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Authors: Younesi, Simin, Mehregan, Iraj, Assadi, Mostafa, Nejadsattari, Taher, and Lidén, Magnus

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Willdenowia
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SIMIN YOUNESI[1], IRAJ MEHREGAN[1*], MOSTAFA ASSADI[2], TAHER NEJADSATTARI[1] & MAGNUS LIDÉN[3]

Dionysia robusta (*Primulaceae*), a new species from W Iran

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Abstract: A new species from the W part of the Iranian Zagros Mountains in Ilam province, *Dionysia robusta* (*Primulaceae*), is described, illustrated and compared with similar and related species. It differs from these relatives in leaf shape, length and density of glandular hairs, and shape of the calyx. The DNA sequence of the nuclear ribosomal ITS region of *D. robusta* is most similar to that of *D. gaubae*.

Key words: Irano-Turanian region, Iran, Zagros Mountains, new species, *Primulaceae*, *Dionysia*, ITS sequences

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Introduction

Dionysia Fenzl, one of the larger genera of *Primulaceae*, consists of more than 50 species. They are suffrutescent herbs that form loose tufts or dense cushions in crevices of rocks and cliffs. During spring, these plants are covered by yellow, purple or pink flowers (Lidén 2007; Grey-Wilson 1989; Melchior 1943; Wendelbo 1961, 1964, 1965, 1971).

The majority of *Dionysia* species (more than 20) are found in the Zagros Mountains of W Iran (Lidén 2007). The number of species of *Dionysia* in other regions of SW Asia is lower: there are eight species in other areas of Iran, 11 species in Afghanistan, three in Turkey, two in Iraq, two in Turkmenistan, two in Tajikistan, one in Oman and one in Pakistan. The majority of species of *Dionysia* occurs in the area covered by *Flora iranica* (Wendelbo 1965). Thanks to the extensive collection of material from different mountainous areas of this region in recent years, new species have been described (Jamzad 1996, 1999; Lidén 2007; Borjian & al. 2014).

Ilam province in W Iran is a poorly studied area, from which several new species have recently been described (Mozaffarian 2008). Two nearby localities of an unknown *Dionysia* species were found in the Dinar-Kouh mountains in the Abdanan region of this province by the first author. Here, we report the finding of a new species and clarify its relationships to other species of *Dionysia*.

Material and methods

Morphology

We compared our unknown species with other *Dionysia* species reported from W Iran and N Iraq, namely *D. bornmuelleri* (Pax) Clay, *D. gaubae* Bornm., *D. odora* Fenzl and *D. tacamahaca* Lidén (Wendelbo 1965; Jam-

1 Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran; *e-mail: iraj@daad-alumni.de (author for correspondence).
2 Research Institute of Forests and Rangelands, Agricultural Research Education and Extension Organization (AREEO), PO Box 13185-116, Tehran, Iran.
3 Uppsala University, EBC, Department of Systematic Biology, Norbyvägen 18D, SE-752 36, Uppsala, Sweden.

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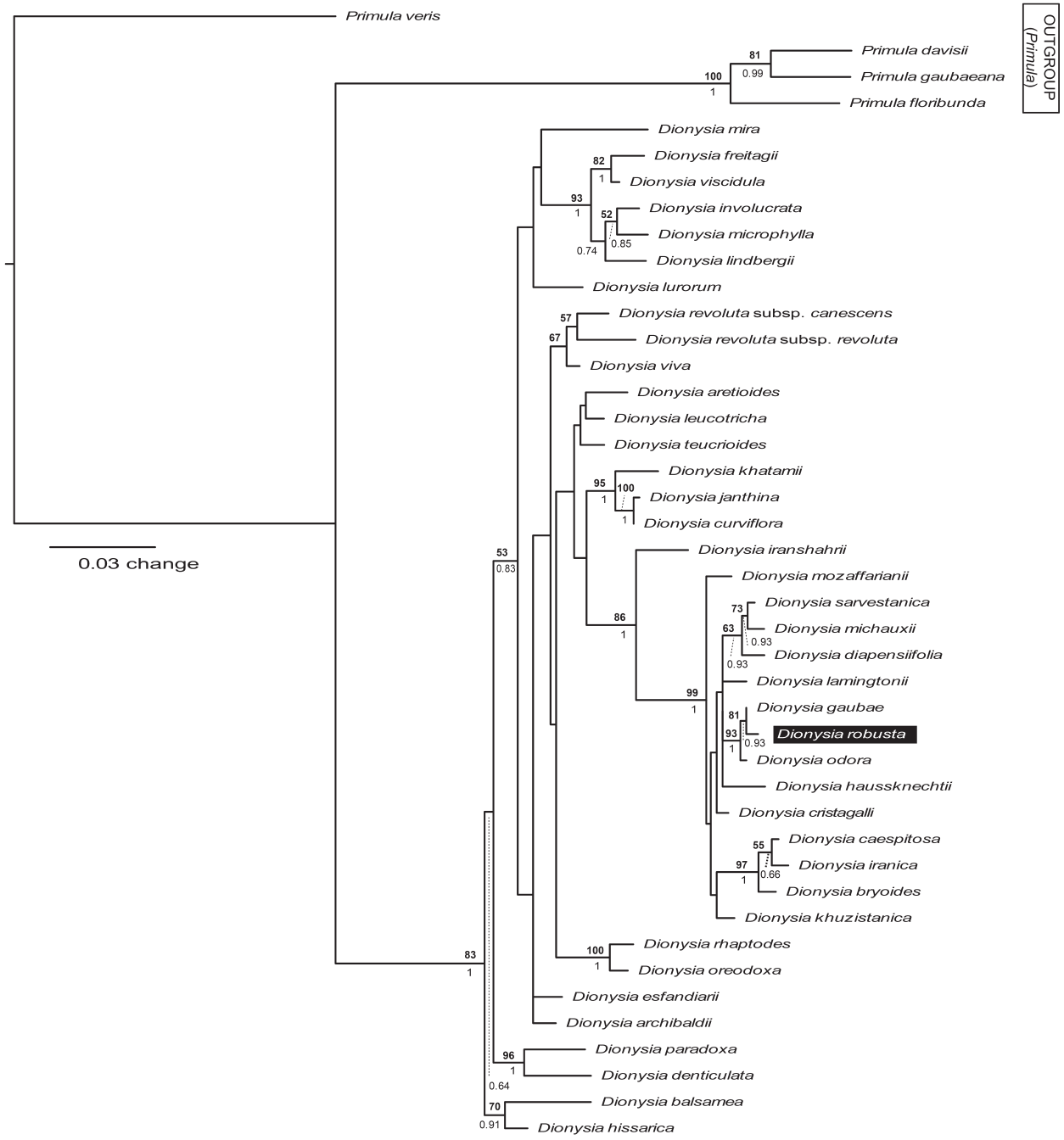

Fig. 1. Phylogram obtained from a maximum likelihood (ML) analysis of ITS DNA sequences. The numbers above the branches are ML bootstrap values, the numbers below the branches are posterior probabilities (PP). Bootstrap values equal to or less than 50 % and PP equal to or less than 0.5 are not shown.

zad 1999; Lidén 2007), as well as all Iranian species with similar morphology. All type specimens of the above-mentioned species were studied. Vegetative and floral parts were observed and measured under a stereomicroscope (NTB-3A, Wuzhou New Found Instrument Co., Guangxi, China). Voucher specimens were prepared and deposited in the herbarium of Science and Research Branch, Islamic Azad University (IAUH), Tehran, with duplicates in B and UPS (herbarium codes according to Thiers [continuously updated]).

Molecular markers

Total DNA was extracted from either silica-gel dried leaves collected from the wild or leaf fragments of herbarium material using the NucleoSpin Plant II kit (Machery-Nagel, Düren, Germany) following the manufacturer's instructions. The complete internal transcribed spacer (ITS) region of the nuclear ribosomal cistron was amplified by using the forward primer AB101 (5′-ACG AAT TCA TGG TCC GGT GAA GTG TTC G-3′) and the reverse primer AB102 (5′-TAG AAT TCC CCG

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GTT CGC TCG CCG TTA C - 3′; Douzery & al. 1999) in a PCR reaction under the following conditions: a pretreatment of 5 min at 95 °C, 35 cycles of 30 sec at 95 °C, 30 sec at 50 °C, and 90 sec at 72 °C, and a final extension of 7 min at 72 °C. Amplicons were sequenced on an ABI 3730 DNA Analyzer (Hitachi-Applied Biosystems, Waltham, Massachusetts, U.S.A.). Sequences were visually checked and edited with the software tool Sequencher 4 (Gene Codes Corporation, Ann Arbor, Michigan, U.S.A.).

We added the ITS DNA sequence of the new species presented here to those of the species already present in the phylogeny of *Dionysia* by Trift & al. (2004). This new dataset was aligned using the software tool MacClade 4.08 (Maddison & Maddison 2000). A maximum parsimony (MP) analysis of the ITS dataset was performed via PAUP* (Swofford 2002) under the following parameters: heuristic search, number of replicates: 100, swapping method: TBR. The shortest trees recovered under MP were combined to form a strict consensus tree. Support for each branch was calculated via bootstrapping (Felsenstein 1985) using 100 replicates and the same setting as described above. Phylogenetic inferences were also conducted under the maximum likelihood (ML) criterion using the software tool RAxML 2.0 (Stamatakis 2014) on CIPRES Science Gateway (Miller & al. 2010). In addition, a Bayesian analysis was performed using the software tool MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003). Prior to phylogenetic analyses, the best-fitting model of DNA nucleotide substitution was selected using the software tool Modeltest 3.7 (Posada & Crandall 1998, 2001). Following this analysis, the following sequence model priors were set for the analysis in MrBayes: TrN+I+G (Nst = 6, rates=gamma, A–C substitution rate = 1.0000, A–G = 4.5110, A–T = 1.0000, C–G = 1.0000, C–T = 9.3590, G–T = 1.0000, proportion of invariable sites = 0.4147, shape parameter of the gamma distribution = 0.6374). A Markov Chain Monte Carlo (MCMC) sampling was conducted for 5 000 000 generations in four simultaneous chains, with trees being sampled every 100 generations for a total of 50 000 trees in the initial sample. The first 25 percent of the sampled trees were discarded, and the posterior probability of the resulting phylogeny and its branches was determined from the remaining trees.

Results and Discussion

Material of the new putative species showed some morphological dissimilarity to those species already described from W Iran, as summarized in Table 1. Morphologically, our material also showed resemblance to *Dionysia iranica* Jamzad and *D. sarvestanica* Jamzad & Grey-Wilson, which were described from localities further south. Genetically, the new material can be distinguished from these and other species, as shown in Table 2.

We found that the ITS DNA sequence of the new species is most similar to that of *Dionysia gaubae* and differs from it by only four bases (Table 2 and Fig. 1). MP analysis identified a set of 142 parsimony-informative characters and resulted in 449 most parsimonious trees with a length of 491 steps, a consistency index (CI) of 0.607 and a retention index (RI) of 0.749. The strict consensus tree displayed the following characteristics: length = 502 steps, CI = 0.594, and RI = 0.735 (tree not shown). In all three analyses of the ITS dataset (Fig. 1), our new putative species showed to be closely related to *D. gaubae*. It did not display close relationships to the morphologically similar species *D. iranica* and *D. sarvestanica*. Hence, we conclude that that our newly gathered material indeed represents a new species.

Dionysia robusta Younesi, **sp. nov.** – Fig. 2 & 3.
Holotype: Iran, Ilam Province, SW of Abdanan, Dinar-Kouh protected region, Pizeleh, 32°56'09.6"N, 47°18'35.4"E, 1720 m, 11 May 2015, *Younesi 14494* (IAUH; isotypes: B, TARI).

Description — Cushions very dense, bluish grey-green, up to 70 cm in diam. Branches tightly packed, very densely leafy, with cream-coloured marcescent leaves long persistent. Basal part of leaf suberect, distal part widely spreading, subspatulate-obovate, 4–7 mm long, 2–3 mm wide, covered with subsessile glands to 0.1 mm long, more sparsely so abaxially, margin entire (rarely slightly lobed at apex), apex obtuse to subacute. Inflorescence with a single sessile flower. Bract 1, oblanceolate, 4–5 mm long, c. 1 mm wide, base very shortly ciliate, margin entire or apically with a few minute denticles. Calyx 4–5 mm long, divided to ⅔–¾; lobes broadly lanceolate, often overlapping in basal ⅓, outside shortly glandular hairy, especially at base, on veins and on margin, inside sparsely glandular, base keeled, margin entire, apex subacute. Corolla yellow, glandular hairy, rather densely so toward base, less so apically, hairs 0.1–0.2 mm long; tube 20–24 mm long; limb c. 10 mm wide; lobes not or slightly overlapping, apex rounded or very slightly emarginate. Stamens in long-styled flowers inserted c. ⅔ from base; anthers c. 2 mm long. Ovary with 3 or 4 ovules; style in long-styled flowers conspicuously exserted; style in short-styled flowers c. ⅔ as long as corolla tube. Capsule broadly ovoid, c. 3 mm long, 3- or 4-seeded; valves thick, not twisted. Seeds 1.4–1.6 × 0.6–0.7 mm.

Phenology — Flowering in May, fruiting in June.

Distribution and ecology — *Dionysia robusta* is known from two localities in Ilam Province, Iran (Fig. 4). Each locality is a strip on the SE face of two parallel, deep gorges between 32°55'N, 47°18'E and 32°57'N, 47°22'E in an altitudinal range of 1550–1800 m. The type material was collected from the more northern strip. The spe-

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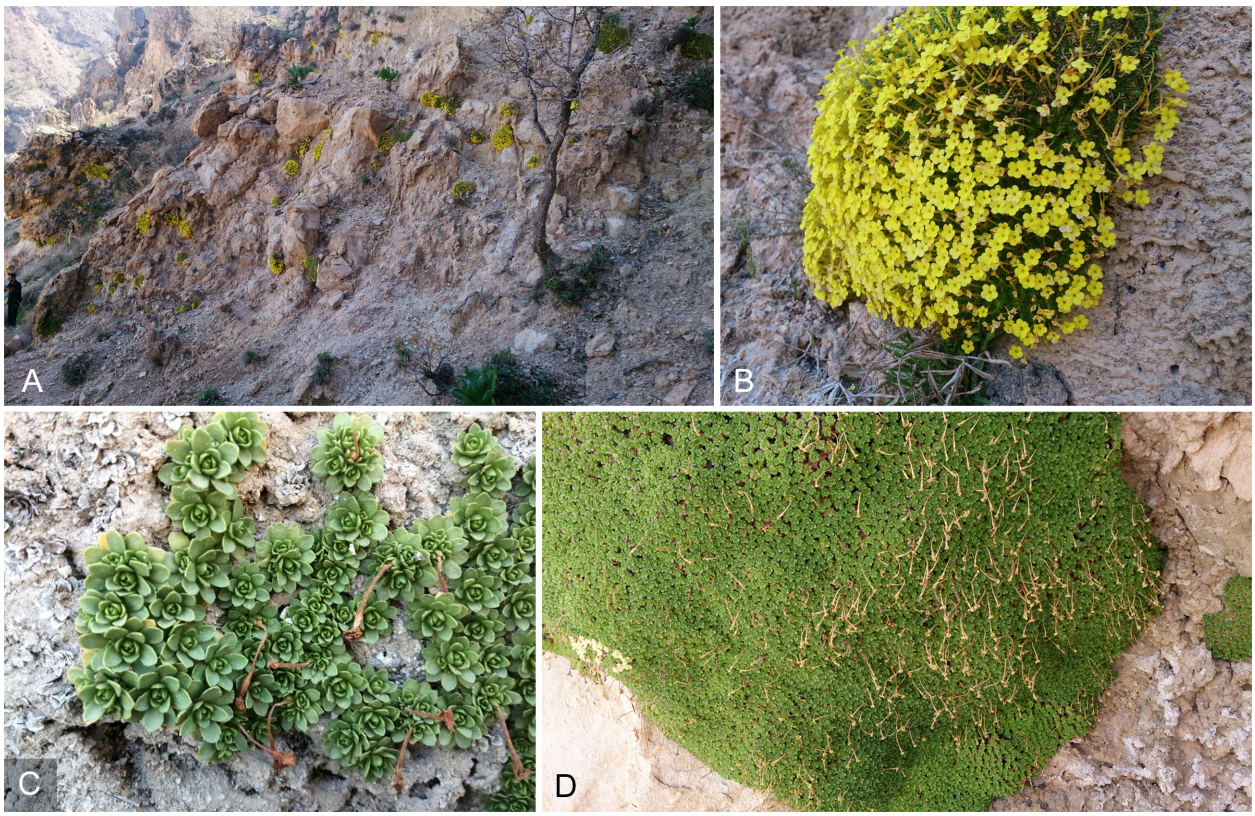

Fig. 2. *Dionysia robusta* in its natural habitat – A, B: at anthesis; C, D: after anthesis. – All photographs taken at the type locality by S. Younesi; A, B: 11 May 2015; C, D: 15 June 2014.

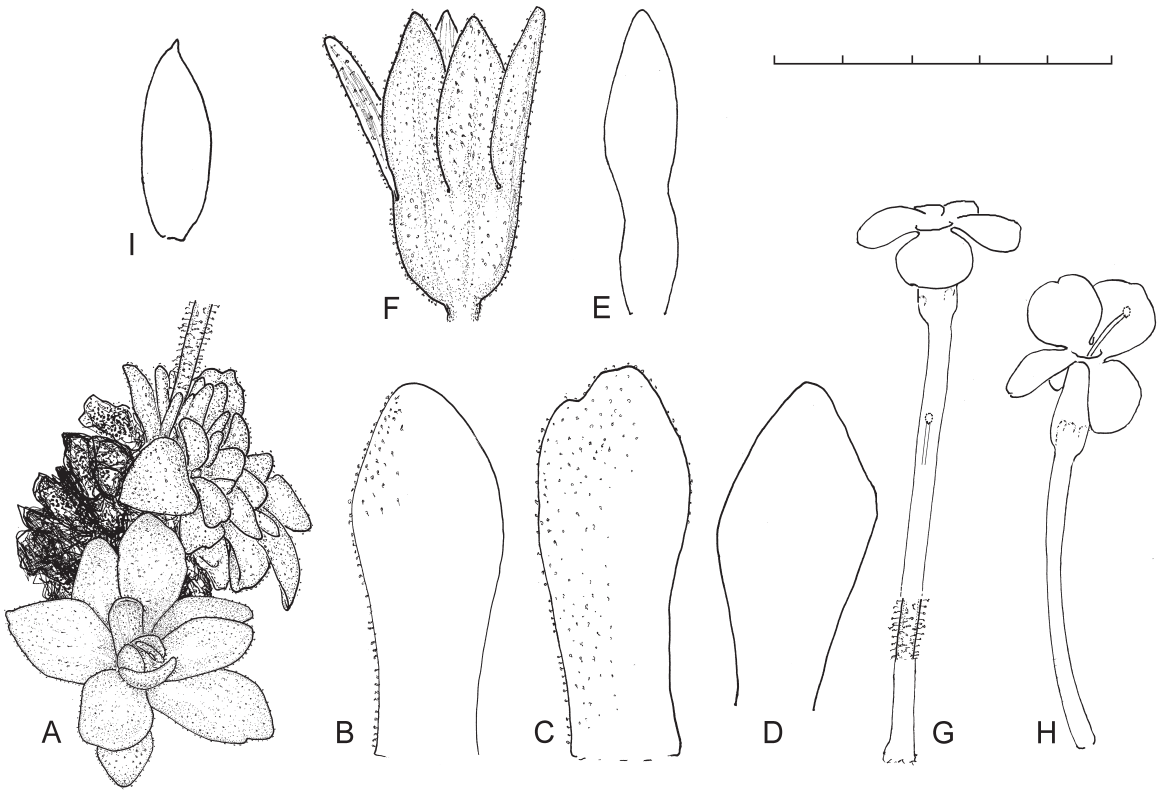

Fig. 3. A–G: *Dionysia robusta* – A: flowering branch; B–D: leaves; E: bract; F: calyx; G: corolla of short-styled flower; H: corolla of long-styled flower; I: fruit valve. – Scale bar: for A, G, H = 10 mm; for B–F, I = 5 mm. – Drawn by M. Lidén from part of the type material (*Younesi 14494*, IAUH).

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Table 1. Comparison of morphological characters of *Dionysia robusta*, *D. gaubae*, *D. iranica*, *D. sarvestanica* and *D. tacamahaca*.

	D. robusta	***D. gaubae***	***D. iranica***	***D. sarvestanica***	***D. tacamahaca***
Plant habit	very dense cushions	lax to moderately dense cushions	moderately dense cushions	dense cushions	dense cushions
Marcescent leaves colour	cream	brown	dark brown	brown	brown
Leaf shape	subspatulate-obovate, apex obtuse to subacute	obovate to spatulate, apex obtuse	obovate to spatulate, apex obtuse	oblong to obovate, apex obtuse	narrowly obovate, apex subacute
Leaf size [mm]	4–7 × 2–3	4–8 × 2–4	2–10 × 2–3	4–5 × 2–3	3–5 × 2
Leaf indumentum	subsessile glandular hairs	short glandular hairs	subsessile glandular hairs	short glandular hairs	short and long glandular hairs
Leaf margin	entire (rarely slightly lobed at apex)	lobed, rarely entire	entire or lobed	entire	entire
Bracts number	1	1	1	1	1 or 2
Bract shape	oblanceolate	narrowly oblong to oblanceolate	linear	oblanceolate	linear
Bract length [mm]	4–5	4–7	c. 4	c. 4	3–4
Calyx length [mm]	4–5	5–7	c. 5.5	c. 4	3.5–4.5
Calyx division	divided to 2/3–3/4	divided to 4/5–5/6	divided to c. 4/5	divided to base	–
Calyx lobes shape	broadly lanceolate, margin entire, apex subacute	oblanceolate	lanceolate to oblanceolate	narrowly oblanceolate	ovate-lanceolate, apex acute

Table 2. Comparison of different bases in ITS DNA sequences of *Dionysia robusta*, *D. gaubae*, *D. iranica*, *D. odora* and *D. sarvestanica*. – Alignment length = 681 bases; each column represents a position with dissimilar bases.

	0	0	0	0	1	1	1	1	2	2	2	3	4	4	4	4	4	5	6	6	6
	6	6	8	9	3	3	7	7	3	4	4	9	4	5	6	7	8	8	0	1	3
	0	9	6	0	0	1	0	3	3	0	4	2	8	9	8	2	5	1	9	5	4
D. robusta	C	G	A	A	G	C	G	C	G	C	G	G	C	C	G	G	T	C	C	T	C
D. gaubae	C	G	A	G	C	C	G	C	A	C	G	G	T	C	G	G	T	C	C	T	C
D. iranica	A	A	G	G	C	C	A	T	A	T	G	T	T	T	T	A	C	C	A	T	T
D. odora	C	G	A	G	G	C	G	C	A	C	G	G	C	C	G	G	T	C	C	T	C
D. sarvestanica	C	A	A	G	G	T	G	C	A	T	A	G	T	C	C	A	T	T	C	C	C

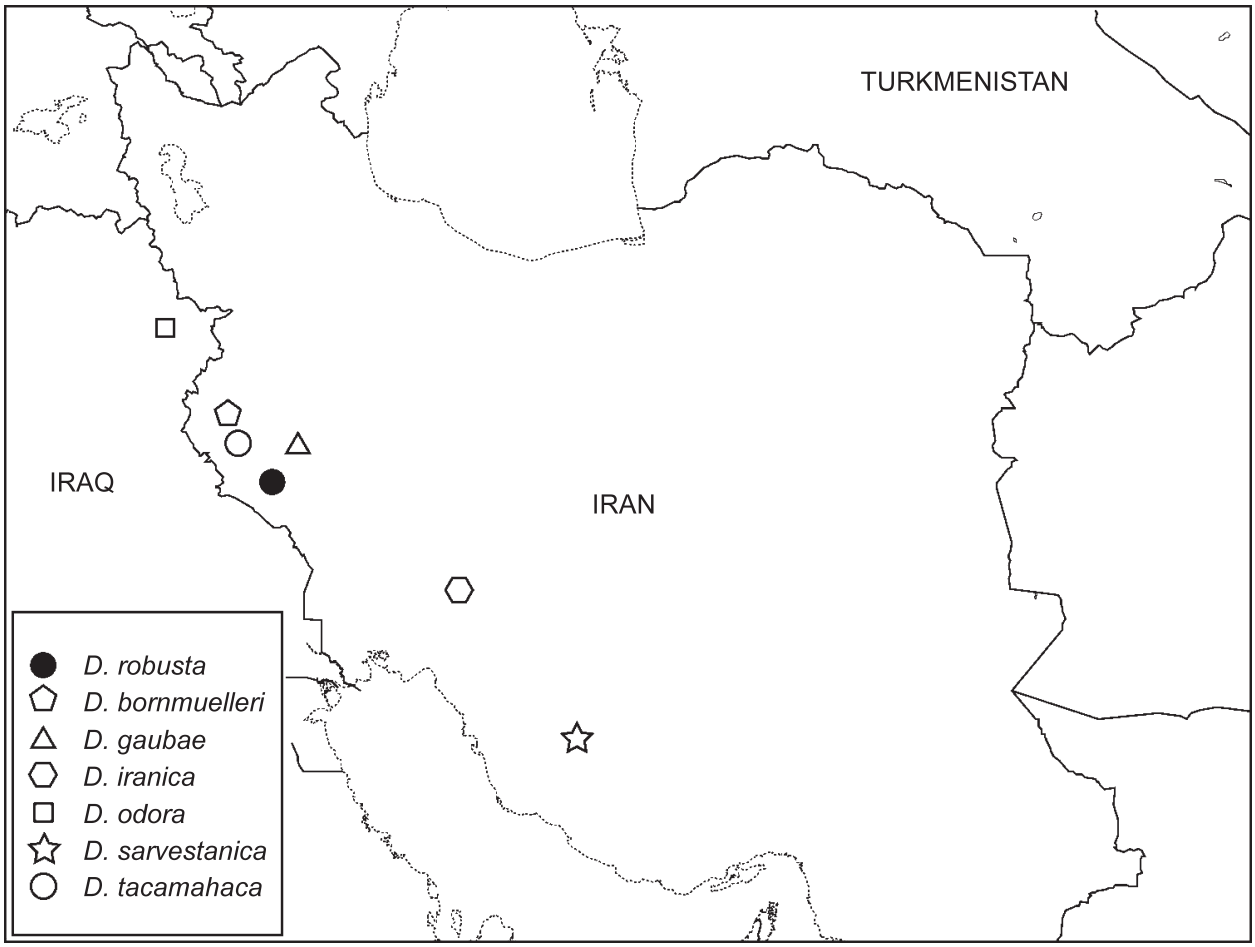

Fig. 4. Geographical distribution of *Dionysia robusta*, *D. bornmuelleri*, *D. gaubae*, *D. iranica*, *D. odora*, *D. sarvestanica* and *D. tacamahaca* in Iran and Iraq.

cies forms large subpopulations at both localities – not common for most species of this genus. The plants grow in crevices of vertical calcareous rocks and N-facing slopes. The general vegetation is dominated by *Quercus brantii* Lindl. accompanied by *Acer monspessulanum* L., *Amygdalus orientalis* Mill., *Crataegus azarolus* var. *aronia* L., *Ficus carica* subsp. *rupestris* (Hausskn. ex Boiss.) Browicz and *Pistacia atlantica* Desf.

Conservation status — According to our observations, the number of mature individuals of *Dionysia robusta* is rather high (probably more than 10 000). We observed the population in two large localities in the form of two strips up to 10 km long and 400 m wide. The area of occupancy of *D. robusta* is less than 10 km^2. There is no information on population size reduction, but we project a decline in the area of occupancy, quality of habitat, and number of mature individuals due to recent severe droughts in the area. We suggest, therefore, that this species should be placed under the IUCN (2012) category Critically Endangered with the criteria CR B2ab(ii,iii,v).

Etymology — The epithet *robusta* refers to the robust habit and large size of the plants.

Comparison with other species — *Dionysia robusta* is easily distinguishable from other species growing in the region by having cushions very large, up to 70 cm in diameter, and leaves entire or slightly divided. It is similar to *D. gaubae*, *D. iranica*, *D. sarvestanica* and *D. tacamahaca* (Table 1). However, *D. gaubae* differs in having leaves longer and usually more divided, marcescent leaves strongly reflexed so as to become more or less coiled, glands on the leaves more clearly stalked, and calyx much more deeply divided. *Dionysia iranica* differs in its dark green colour, usually less dense habit, and more deeply divided calyx. *Dionysia sarvestanica* differs in having leaves not or only slightly spreading, smaller, and always entire, more densely glandular with hairs clearly stalked, and calyx divided to the base. *Dionysia robusta* is similar to *D. tacamahaca* in the outcurved leaves and distinct calyx cup, but is very different in indumentum.

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very accurate scrutiny of the earlier versions of this paper.

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Appendix

Voucher information: GenBank accession numbers of material used in the molecular analysis of the ITS region. Except for two newly sequenced samples (indicated in **boldface**), all other accessions are from Trift & al. (2004) and Lidén (2007).

Dionysia archibaldii Wendelbo	AY680737
D. aretioides (Lehm.) Boiss.	AY680723
D. balsamea Wendelbo & Rech. f.	AY680709
D. bryoides Boiss.	AY680728
D. caespitosa (Duby) Boiss.	AY680738
D. cristagalli Lidén	AY680746
D. curviflora Bunge	AY680739
D. denticulata Wendelbo	AY680721
D. diapensiifolia Boiss.	AY680713
D. esfandiarii Wendelbo	AY680712
D. freitagii Wendelbo	AY680729
D. gaubae Bornm.	AY680740
D. haussknechtii Bornm. & Strauss	AY680720
D. hissarica Lipsky	AY680725
D. involucrata Zaprjag.	AY680705
D. iranica Jamzad	AY680741

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D. iranshahrii Wendelbo	AY680735
D. janthina Bornm. & C. Winkl.	AY680711
D. khatamii Mozaff.	AY680742
D. khuzistanica Jamzad	AY680727
D. lamingtonii Stapf	AY680743
D. leucotricha Bornm.	AY680717
D. lindbergii Wendelbo	AY680748
D. lurorum Wendelbo	AY680718
D. michauxii (Duby) Boiss.	AY680714
D. microphylla Wendelbo	AY680706
D. mira Wendelbo	AY680733
D. mozaffarianii Lidén	AY680716
D. odora Fenzl	AY680719
D. oreodoxa Bornm.	AY680744
D. paradoxa Wendelbo	AY680708
D. revoluta subsp. *canescens* (Boiss.) Wendelbo	AY680730
D. revoluta Boiss. subsp. *revoluta*	AY680731
D. rhaptodes Bunge	AY680745
D. robusta Younesi[1]	KU697386
D. sarvestanica Jamzad & Grey-Wilson	AY680715
D. teucrioides P. H. Davis & Wendelbo	AY680734
D. viscidula Wendelbo	AY680722
D. viva Lidén & Zetterl.	AY680736
Primula davisii W. W. Sm.	AY680710
P. floribunda Wall.	AY680707
P. gaubaeana Bornm.[2]	KU697387
P. veris L.	JQ927145

[1] Type locality (see above).

[2] Iran: Kohgiluyeh and Boyer-Ahmad Province, Sisakht, Meymand, Kataa, Pol-e Kataa, 31°11'24.6"N, 51°14'57.0"E, 1560 m, 21 Apr 2012, *Mehregan & Yeganeh 14883* (IAUH).

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