populations at our disposal.

Pollen SEM in the *Alliaria* complex. — *Alliaria petiolata* is a cosmopolitan weed with at least two reported ploidy levels (diploid, hexaploid). Diploid populations were reported from SW Asia (Kiefer & al., 2014) and one hexaploid from Iran (present study), but all other populations in Europe and North America are hexaploid (Kiefer & al., 2014). The Asian vs. European and North American *A. petiolata* accessions are also separated phylogenetically (Fig. 1). Pollen of three diploid populations from Asia (Caucasus, Iran), including the WU voucher of diploid chromosome count (Weiss-Schneeweiss & Schneeweiss, 2003), were examined. The results show substantial differences in shape and size between diploid and hexaploid pollen. Diploid pollen were spherical to broadly ovoid, 16.3–20.5 µm in diameter, and with the length (L) : width (W) ratio of 1.2–1.4. The hexaploid pollen were ellipsoid to oblong, 28.5–33.6 µm in diameter, and with the L : W ratio of 1.9–2.1 (Fig. 4).

■ DISCUSSION

In family-wide phylogenetic studies (e.g., Bailey & al., 2006; Beilstein & al., 2006, 2008; German & al., 2009; Khosravi & al., 2009; Couvreur & al., 2010; Warwick & al., 2010; Huang & al., 2015; Salariato & al., 2016; Guo & al., 2017; Lopez & al., 2017), Aethionemeae were recovered as sister to the remaining Brassicaceae tribes (the core Brassicaceae) forming four major lineages (I, II, expanded II, and III). Our phylogenetic analyses confirmed the monophyletic position of Thlaspideae within expanded lineage II. Although in both ITS and cpDNA phylogenetic trees all genera were well resolved (Electr. Suppl.: Figs. S1, S2), the concatenated tree (Fig. 1) had a considerably better resolution and support values and is thus used as the basis for the following discussion.

Molecular dating of Thlaspideae. — One of the alleged Brassicaceae fossils is the fruit of *Thlaspi primaevum* dated some 29.2–30.8 Ma (Beilstein & al., 2010). Fruit characters in the Brassicaceae are subjected to considerable homoplasy (Al-Shehbaz & al., 2006), and several authors (e.g., Franzke & al., 2011, 2016; Salariato & al., 2016; Guo & al., 2017) and us questioned the assignment of this fossil to the genus *Thlaspi*. Only two studies, Beilstein & al. (2010) and Arias & al. (2014), incorporated that fossil as a calibration point, and the former authors used a molecular clock model calibrated with that fossil as a minimum age constraint for the stem group age of *Thlaspi* (i.e., a rather terminal split in the Brassicaceae phylogeny between *T. arvense* and *Alliaria petiolata*). They concluded that the crown node age of the Brassicaceae was approximately 54 million years, making clade age estimates two- to three-folds older than previously calculated (see Franzke & al., 2016 and references therein). More recent nuclear transcriptome and multi-gene locus-based studies provided independent evidence for a Brassicaceae crown group age of only 31.8 (Edger & al., 2015) to 37.1 million years (Huang & al., 2015) without constraining the *Thlaspi-Alliaria* split by the *T. primaevum* fossil. Indeed, several authors (e.g., Couvreur & al., 2010; Hohmann & al., 2015; Huang & al., 2015; Franzke & al., 2016; Salariato & al., 2016; Guo & al., 2017; Lopez & al., 2017) demonstrated that the split of core Brassicaceae from Aethionemeae was between 32 and 38 Ma.

When we used the *T. primaevum* fossil only to calibrate the origin of the *Thlaspi* clade (including *Didymophysa*, *Mummenhoffia* gen. nov., and *Thlaspi*), we obtained an

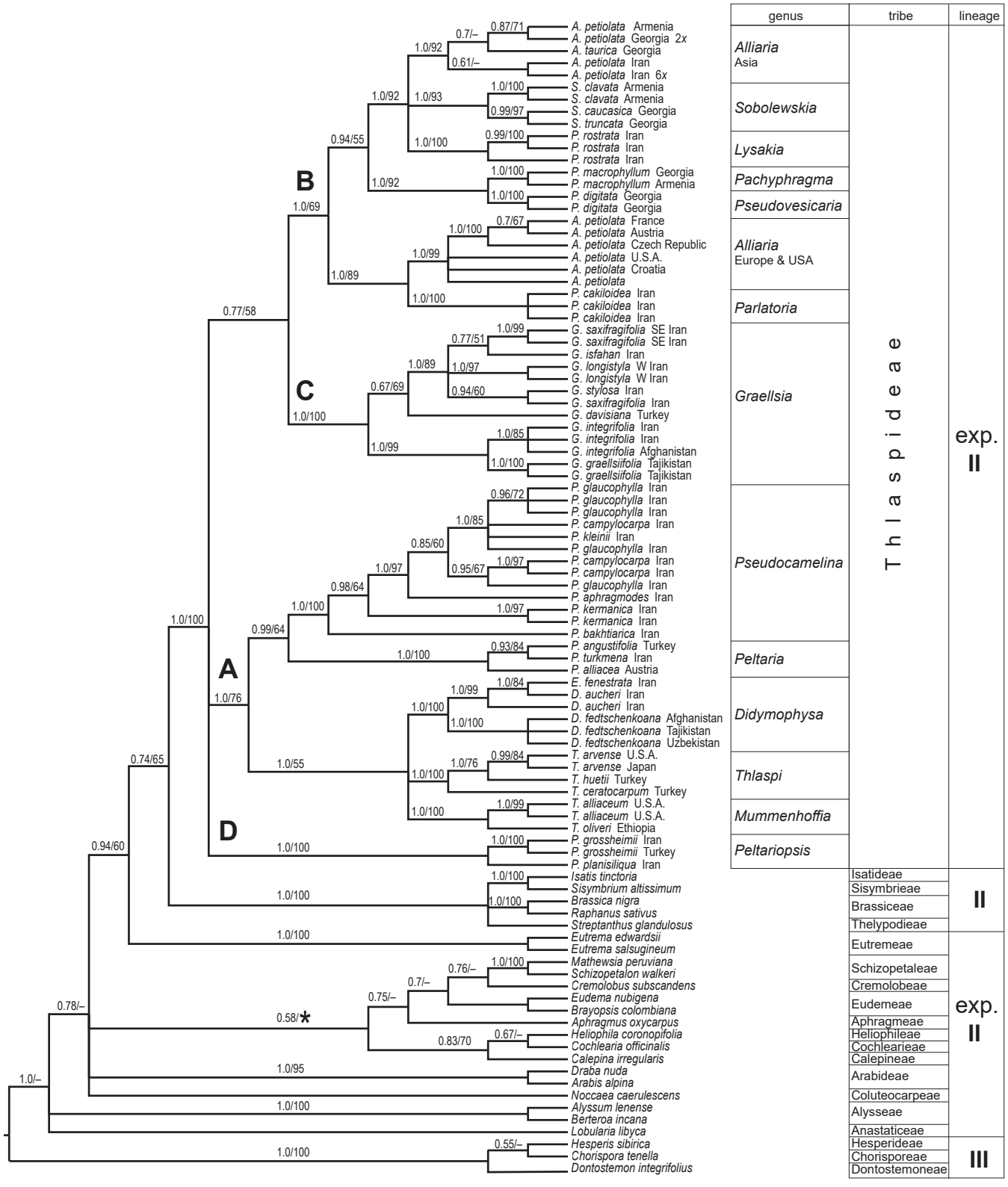

Fig. 1. Bayesian 50% majority-rule consensus tree inferred from combined data (ITS, *trnL-F*). Numbers above branches are Bayesian posterior probability and maximum likelihood bootstrap support values. A dash (–) represents <50% branch support. The branch marked with a star represents the collapsed node in maximum likelihood analysis. The tribal classification is based on Al-Shehbaz (2012); the generic names reflect the new taxonomy.

Table 1. Estimated ages in millions of years for nodes (based on Fig. 4), and their Bayesian posterior probability (PP) values.

Clade	Secondary calibration (median, 95% HPD)	Fossil calibration (median, 95% HPD)	Support (PP)
Lineage III	25.78 (31.25–19.69)	74.79 (105.96–47.3)	1
Lineage III + expanded lineage II & lineage II	31.80 (33.25–30.3)	88.64 (115.57–63.51)	1
Lineage II + expanded lineage II	30.31 (32.7–27.6)	80.27 (104.15–58.63)	1
Lineage II	18.80 (20.95–16.57)	36.11 (48.83–23.37)	1
tribe Thlaspidae	15.56 (18.95–12.3)	36.51 (43.76–31.0)	1
tribe Thlaspideae + lineage II	21.74 (24.37–18.79)	49.55 (62.01–38.13)	0.75
Peltariopsis (Boiss.) N.Busch	2.88 (5.01–1.09)	6.94 (12.11–2.54)	1
Thlaspi L.	3.72 (5.92–1.76)	8.91 (14.15–4.51)	1
Mummenhoffia gen. nov.	2.13 (3.97–0.66)	5.13 (9.3–1.69)	1
Didymophysa Boiss.	4.88 (7.29–2.7)	11.50 (17.02–6.56)	1
Peltaria Jacq.	6.62 (9.74–3.71)	15.80 (22.7–8.9)	1
Pseudocamelina (Boiss.) N.Busch	5.44 (7.93–3.3)	13.02 (18.91–7.91)	1
Graellsia Boiss.	4.74 (6.99–2.81)	11.35 (16.92–6.45)	1
Parlatoria Boiss.	0.91 (1.8–0.23)	2.18 (4.29–0.51)	1
Alliaria Heist. ex. Fabr. (Europe & U.S.A.)	0.88 (1.73–0.22)	2.14 (4.1–0.56)	1
Pseudovesicaria (Boiss.) Rupr.	0.18 (0.57–0.00)	0.44 (1.35–0.0)	1
Pachyphragma (DC.) Rchb.	0.19 (0.59–0.00)	0.45 (1.4–0.0)	1
Lysakia gen. nov.	1.24 (2.34–0.31)	3.03 (5.82–0.82)	1
Sobolewskia M.Bieb.	2.35 (3.99–1.1)	5.60 (9.35–2.29)	1
Alliaria Heist. ex Fabr. (Asia)	1.96 (3.34–0.76)	4.75 (8.23–1.83)	1

unrealistic old age estimate (ca. ~88 million years) for the clade consisting of lineage II, expanded lineage II, and III (Table 1). Therefore, we did not rely on the *T. primaevum* fossil, and instead used the secondary calibration points mentioned above. Based on that approach, the age estimate for Thlaspideae clade was 15.56 million years, compared to 36.51 million years when the fossil calibration was used, a difference of more than 20 million years. All age estimates in the present study (Table 1) are in agreement with Couvreur & al. (2010), Hohmann & al. (2015), Huang & al. (2015), and Salariato & al. (2016), but somewhat older than those estimated by Guo & al. (2017) and Lopez & al. (2017). We believe that Thlaspideae originated about Mid-Miocene, but subsequent increasing temperature and appearance of open dry habitats in the Northern Hemisphere in the Pliocene/Pleistocene (Fig. 2; Table 1) lead to radiation and diversification of most genera of the tribe.

Phylogenetic relationships within Thlaspideae. — *Alliaria*, *Didymophysa*, *Parlatoria*, *Pseudocamelina*, and *Thlaspi* are either paraphyletic or polyphyletic, but the remaining genera are monophyletic. The discussion will focus primarily on the problematic genera excluding *Pseudocamelina* (see Esmailbegi & al., 2017b). The phylogenetic position and taxonomic relationships within the monophyletic genera are straightforward and will not be discussed further.

***Alliaria*. —** *Alliaria petiolata* is widely distributed in Europe and W Asia and is also naturalized in North America, Argentina, and N Africa. The present phylogenetic study clearly shows that *A. petiolata* formed two separate groups in the ITS, *trnL-F*, and concatenated sequence data phylogenies. The group from SW Asia (Caucasus, Iran, Iraq, Lebanon, Syria, E Turkey) formed with Caucasian *Parlatoria taurica* a monophyletic clade sister to well-resolved *Sobolewskia* and the clade consisting of both genera was sister to the well-supported *P. rostrata* clade. By contrast, the European (and naturalized North American; see Durka & al., 2005) hexaploid *A. petiolata* accessions grouped with the morphologically very distinctive and diploid *P. cakiloidea*. Since the Iranian hexaploid *A. petiolata* populations are allied to SW Asian diploids, it is reasonable to conclude that the Asian and European hexaploids experienced different evolutionary histories. The current data does not provide any evidence for an auto- vs. allohexaploid origin of *A. petiolata*, and further studies are needed to elucidate this issue.

An exhaustive and critical morphological study to distinguish the diploid (SW Asia/Caucasus) and hexaploid (throughout Eurasia) populations did not identify reliable characters that would separate them consistently. Only our palynological analysis showed that the diploid and hexaploid plants can be discriminated based on pollen shape and size (Fig. 4), though, the hexaploid population from Iran had similar pollen morphology to the hexaploids of Europe and North America. The present phylogenetic, geographic, palynological, and ploidy data showed that the *Alliaria* complex is much in need of extensive cytogeographic and phylogenetic analysis, focused particularly on the Caucasus and SW Asia.

In conclusion, the present findings came as a surprising outcome of molecular and cytological studies. The most puzzling question is why the hexaploid European plants are sister to the diploid *Parlatoria cakiloidea* but in fruit and seed morphology they are indistinguishable from the Asian *A. petiolata* and substantially different from *P. cakiloidea*. Furthermore, it is unknown how many ancestral species were involved in the origin of hexaploid *A. petiolata*, and what is their relationship to the extant diploid species of *Alliaria*, *Sobolewskia*, and *Parlatoria*. Species of all these genera are indistinguishable from each other in their vegetative and floral morphologies, but their fruits are extremely different.

Alliaria taurica was recently transferred by German & Al-Shehbaz (in Al-Shehbaz, 2012) to *Parlatoria* based on morphological similarities in fruit curvature and tardy dehiscence. However, the present phylogenies strongly support the retention of this species in *Alliaria*, as was done by Schulz (1924) and followed by subsequent workers, including the latest monograph of the family by Appel & Al-Shehbaz (2003). Therefore, the genus *Alliaria* should remain to include *A. taurica* and SW Asian *A. petiolata*.

***Didymophysa* and *Elburzia*. —** Khosravi & al. (2009) were the first to show that *Didymophysa* belongs to the Thlaspideae. The genus is readily distinguished by its indehiscent, didymous,

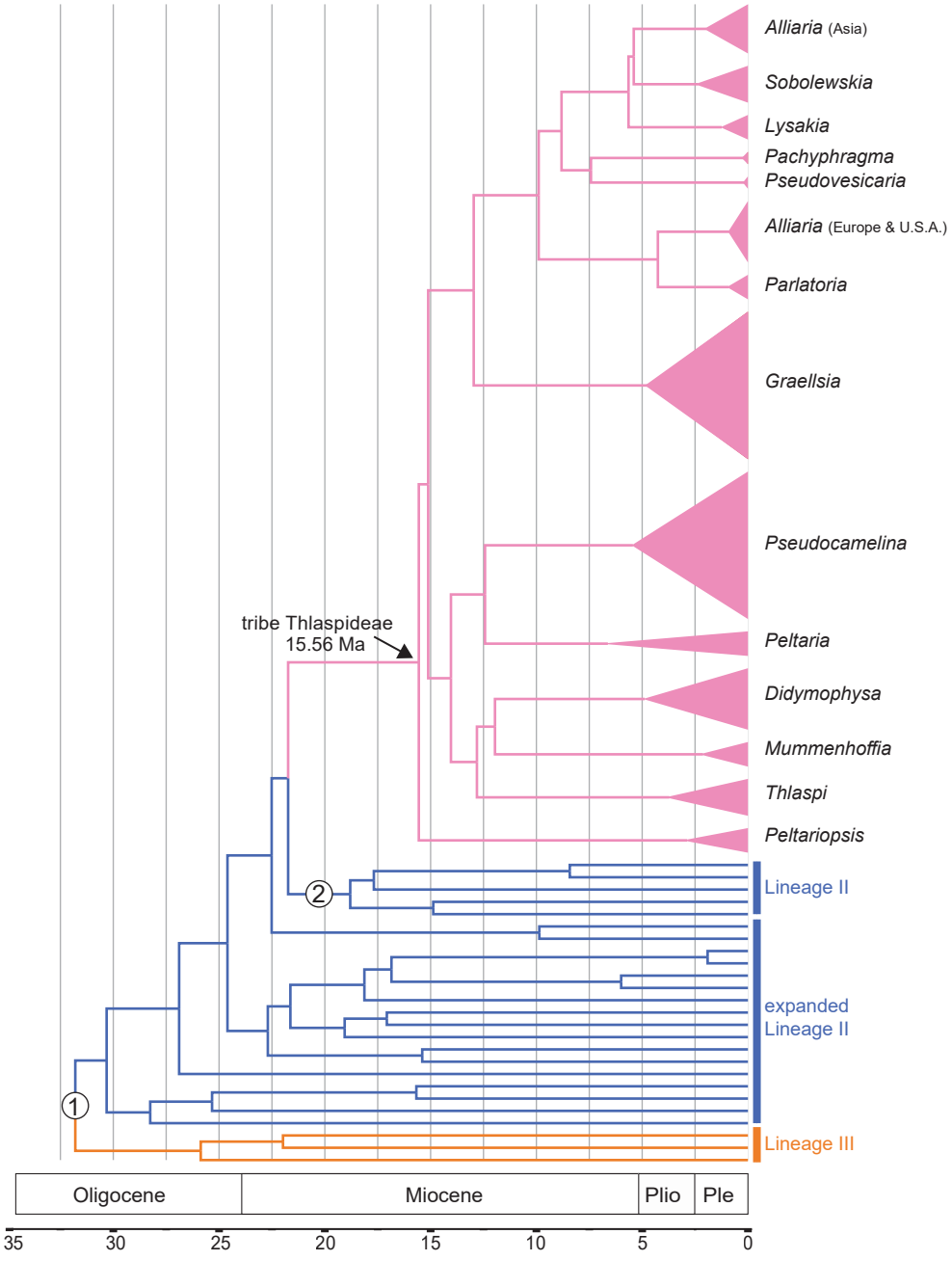

Fig. 2. A dated maximum clade credibility tree from BEAST analyses based on concatenated ITS and *trnL-F* sequences with two secondary calibration points under uniform prior distribution. Two calibration points include (1) 31.5 Ma and (2) 21.5 Ma. See Table 1 for time estimations of other nodes. Ple, Pleistocene; Plio, Pliocene. For details on time estimation and tree calibration, see Materials and Methods.

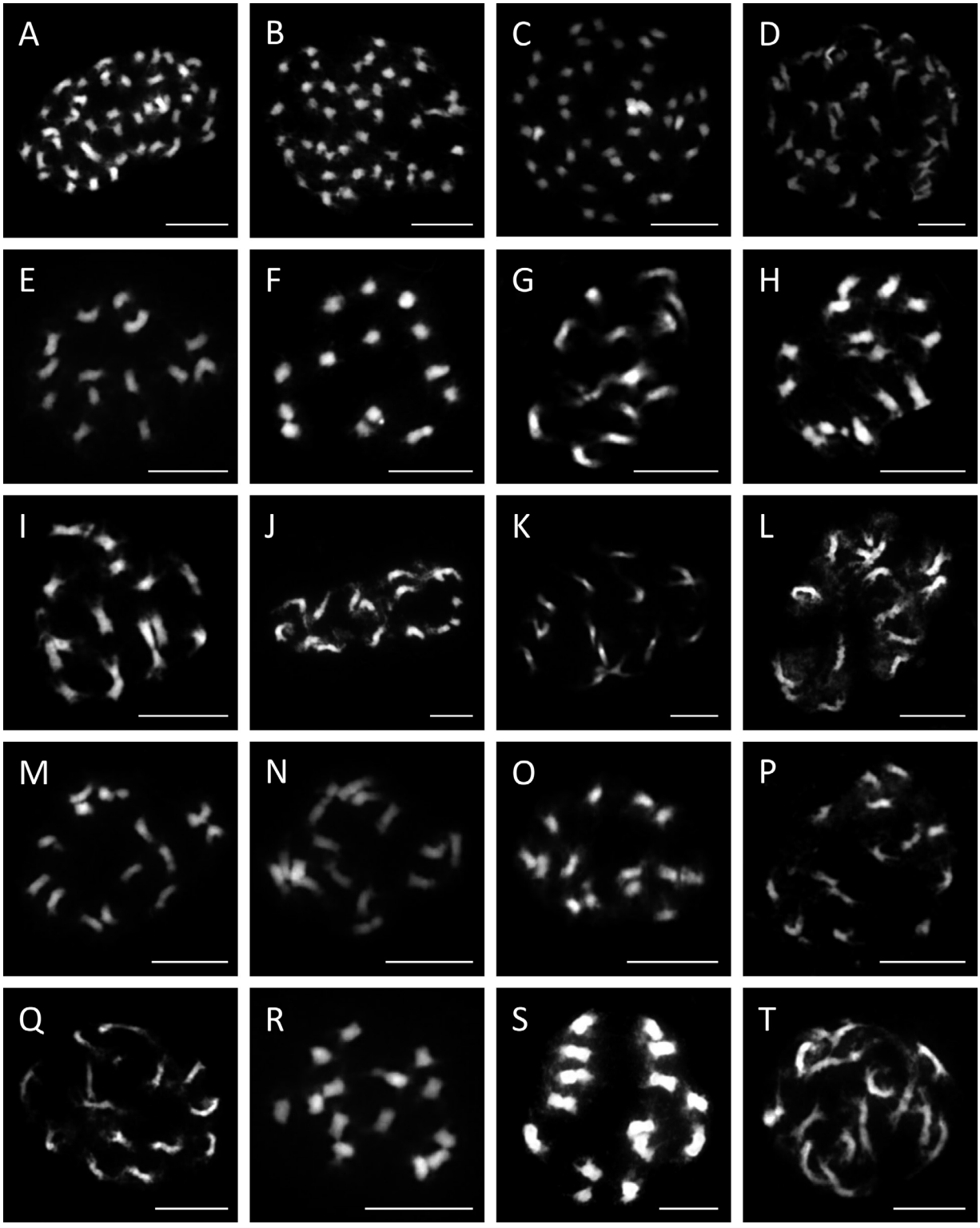

Fig. 3. Chromosome counts of selected Thlaspideae species (including countries of origin and herbarium vouchers). Chromosome numbers equal to $2n = 6x = 42$ **(A–D)** or $2n = 2x = 14$ **(E–T)**. **A,** *Alliaria petiolata*, Czech Republic, *2093* (MIR); **B,** *A. petiolata*, Austria, *2092* (MIR); **C,** *A. petiolata*, Iran, *2090* (MIR); **D,** *A. petiolata*, France, *2091* (MIR); **E,** *Didymophysa aucheri*, Iran, *1759* (MIR); **F,** *D. fenestrata*, Iran, *1558a* (MIR); **G,** *Graellsia saxifragifolia*, Iran, *1780c* (MIR); **H,** *G. stylosa*, Iran, *1744* (MIR); **I,** *Parlatoria cakiloidea*, Iraq, *17-1107* (MIR); **J,** *P. cakiloidea*, Turkey, *s.n.* (Lysak); **K,** *Peltaria angustifolia*, Turkey, *s.n.* (Lysak); **L,** *P. turkmena*, Iran, *45111* (FUMH); **M,** *Peltariopsis grossheimii*, Iran, *1827e* (MIR); **N,** *P. planisiliqua*, Iran, *1777e* (MIR); **O,** *Pseudocamelina aphragmodes*, Iran, *1787* (MIR); **P,** *P. campylocarpa*, Iran, *1754* (MIR); **Q,** *P. glaucophylla*, Iran, *12763* (Sanandaj); **R,** *P. kermanica*, Iran, *1784* (MIR); **S,** *Thlaspi arvense*, Czech Republic (Lysak); **T,** *T. ceratocarpum*, Turkey, *3661* (HUB). — Scale bars: 10 µm.

bladdery fruit, and its type, *D. aucheri*, has flabellate, deeply 3- to 5-lobed leaves, whereas *D. fedtschenkoana* has entire leaves that are sometimes apically 1- to 3-toothed. The present study demonstrates that *Didymophysa* is paraphyletic because monospecific *Elburzia* is nested within it. Warwick & al. (2010) assigned *Elburzia* to the Thlaspideae, but their study did not include *Didymophysa*. *Elburzia* is very similar to *D. aucheri*, especially in perennial habit and leaf morphology, but differs from *Didymophysa* by having slightly inflated and latiseptate (vs. didymous and angustiseptate) fruit and suffruticose (vs. surculose) habit. Therefore, *Elburzia* is reduced to synonymy of *Didymophysa* (see Taxonomic Considerations).

***Graellsia*. —** Based on Esmailbegi & al. (2017a), *Graellsia* includes nine species, of which three are endemic to Iran, two to Tajikistan, and one each to Turkey and Pakistan. Two species, *G. chitralensis*, and *G. hissarica*, are known only from the type collection, and the seven species analyzed here formed a highly supported clade with two subclades. One subclade includes *G. integrifolia* (NE Iran, Turkmenistan, Afghanistan) and *G. graellsiifolia* Lipsky (Tajikistan). The second consists of the Iran-endemic *G. saxifragifolia* (DC.) Boiss., *G. isfahan* Esmailbegi & Al-Shehbaz, *G. stylosa* Boiss., *G. longistyla* (Poulter) Esmailbegi & Al-Shehbaz (Iran, Iraq), and *G. davisiana* Poulter (Turkey) (Electr. Suppl.: Fig. S3. Both *G. graellsiifolia* and *G. stylosa* have angustiseptate fruits (flattened at a right angle to the septum), and our phylogenetic data supports multiple origins of this character in this small genus.

***Parlatoria*. —** *Parlatoria* (sensu Al-Shehbaz, 2012) consists of three species, including *P. cakiloidea*, the type (Iran, Iraq, Turkey), the Iranian endemic *P. rostrata* Boiss., and *P. taurica* (Azerbaijan, Georgia). As discussed above, the last species belongs to true *Alliaria* and should be retained as *A. taurica* (Adams) V.I.Dorof. The diploid ($2n = 2x = 14$) *P. cakiloidea* is sister to hexaploid ($2n = 6x = 42$) European *A. petiolata*, and in the absence of fruits, the two are so similar that they are frequently misidentified. On the other hand, *P. rostrata* more resembles *A. taurica* in fruit morphology than it does to *P. cakiloidea*. It is sister to *Sobolewskia*, but can not be assigned to any of the above genera based on significant differences in fruit morphology and its formation of an unresolved relationship in our molecular phylogeny (Fig. 1). Therefore, *P. rostrata* (Fig. 5F) forms a well-delimited new genus recognized herein as *Lysakia* gen. nov. endemic and highly restricted to Elburz mountain range in N Iran (Electr. Suppl.: Fig. S3).

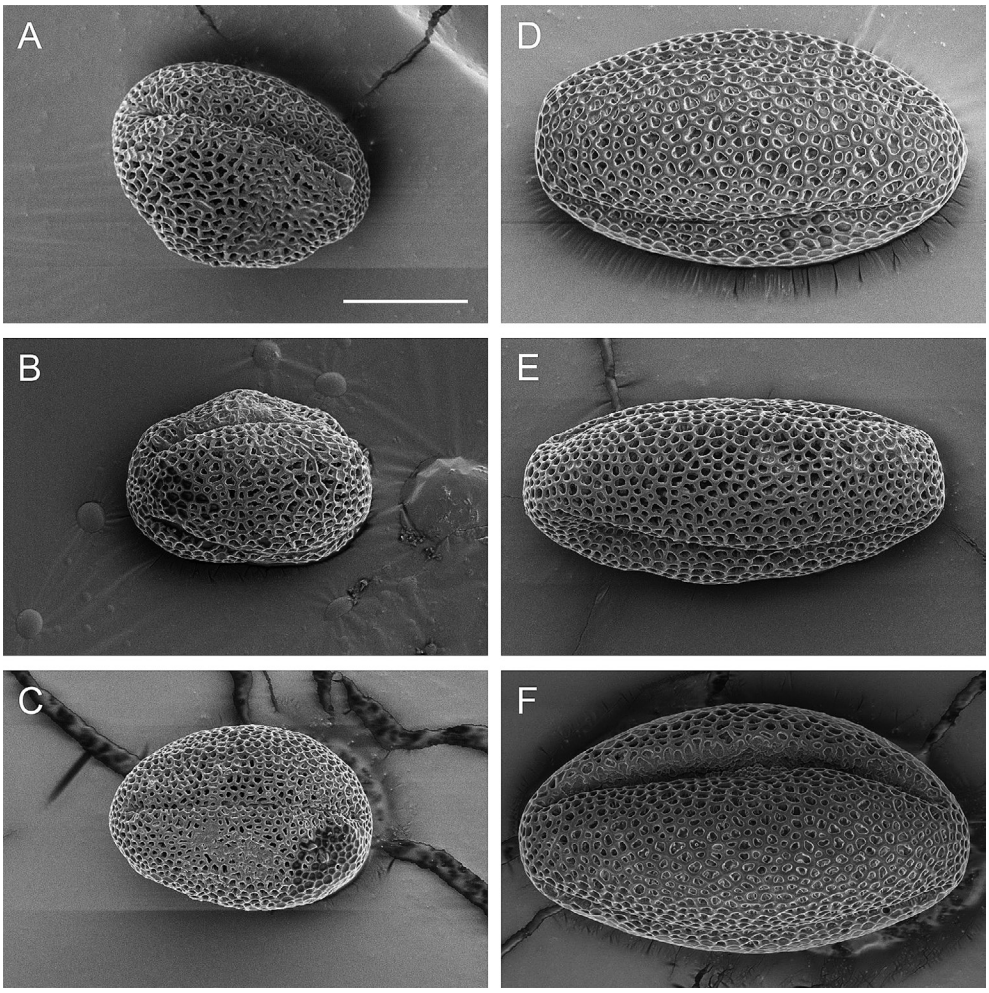

Fig. 4. Pollen morphology of diploid **(A–C)** and hexaploid **(D–F)** accessions in the *Alliaria petiolata* complex. Country of origin and herbarium voucher for analyzed populations: **A,** Georgia, *6924* (WU); **B,** Georgia, 1992-0016380 (W); **C,** Iran, 1965-0018048 (W); **D,** Austria, *2092* (MIR); **E,** Czech Republic, *2093* (MIR); **F,** France, *2091* (MIR). — Scale bars, 10 µm.

***Peltaria*. —** *Peltaria* is a distinct genus both morphologically and phylogenetically. It includes *P. turkmena* (restricted to Kopet-Dagh mountain in NE Iran and S Turkmenistan), *P. angustifolia* (Iran, Iraq, Turkey, Syria, Lebanon), and *P. alliacea* (SE and C Europe). Although *P. turkmena* is not as sharply distinct morphologically from *P. angustifolia*, our molecular data showed them to be quite distinct and both taxa should be recognized as distinct species (Electr. Suppl.: Fig. S3).

***Pseudocamelina* and *Camelinopsis*. —** *Camelinopsis* A.G.Mill. was most recently reduced by Esmailbegi & al. (2017b) to synonymy of *Pseudocamelina*. That transfer was based on the present molecular data in which the former genus is nested within the latter, as well as on the lack of any morphological character that supports the retention of two genera. *Pseudocamelina* includes nine species, of which seven are endemic to Iran and one each to Pakistan and Kurdistan Iraq. The reduction of *Camelinopsis* to synonymy of *Pseudocamelina* was highly supported (Fig. 1). One species, *P. conwayi*, is known only from the fragmentary holotype and was not included in the present study.

***Thlaspi*. —** The most taxonomically controversial member of the Thlaspideae is the type *Thlaspi* s.l. It was said to include 75 species (Appel & Al-Shehbaz, 2003). However, on the basis of primarily seed-coat anatomy, Meyer (1973, 1979) split the genus into 12 genera and retained only six species in *Thlaspi* s.str. The taxonomic status of Meyer's segregates was completely rejected by Greuter & al. (1986), partially accepted by Al-Shehbaz (2012), and fully maintained by Czerepanov (1995). Extensive molecular and comparative morphological studies (e.g., Mummenhoff & Zunk, 1991; Mummenhoff & Koch, 1994; Zunk & al., 1996; Mummenhoff & al., 1997, 2001; Koch & al., 1998; Koch & Hurka, 1999; Koch & Mummenhoff, 2001; Koch & Al-Shehbaz, 2004; Koch & Bernhardt, 2004) supported Meyer (1973) in treating *Thlaspi* as a small genus and in removing the majority of its species to other genera, especially *Noccaea* Moench (Meyer, 1973, 2006). These DNA-based studies clearly demonstrated that *Thlaspi* s.str. belongs to Thlaspideae, whereas the remaining segregates belong to Coluteocarpeae (Al-Shehbaz & al., 2006; Al-Shehbaz, 2012, 2014; Firat & al., 2014).

Meyer (1973, 2001) assigned the six species of *Thlaspi* s.str. to two sections and three series. However, such infrageneric classification for a small genus does not seem to be rational. As shown by Koch & Mummenhoff (2001) and Firat & al. (2014), the genus is polyphyletic, and our comprehensive phylogenetic study confirmed this. Our results clearly show that two species (*T. alliaceum*, *T. oliveri*), which were placed by Meyer (1973, 2001) in sect. *Chaunothlaspi* O.E.Schulz, formed a distinct clade well separated from the clade including the remaining four *Thlaspi* s.str. species. The above-mentioned species pair are excluded from the genus and placed here in the new genus *Mummenhoffia*. The latter differs from the four species of *Thlaspi* (*T. arvense*, *T. ceratocarpum*, *T. kochianum*, *T. huetii*) by having reticulate-foveolate (vs. longitudinally striate) seeds and fruits with a narrow apical wing (vs. broadly winged all around or extended into long apical horns).

■ TAXONOMIC CONSIDERATIONS

As delimited here, the Thlaspideae consists of 13 genera and 42 species distributed primarily in SW Asia and S and C Europe, and representative taxa are shown in Fig. 5. Of these, 39 species (93%) are native to SW Asia, 22 (52%) to Iran, and 13 (31%) to the Caucasus. Therefore, it is safe to assume a SW Asian origin and/or diversification center of the tribe (Electr. Suppl.: Fig. S3). For an expanded taxonomic account see Appendix S1 (Electr. Suppl.).

Key to genera of Thlaspideae

1 Fruit winged at least apically, often strongly keeled …. 2
1 Fruit wingless, not keeled …… 4
2 Plants rhizomatous perennial; cauline leaves petiolate, not auriculate; seeds up to 4 per fruit …… ***Pachyphragma***
2 Plants annual or biennial; cauline leaves sessile, auriculate or sagittate; seeds 5–16 per fruit …… 3
3 Seeds reticulate-foveolate; fruit minutely winged apically …… ***Mummenhoffia***
3 Seeds longitudinally striate; fruit winged all around or with distinct apical wing-like horns …… ***Thlaspi***
4 Racemes with strongly flexuous rachis …… ……***Pseudocamelina***
4 Racemes with straight rachis …… 5
5 Fruit didymous, strongly inflated, bladder-like …… …… ***Didymophysa***
5 Fruit neither didymous nor inflated, variously shaped .. 6
6 Fruit indehiscent; seeds 1 or 2, rarely 4 …… 7
6 Fruit dehiscent; seeds 4–20 …… 10
7 Fruit orbicular, elliptic or obovate samara, strongly latiseptate; cotyledon accumbent …… 8
7 Fruit oblong or clavate siliques, terete or slightly 4-angled; cotyledon incumbent …… 9
8 Leaves pinnately veined …… ***Peltaria***
8 Leaves palmately veined …… ***Graellsia***
9 Plants annual; anthers not apiculate; seeds 1; pedicels articulate …… ***Parlatoria***
9 Plants perennial; anthers apiculate; seeds 2–4; pedicels not articulate …… ***Sobolewskia***
10 Fruit linear, terete or 4-angled; seeds striate …… 11
10 Fruit elliptic, oblong or obovate, latiseptate or angustiseptate; seeds reticulate …… 12
11 Fruit beaked, often curved and indehiscent distally, dehiscent proximally; ovules 6–8 per ovary; seeds minutely striate …… ***Lysakia***
11 Fruit not beaked, straight apically, dehiscent throughout; ovules 14–20 per ovary; seeds coarsely striate .. ***Alliaria***
12 Leaves pinnately veined, entire …… ***Peltariopsis***
12 Leaves palmately veined; dentate or 3–6 lobed …… 13
13 Biennials; seeds biseriate …… ***Pseudovesicaria***
13 Perennials; seeds uniseriate …… 14
14 Basal leaves not rosulate, 3-lobed, short petiolate; fruit valves not veined …… ***Didymophysa***
14 Basal leaves rosulate, dentate or entire, long petiolate; fruit valves prominently veined …… ***Graellsia***

Didymophysa Boiss. in Ann. Sci. Nat., Bot., sér. 2, 16: 379. 1841 – Type: *D. aucheri* Boiss.

= *Elburzia* Hedge in Notes Roy. Bot. Gard. Edinburgh 29: 181. 1969, **syn. nov.** – Type: *E. fenestrata* (Boiss. & Hohen.) Hedge (≡ *Petrocallis fenestrata* Boiss. & Hohen.).

Didymophysa fenestrata (Boiss. & Hohen.) Esmailbegi, D.A. German & Al-Shehbaz, **comb. nov.** ≡ *Petrocallis fenestrata* Boiss. & Hohen. in Boissier, Diagn. Pl. Orient., ser. 1, 8: 27. 1849 ≡ *Elburzia fenestrata* (Boiss. & Hohen.) Hedge in Notes Roy. Bot. Gard. Edinburgh 29: 181. 1969 – Lectotype (designated by Hedge in Rechinger, Fl. Iranica 57: 175. 1968): as No. 493: Iran, "In saxosis meidan Abdullah in valle Loura m. Elbrus. 12 Jul. 1843. [T. Kotschy] 493. A. 795." (G-BOIS barcode G00332464!; isolectotypes: P barcode P02272395!, W No. W 0053489!).

Lysakia Esmailbegi & Al-Shehbaz, **gen. nov.** – Type: *L. rostrata* (Boiss. & Hohen.) Esmailbegi & Al-Shehbaz (≡ *Parlatoria rostrata* Boiss. & Hohen.).

Diagnosis. – Differs from *Parlatoria* by having persistent, non-articulate (vs. deciduous and articulate) fruiting pedicels, basally dehiscent and distally indehiscent, long-beaked, and linear (vs. indehiscent, beakless, oblong-lanceolate) fruits, basally bracteate (vs. ebracteate) racemes, striate (vs. reticulate) seeds, and 6–8 (vs. 1 or 2) ovules per ovary.

Description. – Herbs annual or biennial. Basal leaves rosulate, dentate or crenate, palmately veined; cauline leaves petiolate, cuneate or cordate at base, not auriculate. Racemes bracteate along lowermost few flowers, ebracteate above, elongated in fruit; fruiting pedicels divaricate to horizontal, persistent, not articulate. Sepals oblong, deciduous, ascending; petals white, oblanceolate, claw shorter than sepals; stamens strongly tetradynamous, filaments wingless, anthers not apiculate; nectar glands confluent; ovules 6–8 per ovary. Fruit siliques, dehiscent proximally, indehiscent distally, linear, terete, attenuate into curved beak; valves with a distinct midvein, smooth; septum complete; style to 1 mm long; stigma capitate, entire. Seeds uniseriate, wingless, narrowly oblong, minutely striate, not mucilaginous when wetted; cotyledons obliquely incumbent.

Distribution. – A monospecific genus endemic to northern Iran (Electr. Suppl.: Fig. S3).

Etymology. – The genus is named in honor of Dr. Martin A. Lysak (30 March 1973–) in recognition of his major contributions to the cytogenetics, genome evolution, and phylogenetics of the Brassicaceae.

Lysakia rostrata (Boiss. & Hohen.) Esmailbegi & Al-Shehbaz, **comb. nov.** ≡ *Parlatoria rostrata* Boiss. & Hohen. in Diagn. Pl. Orient., ser. 1, 8: 22. 1849 – **Lectotype** (first-step designated by Hedge in Rechinger, Fl. Iranica 57: 311. 1968), **second step (designated here):** Iran, "In valle Wesbach m. Elbrus pr. Derbend. D. 3. Jun. 1843", *T. Kotschy 236* (G-BOIS barcode G00332257!; isolectotypes: BM barcodes BM001254051! & BM001254073!, FI barcode FI005715!, G barcodes G00446112!, G00446113! & G00446114!, P barcodes P00741766! & P00741767!, W Nos. W 0075971! & W 0075972!).

Note. – Hedge (1968) did not specify which one of the four duplicates in the combined Geneva herbaria is the lectotype. For this reason, a second-step lectotypification became necessary.

Mummenhoffia Esmailbegi & Al-Shehbaz, **gen. nov.** – Type: *M. alliacea* (L.) Esmailbegi & Al-Shehbaz (≡ *Thlaspi alliaceum* L.).

Diagnosis. – *Mummenhoffia* is readily distinguished from *Thlaspi* by having a rudimentary (vs. well-developed) fruit wing and reticulate-foveolate (vs. longitudinally striate) seeds.

Description. – Herbs annual. Basal leaves petiolate, not rosulate, entire or dentate; cauline leaves sessile, auriculate to sagittate at base. Racemes ebracteate, elongated in fruit; fruiting pedicels divaricate, persistent. Sepals ovate or oblong, suberect, equal; petals white, spatulate, claw obscurely differentiated; stamens 6, included, filaments wingless, anthers not apiculate; nectar glands 2, lateral; ovules 6–10 per ovary. Fruit dehiscent silicles, obovate, angustiseptate; valves keeled, with rudimentary apical wing; septum complete; style to 0.5 mm long; stigma capitate, entire. Seeds uniseriate, wingless, reticulate-foveolate, not mucilaginous when wetted; cotyledons incumbent.

Distribution. – Two species in C and S Europe and alpine E Africa (Electr. Suppl.: Fig. S3).

Etymology.– The genus is named in honor of Dr. Klaus Mummenhoff (12 Nov. 1956–) for his major contributions to phylogenetics of the Brassicaceae, including three papers (Koch & Mummenhoff, 2001; Mummenhoff & al., 2001; Khosravi & al., 2009) showing that *Thlaspi alliaceum* did not fall from a monophyletic clade with *T. arvense*, the type of the genus name.

Key to the species of *Mummenhoffia*

1 Stems (22–)30–75(–90) cm tall, erect to ascending; racemes with numerous flowers; fruiting pedicels (6–)9–14(–17) mm long; petals 2.5–4 mm long; style 0.1–0.3 mm long; C & S Europe, naturalized in E & C U.S.A. …… ***M. alliacea***

1 Stems 3–12 cm tall, several from base, decumbent, rarely erect when single; racemes with few to several flowers; fruiting pedicels 2–4.5 mm long; petals 2–2.5 mm long; style 0.3–0.5 mm long; Ethiopia, Kenya, Tanzania ……. …… ***M. oliveri***

Mummenhoffia alliacea (L.) Esmailbegi & Al-Shehbaz, **comb. nov.** ≡ *Thlaspi alliaceum* L., Sp. Pl.: 646. 1753 – Lectotype (designated by Marhold & Mártonfi in Novon 11: 189. 2001): "Habitat in Europa australi"; [illustration] "SCORODOTHLASPI ULYSSIS Aldroandi" in Bauhin & al., Hist. Pl. 2: 932. 1651.

Note. – Meyer (2001) designated a collection by Van Royen in Leiden (L-901.257-174) as the lectotype, but his lectotypification was predated by that of Marhold & Mártonfi (2001).

Distribution. – Native of S and C Europe from N Spain into Hungary, Romania, and European Turkey; naturalized elsewhere in Europe and E and C U.S.A. (Greuter & al., 1986; Jalas & al., 1996; Al-Shehbaz, 2010).

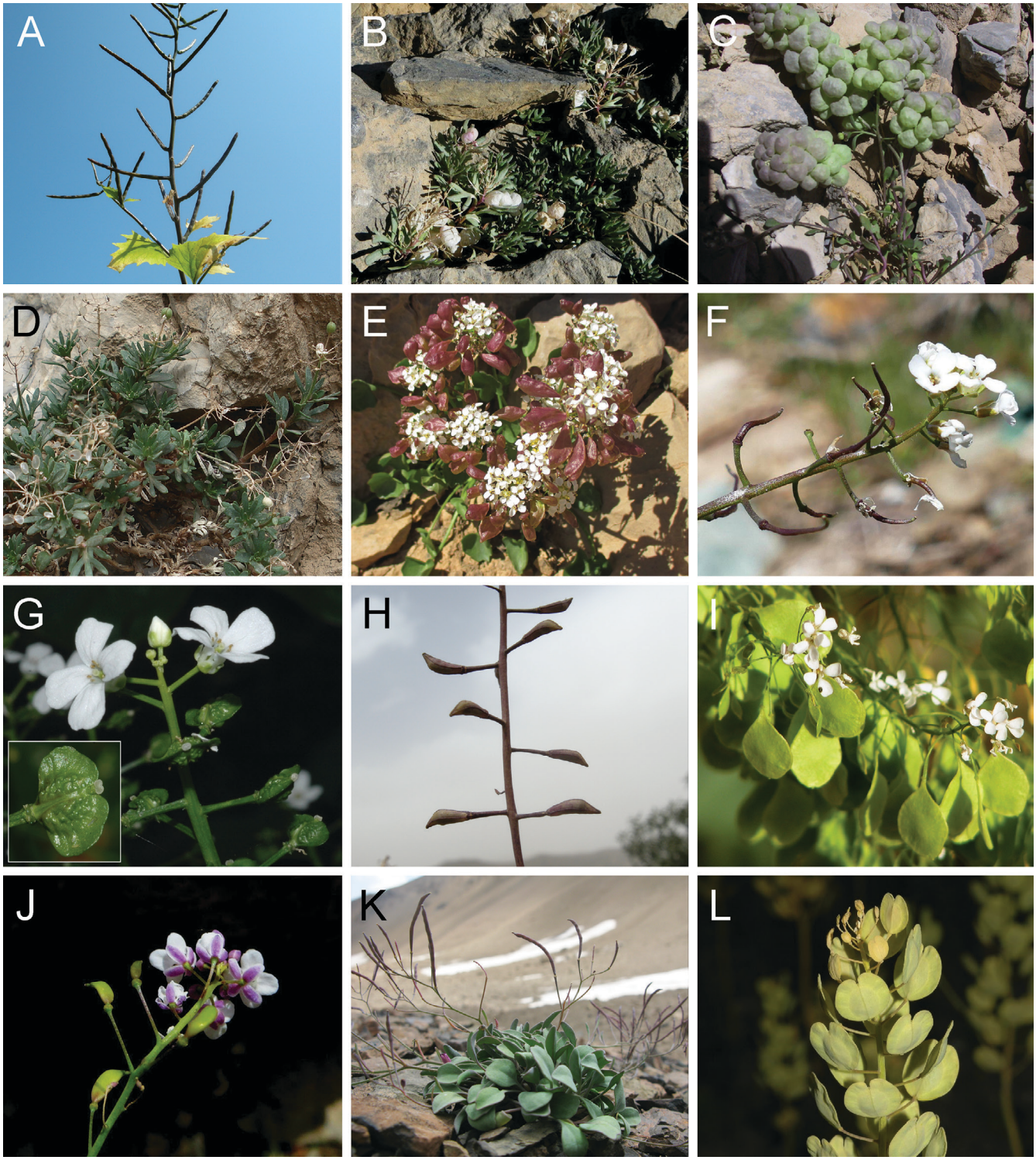

Fig. 5. Images of 12 representative species of tribe Thlaspideae. **A,** *Alliaria petiolata*; **B,** *Didymophysa aucheri*; **C,** *D. fedtschenkoana*; **D,** *D. fenestrata*; **E,** *Graellsia saxifragifolia*; **F,** *Lysakia rostrata*; **G,** *Pachyphragma macrophyllum*; **H,** *Parlatoria cakiloidea*; **I,** *Peltaria angustifolia*; **J,** *Peltariopsis planisiliqua*; **K,** *Pseudocamelina kleinii*; **L,** *Thlaspi arvense*. — Photographs by Ihsan A. Al-Shehbaz (A, C, H, L), Shokouh Esmailbegi (D), Kourosh Kavoosi (K), Jan De Laet (G), Mansour Mirtadzadini (B, I, J), Jalil Noroozi (F), and Hamed Parizi (E)].

Mummenhoffia oliveri (Engl.) Esmailbegi & Al-Shehbaz, **comb. nov.** ≡ *Thlaspi oliveri* Engl., Hochgebirgsfl. Afr.: 223. 1892 – Lectotype (designated by Jonsell in Polhill, Fl. Trop. East Africa: 27. 1982): Ethiopia, Begemdir, 4500 m, 1850, *W. Schimper 216* (P barcode P00486650!; isolectotype: B barcode B 10 0154915!).

Distribution. – E African mountains at 3050–4600 m in Ethiopia, Kenya, and Tanzania.

Notes. – Although Warwick & Al-Shehbaz (2006) did not list a chromosome number of the species, the label of *Hedberg & Aweke 5427* (MO) indicated a chromosome count of $2n = 14$. Following Hedberg (1957), Jonsell (1982, 2000) also misidentified the Ethiopian and other afro-alpine plants as the European *Thlaspi alliaceum* instead of *T. oliveri*, listed the latter in the synonymy of the former, and provided a species description that covers both taxa. However, Meyer (2001) was the first to point out that error.

■ AUTHOR CONTRIBUTIONS STATEMENT

SE, IAAS, KM and MAL designed the study. SE, IAAS, MRR and MM performed field and herbarium studies. SE, MP and TM conducted laboratory studies and anlyzed the data. SE, IAAS and MAL wrote the manuscript. — SE, https://orcid.org/0000-0002-5589-5580, esmaielbegi@yahoo.com; IAAS, ihsan.al-shehbaz@mobot.org; MP, milpouch@centrum.cz; TM, https://orcid.org/0000-0001-6485-0563, terezie.mandakova@ceitec.muni.cz; KM, klaus.mummenhoff@biologie.uni-osnabrueck.de; MRR, https://orcid.org/0000-0003-2459-482X, mrr@sci.ui.ac.ir; MM, https://orcid.org/0000-0003-1101-3344, mirtadz@uk.ac.ir; MAL, https://orcid.org/0000-0003-0318-4194

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Appendix 1. Voucher information and GenBank accession numbers of taxa used in the present study.

Species names are followed by country of origin, collector and number, and herbarium code (Three herbaria are not registered in Index Herbariorum including "Lysak": Martin A. Lysak private herbarium, Czech Republic; "Isfahan": Isfahan University herbarium, Iran; "Sanandaj": Herbarium of Kurdistan, Sanandaj, Iran.), GenBank accessions are presented as (ITS / *trnL-F*). Sequences newly obtained in this study are followed by an asterisk (*). Samples that did not amplify for *trnL-F* or ITS are presented as "/–)". Missing information of sequences taken directly from GenBank is presented as " ?".

Alliaria petiolata (M.Bieb.) Cavara & Grande, Iran, Asalem to Khalkhal, *Khosravi 1772*, MIR (LC317153*/–); U.S.A., Wisconsin, *Nee 61138*, MO (LC317142*/LC317234*); Iran, Saghez to Baneh, *Maroofi & Moradi 6730*, Sanandaj (LC317155*/LC317233*); Austria, Kalkvoralpen, *Barta 2128*, W (LC317141*/–); Austria,Vienna, *Staudinger 99-16a2*, W (LC317144*/–); Armenia, Lake Parzlich, *Oganesian 4*, W (LC317154*/LC317232*); Croatia, Istria, *Starmühler & al. s.n.*, W (LC317143*/LC317235*); GenBank, ? (KJ748666/JN189781); Georgia, Trialetic Mt., Bakuriani, *Schneeweiss & Tribsch 6924*, WU (LC317149*/LC317267*); France, Paris, *Esmailbegi & Al-Shehbaz 2091*, MIR (LC317145*/LC317266*); Austria, Vienna, *Esmailbegi 2092*, MIR (LC317147*/LC317269*); Czech Republic, Brno, Lesni bus, *Esmailbegi 2093*, MIR (LC317146*/LC317270*); Iran, Tehran, Darakeh, *Doostmohammadi 2090c*, MIR (LC317148*/LC317268*); ***A. taurica*** (Adams) V.I.Dorof., Georgia, *Eristavi 926*, MO (LC317156*/LC317237*); ***Didymophysa aucheri*** Boiss., Iran, NW of Tar lake, *Mirtadzadini 1759*, MIR (LC317188*/LC317259*); GenBank, Iran, ? (FJ187877/FJ188023); ***D. fedtschenkoana*** Regel, Afghanistan, Wakhan, *Renz 14*, W (LC317189*/LC317258*); Uzbakistan, *Fedtschenko 294*, MO (LC317190*/LC317257*); GenBank, Tajikistan, ? (EF514648/FJ188024); ***D. fenestrata*** (Hedge) Esmailbegi, D.A.German & Al-Shehbaz, Iran, 5 km W of Gachsar, *Mirtadzadini 1758a*, MIR (LC317187*/LC317260*); GenBank, Iran, ? (GQ424533/–); ***Graellsia davisiana*** Poulter, Turkey, *Zaore 123*, M (LC317138*/LC317247*); Turkey, Maraş, Yalak, *Davis & al. 19972*, W (LC317139*/–); ***G. graellsiifolia*** (Lipsky) Poulter, Tajikistan, kishlak Vuzh, *Dengubenko 3076*, LE (LC317132*/LC317243*); Tajikistan, kishlak Bartang, *Ikonnikov 16259*, LE (LC317133*/LC317242*); ***G. integrifolia*** (Rech.f.) Rech.f., Iran, *Faghihnia & Zangooei 23925*, FUMH (LC317127*/LC317246*); Iran, SW of Bojnord, *Joharch & Zangooei 40639*, FUMH (LC317126*/LC317245*); Afghanistan, Kabol, *Rasoul 582*, MSB (LC317128*/LC317244*); ***G. isfahan*** Esmailbegi & Al-Shehbaz, Iran, Isfahan, S of Kord-e Olia, *Mirtadzadini 1748c*, MIR (LC317135*/LC317248*); ***G. longistyla*** (Poulter) Esmailbegi & Al-Shehbaz, Iran, Maryvan to Paveh, Darband Dezli, *Maroofi & Mohammadi 6483*, Sanandaj (LC317140*/LC317254*); Iran, Maryvan, Tata pass, *Maroofi & Kargar 456*, Sanandaj (LC317129*/LC317253*); ***G. saxifragifolia*** (DC.) Boiss., Iran, Kerman, Kuhpayeh, *Peyravian 1738*, MIR (LC317136*/LC317252*); Iran, Kerman, Rabor, *Mirtadzadini 1780c*, MIR (LC317137*/LC317250*); GenBank, Iran, ? (GQ424572/–); Iran, Isfahan, *Noroozi 3841*, W (LC317134*/LC317249*); ***G. stylosa*** (Boiss. & Hohen.) Poulter, Iran, Darband, *Esmailbegi 1744*, MIR (LC317130*/LC317251*); Iran, Karaj to Chalus, S of Gachsar, *Mirtadzadini 1779*, MIR (LC317131*/–); ***Lysakia rostrata*** (Boiss. & Hohen.) Esmailbegi & Al-Shehbaz, Iran, Tehran, *American-Iranian Botanical Delegation 34056*, MO (LC317157*/LC317229*); Iran, Tehran, Tochal, *Sojak s.n.*, PR (LC317158*/LC317230*); Iran, Tehran, W of Emamzadeh Davood, *Noroozi 2907*, W (LC317159*/LC317231*); GenBank, Iran, ? (GQ424552/–); ***Mummenhoffia alliacea*** (L.) Esmailbegi & Al-Shehbaz, U.S.A., Delware, *Longbottom 16715*, MO (LC317171*/LC317211*); U.S.A., Pennsylvania, *Cusick 36555*, MO (LC317170*/LC317212*); GenBank, ? (AH010912/–); ***M. oliveri*** (Engl.) Esmailbegi & Al-Shehbaz, Ethiopia, Bale Mt. Natl. Park, *Morton E48*, MO (LC317172*/LC317210*); ***Pachyphragma macrophyllum*** (Hoffm.) N.Busch, Georgia, Kakheti, *Schneeweiss & al. 1885*, W (LC317166*/LC317256*); Armenia, *Fayvush & al. 05-1068*, MO (LC317167*/LC317255*); GenBank, Georgia, ? (AF283486/–); ***Parlatoria cakiloidea*** Boiss., Iran, Shahoo Mt., Biashosh village, *Manoochehri 7850*, Sanandaj (LC317151*/LC317239*); Iran, Kamyaran, Qale Tkhte Zangi Village, *Esmailbegi 1828c*, MIR (LC317150*/LC317241*); Iran, Maryvan to Paveh, *Maroofi & Karami 7374*, Sanandaj (LC317152*/LC317240*); ***Peltaria alliacea*** Jacq., Austria, *Barta 2128*, G (LC317194*/LC317214*); Austria, Hohe Wand, *Pull s.n.*, W (LC317195*/–); GenBank, ? (KF022717/DQ479879); ***P. angustifolia*** DC., Iran, Yasuj, *Sahebi 14602-2*, Isfahan (LC317197*/–); Iran, Isfahan, between Damaneh and Khunsar, *Aryavand & Sahebi 2927*, Isfahan (LC317198*/–); Turkey, Antalya, Gazipaşa, Sugözü village, *Lysak & al. s.n.*, Lysak (LC317196*/LC317215*); ***P. turkmena*** Lipsky, Iran, NW of Neyshabur, Bar waterfall, *Joharchi 45111*, FUMH (LC317199*/LC317213*); GenBank, ? (AF283490, AF283491/–); ***Peltariopsis grossheimii*** N.Busch, Turkey, B9, Van, Tendurek Da, *Sorger & Buchner 82-66-14*, W (LC317201*/LC317261*); Iran, Azarbaijan, 15 km S of Tshaldoran, *Mirtadzadini 1827e*, MIR (LC317200*/LC317263*); ***P. planisiliqua*** (Boiss.) N.Busch, Iran, between Sufian and Marand, *Mirtadzadini 1777e*, MIR (LC317202*/LC317262*); Turkey, Prov. Bitlis, Nemrut Dağ, *McNeill 582*, W (LC317203*/–); GenBank, ? (GQ424553/–); ***Pseudocamelina aphragmodes*** (Boiss.) N.Busch, Iran, *Mirtadzadini 1787*, MIR (LC317173*/LC317219*); ***P. bakhtiarica*** Esmailbegi, Al-Shehbaz & Mirtadzadini, Iran, Bakhtiari, SW of Samsami village, Mt. Milli, *Mirtadzadini 1786d*, MIR (LC317175*/LC317227*); ***P. campylocarpa*** (Boiss.) N.Busch, Iran, Kerman, 10 km W of Deh-e Rastegar, *Mirtadzadini 1781a*, MIR (LC317180*/LC317221*); Iran, Luristan, Kebara, *Koelz 18249*, W (LC317177*/LC317226*); Iran, Kerman, Khane-Sorkh neck,

Appendix 1. Continued.

Mirtadzadini 1754, MIR (LC317181*/LC317217*); ***P. glaucophylla*** (DC.) N.Busch, Iran, Kurdistan, *Maroofi 12763*, Sanandaj (LC317179*/LC317225*); Iran, Kashan, Niasar to Delijan, mt. Karkas, *Mirtadzadini 1805b*, MIR (LC317174*/LC317222*); Iran, Azarbaijan, E of Hir, Darband region, *Mirtadzadini & al. 1806b*, MIR (LC317175*/LC317227*); GenBank, ? (DQ357582/–); Iran, Kurdistan, *Maroofi 11053*, Sanandaj (LC317182*/LC317224*); Iran, Bijar to Zanjan, 5 km up to Bianlo village, *Maroofi & Naseri 5574*, Sanandaj (LC317176*/LC317223*); ***P. kermanica*** Esmailbegi, Al-Shehbaz & Mirtadzadini, Iran, Kerman, 3 km SW of Madun village, *Mirtadzadini 1782b*, MIR (LC317185*/LC317220*); Iran, Kerman, Shahrbabak, Meymand, *Mirtadzadini 1784*, MIR (LC317183*/LC317216*); ***P. kleinii*** Rech.f., Iran, Alam Kuh, Khersan glacial, *Kavousi 1788*, MIR (LC317178*/LC317228*); ***P. kurdica*** (A.G.Mill.) Esmailbegi & Al-Shehbaz, Iraq, Sulaimaniya, *Rechinger 10435*, W (LC317184*/–); ***Pseudovesicaria digitata*** (C.A.Mey.) Rupr., Georgia, mt. range SE Juta and NE peak Chaukhi, *Schneeweiss & al. 8333*, W (LC317168*/LC317205*); Georgia, Mtskheta-Mtianeti, *Schneeweiss & al. 8302*, W (LC317169*/LC317204*); ***Sobolewskia caucasica*** (Rupr.) N.Busch, Georgia, Chewi, *Schneeweiss 4491*, W (LC317163*/LC317238*); Georgia, Tushetia, *Grudzinskaya & al. s.n.*, LE (LC317165*/–); GenBank, Georgia, ? (KF022718/–); ***S. clavata*** (Boiss.) Fenzl, Armenia, Province Syunik, *Gonzalo & al. 38*, W (LC317162*/LC317265*); Armenia, Sisian, *Oganesian & al. 07-0367*, W (LC317160*/LC317264*); ***S. sibirica*** (Willd.) P.W.Ball, –, *Anonymous*, *1706*, G, (LC317161*/–); ***S. truncata*** N.Busch, Russia, Daghestan, Rutul district, *Klochkova & al. 523*, LE (LC317164*/LC317236*); ***Thlaspi arvense*** L., U.S.A., Missouri, *Tadych & Sonderman OA-2*, MO (LC317191*/LC317208*); GenBank, Japan, ? (LC090020/KJ480809); ***T. ceratocarpum*** (Pall.) Murr., Turkey, Erzincan, Refahiye, SW of Sağlık Village, *Özüdoğru 3661*, HUB (LC317193*/LC317209*); GenBank, ? (AH010911/–); ***T. huetii*** Boiss., Turkey, A9, Kars: Göle, Karlıyazı Village, *Denirkus 1012c*, HUB (–/LC317206*); Turkey, A8, Rize: 2 km from Fındıklı to Ardeşen, *Güner 5563*, HUB (LC317192*/LC317207*); ***Alyssum lenense*** Adams, GenBank, ? (EF514610, FN677633/FN677633); ***Berteroa incana*** (L.) DC., GenBank, ? (EF514632/KX668100, KJ623445); ***Lobularia libyca*** (Viv.) Meisn., GenBank, ? (EF514680/DQ479876, DQ518372); ***Aphragmus oxycarpus*** (Hook.f. & Thomson) Jafri, GenBank, ? (DQ165337/DQ479853, DQ518350); ***Arabis alpina*** L., GenBank, ? (DQ060100/JF705252); ***Draba nuda*** (Bél.) Al-Shehbaz & M.A.Koch, GenBank, ? (GU202513/GU202757, GU202635); ***Brassica nigra*** (L.) W.D.J.Koch, GenBank, ? (DQ003644/AY958575); ***Raphanus sativus*** L., GenBank, ? (GQ268079/GQ268055); ***Calepina irregularis*** (Asso) Thell., GenBank, ? (AM905715/AY751760); ***Cochlearia officinalis*** L., GenBank, ? (HQ268642/HQ268697); ***Noccaea caerulescenc*** (J.Presl & C.Presl) F.K.Mey., GenBank, ? (DQ518397/KT253346); ***Cremolobus subscandens*** Kuntze, GenBank, ? (EU620291/EU620348); ***Eudema nubigena*** Humb. & Bonpl., GenBank, ? (EU620298/EU620355); ***Brayopsis colombiana*** Al-Shehbaz, GenBank, ? (EU620283/EU620339); ***Eutrema edwardsii*** R.Br., GenBank, ? (DQ165350/KR303791); ***Eutrema salsugineum*** (Pall.) Al-Shehbaz & S.I.Warwick, GenBank, ? (AF531626/KR303797); ***Heliophila coronopifolia*** L., GenBank, ? (DQ249846/DQ479873, DQ518369); ***Isatis tinctoria*** L., GenBank, ? (AF384104/KJ765852); ***Mathewsia peruviana*** O.E.Schulz, GenBank, ? (EU620303/EU620362); ***Schizopetalon walker*** Sims, GenBank, ? (EU620315/EU620378); ***Sisymbrium altissimum*** L., GenBank, ? (AF531559/AY958545); ***Streptanthus glandulosus*** Hook., GenBank, ? (AF346651/AY958575); ***Chorispora tenella*** (Pall.) DC., GenBank, ? (DQ357526/FN677721); ***Dontostemon integrifolius*** (L.) C.A.Mey., GenBank, ? (AY558926, AY558954/LK021285); ***Hesperis sibirica*** L., GenBank, ? (FM164658/FN677642).