RESEARCH ARTICLE

# Phylogenetic relationships and biogeography in *Hedysarum* (Hedysareae, Fabaceae) with a focus on Central Asian taxa

**Inom Juramurodov,**[1,2,3,4] **Dilmurod Makhmudjanov,**[1,2,3,4] **Pei-Liang Liu,**[5] **Ziyoviddin Yusupov,**[1,2,6] **Elena Nikitina,**[7] **Tao Deng,**[1,2] **Komiljon Tojibaev**[2,3] **& Hang Sun**[1,2]

1 *CAS Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, Yunnan, China*
2 *Yunnan International Joint Laboratory for Biodiversity of Central Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, Yunnan, China*
3 *Flora of Uzbekistan laboratory, Institute of Botany of the Academy of Sciences of the Republic of Uzbekistan, 32 Durmon Yuli St., Tashkent, 100125, Uzbekistan*
4 *University of Chinese Academy of Sciences, Beijing, China*
5 *Key Laboratory of Resource Biology and Biotechnology in Western China, Ministry of Education, Northwest University, Xi'an, Shaanxi 710069, China*
6 *International Joint Lab for Molecular Phylogeny and Biogeography, Institute of Botany, Academy Sciences of Uzbekistan, Tashkent, 100125, Uzbekistan*
7 *Cadastre and Monitoring of rare plant species laboratory, Institute of Botany of the Academy of Sciences of the Republic of Uzbekistan, 32 Durmon Yuli St., Tashkent, 100125, Uzbekistan*
Addresses for correspondence: *Tao Deng*, dengtao@mail.kib.ac.cn; *Komiljon Tojibaev*, ktojibaev@mail.ru; *Hang Sun*, sunhang@mail.kib.ac.cn

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**Abstract** Central Asia, especially the Tian-Shan and Pamir-Alay, is a biodiversity hotspot for *Hedysarum* (Fabaceae) with a high number of endemic species. However, to date, studies of the genus *Hedysarum* based on molecular data have included only a few species distributed in this region. To fill this gap, we analysed 110 *Hedysarum* species, 36 of which were from the Tian-Shan and Pamir-Alay Mountains of Central Asia. We performed phylogenetic analyses, divergence time estimates, and biogeographic analyses using both the internal transcribed spacers of nuclear ribosomal DNA (nrDNA ITS) and three plastid DNA (cpDNA) sequences (*trnL-trnF*, *matK*, *psbA-trnH*). Our phylogenetic analysis grouped the species of *Hedysarum* into three sections: *H.* sect. *Hedysarum*, sect. *Multicaulia*, and sect. *Stracheya*, which are largely consistent with previous phylogenetic classifications. Accordingly, most of the Central Asian species we studied (28 spp.) were placed in *H.* subsect. *Crinifera* within *H.* sect. *Multicaulia*. Our divergence time and biogeographic analyses indicate that *Hedysarum* likely originated in West Asia and/or East Asia during the Early Miocene or Middle Miocene and subsequently dispersed to adjacent areas of Eurasia, as well as North America via the Bering Land Bridge. The likely area of origin for *H.* sect. *Multicaulia* is West Asia, while East Asia appears to be the center of origin for *H.* sect. *Hedysarum* and sect. *Stracheya*. In addition, we suggest that the rapid radiation of the species of *H.* subsect. *Crinifera* occurred in the Tian-Shan and Pamir-Alay Mountains, as well as the Turkish-Iranian Plateau, and that geological and climatic changes were the main causes of speciation in *Hedysarum*.

**Keywords** biogeography; divergence time; diversification; East Asia; Pamir-Alay Mountains; Tian-Shan Mountains; phylogeny; West Asia

**Supporting Information** may be found online in the Supporting Information section at the end of the article.

## INTRODUCTION

*Hedysarum* L. is the largest genus of the tribe Hedysareae (Fabaceae), comprising more than 160 species distributed in Eurasia, North Africa, and North America (Lock, 2005; Liu & al., 2017). Central Asia is considered one of the hotspots for *Hedysarum* diversity (Choi & Ohashi, 2003; Zhang & al., 2020). According to our estimates, at least 81 *Hedysarum* species occur in Central Asia, 46 of which are endemic to the Tian-Shan and Pamir-Alay Mountains. The species occur in meadows, clayey and stony places, deserts, steppes, forests, tundras, river valleys, and on mountain slopes (Kovalevskaya, 1981; Choi & Ohashi, 2003). *Hedysarum* includes perennial herbs (rarely semishrubs) and can be distinguished from closely related genera by the morphology of the jointed pods (Fig. 1), that are flat-compressed or slightly inflated, smooth, glabrous or pubescent, reticulate or transversely ribbed, and often covered with short or longer bristles, and these characters are used to diagnose and delimit infrageneric taxa (Basiner, 1845; Fedtschenko, 1902, 1948; Choi & Ohashi, 2003; Nafisi & al., 2019, 2021).

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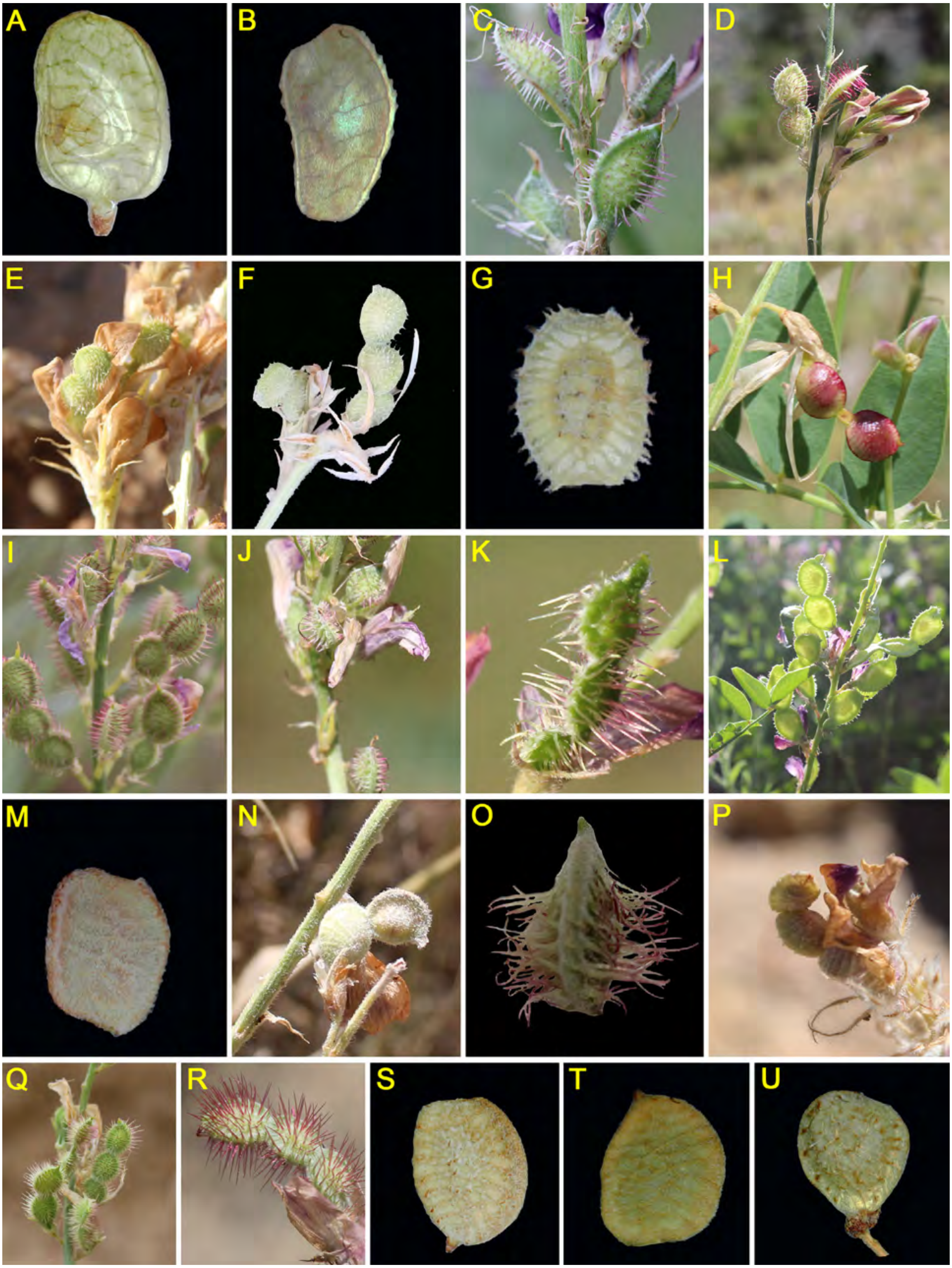

**Fig. 1.** Pod types in *Hedysarum* species. **A**, *H.* sect. *Hedysarum*; **B**, *H.* sect. *Stracheya*; **C–U**, *H.* sect. *Multicaulia* (*H.* subsect. *Crinifera*). **A**, *H. flavescens* **B**, *H. lehmannianum*; **C**, *H. baldshuanicum*; **D**, *H. bucharicum*; **E**, *H. drobovii*; **F**, *H. gypsaceum*; **G**, *H. iomuticum*; **H**, *H. magnificum*; **I**, *H. mogianicum*; **J**, *H. montanum*; **K**, *H. nuratense*; **L**, *H. olgae*; **M**, *H. plumosum*; **N**, *H. pskemense*; **O**, *H. sunhangii*; **P**, *H. talassicum*; **Q**, *H. taschkendicum*; **R**, *H. turkestanicum*; **S**, *H. jaxarticum*; **T**, *H. popovii*; **U**, *H. santalaschi*. — Images: Inom Juramurodov.

*Hedysarum* has a long history of taxonomy. The first classification of *Hedysarum* was made by Linnaeus (1753), who divided the genus into four sections based on the shape of the leaves. Later, Candolle (1825), Boissier (1872), and Fedtschenko (1902) produced classifications based on the presence or absence of bristles and setae on the joints of the pod and on the habit of the species. The last comprehensive morphological classification was carried out by Choi & Ohashi (1996, 2003). They revised the genus based on pollen characters and morphological evidence. A classification based on molecular data was conducted by Amirahmadi & al. (2014). They separated *H.* sect. *Membranacea* from *Hedysarum* based on their phylogenetic reconstruction and proposed a new genus, *Greuteria* Amirahmadi & Kaz.Osaloo. More information on the history and development of the infrageneric classification of *Hedysarum* is given in Table 1.

The botanical exploration of *Hedysarum* in Central Asia has a history of more than 150 years (Juramurodov & al., 2021a). The first significant studies of the genus in the region were carried out by Baitenov (1964), who investigated the evolution of the genus based on morphology and also described more than 10 new species (Baitenov, 1956, 1961, 1964). Furthermore, the systematics, ecology, and geography of *Hedysarum* in Kyrgyzstan were studied by Sultanova (1971), who indicated that the species in *H.* sect. *Multicaulia* are variable and polymorphic. Kovalevskaya (1981) provided information on the distribution and habitats of 72 *Hedysarum* species in Central Asia in *Conspectus Florae Asiae Mediae*. Recently, Juramurodov & al. (2022) reviewed the type specimens of *Hedysarum* collected in Central Asia and stored in the National Herbarium of Uzbekistan (TASH). Although these studies are crucial for understanding the systematics, morphology, geographic distribution, and habitat of *Hedysarum* species in Central Asia, none of them included molecular phylogenetic analyses.

In recent years, several molecular systematic studies have been published, including Ahangarian & al. (2007), Amirahmadi & al. (2014), Duan & al. (2015), Liu & al. (2017), and Nafisi & al. (2019) to clarify the phylogenetic relationships among *Hedysarum* species. In all these studies, it was found that the genus is paraphyletic based on the phylogeny of nrDNA ITS. This inference was first determined by Ahangarian & al. (2007) based on five sampled species of the genus. Subsequently, phylogenetic studies of *Hedysarum* conducted by Amirahmadi & al. (2014) showed that the genus is paraphyletic based on analyses of both nuclear and plastid datasets. In contrast, the phylogenetic studies by Duan & al. (2015), Liu & al. (2017), and Nafisi & al. (2019) showed that *Hedysarum* is paraphyletic only in the nuclear tree, but those phylogenetic analyses based on plastid data revealed that *Hedysarum* is monophyletic. The incongruence between the nuclear and plastid phylogenies in *Hedysarum* was explained by an ancient chloroplast capture hypothesis by Liu & al. (2017) using multiple nuclear (ITS, ETS, PGDH, TRPT, SQD1) and plastid DNA regions (*trnH-psbA*, *trnC-petN*, *trnL-trnF*, *trnS-trnG*, *petN-psbM*). However, in these studies, very few species were sampled from the Tian-Shan and Pamir-Alay Mountains. Dating analyses by Nafisi & al. (2019), which included 93 species of *Hedysarum*, showed that the origin and subsequent divergence of the genus started in the Middle Miocene and extended into the Pliocene and Pleistocene. However, this study did not investigate the biogeography of the whole genus, again lacking samples from the Tian-Shan and Pamir-Alay Mountains of Central Asia.

In this study, we analysed about 110 *Hedysarum* taxa, 36 of which were from the Tian-Shan and Pamir-Alay Mountains in Central Asia. We generated new nrDNA ITS (46 accessions) and plastid marker (*trnL-F*, *matK*, *psbA-trnH*; 59 accessions in total) sequences belonging to a total of 25 species, of which 19 are endemic to Central Asia. Our study builds upon previous research on the genus *Hedysarum* and aims to fill phylogenetic gaps in the genus and explain its biogeography. Thus, the main objectives of this study were to: (1) estimate the molecular evolution and phylogenetic relationships of the species of *Hedysarum*, especially with new data from Central Asia; and (2) examine biogeographic events and divergence times for *Hedysarum* in an expanded context of species sampling.

## ■ MATERIALS AND METHODS

**Study area. —** The Tian-Shan and Pamir-Alay Mountains in Central Asia are defined according to the *Conspectus Florae Asiae Mediae* (Kovalevskaya, 1981) (Fig. 2). From 2018 to 2022, we conducted field observations in the western Tian-Shan and southwestern Pamir-Alay Mountains in Uzbekistan. Due to the fact that the territory of Uzbekistan is located in both the Tian-Shan and Pamir-Alay mountain systems, it is an important region for conducting biogeographical and taxonomic research on *Hedysarum* in Central Asia. This study included 36 species distributed in the Tian-Shan and Pamir-Alay Mountains, 20 of which are endemic to Central Asia (Kovalevskaya, 1981; Tojibaev & al., 2020). The species used in this study occur in the floras of Uzbekistan (25 species were used in this study; 18 of them are endemic to Central Asia), Kyrgyzstan (19; 8), Kazakhstan (17; 4), and Tajikistan (13; 6) (Baitenov, 1961; Sultanova, 1971; Karimova, 1978; Juramurodov & al., 2021b).

**Taxon sampling. —** In this study, 129 taxa and 159 accessions (110 taxa and 140 accessions belonging to *Hedysarum*) were sampled for nrDNA ITS, and 124 species and 311 accessions were sampled for cpDNA (107 taxa, 266 accessions belonging to *Hedysarum*: 103 *trnL-F*; 96 *matK*; 67 *psbA-trnH*). Some new nrDNA ITS (46 accessions) and cpDNA (59 accessions: 22 *trnL-F*, 17 *matK*, 20 *psbA-trnH*) sequences for 25 species were generated (Appendix 1). The collection sites of the 25 species (including 7 herbarium and 18 fresh materials) used to generate new sequences for this study are shown in Fig. 3. The herbarium material was taken from the National Herbarium of Uzbekistan (TASH). All available sequences of *Hedysarum* that had at least three of the DNA regions we used

**Table 1.** Infrageneric classification history of *Hedysarum*.

| Candolle (1825) | Basiner (1845) | Boissier (1872) | Fedtschenko (1902) | Choi & Ohashi (2003) | Amirahmadi & al. (2014) | Duan & al. (2015) | Liu & al. (2017) | Nafisi & al. (2019) and current classification |
|---|---|---|---|---|---|---|---|---|
| sect. *Echinolobium* DC. | tribe *Isoloma* sect. *Eleutherotion* | tribe *Isoloma* sect. *Eleutherotion* group *Annua* | tribe *Isoloma* subtribe *Eleutherotion* sect. *Spinosissima* B.Fedtsch. | Transferred to genus *Sulla* Medik. | *Sulla* | *Sulla* | *Sulla* | *Sulla* |
|  | sect. *Gamotion* | sect. *Gamotion* | subtribe *Gamaation* *H.* sect. *Obscura* B.Fedtsch. | *H.* sect. *Hedysarum* | *H.* sect. *Hedysarum* | *H.* sect. *Hedysarum* | *Hedysarum* s.str. clade (incl. *Stracheya* clade) | *H.* sect. *Hedysarum* |
|  |  |  |  | *H.* sect. *Multicaulia* | *H.* sect. *Multicaulia* | *H.* sect. *Multicaulia* | *Sartoria* clade | *H.* sect. *Multicaulia* |
|  |  | group *Multicaulia* Boiss. | *H.* sect. *Multicaulia* (Boiss.) B.Fedtsch. | *H.* subsect. *Multicaulia* (Boiss.) B.H.Choi & H.Ohashi | *H.* subsect. *Multicaulia* | – | – | *H.* subsect. *Multicaulia* |
|  |  | group *Crinifera* Boiss. | *H.* sect. *Crinifera* (Boiss.) B.Fedtsch. | *H.* subsect. *Crinifera* (Boiss.) B.H.Choi & H.Ohashi | *H.* subsect. *Crinifera* | – | – | *H.* subsect. *Crinifera* p.p. |
|  |  | group *Subacaulia* Boiss. | *H.* sect. *Subacaulia* (Boiss). B.Fedtsch. | *H.* subsect. *Subacaulia* (Boiss.) B.H.Choi & H.Ohashi | *H.* subsect. *Subacaulia* | – | – | *H.* subsect. *Crinifera* p.p. |
|  |  |  | *H.* sect. *Membranacea* B.Fedtsch. | *H.* sect. *Membranacea* | *Greuteria* Amirahm. & Kaz.Osaloo | *Greuteria* | *Greuteria* | *Greuteria* |
|  |  |  |  | *Sartoria* | *H.* sect. *Multicaulia* p.p | *H.* sect. *Multicaulia* p.p. | *Sartoria* clade | *H.* subsect. *Crinifera* p.p. |
|  |  |  |  | *H.* sect. *Stracheya* (Benth.) B.H.Choi & H.Ohashi | *H.* sect. *Stracheya* | *H.* sect. *Stracheya* | *H.* sect. *Stracheya* | *H.* sect. *Stracheya* |
| sect. *Leiolobium* DC. | tribe *Heteroloma* | tribe *Heteroloma* | tribe *Heteroloma* *H.* sect. *Fruticosa* B.Fedtsch. | *Corethrodendron* Fisch. & Basiner | *Corethrodendron* | *Corethrodendron* | *Corethrodendron* | *Corethrodendron* |

(95 species, 301 accessions: 94 nrDNA ITS; 81 *trnL-F*; 79 *matK*; 47 *psbA-trnH*), as well as outgroup species (19 species, 64 accessions: 19 nrDNA ITS; 17 *trnL-F*; 15 *matK*; 13 *psbA-trnH*), were retrieved from GenBank (www.ncbi.nlm.nih.gov/Genbank) (Appendix 1). For this study, the species of the genera *Alhagi* Gagnebin, *Astragalus* L., *Caragana* Fabr., and *Oxytropis* DC. were used as outgroups, following Duan & al. (2015), Liu & al. (2017), and Nafisi & al. (2019).

**DNA extraction, amplification and sequencing. —** Total genomic DNA was isolated from silica gel-dried leaf material using the commercial set, Plant Genomic DNA Kit (TIANGEN Biotech, Beijing, China), according to the manufacturer's protocol. The nrDNA ITS and three plastidial regions (*matK*, *psbA-trnH*, *trnL-trnF*) were selected based on previous studies (Duan & al., 2015; Liu & al., 2017; Nafisi & al., 2019). The nrDNA ITS sequences were amplified using primers ITS1 and ITS4 (White & al., 1990). The cpDNA regions were amplified with the following primers: matK-390F and matK-1326R (Cuenoud & al., 2002), psbA 3_F (Sang & al., 1997) and trnHf_05 (Tate & Simpson, 2003), trnL-c and trnL-f, respectively (Taberlet & al., 1991). PCR amplifications were performed according to the protocols of Nafisi & al. (2019). Sequencing was performed using forward and reverse primer sets (TsingKe, China).

**Phylogenetic analyses. —** Sequencher v.4.1.4 (Gene Codes Corporation) was used to assemble and edit the raw sequence data. Sequence alignments were performed using MUSCLE v.3.8.31 (Edgar, 2004) as implemented in MEGA X software (Kumar & al., 2018). The plastid datasets were combined using SequenceMatrix software v1.7.8 (Vaidya & al., 2011). Based on previous studies and our preliminary analyses, we identified several potential sources of conflict between these two datasets, specifically relationships within *Hedysarum* sect. *Hedysarum* and sect. *Multicaulia* and the placement of *H.* sect. *Stracheya*. Therefore, phylogenetic reconstructions were conducted based on the nuclear and the plastid dataset separately.

Phylogenetic trees were reconstructed using Bayesian inference (BI), maximum parsimony (MP) and maximum likelihood (ML) methods. For BI, we used MrBayes v.3.2.7a (Ronquist & al., 2012). The nrDNA ITS and the plastid datasets were each run with two replicates for 10 million generations, sampling one tree every 1000 generations (10,000 trees sampled in total). The first 2500 trees (25%) were discarded as burn-in, and a 50% majority-rule consensus tree

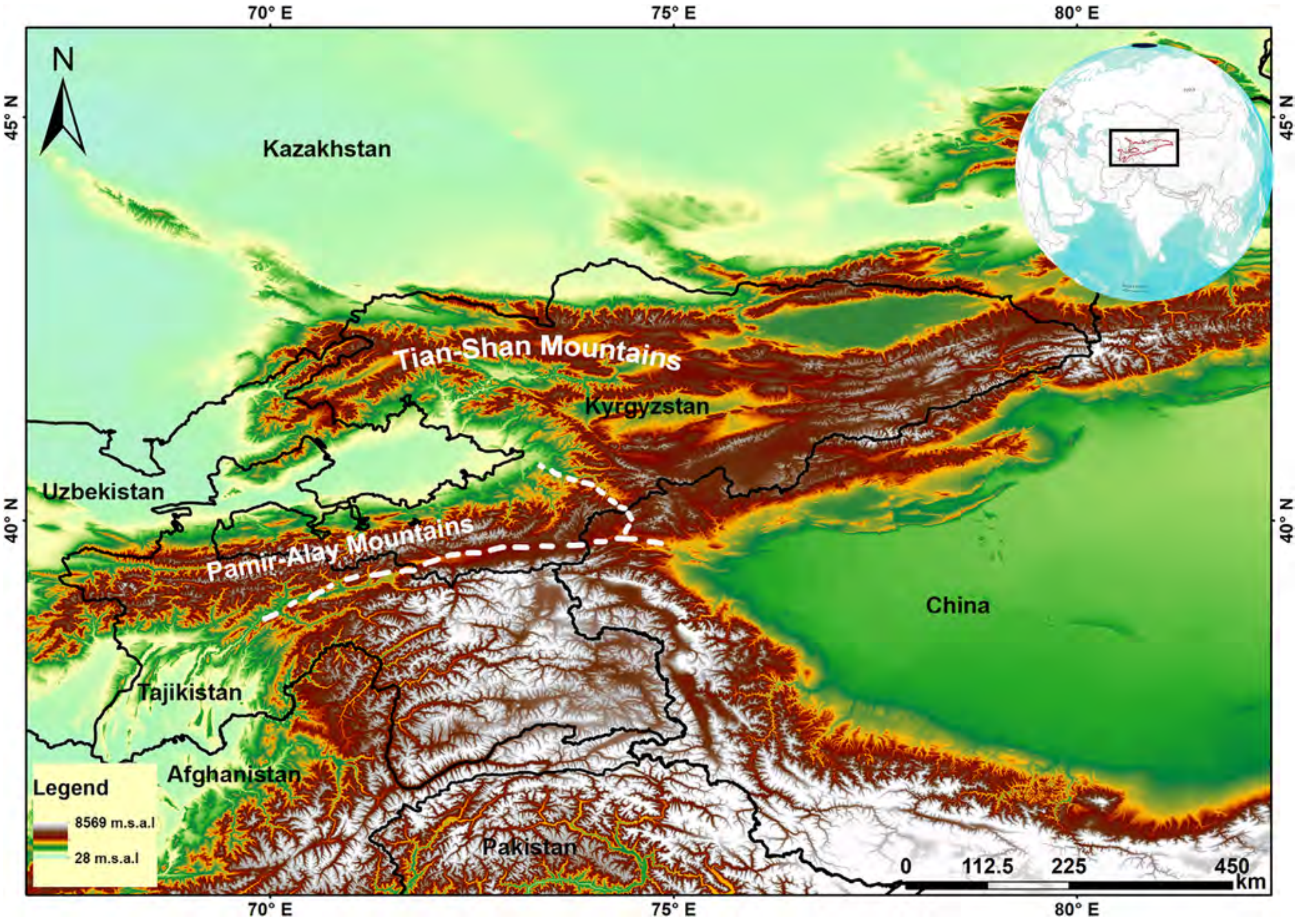

**Fig. 2.** Map of the study area. The borders of the mountains are marked by dashed lines

was constructed from the remaining trees to estimate posterior probabilities (PP). For each analysis, the best-fit model of nucleotide substitution was selected based on the Akaike information criterion (AIC) using jModelTest2 on XSEDE (www.phylo.org). Thus, GTR + I + Γ was identified as the best model for nrDNA ITS, whereas TVM + Γ was identified as the best model for the combined plastid markers. The ML phylogeny was reconstructed using IQ-TREE v.2.1.2 software (Minh & al., 2020) under the above-mentioned models with 1000 bootstrap replicates. The MP bootstrap analysis was performed using PAUP* v.4.0a169 (Swofford, 2002) with heuristic search, TBR branch-swapping, 1000 bootstrap replicates, a random addition sequence with 10 replicates, and a maximum of 1000 trees saved per round. The MP and ML bootstrap percentages were labeled on the corresponding branches of the BI tree. Trees were visualized in FigTree v1.4.0 (Rambaut, 2012).

**Estimation of divergence times. —** The analyses of divergence time were performed using BEAST2 v.2.6.6 (Bouckaert & al., 2014). XML command files were generated in BEAUti 2 v.2.6.6 (Bouckaert & al., 2019). We implemented two independent runs separately for the nrDNA ITS and the combined plastid dataset. The priors for the analyses were: a relaxed uncorrelated lognormal clock; the Yule Model of speciation; and a random starting tree. We performed Markov chain Monte Carlo (MCMC) with a chain length of 80 million and 120 million for the nrDNA ITS and the combined plastid data, respectively. Samples were saved every 1000 generations. MCMC samples were checked in TRACER v.1.7.2 (Rambaut & al., 2018) to confirm sampling adequacy and convergence of the chains to a stationary distribution. All parameters of each run ended with an effective sample size (ESS) >200. A maximum clade credibility (MCC) tree with mean and 95% HPD node ages was calculated using TreeAnnotator v.2.6.6 (Bouckaert & al., 2014) with 10% burn-in and a zero posterior probability limit.

The nrDNA ITS and the combined plastid trees were calibrated separately by setting a normal distribution for the

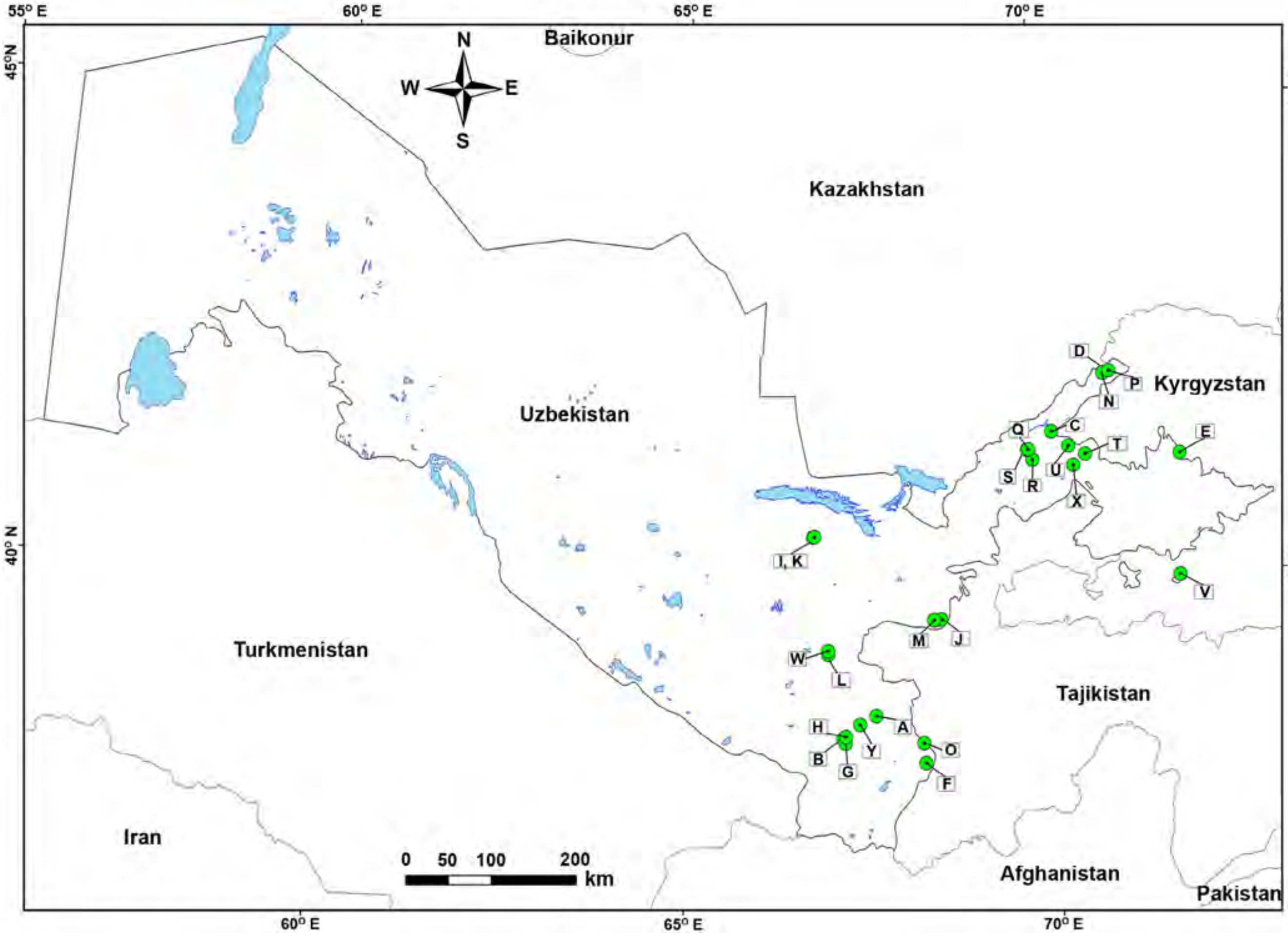

**Fig. 3.** Collection sites of *Hedysarum* species in Uzbekistan. **A**, *Hedysarum baldshuanicum*; **B**, *H. bucharicum*; **C**, *H. drobovii*; **D**, *H. flavescens*; **E**, *H. gypsaceum*; **F**, *H. iomuticum*; **G**, *H. lehmannianum*; **H**, *H. magnificum*; **I**, *H. mogianicum*; **J**, *H. montanum*; **K**, *H. nuratense*; **L**, *H. olgae*; **M**, *H. plumosum*; **N**, *H. pskemense*; **O**, *H. sunhangii*; **P**, *H. talassicum*; **Q**, *H. taschkendicum*; **R**, *H. turkestanicum*; **S**, *H. jaxarticum*; **T**, *H. popovii*; **U**, *H. santalaschi*; **V**, *H. alaicum*; **W**, *H. amankutanicum*; **X**, *H. angrenicum*; **Y**, *H. minjanense*.

divergence in *Hedysarum* as estimated by Nafisi & al. (2019). Based on Nafisi & al.'s study, the split between Hedysareae and *Caragana* was set to 26.5 Ma and 23.98 Ma (1), and the crown age of Hedysareae was set to 22.81 Ma and 21.1 Ma (2) for the nrDNA ITS and plastid datasets, respectively.

**Ancestral area reconstruction. —** Ancestral area reconstruction analyses were conducted separately for the nrDNA ITS and plastid datasets using RASP v.4.3 (Reconstruct Ancestral State in Phylogenies; Yu & al., 2015). Initially, we removed all outgroups from both nrDNA ITS and plastid BEAST MCMC trees, i.e., all taxa that do not belong to *Hedysarum*, prior to analysis using the outgroup-removal tool provided by RASP. The Bayesian Binary MCMC (BBM) method was used for ancestral area reconstruction and estimation of the spatial patterns of geographic diversification within *Hedysarum*. BBM was performed using 5 million generations with 10 chains, samples were saved every 1000 generations. The first 1000 trees were eliminated (burn-in). State frequencies were set to fixed, Jukes-Cantor (JC) and among-site rate variation was set to equal. The maximum number of areas was set to four.

The best models selected via model comparison of BioGeoBEARS (Matzke, 2014) implemented in RASP were the likelihood version of the BayArea (BAYAREALIKE; Landis & al., 2013) model and the dispersal-extinction-cladogenesis (DEC; Ree & Smith, 2008) model for the nrDNA ITS and the plastid datasets, respectively (suppl. Tables S1, S2). The distribution information of the *Hedysarum* species was compiled from literature sources, an online database, and herbarium specimens. We divided the entire geographic range of the genus *Hedysarum* into six areas corresponding to the floristic region designations of Takhtajan (1986): A, East Asia; B, Central Asia; C, West Asia; D, Mediterranean; E, North America (North American Atlantic, Rocky Mountain, and Madrean Regions are combined); F, Circumboreal.

## ■ RESULTS

**Dataset characteristics. —** The total nrDNA ITS dataset consisted of 545 aligned base pairs (bp) and 159 accessions of the 129 taxa, including 140 accessions of 110 taxa in *Hedysarum*. The combined plastid dataset contained 1925 aligned base pairs and 312 accessions of a total of 124 taxa (107 taxa and 266 accessions belong to *Hedysarum*). The characteristics of individual loci are shown in Table 2, and the alignment files are available as suppl. Appendices 1 and 2.

**Nuclear DNA-based phylogeny. —** Phylogenetic analyses of the nrDNA ITS (Fig. 4) showed that the selected species from the study area, as well as other members of the genus *Hedysarum*, were placed in three well-supported clades corresponding to *H.* sect. *Hedysarum*, sect. *Stracheya*, and sect. *Multicaulia*. However, the genus was paraphyletic and was clustered with the genera *Ebenus*, *Onobrychis*, and *Eversmannia*. *Hedysarum* sect. *Hedysarum* comprised 31 taxa (32 accessions), including 4 species from the Tian-Shan and Pamir-Alay Mountains (Fig. 4). The clade containing *Ebenus*, *Eversmannia*, and *Onobrychis* was sister to the clade containing *H.* sect. *Multicaulia* and *H.* sect. *Stracheya* with weak support. *Hedysarum cisdarvasicum* Kamelin & Karimova, *H. denticulatum* Regel, *H. kumaonense* Benth. ex Baker, *H. lehmannianum* Bunge (3 accessions), *Hedysarum minjanense* Rech.f. (2 accessions), and *H. tibeticum* (Benth.) B.H. Choi & H.Ohashi were grouped in the *H.* sect. *Stracheya* clade, most of which are distributed in Central Asia. Additionally, *H.* subsect. *Crinifera* and *H.* subsect. *Multicaulia* formed a sister groups within *H.* sect. *Multicaulia*, which was strongly supported. Our analysis showed that none of the Central Asian species we examined belonged to *H.* subsect. *Multicaulia*, which is composed mainly of West Asian and Mediterranean species. Within *H.* subsect. *Crinifera*, some subgroups (Fig. 4; subgroups A, B, C) were identified among the 28 species (53 accessions) of *Hedysarum* found in the Tian-Shan and Pamir-Alay Mountains. Nevertheless, most species found in the Tian-Shan and Pamir-Alay Mountains belonging to *H.* subsect. *Crinifera* were not resolved.

**Plastid DNA-based phylogeny. —** Our phylogenetic reconstruction based on the plastid dataset supported the monophyly of the genus *Hedysarum* (Fig. 5). The clade comprising the genera *Onobrychis* and *Eversmannia* was a sister clade to *Hedysarum* based on the BI, ML, and MP analyses. Despite differences in the placement of sections and species intersectional relationships between the phylogenetic tree based on the plastid dataset and the tree constructed using data from nrDNA ITS, section assignments of individual species in both trees showed similarities. From the plastid dataset, the placement of *H.* sect. *Stracheya* as a sister of *H.* sect. *Hedysarum* plus *H.* sect. *Multicaulia* was well supported by all analyses. Our analysis revealed that species from *H.* sect. *Stracheya*, which are distributed in the study area, including *H. cisdarvasicum* and *H. denticulatum*, as well as *H. lehmannianum* and *H. minjanense*, are closely related. *Hedysarum* sect. *Hedysarum* was sister to *H.* sect. *Multicaulia*, which was weakly supported by the BI and MP analyses and well

**Table 2.** Characteristics of the individual datasets.

| Dataset | Length (bp) | Conserved (%) | Variable sites (%) | Parsimony-informative sites (%) | Singleton sites (%) |
|---|---|---|---|---|---|
| ITS | 545 | 273 (50.1) | 272 (49.9) | 210 (38.5) | 57 (10.5) |
| *trnL-F* | 814 | 503 (61.8) | 311 (38.2) | 190 (23.3) | 120 (14.7) |
| *matK* | 772 | 503 (65.2) | 269 (34.8) | 119 (15.4) | 149 (19.3) |
| *psbA-trnH* | 339 | 189 (55.8) | 150 (44.2) | 96 (28.3) | 53 (15.6) |

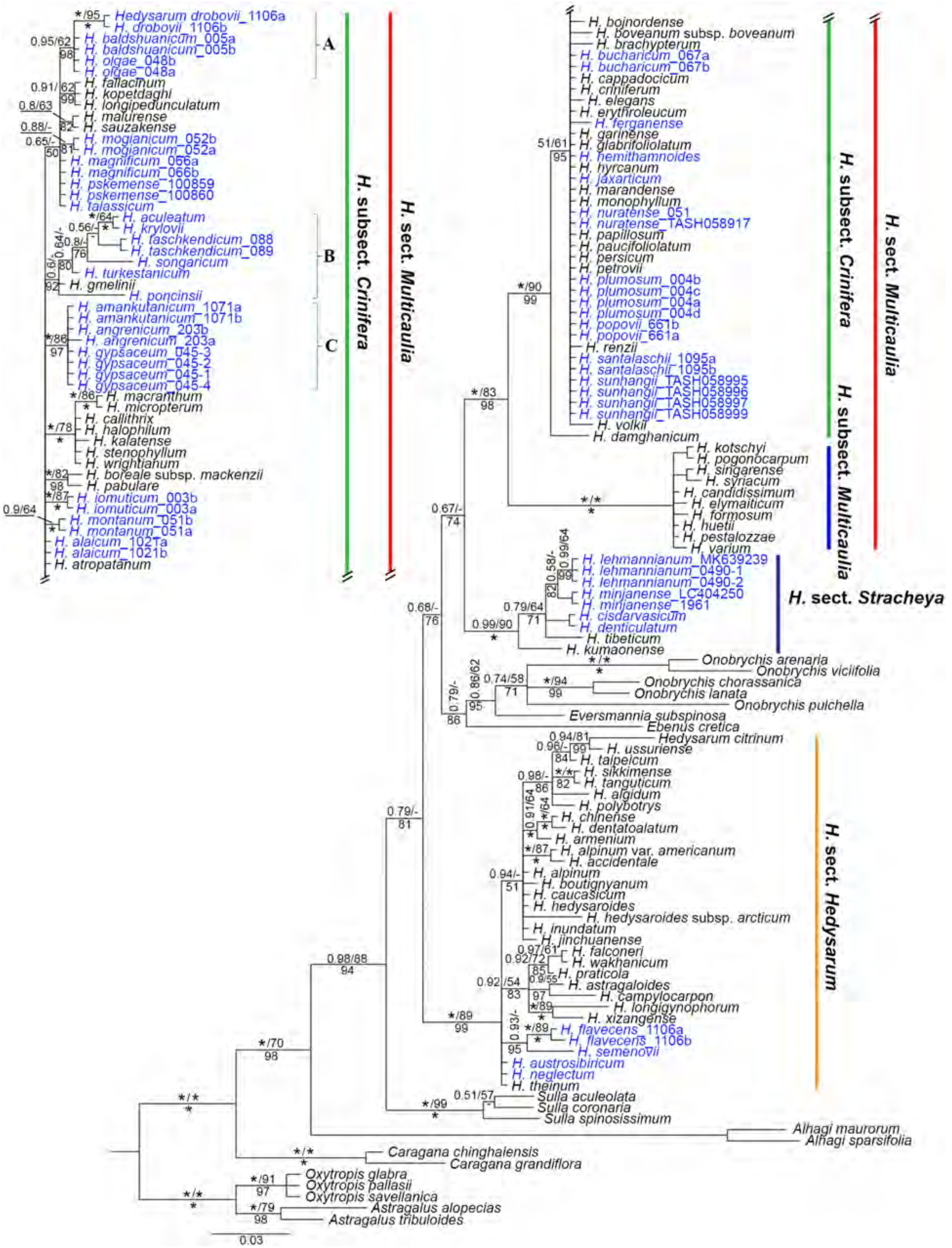

**Fig. 4.** Fifty percent majority-rule consensus tree resulting from Bayesian inference of nrDNA ITS sequences. Bayesian posterior probability (PP)/ maximum parsimony (MP) percentages are given above each branch; maximum likelihood (ML) percentage is given below each branch. Species occurring in Central Asia (Tian Shan and Pamir Alay Mountains) are written in blue. Asterisk indicates branches maximally supported by either method.

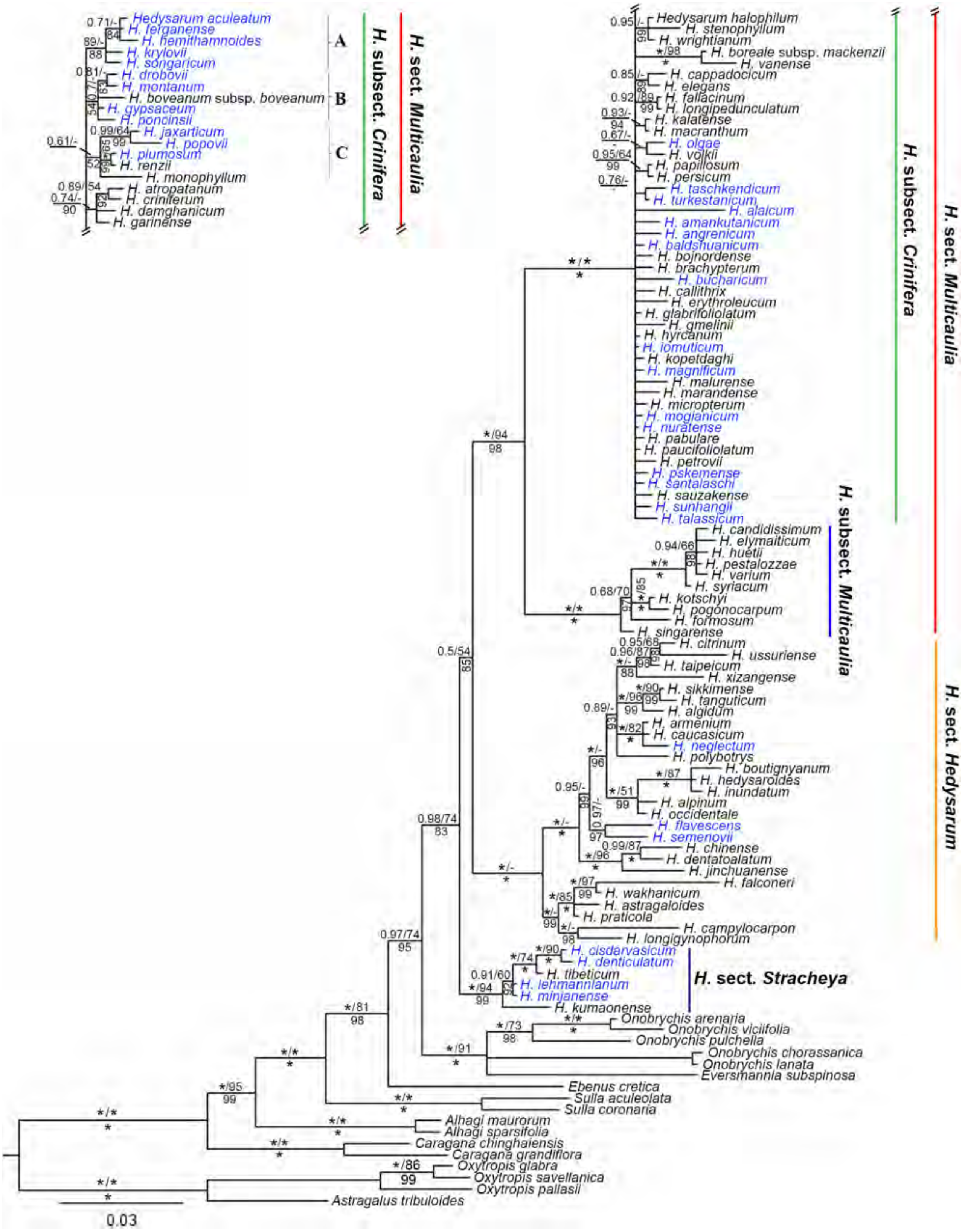

**Fig. 5.** Fifty percent majority-rule consensus tree resulting from Bayesian inference of the combined plastid *trnL-trnF*, *matK* and *psbA-trnH* sequences. Bayesian posterior probability (PP)/maximum parsimony (MP) percentage are given above each branch; maximum likelihood (ML) percentage is given below each branch. Species occurring in Central Asia (Tian Shan and Pamir Alay Mountains) are written in blue. Asterisk indicates branches maximally supported by either method.

supported by the ML analysis. Our analysis showed that *H. flavescens* Regel & Schmalh. and *H. semenovii* Regel & Herder, which belong to *H.* sect. *Hedysarum* and are distributed in the study area, show a close relationship that was strongly supported by both the BI and ML analyses. Only *H. neglectum* Ledeb. formed a subgroup with species distributed in other regions, such as *H. armenium* Boiss. ex Tchich. and *H. caucasicum* M.Bieb., which was strongly supported by the BI and ML analyses and well supported by the MP analysis. *Hedysarum* subsect. *Multicaulia* and subsect. *Crinifera* were sisters forming the *H.* sect. *Multicaulia* clade, which was strongly supported by the analyses using BI, MP, and ML. Our phylogenetic analyses based on the plastid dataset confirmed that the species we studied, which are distributed in the Tian-Shan and Pamir-Alay Mountains, do not belong to *H.* subsect. *Multicaulia*. Furthermore, the plastid dataset also confirmed that the majority of the species we examined in the study area belong to *H.* subsect. *Crinifera*. However, most of these species also had an unresolved position in the plastid tree, with the exception of some subgroups (subgroups A, B, C; Fig. 5). These subgroups were only weakly supported by BI and ML and not supported by MP.

**Divergence times estimations. —** The chronogram based on nrDNA ITS (Fig. 6) revealed that *Hedysarum* and related genera split from *Sulla* in the Early Miocene (19.64 Ma; 16.35–22.81 Ma; 95% HPD), whereas the plastid dataset (Fig. 7) suggested that *Hedysarum* split from *Onobrychis* and *Eversmannia* in the Middle Miocene (14.37 Ma; 11.18–17.43 Ma; 95% HPD). According to nrDNA ITS dating, the crown node ages of the *H.* sect. *Multicaulia*, sect. *Hedysarum*, and sect. *Stracheya* were inferred to be 11.03 Ma (7.38–14.62 Ma; 95% HPD), 9.63 Ma (5.83–13.8 Ma; 95% HPD), and 6.51 Ma (3.04–10.36 Ma; 95% HPD), respectively. The ages of *H.* subsect. *Multicaulia* and subsect. *Crinifera* were estimated to be 4.45 Ma (1.76–7.53 Ma; 95% HPD) and 7.28 Ma (4.39–10.29 Ma; 95% HPD), respectively. The chronogram of the plastid dataset indicated that *Hedysarum* diversified into two lineages around 12.81 Ma (9.75–15.85 Ma; 95% HPD; Fig. 7). One lineage was formed by *H.* sect. *Multicaulia*, which split into the *H.* subsect. *Crinifera* and subsect. *Multicaulia* in the Late Miocene (10.53 Ma; 7.6–13.51 Ma; 95% HPD). The crown node ages of these two subsections were estimated to be 6.55 Ma (4.13–9.06 Ma; 95% HPD) and 7.19 Ma (4.67–9.82 Ma; 95% HPD), respectively. The second lineage was formed by *H.* sect. *Stracheya* and sect. *Hedysarum*, which separated from each other at about 11.91 Ma (8.83–15 Ma; 95% HPD). The crown node ages of these two sections were 4.99 Ma (1.76–8.83 Ma; 95% HPD) and 10.23 Ma (7.26–13.26 Ma; 95% HPD), respectively. The ages of species distributed in the Tian-Shan and Pamir-Alay Mountains, which belong to *H.* sect. *Stracheya* were estimated to be 3.71 Ma (1.17–6.62 Ma; 95% HPD) or 3.21 Ma (0.96–5.95 Ma; 95% HPD) by the nrDNA ITS and plastid dating, respectively (Figs. 6, 7). As shown in Figs. 6 and 7, the mean divergence times of the species of *H.* sect. *Hedysarum* found in the study area were estimated to be around 4.02–0.53 Ma or 3.2–1.84 Ma, respectively. According to the nrDNA ITS and the plastid dataset chronograms, the species belonging to *H.* subsect. *Crinifera*, which occur in the Tian-Shan and Pamir-Alay Mountains, originated in the Pliocene and Pleistocene.

**Biogeographical analyses. —** Despite the prediction that the BAYAREALIKE model would be the best-fit model for nrDNA ITS, the region of origin for significant nodes in this model-based analysis remained largely unresolved (Table 3). Therefore, we focused more on the BBM analysis, which suggests single-distribution areas for ancestral nodes, in representing biogeographic events based on nrDNA ITS data for the genus *Hedysarum*. Additionally, the results of the separate analyses based on the nrDNA ITS and the combined plastid data using the BBM method showed similar results, except for nodes I and III (Fig. 8A–B and Table 3). Based on the BBM analysis of the nrDNA ITS, the region of origin for *Hedysarum* was East Asia (area A; Fig. 8A node I), while the analysis based on the plastid dataset indicated that the genus originated from West Asia (area C; Fig. 8B, node I). Moreover, the results from DEC and BBM based on the plastid dataset were mostly in agreement, differing only at two nodes: node I (which DEC estimates as the genus *Hedysarum* originated from East Asia+West Asia), and node III (which BBM estimated as *H.* sect. *Stracheya* originated in East Asia only, while DEC estimated it originated in East Asia+West Asia). Notably, BBM analysis based on nrDNA ITS showed that the species of *H.* sect. *Stracheya* were dispersed from West Asia only. Furthermore, both the BBM and DEC analyses based on the plastid dataset and the BBM analysis based on nrDNA ITS were consistent between nodes II, IV, V, and VI, indicating that *H.* sect. *Hedysarum* originated in East Asia (area A; Fig. 8A–B, both nodes II), while species of *H.* sect. *Multicaulia*, including *H.* subsect. *Multicaulia* and *H.* subsect. *Crinifera*, were colonized from West Asia (area C; Fig. 8A–B, nodes IV–VI, respectively).

The ancestral area reconstruction based on the plastid dataset indicated that *Hedysarum* required a total of either 49 global dispersals or 14 global vicariance events (inferred by BBM) or 44 global dispersals and 12 global vicariances (inferred from the DEC model) to reach its current distribution. According to the ancestral area reconstruction analysis using the nrDNA ITS-based BBM method, 51 global dispersals and 10 global vicariances have occurred in *Hedysarum* so far.

## ■ DISCUSSION

***Hedysarum* phylogeny. —** Amirahmadi & al. (2014) found that *Hedysarum* was paraphyletic in any of the nrDNA ITS, plastid *trnL-F*, and *matK* trees. Then they separated the genera *Sulla* and *Greuteria* from *Hedysarum* and merged the genus *Sartoria* Boiss. into *Hedysarum*. After these treatments, *Hedysarum* was monophyletic in the plastid trees but paraphyletic in the nrDNA ITS tree. This incongruence

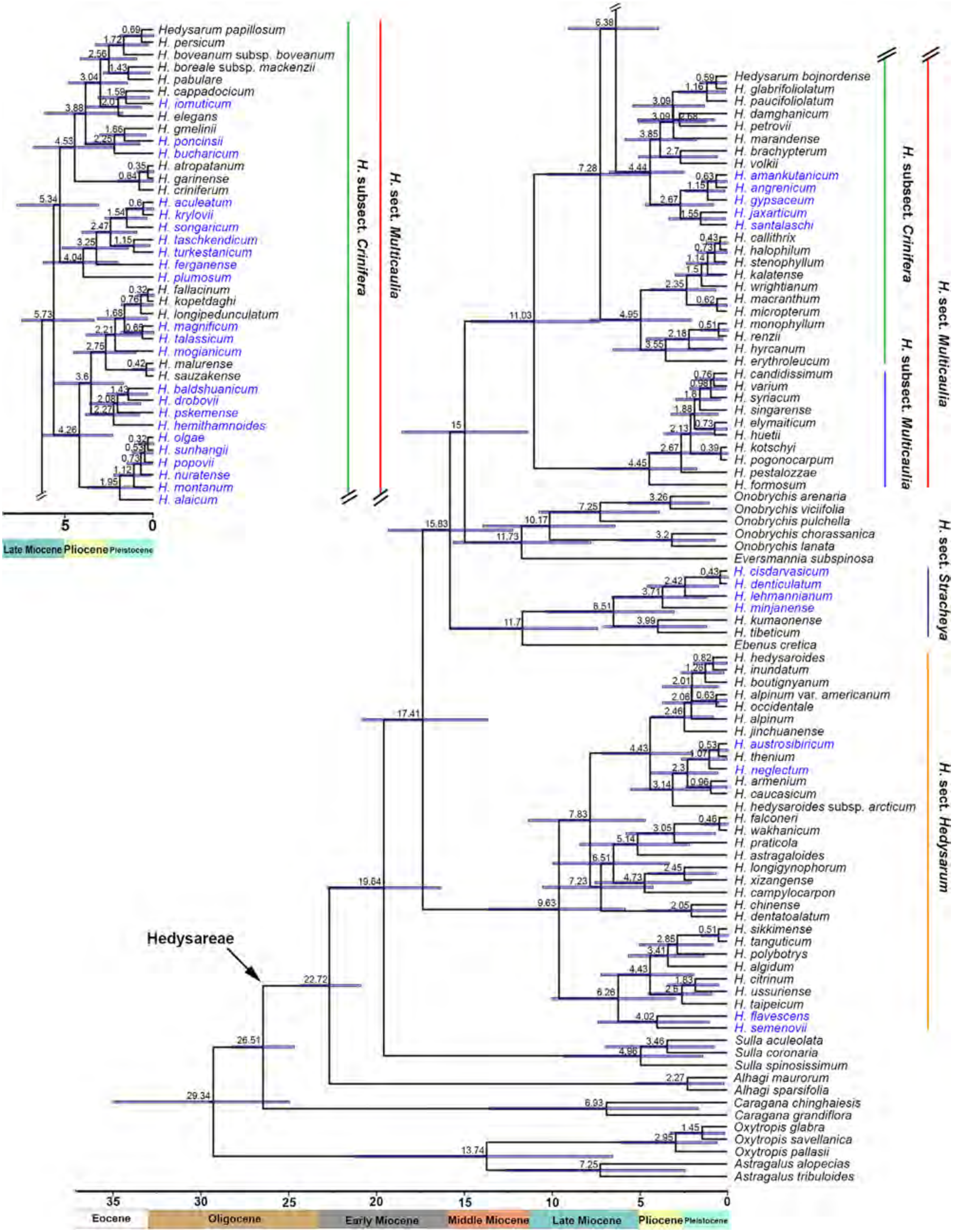

**Fig. 6.** Chronogram inferred from BEAST analysis of nrDNA ITS. Mean divergence time is shown at each node; gray bars represent the 95% highest posterior density intervals. Species occurring in Central Asia (Tian-Shan and Pamir-Alay Mountains) are written in blue.

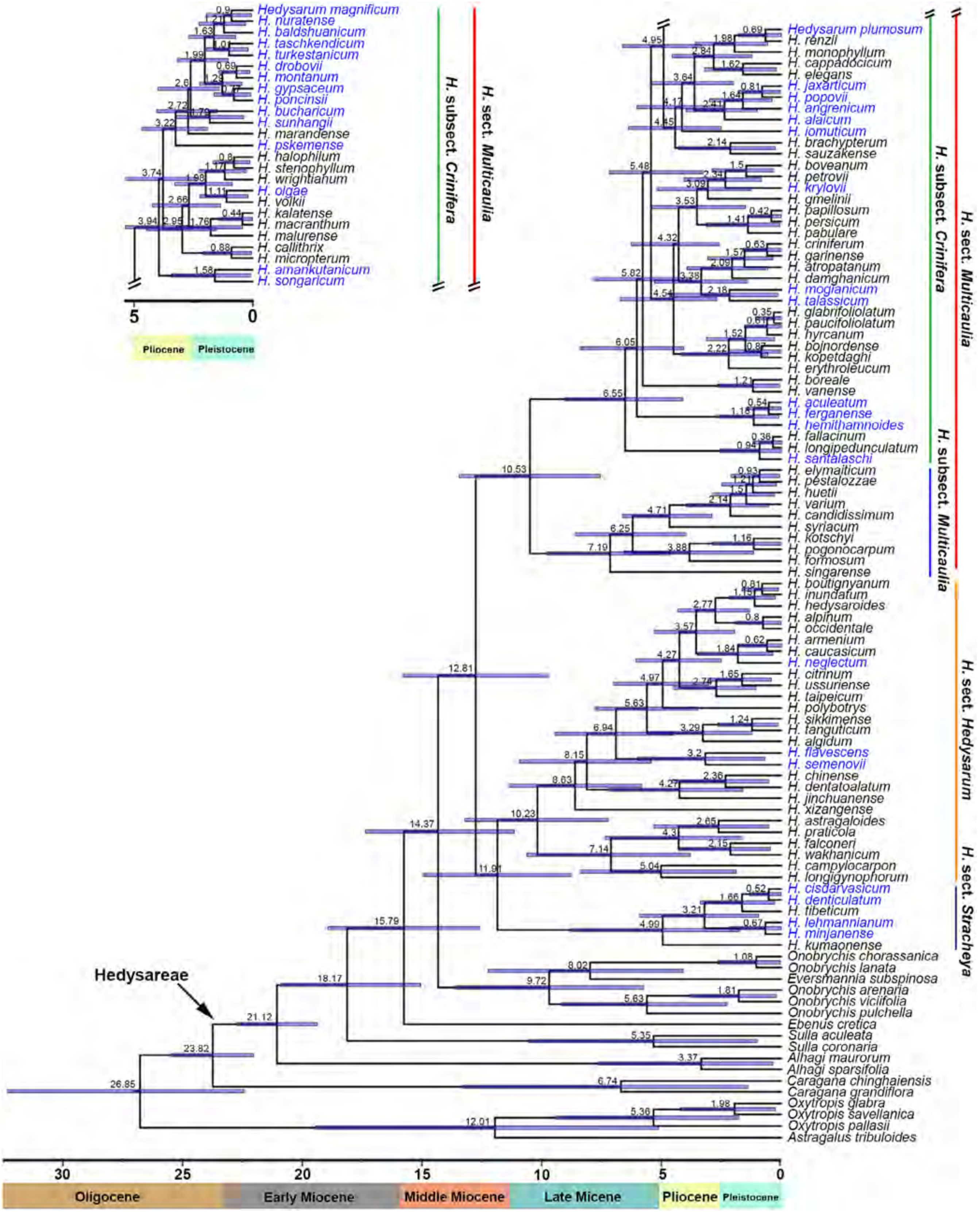

**Fig. 7.** Chronogram inferred from BEAST analysis of the combined plastid datasets (*matK*, *trnL-F*, *psbA-trnH*). Mean divergence time is shown at each node; gray bars represent 95% highest posterior density intervals. Species occurring in Central Asia (Tian Shan and Pamir Alay Mountains) are written in blue.

between plastid and nuclear trees was also observed by Duan & al. (2015), Liu & al. (2017), and Nafisi & al. (2019). Liu & al. (2017) proposed the hypothesis of chloroplast capture via introgression to explain this incongruence. In any case, three well-supported clades of *Hedysarum* were recognized: the *H.* sect. *Hedysarum* clade, the *H.* sect. *Stracheya* clade, and the *H.* sect. *Multicaulia* clade (Duan & al., 2015; Liu & al., 2017). Nafisi & al. (2019) also recognized the *H.* subsect. *Multicaulia* clade and the *H.* subsect. *Crinifera* clade, which together form the *H.* sect. *Multicaulia* clade.

In agreement with Duan & al. (2015) and Nafisi & al. (2019), our nrDNA ITS dataset (Fig. 4) placed *Hedysarum* sect. *Stracheya* as sister to *H.* sect. *Multicaulia*, while the nuclear markers used by Liu & al. (2017), placed *H.* sect. *Stracheya* together with *H.* sect. *Hedysarum*. However, our analysis based on the combined plastid dataset revealed *H.* sect. *Stracheya* as sister to *H.* sect. *Hedysarum* and *H.* sect. *Multicaulia* (Fig. 5), which is different from all previous phylogenetic studies (Duan & al., 2015; Liu & al., 2017; Nafisi & al., 2019). Therefore, the close relationship between *H.* sect. *Stracheya* and these two sections (*H.* sect. *Hedysarum*, *H.* sect. *Multicaulia*) should be further investigated using the whole plastid genome. The monophyly of the three sections that comprised the Central Asian species was supported by the morphological characters observed in previous studies (Ahangarian & al., 2007; Amirahmadi & al., 2014; Duan & al., 2015; Liu & al., 2017; Nafisi & al., 2019).

The results of our phylogenetic analyses including both nrDNA ITS and combined plastid datasets revealed that *Hedysarum austrosibiricum* B.Fedtsch., *H. flavescens*, *H. neglectum* Ledeb., and *H. semenovii*, which are distributed in the Tian-Shan and Pamir-Alay Mountains, belong to *H.* sect. *Hedysarum* (except for *H. austrosibiricum*, which was used only for nrDNA ITS analyses). These species exhibit characteristics of *H.* sect. *Hedysarum*, including a strongly developed stem, downcurved flowers in the inflorescence, triangular calyx teeth that are shorter than or as long as the calyx tube, and compressed, more or less reticulate veined pods without setae (Choi & Ohashi, 2003; Nafisi & al., 2019, 2021).

Phylogenetic analyses based on both the nrDNA ITS and the plastid datasets supported the close relationship of *Hedysarum cisdarvasicum* and *H. denticulatum*, as well as *H. lehmannianum* and *H. minjanense* within *H.* sect. *Stracheya*. The distribution of these species in the mountains of Pamir-Alay (Kovalevskaya, 1981) likely contributed to their close phylogenetic relationship. Additionally, these species have features of *H.* sect. *Stracheya*, including an inconspicuous or strongly reduced stem, all leaves radical, flowers on leafless peduncles, and pods with prickly margins (Choi & Ohashi, 2003; Nafisi & al., 2019).

Phylogenetic analyses using both the nrDNA ITS and the plastid dataset indicated that the majority of the species (28 species) analysed in the study area belong to *Hedysarum* subsect. *Crinifera*. However, relationships within *H.* subsect. *Crinifera* were largely unresolved in both phylogenetic trees, as reported earlier by Duan & al. (2015) and Nafisi & al. (2019). The low variability in the selected DNA sequences might be the main reason for the polytomy of *H.* subsect. *Crinifera* (Whitfield & Lockhart, 2007). This was previously noted by us in the case of *H. sunhangii* Juram. & Tojibaev and *H. nuratense* Popov (Juramurodov & al., 2021a), suggesting explosive speciation as a possible explanation.

**Table 3.** Comparison of results derived from biogeographic analyses.

| | BBM$_1$ | | | BBM$_2$ | | | BAYAREALIKE | | | DEC | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Nodes | Area | RP | Event | Area | RP | Event | Area | RP | Event | Area | RP | Event |
| Node I | A<br>C<br>B | 42<br>28<br>18 | 1/1/0/0.18 | C<br>A | 73<br>19 | 1/1/0/0.56 | ABC<br>ABCD<br>ABCF | 20<br>18<br>18 | 3/0/0/0.02 | AC | 87 | 1/0/0/0.46 |
| Node II | A<br>AB | 54<br>26 | 1/0/0/0.11 | A | 93 | 0/0/0/0.8 | ABC<br>AB<br>ABCF | 38<br>20<br>20 | 0/0/0/0.15 | A<br>AC | 73<br>27 | 1/0/0/0.39 |
| Node III | C | 66 | 1/1/0/0.37 | A | 85 | 1/1/0/0.32 | AC<br>ABC | 43<br>19 | 0/1/0/0.13 | AC<br>A | 78<br>22 | 1/0/0/0.67 |
| Node IV | C | 96 | 0/0/0/0.88 | C | 97 | 0/0/0/0.83 | BC<br>BCD | 33<br>16 | 2/0/0/0.14 | C | 100 | 0/0/0/0.79 |
| Node V | C | 94 | 0/0/0/0.43 | C | 95 | 0/0/0/0.5 | CD<br>C | 59<br>29 | 1/0/0/0.57 | C | 100 | 0/0/0/0.85 |
| Node VI | C | 97 | 0/0/0/0.86 | C | 90 | 0/0/0/0.51 | BC | 74 | 1/0/0/0.5 | C<br>BC | 79<br>21 | 1/0/0/0.52 |

BBM$_1$ and BBM$_2$ are based on the nrDNA ITS and the combined plastid datasets, respectively. BAYAREALIKE and DEC are based on the nrDNA ITS and the combined plastid datasets, respectively. Relative probabilities (RP) of the ancestral areas are given as a fraction of the global likelihood of a split. Only ancestral areas with RP > 15% are shown. Most likely events (likelihood) and probability for each node are provided (dispersal/vicariance/extinction/probability, respectively). The areas are coded as follows: A, East Asia; B, Central Asia; C, West Asia; D, Mediterranean; E, North America; F, Circumboreal.

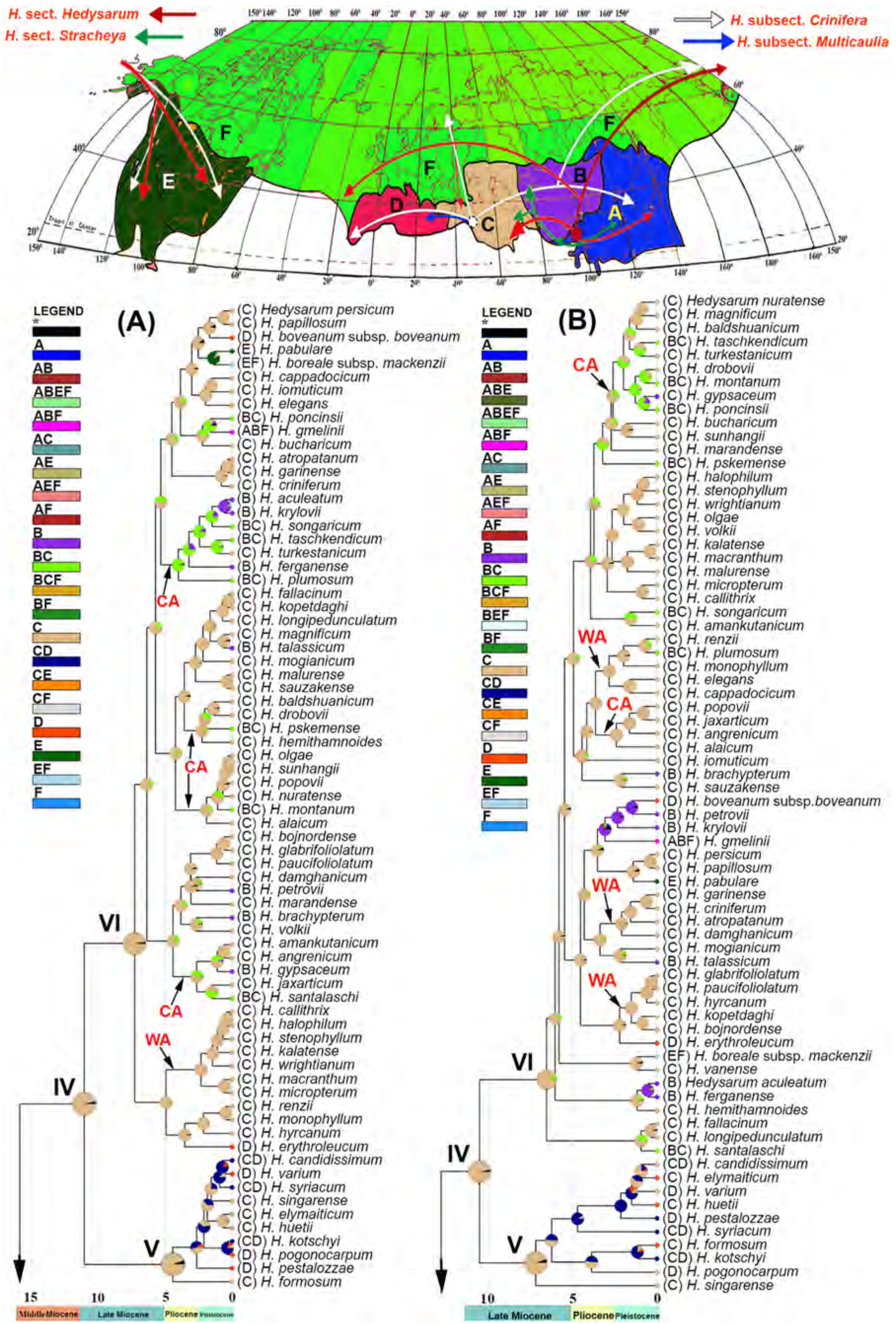

**Fig. 8.** Continues.

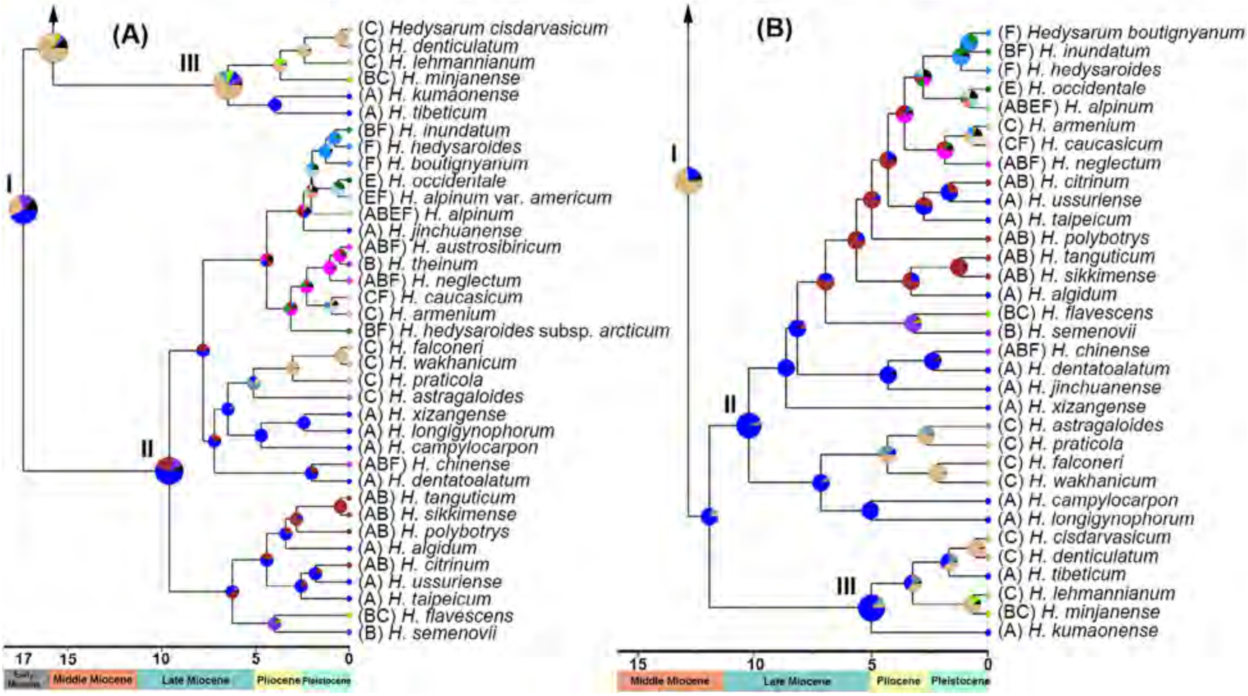

**Fig. 8.** Biogeographic reconstruction of the *Hedysarum* species in the World using Bayesian Binary MCMC (BBM) in RASP based on the nrDNA ITS (**A**) and the combined plastid dataset (**B**) phylogenies derived from BEAST analysis. The nodes are shown in Roman numerals. Pie charts indicate the most likely distribution areas of the most recent common ancestors. Black with an asterisk represents other ancestral ranges. The current distribution areas of accessions are provided at the tips of the branches. The areas are coded as follows: A, East Asia; B, Central Asia; C, West Asia; D, Mediterranean; E, North America; F, Circumboreal. The curved arrows show the possible migrating routes of (sub)sections in *Hedysarum*. Branches containing only Central Asian or West Asian species are represented by CA and CW, respectively.

When many speciation events occur in a short period of time, species may not differ sufficiently in their DNA sequences. As a result, species may differ little genetically despite morphological differences. The explosive-speciation hypothesis is similar to species radiation, meaning that many species emerge (almost) simultaneously from a common ancestor. Such a phenomenon has been observed in *Oxytropis* (Fabaceae), which shares similar unresolved clades (Kholina & al., 2016; Shahi Shavvon & al., 2017).

In our phylogenetic analysis based on nrDNA ITS, although many species within *Hedysarum* subsect. *Crinifera* remained unresolved, some subgroups of specific species were formed, such as *H. drobovii* Korotkova, *H. baldshuanicum* B.Fedtsch., and *H. olgae* B.Fedtsch. (Fig. 4; subgroup A). This subgroup is characterized by a well-developed stem, bracts longer than 5 mm, standard longer than keel or rarely equal in length (*H. drobovii*), and up to three jointed pods. Also, the subgroup that includes *H. aculeatum* Golosk. and *H. poncinsii* Franch. contains a total of seven species (Fig. 4; subgroup B), all of which occur in the Tian Shan Mountains, with the exception of *H. gmelinii* Ledeb., which occurs in eastern Kazakhstan near the Tian Shan. These species share common morphological characters, including more than 4 pairs of oblong or oblong-elliptical leaflets (except for *H. poncinsii*, which has 2–4 pairs of leaflets). Finally, the subgroup consisting of *H. amankutanicum* B.Fedtsch., *H. angrenicum* Korotkova, and *H. gypsaceum* Korotkova (Fig. 4; subgroup C) is characterized by having calyx teeth of equal length and teeth four times as long as the tube.

The subgroups in the phylogenetic tree derived from the plastid dataset (Fig. 5; subgroups A, B, C) were supported by a greater number of morphological characters than the subgroups in the phylogenetic tree based on nrDNA ITS (Fig. 4; subgroups A, B, C). For instance, a subgroup that includes *Hedysarum aculeatum*, *H. ferganense* Korsh., *H. hemithamnoides* Korotkova, *H. krylovii* Sumnev, and *H. songaricum* Bong. (Fig. 5; subgroup A) has features such as more than four pairs of leaflets, calyx teeth 2–3 times longer than the tube, and pods without long setae. Another subgroup (Fig. 5; subgroup B) consisting of *H. drobovii*, *H. montanum* (B.Fedtsch.) B.Fedtsch., *H. boveanum* subsp. *boveanum*, *H. gypsaceum*, and *H. poncinsii* can be distinguished by characteristics such as calyx teeth of equal length (except in *H. montanum*), wings longer than half the length of the keel, and pods with long setae (except in *H. gypsaceum*). Additionally, the following subgroup (Fig. 5; subgroup C) consists of *H. jaxarticum* Popov, *H. popovii* Korotkova, and *H. plumosum* Boiss. & Hausskn. ex Boiss., along with some northeastern Iranian species (*H. renzii* Rech.f., *H. monophyllum* Boriss.), characterized by a short plant and dense pubescence (epidermis not visible), a short or undeveloped stem, long bracts and bracteoles, calyx teeth several times longer than the tube, and tomentose pod joints without bristles.

Overall, the species of *Hedysarum* subsect. *Crinifera* distributed in the study area are generally consistent with the taxonomic treatment of Nafisi & al. (2019), with some additional features. For instance, our observations revealed that certain features such as calyx teeth shorter than tube, white or pink corolla, and wings longer than half the standard are also characteristic of this subsection, as in the species we studied. Finally, the present study has reaffirmed the high morphological polymorphism among the species in *H.* subsect. *Crinifera*, a characteristic that has been previously emphasized by Dehshiri & Goodarzi (2016), as well as Nafisi & al. (2019, 2021).

**Divergence time and biogeography. —** In the study conducted by Nafisi & al. (2019), the first comprehensive analysis of divergence time estimation was performed for the genus *Hedysarum*. Their results suggest that the genus *Hedysarum* and related genera originated approximately 20.63 Ma, as indicated by nrDNA ITS data, while plastid data suggest that the genus originated around 12.83 Ma. In our current study, which included more species, nrDNA ITS data resulted in slightly younger age estimates for *Hedysarum* and its closest genera, such as *Ebenus*, *Eversmannia*, and *Onobrychis* (ca. 19.64 Ma; Fig. 6). However, the crown ages of the main clades within *Hedysarum*, as determined by the nrDNA ITS-based divergence time estimation, were consistent with previous studies. Notably, our molecular dating analyses based on the plastid dataset revealed that the time of origin of the genus *Hedysarum* was earlier than previously reported (ca. 14.37 Ma; Fig. 7). Similarly, our analysis based on the plastid dataset indicated that also the diversification of the major clades within the genus *Hedysarum* began earlier than previously reported. Below, we discuss our results with respect to biogeographic phenomena.

Mountain formation and associated climatic changes have been identified as significant drivers of biological evolution in plants (Hoorn & al., 2013; Sun & al., 2017; Antonelli & al., 2018). Our study contributes to this understanding by suggesting that an early vicariance event, possibly related to mountain formation, climate, or a combination of both, played a role in the initial divergence of lineages within *Hedysarum*. *Hedysarum* species occur primarily in mountainous regions of the Northern Hemisphere and occupy diverse ecological niches. Our biogeographic reconstructions based on the nrDNA ITS (with the BBM analysis) and plastid datasets (with the BBM and DEC analyses) yielded different results, with East Asia (A) and/or West Asia (C) identified as the most likely ancestral region(s) for *Hedysarum* (Fig. 8A–B, both nodes I; Table 3). Considering that both West Asia and East Asia are rich in endemic *Hedysarum* species, it is likely that both regions served as the area of origin for this genus. However, subsequent analyses consistently revealed East Asia as the region of origin for *H.* sect. *Hedysarum* (Fig. 8A–B, both nodes II). The crown age of *H.* sect. *Hedysarum* was inferred from plastid and nrDNA ITS datasets to be 10.23 Ma and 9.63 Ma, respectively, corresponding to the time of climate change due to the uplift of the Qinghai-Tibet Plateau (QTP). It is known from previous studies that the uplift of the QTP has intensified the cooling and drying trend since the Late Miocene (Srivastava & al., 2018; Cao & al., In press). In addition, several taxa, such as *Gentiana* sect. *Cruciata* Gaudin (Zhang & al., 2009), *Hippophaë rhamnoides* L. (Jia & al., 2012), and *Lagotis* J.Gaertn. (Li & al., 2014), also diversified due to climate change after the uplift of the QTP. Furthermore, our analyses of BBM and DEC based on nrDNA ITS and plastid datasets, respectively, estimated that the dispersal events in *H.* sect. *Hedysarum* caused its initial diversification (Table 3). Thus, we suggest that initial dispersal and later vicariance events within the QTP may have driven additional species divergence and lineage diversification in this section. Currently, more than half of the species in this section occur in the QTP, as well as in the neighboring Tian-Shan, Pamir-Alay, and Western Himalayas Mountains. Furthermore, some species of *H.* sect. *Hedysarum* are distributed in the Circumboreal and North American regions. The Bering Land Bridge (BLB) and the North Atlantic Land Bridge (NALB) served as important migration routes for species from Eurasia to North America (Nie & al., 2005; Nie & al., 2006; Denk & al., 2010; Zhu & al., 2013). The NALB is assumed to have served as a corridor for plant migration only from the early Eocene (~50 Ma) to the middle to late Miocene (~10–8 Ma) (Tiffney, 1985; Parks & Wendel, 1990; Tiffney & Manchester, 2001; Denk & al., 2010, 2011). Our molecular clock analysis revealed that the split between the New World taxa (*H. alpinum* var. *americanum* Michx. ex Pursh., *H. occidentale* Greene) and the Old World species in *H.* sect. *Hedysarum* occurred at 2.01 Ma (0.53–3.72 Ma; 95% HPD; Fig. 6) or at 2.77 Ma (1.35–4.35 Ma; 95% HPD; Fig. 7). Therefore, we suggest that the species of *H.* sect. *Hedysarum* migrated from Eurasia to North America via the BLB during this period. Beringian migrations have been proposed for several flowering plant groups during this time, including *Rhodiola* L. at 5.3 Ma (95% HPD: 2.3–9.1 Ma; Zhang & al., 2014), *Lysichiton* Schott at 4.02 or 7.18 Ma (95% HPD: 1.6–4.02 Ma or 4.33–7.18 Ma; Nie & al., 2006); *Kelloggia* Torr. ex Benth. & Hook.f. at 5.42 Ma (95% HPD: 3.1–7.74; Nie & al., 2005); *Astilbe* Buch.-Ham. ex D.Don at 3.54 Ma (95% HPD: 1.29–6.18 Ma; Zhu & al., 2013). Additionally, the species of *H.* sect. *Hedysarum* prefer mesic habitats and are widely distributed in temperate mountain forests, alpine, and arctic regions (Choi & Ohashi, 2003; Duan & al., 2015). Therefore, we suggest that some species of *H.* sect. *Hedysarum* (*H. armenium*, *H. caucasicum*) may have migrated to Transcaucasia and northwestern Iran through the Circumboreal floristic region at 1.84 Ma (0.36–3.51 Ma; 95% HPD Fig. 7; Fig. 8B) or earlier at 2.3 Ma (0.54–4.38 Ma; 95% HPD; Fig. 6; Fig. 8A).

Our analysis of ancestral area reconstruction yielded different results for the most likely ancestral region of *Hedysarum* sect. *Stracheya*, as shown in Table 3. Here, we estimated that East Asia (specifically the southern QTP region) is the most probable area of origin for this section, which is supported by BBM analysis based on the plastid dataset. The divergence time between *H.* sect. *Stracheya* and *H.* sect. *Hedysarum*, according to the plastid dataset chronogram, was estimated to be 11.98 Ma (8.83–15 Ma; 95% HPD), which coincides with the second uplift of the QTP region (~13–15 Ma) (Harrison & al., 1992; Li & al., 1995; Shi & al., 1998a; Spicer & al., 2003). Later, the cooler climate and aridification of inland Asia caused by the extensive uplift of the QTP from the Pliocene to the Pleistocene (Shi & al., 1998b) may have led to environmental changes that drove the diversification of *H.* sect. *Stracheya*. Additionally, this result supports the long-standing hypothesis that the biogeographic significance of the QTP for alpine plant evolution, as species of *H.* sect. *Stracheya* may have migrated/dispersed from the QTP to the southern and eastern parts of Central Asia after the Pliocene, when global temperatures decreased. Currently, seven species are known from *H.* sect. *Stracheya* (in the current study not included is *H. wangii* P.L.Liu & Zhao Y.Chang, but see Liu & al., 2019), which have adapted to cold and arid habitats in the Pan-Himalayan region (*H. kumaonense*, *H. tibeticum*), and adjacent southern and eastern parts of Central Asia (*H. cisdarvasicum*, *H. denticulatum*, *H. lehmannianum*, *H. minjanense*) (Xu & Choi, 2010; Kovalevskaya, 1981). Only *H. wangii*, which is distributed along the northeastern edge of the QTP, is isolated. Species in this section occur mainly above 2200 m above sea level (Kovalevskaya, 1981; Xu & Choi, 2010; Nafisi & al., 2019; Liu & al., 2019).

Based on our analysis of both the nrDNA ITS and plastid datasets, we determined that West Asia (C) was the most probable region of origin for *Hedysarum* sect. *Multicaulia*, with an estimated divergence time of 15 Ma (Fig. 6; Fig. 8A, node V) or 12.81 Ma (Fig. 7; Fig. 8B, node V), respectively. This result is consistent with previous studies that identified West Asia as the center of origin for several other genera, including *Gagea* Salisb. (Peterson & al., 2019), *Astragalus* L. (Azani & al., 2019), *Cousinia* Cass. (Djamali & al., 2012), *Klasea* Cass. (Martins, 2006), *Acantholimon* Boiss. (Moharrek & al., 2019), and *Acanthophyllum* Hook. & Arn. (Mahmoudi Shamsabad & al., 2021). Moreover, our findings are consistent with the theory of Nafisi & al. (2019) that the Turkish-Iranian Plateau may have been the center of origin for *H.* sect. *Multicaulia*. This plateau, located in the Arabian-Eurasian collision zone and extending across the Zagros Mountains, encompasses eastern Turkey, the Caucasus, and the northwestern part of Iran (Allen & al., 2013; Sançar, 2021). Our analysis also suggests that the split of *H.* sect. *Multicaulia* into the *H.* subsect. *Crinifera* and subsect. *Multicaulia* occurred around 11.03 Ma (Fig. 6) or 10.53 Ma (Fig. 7), coinciding with the uplift of the Iranian and Zagros Plateaus between 15 and 12 Ma (Mouthereau & al., 2012). Thus, it is possible that climatic and topographic changes caused by the uplift of these plateaus played a crucial role in the divergence of *H.* sect. *Multicaulia* into two subsections (*H.* subsect. *Multicaulia*, subsect. *Crinifera*).

Most species of *Hedysarum* subsect. *Multicaulia* are distributed in the western part of Western Asia (northwestern Iran, northern part of Iraq and Syria, Transcaucasia, eastern part of Turkey) and in the Mediterranean region (Lebanon, Syria, western Turkey) (Akpinar & Yildiz, 1999; Hoşgören & Ertekin, 2018; Nafisi & al., 2021) in arid and semiarid habitats (Duan & al., 2015). It is likely that these species of *H.* subsect. *Multicaulia* originated in the Turkish-Iranian Plateau and migrated to the Mediterranean region (see map in Fig. 8), with the intense drought and falling sea levels caused by the Messinian salinity crisis (~5.96–5.33 Ma; Fauquette & al., 2006) possibly leading to the early diversification of the *H.* subsect. *Multicaulia* in the Mediterranean region. The diversification and distribution of *H.* subsect. *Multicaulia* may have continued during the appearance of modern Mediterranean floral elements about 3.2 Ma (Suc, 1984). Some species, such as *H. candidissimum* Freyn, *H. pestalozzae* Boiss., and *H. varium* Willd., which are endemic to the Mediterranean, may have diversified during this period.

Our biogeographic reconstruction and molecular dating analysis revealed that the ancestor of *Hedysarum* subsect. *Crinifera* likely originated in West Asia during the Late Miocene (see Figs. 6, 7 and Fig. 8A–B, nodes VII). *Hedysarum* subsect. *Crinifera* has a much higher species number than *H.* subsect. *Multicaulia*, most of which are distributed in Central Asia (Tian-Shan and Pamir-Alay Mountains) and Western Asia (around the Turkish-Iranian Plateau). Only a few species are known from the Mediterranean, Circumboreal, East Asia, and North America regions. Our phylogenetic analyses based on nrDNA ITS and plastid data (see Figs. 4, 5) showed that the species of *H.* subsect. *Crinifera* formed polytomies, suggesting a possible explosive speciation or radiation event within this subsection. However, some Central (including Tian-Shan and Pamir-Alay Mountains) and West Asian (including Turkish-Iranian Plateau) species formed independent lineages from the late Miocene to the Pleistocene (see Fig. 8A–B; clades CA and WA). As mentioned earlier, West Asia is known to be the center of origin for many plants, as well as the mountains of Central Asia are also the regions of origin for several other taxa, including *Calophaca* Fisch. (Zhang & al., 2015a), *Caragana* Lam. (Zhang & al., 2015b), *Incarvillea* Juss. (Rana & al., 2021), and *Klasea* sect. *Klasea* (Martins, 2006), among others. Additionally, our biogeographic reconstruction and molecular dating analyses showed the rapid radiation of the species within *H.* subsect. *Crinifera* occurred from the Late Miocene to Pleistocene periods in both Central and western Asia (Figs. 6–8). Deformation of the entire Tian-Shan Mountains during the Late Miocene (Jia & al., 2020) and major regional kinematic changes in the Pamir at the Miocene-Pliocene boundary (Thompson & al., 2015) may have been key factors in the rapid radiation of the species across Central Asia. Similarly, on the Turkish-Iranian Plateau, tectonic rearrangement of the Iranian Plateau and its uplift by 4–6 Ma likely contributed to the radiation of *H.* subsect. *Crinifera* species in this area (Djamali & al., 2012; Nafisi & al., 2019). Furthermore, global climate change may also have played a role in species diversification within *H.* subsect. *Crinifera*, as extensive uplift of the QTP during the Miocene led to drying and cooling of the climate in Asia (Miao & al., 2012; Favre & al., 2015). Most species in *H.* subsect. *Crinifera* are adapted to xeric habitats and have morphological traits that enable them to withstand drought, such as a well-developed root system, a thicker leaf blade than in other sections of the genus, and dense or sparse pubescence of the plant, as well as loments with long or short setae. Similar species radiations during and after the Miocene have been observed in other plant genera, such as *Caragana* (Zhang & Fritsch, 2010; Zhang & al., 2015b) and *Oxytropis* (Shahi Shavvon & al., 2017). Furthermore, some species of *H.* subsect. *Crinifera* have migrated to other parts of the Northern Hemisphere, including East Asia (A), the Mediterranean (D), North America (E), and the Circumboreal region (F). However, our current results are limited in determining the details of species migration in *H.* subsect. *Crinifera* due to unresolved polytomies.

## ■ CONCLUSIONS

Our study investigated the phylogenetic framework of about 110 *Hedysarum* species worldwide, focusing on the flora of the Tian-Shan and Pamir-Alay Mountains. We utilized the nrDNA ITS and the combined plastid datasets (*trnL-trnF*, *matK*, *psbA-trnH*) separately. Our nuclear data showed that *Hedysarum* is paraphyletic, whereas the combined plastid dataset showed the genus to be monophyletic. The majority of Central Asian species (28 spp.) studied belonged to *H.* subsect. *Crinifera* and were confirmed by both datasets.

Our biogeographic reconstruction and molecular dating analyses revealed that *Hedysarum* originated in East Asia and/or West Asia during the Early Miocene or Middle Miocene. *Hedysarum* sect. *Hedysarum* and *H.* sect. *Stracheya* originated in East Asia (area A), while *H.* sect. *Multicaulia* and its two subsections were from West Asia (area C). However, our results suggest a complex biogeographic history of *H.* subsect. *Crinifera* within *H.* sect. *Multicaulia*, which are important floristic elements of xeric habitats in the mountains of western and Central Asia. They originated at the Middle Miocene-Late Miocene boundary in West Asia, followed by later diversification in Central Asia, the Circumboreal, the Mediterranean, and North America, with repeated dispersals into neighboring areas. Our study provided further evidence for the hypothesis that an early vicariance event may have occurred through mountain building and climate change, or a combination of both. Additionally, we proposed that rapid radiation of species within *H.* subsect. *Crinifera* occurred in the Tian-Shan and Pamir-Alay Mountains and on the Turkish-Iranian Plateau, primarily due to geological and climatic changes in these areas.

Despite the distribution of *Hedysarum* subsect. *Crinifera* species in all regions studied, the details of possible intercontinental migration of them remain "open" in this paper due to the unresolved polytomy. Therefore, we suggest further research to explain the intercontinental migration of *H.* subsect. *Crinifera* through biogeographic analysis based on whole nuclear and plastid genomes.

## ■ AUTHOR CONTRIBUTIONS

IJ: Conceptualization, methodology, data analysis, identification, visualization, writing, original draft preparation, reviewing, editing, and discussing. DM: Methodology, data analysis, visualization. P-LL: Methodology, reviewing, and discussing. ZY: Methodology, collection. EN: Data provision. TD: Reviewing and editing, design of the research. KT: Supervision, investigation, identification, reviewing, editing, and discussing. HS: Supervision, conceptualization, and discussing. IJ, https://orcid.org/0000-0003-4972-8919; DM, https://orcid.org/0000-0002-3137-7308; P-LL, https://orcid.org/0000-0001-6566-7824; ZY, https://orcid.org/0000-0003-2278-542X; EN, https://orcid.org/0000-0003-2113-9362; TD, https://orcid.org/0000-0003-4163-3368; KT, https://orcid.org/0000-0003-2846-5777; HS, https://orcid.org/0000-0001-5127-4579

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**Appendix 1.** Voucher information for the sequences used in this study.

Taxon name, followed by [if available]: geographic origin, collector and collection number (herbarium) and GenBank accession numbers. Order: ITS, *trnL-F*, *matK*, *psbA-trnH*. Newly generated sequences are indicated by an asterisk (*); missing sequences are indicated by a dash (–); no information is indicated by a question mark (?).

***Alhagi maurorum*** Medik., Iran, *Kazempour 2008-1* (TMUH); AB854477, AB854520, AB854562, –; ***Alhagi sparsifolia*** Shap. ex Keller & Shap., **Voucher 1:** China, Xinjiang, *Duan TLF001* (TURP), **Voucher 2:** Unpublished data, KP338103$^1$, KP338225$^1$, AY177669$^2$, KP338351$^1$; ***Astragalus alopecias*** Pall., Iran, *Assadi & Maassoumi 50860* (TARI), AB741272, –, –, –; ***Astragalus tribuloides*** Delile, **Voucher 1:** Iran, *S. Zarre, Y. Salmaki & N. Azani 43865* (TUH), **Voucher 2:** Iran, *Maassoumi & Abouhamzeh 52003* (TARI), **Voucher 3:** Iran, *Rafeii & Zangooii 30536* (FUMH), KX955041$^1$, AB485929$^2$, KX955200$^3$, –; ***Caragana***

**Appendix 1.** Continued.

***chinghaiensis*** Y.X.Liou, China, *Chang & al. 2010160* (WUK), KP338110, KP338231, KP338488, KP338358; ***Caragana grandiflora*** DC., Iran, *Assadi & Shahsavari 65834* (TARI), AB051905, AB287412, AB854564, –; ***Ebenus cretica*** L., **Voucher 1:** Greece, *Gadringer & al. 18065* (MSB), **Voucher 2:** Greece, *Duan 2013001* (US), AB854482$^{1}$, AB854525$^{1}$, AB854570$^{1}$, KP338390$^{2}$; ***Eversmannia subspinosa*** (Fisch. ex DC.) B.Fedtsch., **Voucher 1:** Kazakhstan, *Krascheninnikov 5* (US), **Voucher 2:** Iran, *Freitag & Mozaffarian 28397* (TARI), KP338144$^{1}$, AB854527$^{2}$, AB854573$^{2}$, KP338394$^{1}$; ***Hedysarum aculeatum*** Golosk., Kazakhstan, *Goloskokov 4329* (F), KP338148, KP338271, KP338526, KP338399; ***Hedysarum alaicum*** B.Fedtsch., **Voucher 1:** Uzbekistan, *Drobov 1021a* (TASH), **Voucher 2:** Uzbekistan, *Drobov 1021b* (TASH), OM458868$^{1}$*/OM458869$^{2}$*, OM585613$^{1}$*, –, –; ***Hedysarum algidum*** L.Z.Shue ex P.C.Li, China, *Yang 2008010* (WUK), KP338149, KP338272, KP338527, KP338400; ***Hedysarum alpinum*** L., **Voucher 1:** Russia, *Karnaukhova ZUD_29082007* (?), **Voucher 2:** Russia, *K.P. Fedotova NSK0008797* (?), **Voucher 3:** U.S.A., Alaska, *J. Cole & R. Meyers AK025-002* (K), **Voucher 4:** U.S.A., Alaska, *Shetler & al. 4802* (US), MT081332$^{1}$, MG905925$^{2}$, JQ669599$^{3}$, KP338401$^{4}$; ***Hedysarum alpinum*** var. ***americanum*** Michx. ex Pursh., Canada, *Gillespie & al. 8934* (US), KP338150, –, –, –; ***Hedysarum amankutanicum*** B.Fedtsch., **Voucher 1:** Uzbekistan, *Mikhelson 1071a* (TASH), **Voucher 2:** Uzbekistan, *Mikhelson 1071b* (TASH), OM458870$^{1}$*/OM458871$^{2}$*, OM585616$^{1}$*, –, –; ***Hedysarum angrenicum*** Korotkova, **Voucher 1:** Uzbekistan, *Maskevich 203a* (TASH), **Voucher 2:** Uzbekistan, *Maskevich 203b* (TASH), OM458872$^{1}$*/OM458873$^{2}$*, OM585617$^{1}$*, –, –; ***Hedysarum armenium*** Boiss. ex Tchich., Iran, *Amini Rad & Torabi* (TARI), LC404198, LC404283, LC404353, –; ***Hedysarum astragaloides*** Benth. ex Baker, Pakistan, *W. Koelz 5024* (US), KP338153, KP338275, KP338530, KP338405; ***Hedysarum atropatanum*** Bunge ex Boiss., **Voucher 1:** Iran, *Kazempour-Osaloo & Nafisi 2015-01* (TMUH), **Voucher 2:** Azerbaijan, *Grossgeim & Elinskaya s.n.* (US 2394105) LC404199$^{1}$, LC404284$^{1}$, LC404354$^{1}$, KP338406$^{2}$; ***Hedysarum austrosibiricum*** B.Fedtsch., Kazakhstan, *Selyutina KZ_RIP_25071998* (?), MT117952, –, –, –; ***Hedysarum baldshuanicum*** B.Fedtsch., **Voucher 1:** Uzbekistan, *Juramurodov & Maxmudjanov 005a* (TASH), **Voucher 2:** Uzbekistan, *Juramurodov & Maxmudjanov 005b* (TASH), OM458843$^{1}$*/OM458844$^{2}$*, OM585601$^{1}$*, OM898910$^{1}$*, OM898878$^{1}$*; ***Hedysarum bojnordense*** Ranjbar & Joharchi, Iran, *Johartchi 34784* (FUMH), LC404203, LC404287, LC404357, –; ***Hedysarum boreale*** subsp. ***mackenzii*** (Richardson) C.L.Hitchc., **Voucher 1:** Canada, *F.J. Hermann 13479* (US), **Voucher 2:** ?, *L.J. Gillespie & al. 8935* (?), KP338176$^{1}$, KY366139$^{2}$, KP338552$^{1}$, KP338429$^{1}$; ***Hedysarum boutignyanum*** d'Alleiz., **Voucher 1:** France, *Lippert & Merxmuller 20357* (MSB), **Voucher 2:** France, *Villar & 13769* (US), LC404206$^{1}$, LC404289$^{1}$, LC404359$^{1}$, KP338407$^{2}$; ***Hedysarum boveanum*** Bunge ex Basiner subsp. ***boveanum***, Spain, *Jongkind 2300* (MO), KY366154, KY366135, –, KY365758; ***Hedysarum brachypterum*** Bunge, China, Inner Mongolia, *Chang & al. 2003236* (WUK), KP338156, KP338277, KP338533, KP338408; ***Hedysarum bucharicum*** B.Fedtsch., **Voucher 1:** Uzbekistan, *Juramurodov 067a* (TASH), **Voucher 2:** Uzbekistan, *Juramurodov 067b* (TASH), OM030218$^{1}$*/ON124019$^{2}$*, OM585621$^{1}$*, OM898916$^{1}$*, OM898898$^{1}$*; ***Hedysarum callithrix*** Bunge ex Boiss., Iran, *Rechinger 5167-b* (US), KP338157, KP338278, KP338534, KP338410; ***Hedysarum campylocarpon*** H.Ohashi, China, *Chang & al. 2011203* (WUK), KP338158, KP338279, KP338535, KP338411; ***Hedysarum candidissimum*** Freyn, Turkey, *Ganer 15114* (GAZI), LC404209, LC404292, LC404362, –; ***Hedysarum cappadocicum*** Boiss, Turkey, *Akçiçek 3540* (GAZI), LC404210, LC404293, LC404363, –; ***Hedysarum caucasicum*** M.Bieb., Caucase central, *Vosak 6573* (MSB), LC404211, LC404294, LC404364, –; ***Hedysarum chinense*** (B.Fedtsch.) Hand.-Mazz., China, *Jiang 1565* (WUK), KP338159, KP338280, KP338536, KP338412; ***Hedysarum cisdarvasicum*** Kamelin & Karimova, Tajikistan, *Kamelin s.n.* (TAD), MK639233, MK639275, –, MK639289; ***Hedysarum citrinum*** Baker f., China, *Chang & al. QZ-462* (WUK), KP338160, KP338281, KP338537, KP338413; ***Hedysarum criniferum*** Boiss., **Voucher 1:** Iran, *Gentry 14893* (US), **Voucher 2:** Iran, *Nemati & Mirabdali 3530* (RANK), **Voucher 3:** Iran, *Mozaffarian 57360* (TARI), KP338161$^{1}$, LC404295$^{2}$, LC404365$^{3}$, KP338414$^{1}$; ***Hedysarum damghanicum*** Rech.f, Iran, *Kazempour & al. s.n.* (TMUH), AB854493, AB854536, AB854584, –; ***Hedysarum dentatoalatum*** K.T.Fu, China, *Chang & al. 2013267* (WUK), KP338162, KP338283, KP338539, KP338415; ***Hedysarum denticulatum*** Regel, Tajikistan, *Striisova 1975* (TAD), MK639235, MK639277, –, MK639291; ***Hedysarum drobovii*** Korotkova, **Voucher 1:** Uzbekistan, *Juramurodov 1106a* (TASH), **Voucher 2:** Uzbekistan, *Juramurodov 1106b* (TASH), OM030219$^{1}$*/ON124020$^{2}$*, OM585622$^{1}$*, OM898918$^{1}$*, OM898892$^{1}$*; ***Hedysarum elegans*** Boiss. & A.Huet, Turkey, *Vural 8910* (GAZI), LC404218, LC404297, LC404366, –; ***Hedysarum elymaiticum*** Boiss. & Hausskn, Iran, *Ghahraman & al. 22526* (TUH), LC404219, LC404298, LC404367, –; ***Hedysarum erythroleucum*** Schott & Kotschy ex Boiss., Turkey, *Demirkus 6026* (GAZI), LC404220, LC404299, LC404368, –; ***Hedysarum falconeri*** Baker, Pakistan, *Polunin 6096* (F), KP338163, KP338284, KP338540, KP338416; ***Hedysarum fallacinum*** Rech.f. & Aellen, Iran, *Rechinger & Aellen 0005666* (W), LC404222, LC404301, LC404370, –; ***Hedysarum ferganense*** Korsh., China, *Chang & al. 2004300* (WUK), KP338164, KP338285, KP338541, KP338417; ***Hedysarum flavescens*** Regel & Schmalh., **Voucher 1:** Uzbekistan, *Tojibaev & Juramurodov 1008006* (TASH), **Voucher 2:** Uzbekistan, *Juramurodov 0107* (TASH), OM458845$^{1}$*/OM458846$^{2}$*, OM585602$^{1}$*, OM898899$^{1}$*, OM898879$^{1}$*; ***Hedysarum formosum*** Fisch. & C.A.Mey. ex Basiner, Azerbaijan, *Grossgeim & Elinskaya s.n.* (US 2394108), KP338165, KP338286, KP338542, KP338418; ***Hedysarum garinense*** Dehshiri & Maassoumi, Iran, *Nasiri & Zarrini 97460* (TARI), LC404226, LC404304, LC404373, –; ***Hedysarum glabrifoliolatum*** Ranjbar, Iran, *Faghihnia & Zangooii 29350* (FUMH), LC404227, LC404305, LC404374, –; ***Hedysarum gmelinii*** Ledeb., Russia, *Krascheninnikov 2394109* (US), KP338166, KP338287, KP338543, KP338419; ***Hedysarum gypsaceum*** Korotkova, **Voucher 1:** Uzbekistan, *Juramurodov & Gulomov 045-1* (TASH), **Voucher 2:** Uzbekistan, *Juramurodov & Gulomov 045-2* (TASH), **Voucher 3:** Uzbekistan, *Juramurodov & Gulomov 045-3* (TASH), **Voucher 4:** Uzbekistan, *Juramurodov & Gulomov 045-4* (TASH), OM030221$^{1}$*/ON124021$^{2}$*/ON124022$^{3}$*/ON124023$^{4}$*, ON147182$^{1}$*, OM898908$^{1}$*, –; ***Hedysarum halophilum*** Bornm. & Gauba, **Voucher 1:** Iran, *Maassoumi & Ansari 100608* (TUH), **Voucher 2:** Iran, *Rechinger 1007* (US), LC404230$^{1}$, LC404308$^{1}$, LC404376$^{1}$, KP338420$^{2}$; ***Hedysarum hedysaroides*** (L.) Schinz & Thell., **Voucher 1:** U.S.S.R., *Zaslavskaja 6184* (TARI), **Voucher 2:** Russia, *McDonald & Brummitt 23* (US), AB854495$^{1}$, AB558517$^{1}$, AB854586$^{1}$, KP338421$^{2}$; ***Hedysarum hedysaroides*** subsp. ***arcticum*** (B.Fedtsch.) P.W.Ball, **Voucher 1:** Russia, *Skvortsov & al. 10492* (US), **Voucher 2:** Russia, *Yankova NSK0009777* (?), KP338151$^{1}$, MG905928$^{2}$, KP338528$^{1}$, KP338403$^{1}$; ***Hedysarum hemithamnoides*** Korotkova, Tajikistan, *Popov & Vedensky 6247* (TARI), LC404232, LC404309, LC404377, –; ***Hedysarum huetii*** Boiss., Turkey, *Aytaç 86574* (GAZI), LC404233, LC404310, LC404378, –; ***Hedysarum hyrcanum*** Bornm. & Gauba, Iran, *Mozaffarian 103450* (TARI), LC404234, LC404311, LC404379, –; ***Hedysarum inundatum*** Turcz., China, *Huangtudui 01313* (WUK), KP338170, KP338290, KP338546, KP338423; ***Hedysarum iomuticum*** B.Fedtsch., **Voucher 1:** Uzbekistan, *Pulatov & Jabborov 003a* (TASH), **Voucher 2:** Uzbekistan, *Pulatov & Jabborov 003b* (TASH), OM458847$^{1}$*/OM458848$^{2}$*, OM585603$^{1}$*, OM898912$^{1}$*, OM898881$^{1}$*; ***Hedysarum jaxarticum*** Popov, Uzbekistan, *Maylun Z. & al. 782* (TASH), OM458875*, OM585618*, OM898919*, –; ***Hedysarum jinchuanense*** L.Z.Shue, China, *Zhang & Chang 94308* (WUK), KP338171, KP338291, KP338547, KP338424; ***Hedysarum kalatense*** Dehshiri, Iran, *Dehshiri & Hamedi 96149* (TARI), LC404238, LC404314, LC404382, –; ***Hedysarum kopetdaghi*** Boriss., **Voucher 1:** Iran, *Safaei & Ghanavati 6023* (MRCH), **Voucher 2:** Iran, *Rechinger 5143-b* (US), JX455133$^{1}$, KP338292$^{2}$, KP338548$^{2}$, KP338425$^{1}$; ***Hedysarum kotschyi*** Boiss., Turkey, *Aytaç 3957* (GAZI), LC404241, LC404316, LC404383, –; ***Hedysarum krylovii*** Sumnev., China, Xinjiang, *Xu 2011004* (WUK), KP338173, KP338293, KP338549, KP338426; ***Hedysarum kumaonense*** Benth. ex Baker, China, *Chang & al. 2013084* (WUK), KP338174, KP338294, KP338550, KP338427; ***Hedysarum lehmannianum*** Bunge, **Voucher 1:** Tajikistan, *Budkina 68446* (TAD), **Voucher 2:** Uzbekistan, *Turginov 0490-1* (TASH), **Voucher 3:** Uzbekistan, *Turginov 0490-2* (TASH), MK639239$^{1}$/OM458851$^{2}$*/OM458852$^{3}$*, OM585605$^{2}$*, OM898902$^{2}$*, OM898883$^{2}$*; ***Hedysarum longigynophorum*** C.C.Ni, China, *Chang & al. QZ-620* (WUK), KP338175, KP338295, KP338551, KP338428; ***Hedysarum longipedunculatum*** Ranjbar & Karamian, **Voucher 1:** Iran, *Rafeii & Zangooii 31667* (FUMH), **Voucher 2:** Iran, *Rafeii & Zangooii 33858* (FUMH), LC404242$^{1}$, LC404318$^{2}$, LC404385$^{2}$, –; ***Hedysarum macranthum*** (Freyn & Sint.) B.Fedtsch., **Voucher 1:** Turkmenistan, *Nadezhina s.n.* (US), **Voucher 2:** Turkmenistan, *Vincob 0006790* (W), **Voucher 3:** Iran, *Ajeni & Zangooi 34705* (FUMH), KP338177$^{1}$, LC404320$^{2}$, LC404386$^{3}$, KP338430$^{1}$; ***Hedysarum magnificum*** Kudr., **Voucher 1:** Uzbekistan, *Juramurodov 066a* (TASH), **Voucher 2:** Uzbekistan, *Juramurodov 066b* (TASH), OM458849$^{1}$*/OM458850$^{2}$*, OM585604$^{1}$*, OM898901$^{1}$*, OM898882$^{1}$*; ***Hedysarum malurense*** Rech.f., Afghanistan, *Koelz 0012024* (W), LC404246, LC404321, LC404387, –; ***Hedysarum marandense*** Mozaff., Akrami & Maassoumi, Iran, *Mozaffarian 93757* (TARI), AB854497, AB854539, AB854588, –; ***Hedysarum micropterum*** Bunge ex Boiss., Iran, *Johartchi 34785* (FUMH), LC404248, LC404323, LC404389, –; ***Hedysarum minjanense*** Rech.f., **Voucher 1:** Afghanistan, *Koelz 0012036* (W), **Voucher 2:** Uzbekistan, *Pryakhin 1961* (TASH), **Voucher 3:** Tajikistan, *Dickore 18281* (MSB), LC404250$^{1}$/OM458874$^{2}$*, LC404324$^{3}$, LC404391$^{3}$, OM898896$^{2}$*; ***Hedysarum mogianicum*** B.Fedtsch., **Voucher 1:** Uzbekistan, *Juramurodov 052a* (TASH), **Voucher 2:** Uzbekistan,

**Appendix 1.** Continued.

*Juramurodov 052b* (TASH), OM458853$^{1}$*/OM458854$^{2}$*, OM585606$^{1}$*, OM898913$^{1}$*, OM898884$^{1}$*; ***Hedysarum monophyllum*** Boriss., Iran, *Johartchi & Zangooii 20672* (FUMH), AB854498, AB854540, AB854589, –; ***Hedysarum montanum*** (B.Fedtsch.) B.Fedtsch., **Voucher 1:** Uzbekistan, *Juramurodov 051a* (TASH), **Voucher 2:** Uzbekistan, *Juramurodov 051b* (TASH), OM458855$^{1}$*/OM458856$^{2}$*, OM585607$^{1}$*, OM898903$^{1}$*, OM898885$^{1}$*; ***Hedysarum neglectum*** Ledeb., **Voucher 1:** China, *Xu 1533* (WUK), **Voucher 2:** Russia, *Thomas Elias & al. 7222* (US), KY366160$^{1}$, KY366141$^{1}$, KP338554$^{2}$, KY365764$^{1}$; ***Hedysarum nuratense*** Popov, **Voucher 1:** Uzbekistan, *Juramurodov 051* (TASH), **Voucher 2:** Uzbekistan, *Beshko TASH058917* (TASH), MZ647687$^{1}$/MZ656079$^{2}$, MZ647715$^{1}$, MZ647713$^{1}$, ON147184$^{1}$*; ***Hedysarum occidentale*** Greene, U.S.A., *Moseley 2719* (CS), KP338179, –, KP338555, KP338431; ***Hedysarum olgae*** B.Fedtsch., **Voucher 1:** Uzbekistan, *Juramurodov 048a* (TASH), **Voucher 2:** Uzbekistan, *Juramurodov 048b* (TASH), OM030220$^{1}$*/ON124024$^{2}$*, OM585623$^{1}$*, OM898909$^{1}$*, OM898886$^{1}$*; ***Hedysarum pabulare*** A.Nelson, U.S.A., *Hugh Cutlter 2425* (US), KP338180, KP338298, KP338556, KP338432; ***Hedysarum papillosum*** Boiss. Iran, *Kazempour-Osaloo & al. 2008-01* (TMUH), LC404251, LC404325, LC404392, –; ***Hedysarum paucifoliolatum*** Ranjbar & Olanj, Iran, *Faghihnia & Zangooi 31470* (FUMH), LC404253, LC404327, LC404394, –; ***Hedysarum persicum*** Bidarlord, Ghahrem. & Mozaff., Iran, *Bidarlord 15882* (TARI), LC404254, LC404328, LC404395, –; ***Hedysarum pestalozzae*** Boiss., Turkey, *Vural 7517* (GAZI), LC404255, LC404329, LC404396, –; ***Hedysarum petrovii*** Yakovlev, China, *Chang & al. 2012102* (WUK), KP338181, KP338299, KP338557, KP338433; ***Hedysarum plumosum*** Boiss. & Hausskn. ex Boiss., **Voucher 1:** Uzbekistan, *Juramurodov 004a* (TASH), **Voucher 2:** Uzbekistan, *Juramurodov 004b* (TASH), **Voucher 3:** Uzbekistan, *Juramurodov 004c* (TASH), **Voucher 4:** Uzbekistan, *Juramurodov 004c* (TASH), OM458857$^{1}$*/OM458858$^{2}$*/OM458859$^{3}$*/OM458860$^{4}$*, OM585608$^{1}$*, OM898905$^{1}$*, OM898888$^{1}$*; ***Hedysarum pogonocarpum*** Boiss., Turkey, *Şahin 3389* (GAZI), LC404259, LC404332, LC404398, –; ***Hedysarum polybotrys*** Hand.-Mazz., China, *Chang & al. QZ-042* (WUK), KP338182, KP338300, KP338558, KP338434; ***Hedysarum poncinsii*** Franch., **Voucher 1:** Kyrgyzstan, *Sultanova 5927* (MSB), **Voucher 2:** China, *Xizhixinjiangdui 1905* (WUK), LC404260$^{1}$, KY366142$^{2}$, LC404399$^{1}$, KY365765$^{2}$; ***Hedysarum popovii*** Korotkova, **Voucher 1:** Uzbekistan, *Korovin 661a* (TASH), **Voucher 2:** Uzbekistan, *Korovin 661b* (TASH), ON124025$^{1}$*/OR041882$^{2}$*, OM585624$^{1}$*, –, OM898890$^{1}$*; ***Hedysarum praticola*** Rech.f., Afghanistan, *Kersten 0012117* (W), LC404261, LC404334, LC404400, –; ***Hedysarum pskemense*** Popov ex B.Fedtsch., **Voucher 1:** Uzbekistan, *Tojibaev & Juramurodov 1008059* (TASH), **Voucher 2:** Uzbekistan, *Tojibaev & Juramurodov 1008060* (TASH), OM458861$^{1}$*/OM458862$^{2}$*, OM585609$^{1}$*, OM898906$^{1}$*, OM898891$^{1}$*; ***Hedysarum renzii*** Rech.f., Iran, *Rafeii 30763* (FUMH), LC404262, LC404335, LC404401, –; ***Hedysarum santalaschi*** B.Fedtsch., **Voucher 1:** Uzbekistan, *Nabiev 1095a* (TASH), **Voucher 2:** Uzbekistan, *Nabiev 1095b* (TASH), OM458876$^{1}$*/OM458877$^{2}$*, OM585620$^{1}$*, –, OM898897$^{1}$*; ***Hedysarum sauzakense*** Rech.f. & Köie, Afghanistan, *Podlech 21927* (MSB), LC404263, LC404336, LC404402, –; ***Hedysarum semenovii*** Regel & Herder, Kazakhstan, *Roldugin 4823* (US), KP338183, KP338301, KP338559, KP338435; ***Hedysarum sikkimense*** Benth. ex Baker, China, *Chang & al. QZ-786* (WUK), KP338184, KP338302, KP338560, KP338436; ***Hedysarum singarense*** Boiss. & Hausskn. ex Boiss., **Voucher 1:** Iraq, *Rechinger 0007043* (W), **Voucher 2:** Iraq, *Rawi 8533* (US), LC404266$^{1}$, LC404339$^{1}$, LC404405$^{1}$, KP338437$^{2}$; ***Hedysarum songaricum*** Bong., China, *Xu 96-175* (WUK), KP338186, KP338304, KP338562, KP338438; ***Hedysarum stenophyllum*** Rech.f. & Esfand., Iran, *Esfandiari 0006038* (W), LC404267, LC404340, LC404406, –; ***Hedysarum sunhangii*** Juram. & Tojibaev, **Voucher 1:** Uzbekistan, *Beshko, Makhmudov TASH058995* (TASH), **Voucher 2:** Uzbekistan, *Beshko, Makhmudov TASH058996* (TASH), **Voucher 3:** Uzbekistan, *Beshko, Makhmudov TASH058997* (TASH), **Voucher 4:** Uzbekistan, *Beshko, Makhmudov TASH058998* (TASH), MZ647686$^{1}$/MZ646045$^{2}$/MZ646046$^{3}$/MZ646047$^{4}$, MZ647714$^{1}$, MZ647712$^{1}$, ON147183$^{1}$*; ***Hedysarum syriacum*** Boiss., **Voucher 1:** Turkey, *Cankiri & Bornmüller 14030* (US), **Voucher 2:** Turkey, *Turgut 6304* (GAZI), KY366162$^{1}$, KY366143$^{1}$, LC404408$^{2}$, KY365766$^{1}$; ***Hedysarum taipeicum*** (Hand.-Mazz.) K.T.Fu, China, *Chang & al. 2012122* (WUK), KP338187, KP338305, KP338563, KP338439; ***Hedysarum talassicum*** Nikitina & Sultanova, Uzbekistan, *Tojibaev & Juramurodov 1108149* (TASH), OM458867*, OM585612*, OM898907*, OM898895*; ***Hedysarum tanguticum*** B.Fedtsch., China, *Chang & al. 2010230* (WUK), KP338188, KP338306, KP338564, KP338440; ***Hedysarum taschkendicum*** Popov, **Voucher 1:** Uzbekistan, *Juramurodov 088* (TASH), **Voucher 2:** Uzbekistan, *Juramurodov 089* (TASH), OM458863$^{1}$*/OM585610$^{2}$*, OM898914$^{1}$*, OM898893$^{1}$*, OM458864$^{1}$*; ***Hedysarum theinum*** Krasnob., **Voucher 1:** Kazakhstan, ?, *ipbb_33.7.1.1* (?), **Voucher 2:** Russia, *Nuzhdina NSK0009301* (?), OM010360$^{1}$, MG905942$^{2}$, –, MN115789$^{2}$; ***Hedysarum tibeticum*** (Benth.) B.H.Choi & H.Ohashi, China, *Chang & al. 2011111* (WUK), KP338189, KP338307, KP338565, KP338441; ***Hedysarum turkestanicum*** Regel & Schmalh., Uzbekistan, *Juramurodov & Gulomov 011* (TASH), OM458866*, OM585611*, OM898915*, OM898894*; ***Hedysarum ussuriense*** Schischk. & Kom., Japan, *Okamoto 1090* (US), KP338190, KP338308, KP338566, KP338442; ***Hedysarum vanense*** Hedge & Hub.-Mor., Iran, *Alizade & al. 720* (TMUH), –, AB854542, AB854590, –; ***Hedysarum varium*** Willd., Iraq, *Chapman 9351* (US), KP338191, KP338309, KP338567, KP338443; ***Hedysarum volkii*** Rech.f., Afghanistan, *Anders 4434* (MSB), LC404275, LC404348, LC404412, –; ***Hedysarum wakhanicum*** Podlech & O.Anders, Afghanistan, *Anders 0023746* (W), LC404277, LC404350, LC404413, –; ***Hedysarum wrightianum*** Aitch. & Baker, **Voucher 1:** Iran, *Kazempour-Osaloo & al. 2014-02* (TMUH), **Voucher 2:** Iran, *Faghihnia & Zangooii 31461* (FUMH), LC404282$^{1}$, LC404352$^{2}$, LC404415$^{2}$, –; ***Hedysarum xizangensis*** C.C.Ni, China, *Chen & al. 13-811* (WUK), KP338192, KP338310, KP338568, KP338444; ***Onobrychis arenaria*** DC., **Voucher 1:** China, *Xu 2011-010* (WUK), **Voucher 2:** No voucher information, **Voucher 3:** Kazakhstan, ? (?), KY366167$^{1}$, KY366146$^{1}$, MF158685$^{2}$, HM542804$^{3}$; ***Onobrychis chorassanica*** Bunge ex Boiss., Kazakhstan, *Gamajunova 4422* (US), KP338196, KP338315, KP338573, KP338448; ***Onobrychis lanata*** Boiss., Iraq, *Rechinger 9311* (US), KP338199, KP338319, KP338576, KP338451; ***Onobrychis pulchella*** Schrenk, **Voucher 1:** Iran, *Ghanavati 6141* (NPGBI), **Voucher 2:** Iran, *Ghahraman 27318* (TUH), **Voucher 3:** Turkmenistan, ? (?), JX290036$^{1}$, AB558519$^{2}$, AB854599$^{2}$, HM542805$^{3}$; ***Onobrychis viciifolia*** Scop., China, *Chang & al. QZ-266* (WUK), AB854512, AB854555, AB854604, KP338459; ***Oxytropis glabra*** DC., **Voucher 1:** Iran, *Maassoumi & al. 89919* (TARI), **Voucher 2:** Russia, *Tupitsina s.n.* (KRAS), LC213353$^{1}$, LC213492$^{1}$, –, LN898505$^{2}$; ***Oxytropis pallasii*** Pers., **Voucher 1:** Turkey, ?, (?) **Voucher 2:** Russia, *Byalt s.n.* (LE), KM053395$^{1}$, LN898545$^{2}$, KM387610$^{1}$, LN898583$^{2}$; ***Oxytropis savellanica*** Bunge ex Boiss., **Voucher 1:** Iran, *Shahi Shavvon & Kazempoor-Osaloo 2014s30/2* (TMUH), **Voucher 2:** Turkey, ? (?), LC213434$^{1}$, LC213544$^{1}$, KM387612$^{2}$, –; ***Sulla aculeolata*** (Munby ex Boiss.) Amirahm. & Kaz.Osaloo, **Voucher 1:** Morocco, *Chennaoui & Trifi-Farah THAC–0200* (?), **Voucher 2:** Morocco, *Podlech 122215* (MSB), **Voucher 3:** Morocco, *Forther 6930*, AY772222$^{1}$, AB558512$^{2}$, –, KY365772$^{3}$; ***Sulla coronaria*** (L.) Medik. ex B.H.Choi & H.Ohashi, Italy, *Newman & al. 77/2* (K), KP338215, KP338339, KP338594, KP338471; ***Sulla spinosissima*** (L.) B.H.Choi & H.Ohashi, Spain, *Müller & Lippert 23553* (MSB), AB854515, –, –, –.