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RESEARCH ARTICLE

DNA barcoding of the genus *Verbascum* (Scrophulariaceae) in the Arabian Peninsula

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Abstract *Verbascum* and *Rhabdotosperma* are members of the family Scrophulariaceae. The first genus comprises approximately 360 species from almost all parts of the world, while the second contains a total of 8 species from tropical Africa and the Arabian Peninsula. Since 1977, the relationships between *Verbascum* and *Rhabdotosperma* continue to be contested. The present study aims to present the phylogenetic relationships and status among *Verbascum* species in the Arabian Peninsula. For phylogenetic analyses, maximum parsimony and Bayesian inference were performed. In total, 236 DNA sequences from 59 specimens of Arabian *Verbascum* were analysed. The phylogenetic analysis of one nuclear (ITS) and three chloroplastic (*rbcL*, *matK*, *trnL*) markers confirmed the monophyly of *Verbascum*, including the genus *Rhabdotosperma*. In addition to presenting novel phylogenetic relationships among the different *Verbascum* species in the Arabian Peninsula, our study reduced the species count of Arabian *Verbascum* to 16. Moreover, the phylogenetic analysis strongly supports the reinstatement of the genus *Rhabdotosperma* into *Verbascum* based on the Bayesian and maximum parsimony analyses.

Keywords Arabian Peninsula; phylogeny; *Rhabdotosperma*; species status; *Verbascum*

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

■ INTRODUCTION

Verbascum L. and *Rhabdotosperma* Hartl belong to the tribe Scrophularieae in the family Scrophulariaceae (Oxelman & al., 2005). The latter genus was separated from the former based on Hartl's (1977) analysis of seed morphology; Lobin & Porembski (1994) and Fischer (2004) followed this classification, in which its species can be distinguished from their sister species in *Verbascum* by longitudinally furrowed seeds, the lack of accessory flowers and a stigma that is dilated to disciform in shape (Hartl, 1977; Fischer, 2004). The genus *Verbascum* comprises approximately 360 species worldwide (Heywood & al., 2007; Christenhusz & al., 2017), whereas *Rhabdotosperma* consists of 6 species from tropical Africa and 2 species from the Arabian Peninsula (Hartl, 1977; Lobin & Porembski, 1994; Fischer, 2004; Christenhusz & al., 2017; Alzahrani & al., 2022).

Most studies on the systematics of both genera have focused on morphological characteristics (Murbeck, 1925, 1933; Hartl, 1977; Huber-Morath, 1978; Grabias & al., 1991; Juan & al., 1997). In contrast, a few studies have used molecular phylogenetics to understand the evolution of the morphology and the phylogenetic relationships between *Verbascum* and related genera or within the genus (Remal, 2014; Ghahremaninejad & al., 2015; Sotoodeh, 2015; Riahi & Ghahremaninejad, 2019). Until recently, the phylogeny of the genus *Rhabdotosperma* was unknown; however, as shown in the phylogenetic studies by Dong & al. (2022), the separation of this genus is not supported, and it forms a sister clade to other *Verbascum* species.

The genus *Verbascum* is taxonomically challenging and complex (Huber-Morath, 1978). *Verbascum* is represented on the Arabian Peninsula by about 22 species, including the 2 *Rhabdotosperma* species (Huber-Morath, 1984; Collenette, 1985, 1998, 1999; Western, 1989; Ghazanfar, 1992, 2015; Wood, 1997; Chaudhary, 2001; Jongbloed & al., 2003; Alzahrani & al., 2022). The populations of this genus exhibit variations in habitat and morphological characteristics, particularly in terms of the number of stamens, leaf shape and type of indumentum. Nevertheless, neither a complete taxonomic revision nor a phylogenetic analysis of *Verbascum* has been attempted.

Therefore, this study aims to (a) provide the first DNA barcodes for Arabian *Verbascum* species, based on one

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nuclear (ITS) and three chloroplastic (*rbcL*, *matK*, *trnL*) regions; (b) review the separation between *Verbascum* and *Rhabdotosperma* species; (c) understand the relationships among Arabian *Verbascum* species and gain more insights into their evolutionary history; and (d) evaluate Arabian *Verbascum* species, particularly those described by Al-Hemaid (2001), which resulted in additional morphological status issues among *Verbascum* species in Saudi Arabia.

■ MATERIALS AND METHODS

Taxon sampling. — In this study, 69 samples representing 20 *Verbascum* and 2 *Rhabdotosperma* species were collected from a variety of geographical locations and subpopulations on the Arabian Peninsula. Whenever possible, 2 to 5 specimens of each species were collected; thus, 32 leaf samples were collected in the field between 2020 and 2021, and 37 were from herbarium specimens. All field samples and herbarium specimens are listed in Appendix 1. Each sample was inserted in a teabag with a label in a container containing silica gel (Kress & Erickson, 2012; Wilkie & al., 2013). Outgroup taxa were selected from *Scrophularia* Tourn. ex L. and *Teedia* Rudolphi, which are sister genera of *Verbascum* and *Rhabdotosperma*.

Molecular methods. — The molecular analysis was conducted in a laboratory of the University of Guelph, Canada. Genomic DNA was extracted from plant materials using the Maxwell RSC PureFood GMO and Authentication Kit and the Maxwell RSC system (Promega, Madison, Wisconsin, U.S.A.). The primers used for each region are listed in Table 1.

The PCRs were carried out in 25 µl reactions containing 1× HotStarTaq master mix (Qiagen, Mississauga, Ontario, Canada), 400 µM of each primer, 0.15 µg of BSA and 2 µl of the DNA template. The GeneAmp PCR System 9700 (Applied Biosystems, Foster City, California, U.S.A.) was used to conduct the PCRs. The PCR cycling programmes were 95°C for 10 min of denaturation, 42 cycles of 95°C for 15 s of annealing, 52°C or 55°C for 1 min and 72°C for 2 min of extension, followed by 7 min of final extension at 72°C.

PCR products were visualised on 2% agarose gels, and then the NucleoFast 96 PCR clean-up kit (Macherey-Nagel, Düren, Germany) was used to purify the successful products. The purified PCR fragments were sequenced bidirectionally using an ABI 3730xl Genetic Analyzer (Applied Biosystems) and the same primers as those used for the PCRs. The ABI Prism DNA Sequencing Analysis Software (Applied Biosystems) assembled the sequences into a consensus sequence. The Molecular Evolutionary Genetics Analysis (MEGA11) was used to align and then combine the sequences (Tamura & al., 2021). All alignments are provided in the supplementary material (suppl. Appendix S1–S4).

Phylogenetic analyses. — The nuclear and chloroplast data matrices were separately and jointly analysed using both maximum parsimony (MP) and Bayesian inference (BI).

The jModelTest v.2.1.2 (Darriba & al., 2012) was used to determine the best nucleotide substitution model for each dataset. By the Akaike information criterion (AIC), the GTR+Γ model was selected for the chloroplast and nuclear regions in separate analyses, while the GTR+I+Γ model was selected for the combined regions.

The MP analysis was performed with PAUP* v.4.0a169 (Swofford, 2002), a heuristic search was used with 1000 random addition sequence replicates, tree-bisection reconnection (TBR) branch swapping, the steepest descent turned on and MulTrees enabled with a maximum of 10,000 shortest trees. In addition, a bootstrap analysis was conducted with 1000 replicates, TBR branch swapping with five replicates and the steepest descent option enabled, with a maximum of 10 trees saved per replicate.

Bayesian analyses were performed in MrBayes v.3.1.2 (Ronquist & Huelsenbeck, 2003), with 1 million Markov Chain Monte Carlo (MCMC) generations, with two independent runs consisting of three heated chains and one cold chain. The tree sampling frequency was set to 1000, which resulted in 1000 trees. Then, 25% burn-in was applied to the 1000 trees, which discarded the first 250 sampled trees.

■ RESULTS

Only 236 out of 276 sequences of all 4 regions (ITS, *matK*, *rbcL*, *trnL*) were successfully completed in the DNA

Table 1. PCR Primers used for amplification in DNA regions.

Region	Primer	Sequence (5′–3′)	Reference
rbcL	rbcLa-F	ATGTCACCACAAACAGAGACTAAAGC	Levin & al. (2003)
	rbcLa-R	GTAAAATCAAGTCCACCRCG	
matK	matK472F	CCCRTYCATCTGGAAATCTTGGTTC	Yu & al. (2011)
	matK1248R	GCTRTRATAATGAGAAAGATTTCTGC	
trnL	trnL-f	ATTTGAACTGGTGACACGAG	Taberlet & al. (1991)
	trnL-c	CGAAATCGGTAGACGCTACG	
ITS	ITS2F	ATGCGATACTTGGTGTGAAT	Chen & al. (2010)
	ITS3R	GACGCTTCTCCAGACTACAAT	

barcoding of *Verbascum* species from the Arabian Peninsula (Appendix 1). The combined nuclear (ITS) and chloroplastic (*rbcL*, *matK*, *trnL*) matrices consisted of 2564 characters, of which 375 (14.6%) were variable, and 342 (13.3%) were informative. Together, the three chloroplastic (*matK*, *rbcL*, *trnL*) matrices contained 2115 characters, 39 (1.8%) of which were variable, and 99 (4.6%) were informative. The ITS matrix contained 449 characters, of which 239 (53.2%) were variable, and 50 (11.1%) were informative (Table 2).

There was discordance among BI and MP trees of each individual marker, which had less resolution and lower support values than those of the combined markers. The parsimony analysis of the combined data resulted in a strict consensus on the 10,000 most equally parsimonious trees, with a 939-tree length, a consistency index of 0.8807 and a retention index of 0.8724 (Table 2). In a few cases, the bootstrap values obtained from the MP analyses were either unresolved or less resolved than the posterior probability values obtained from the Bayesian analyses. The Bayesian and the MP analyses of the combined chloroplast and ITS genes are provided in Fig. 1. The phylogenetic trees from separate analyses are available in the supplementary material (suppl. Figs. S1–S8).

The Bayesian and the MP analyses of the concatenated chloroplast and ITS genes resulted in the same topologies for the phylogenetic relationships among *Verbascum* species, and strongly supported the genus *Verbascum* as monophyletic including *Rhabdotosperma* (Bayesian posterior probability [PP] = 1/maximum parsimony bootstrap [PB] = 100; Fig. 1). In addition, the phylogenetic tree was divided into 2 major branches and 11 clades (indicated by nodes 1–2 and A–K, respectively).

In the first major branch, clades A to D are formed in a polytomy with endemic species that share certain characteristics, including clustered flowers and stellate hairs. They are found from northwest Saudi Arabia to southern Yemen. Clade A (PP = 0.98/PB = 56) comprises species found in the southwest region of the Arabian Peninsula. Clade B was supported by the Bayesian analysis (PP = 0.74) but unresolved by the MP analysis; this clade consists of a complex species with a high variation, found in the Asir Mountains of southwest Saudi Arabia and the southern region of Yemen. Clade C (PP = 0.99/PB = 85) consists of species found in variable habitats and with a wide distribution from western to northwest Saudi Arabia. Clade D was well supported by the Bayesian analysis (PP = 0.87) but not by the MP analysis; its species are endemic to the Hijaz Mountains.

Clade E was strongly supported by the Bayesian and MP analyses (PP = 1/PB = 100). It includes a species that has four stamens, solitary flowers and forked hairs (rarely stellate hairs) and is found from northwest Saudi Arabia to the eastern Mediterranean. Clade F (PP = 1/PB = 60) consists of species with set-apart distributions in the north, west and south regions of the Arabian Peninsula; this clade is sister to the remainder of major branch 1.

The Bayesian analysis strongly supported Clade G (PP = 1), whereas the MP analysis weakly supported it (PB = 62). This clade's species can be recognised by five stamens, often solitary or rarely dichasium inflorescence, and glandular or stellate hairs, and their distributions range from northwest Saudi Arabia to the eastern Mediterranean. Clade H (PP = 0.95/PB = 53) is sister to the previous clade; however, its species have four stamens, solitary flowers, dense glandular hairs above and stellate hairs below, and its species are endemic from west to northwest Saudi Arabia. Clade I (PP = 1/PB = 99) consists of species endemic to the foothills of the Hajar Mountains in Oman and the U.A.E., with five stamens, flower clusters, bracteoles and glandular-stellate hairs.

Clade J can be divided into two strongly supported subclades (J1 and J2); both *Verbascum* and *Rhabdotosperma* species within this clade have four stamens, solitary flowers and glandular hairs; however, their seeds have distinct appearances. Subclade J1 (PP = 1/PB = 100) consists of

Table 2. A comparison of the individual and combined datasets from parsimony analysis.

	ITS	*matK*	*rbcL*	*trnL*	Combined chloroplastic	Combined chloroplastic and ITS
No. of sequences	62	62	62	62	62	62
Alignment length (bp)	449	735	561	819	2115	2564
No. of variable characters (%)	239 (53.2)	16 (2.1)	7 (1.2)	16 (2.3)	39 (1.8)	375 (14.6)
No. of informative characters (%)	50 (11.1)	54 (7.3)	10 (1.7)	35 (4.2)	99 (4.6)	342 (13.3)
No. of most equally parsimonious trees	10,000	7	6	1455	390	10,000
Tree length	380	73	20	58	156	939
Consistency index	0.8763	0.9726	1.0000	0.9310	0.9295	0.8807
Retention index	0.8309	0.9835	1.0000	0.9728	0.9618	0.8724
Rescaled consistency index	0.7282	0.9565	1.0000	0.9057	0.8940	0.7684

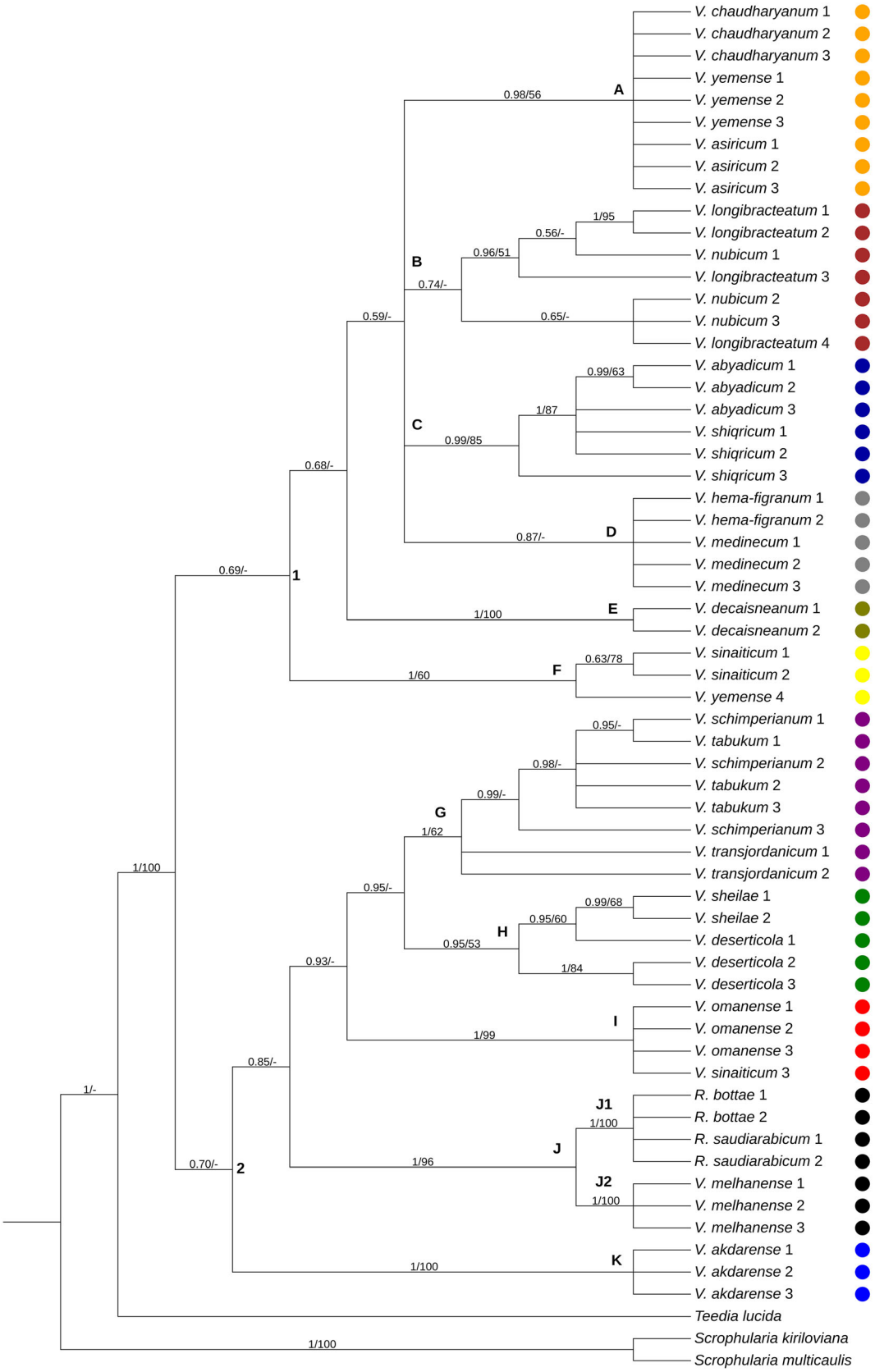

Fig. 1. Bayesian majority-rule (50%) consensus tree of the combined chloroplast and ITS sequence data matrix. Support values on branches are Bayesian posterior probability/maximum parsimony bootstrap. Clades and species (including study samples) are colour-coded; their geographic distribution is indicated in Fig. 2.

Rhabdotosperma species with longitudinally furrowed seeds, whereas subclade J2 (PP = 1/PB = 100) comprises *Verbascum* species with transversally elongated seeds; both subclades are endemic to the southwestern Arabian Peninsula.

Clade K's (PP = 1/PB = 100) species can be recognised by four stamens, solitary flowers and glandular-pubescent hairs; they are found in the foothills and mountains northeast of Oman's Hajar region. This clade is sister to all the other *Verbascum* and the *Rhabdotosperma* taxa on the second main branch.

■ DISCUSSION

The present phylogenetic study shows that the genus *Verbascum* is monophyletic, which is consistent with the findings of previous studies (Ghahremaninejad & al., 2015; Sotoodeh, 2015; Riahi & Ghahremaninejad, 2019). In addition to revealing a novel phylogenetic relationship among the various species of *Verbascum* on the Arabian Peninsula, this study also provides 236 DNA sequences from 59 specimens from each region, representing 16 species of this genus. The status of the *Verbascum* species from the Arabian Peninsula, particularly Saudi Arabia, for which the morphological analysis revealed difficulties in taxon status, is discussed in the following sections.

***Verbascum abyadicum* and *V. shiqricum*. —** Al-Hemaid (2001) described *Verbascum abyadicum* Hemaid and *V. shiqricum* Hemaid from Saudi Arabia based on a single collection for each species. The former has four stamens and is found in Harrat Khaybar in western regions, whereas the latter has five stamens and is found near Shigry in northwestern regions. However, Alzahrani & al. (submitted) treated *V. abyadicum* as synonym of *V. shiqricum* since they share similar morphological characteristics and geographic distributions. *Verbascum shiqricum* is a highly variable species that can be found in a wide range of habitats and is located in west and northwest Saudi Arabia. In the combined analysis, *V. shiqricum* (1, 2 and 3) and *V. abyadicum* (1, 2 and 3) formed a monophyletic clade with strong support (PP = 0.99/PB = 0.85; Figs. 1 & 2 clade C).

***Verbascum akdarense*. —** A distinct species, *Verbascum akdarense* (Murb.) Hub.-Mor. has solitary flowers and glandular-pubescent hairs and is endemic to the foothills and mountains of Hajar to the northeast of Oman. The phylogenetic analysis showed that *V. akdarense* (1, 2 and 3) formed

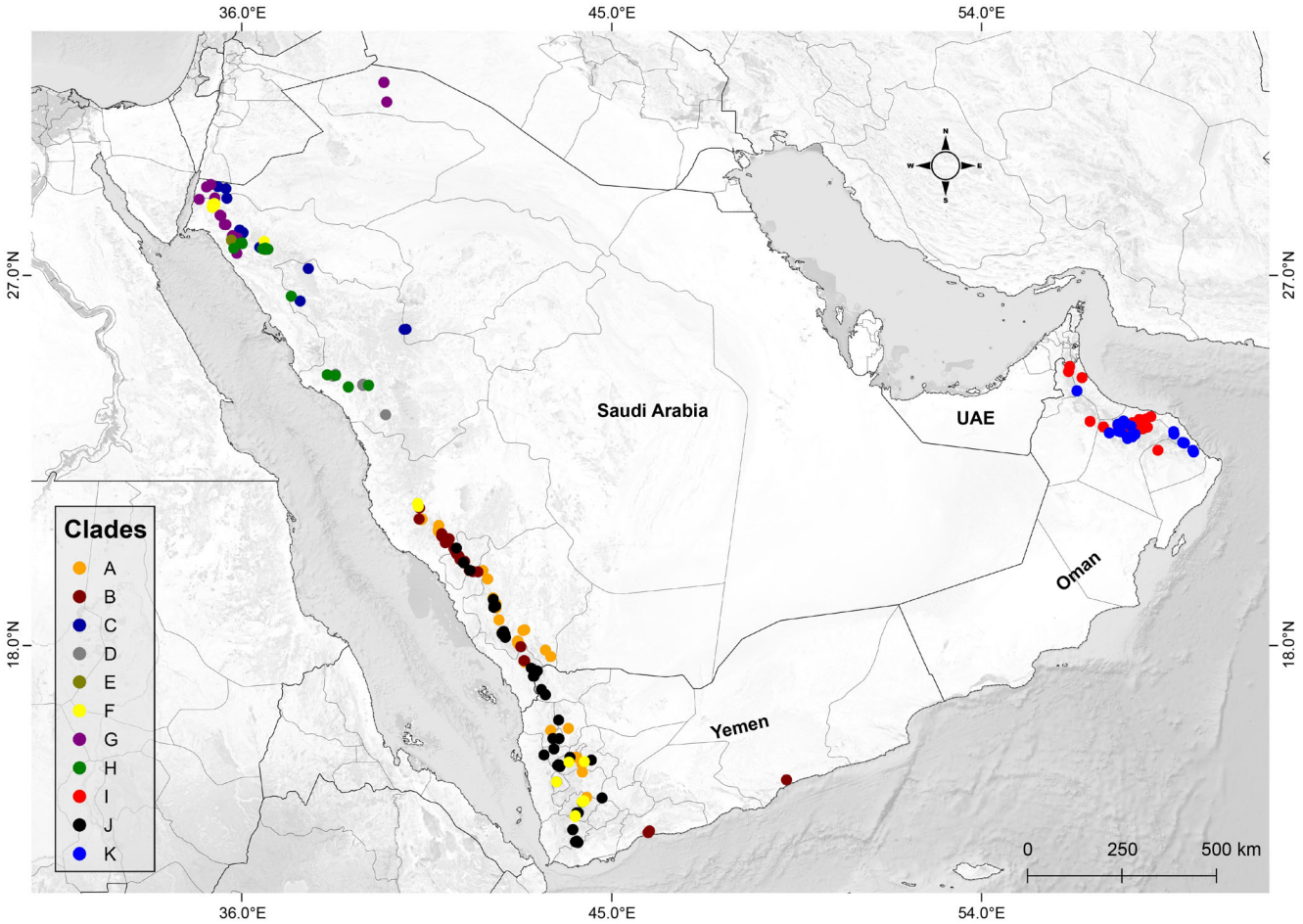

Fig. 2. Distribution of the clades and species, colours correspond to those used in Fig. 1.

a monophyletic clade with strong support (PP = 1/PB = 100; Figs. 1 & 2 clade K).

***Verbascum asiricum, V. chaudharyanum* and *V. yemense*. —** Deflers (1889) described *Verbascum yemense* Deflers as a species endemic to Yemen; much later, Collenette (1985) documented it in Saudi Arabia. Al-Hemaid (2001) recognised it as a distinct species from the newly described *V. asiricum* Hemaid and *V. chaudharyanum* Hemaid from Saudi Arabia. Due to their similarities in morphology and geographic distribution, Alzahrani & al. (submitted) determined *V. chaudharyanum* and *V. asiricum* to be conspecific with *V. yemense*. The phylogenetic tree of *V. yemense* (1, 2 and 3), *V. chaudharyanum* (1, 2 and 3) and *V. asiricum* (1, 2 and 3) is consistent with this interpretation (PP = 0.98/PB = 56; Figs. 1 & 2 clade A).

***Verbascum decaisneanum*. —** *Verbascum decaisneanum* Kuntze can be recognised by its four filaments, solitary flowers and forked (occasionally stellate) hairs, and it is found in northwest Saudi Arabia, Jordan, Egypt (Sinai), Palestine, Lebanon and Syria. In the combined analysis, *V. decaisneanum* (1 and 2) formed a strongly supported monophyletic clade (PP = 1/PB = 100; Figs. 1 & 2 clade E).

***Verbascum deserticola* and *V. sheilae*. —** Murbeck (1925) treated *Verbascum deserticola* (Vatke ex Murb.) Hub.-Mor. as a distinct species and described it from Saudi Arabia, where it is found in the western and northwestern regions. Due to its extreme variability, it has been considered either a synonym of *V. schimperianum* Boiss. or an unaccepted species. In 2001, Al-Hemaid described *V. sheilae* Hemaid and differentiated it from *V. deserticola*; however, Alzahrani & al. (submitted) considered the two taxa to be conspecific due to their similarities in morphology and geographic distribution. Moreover, the chloroplast and ITS analyses revealed that *V. sheilae* (1 and 2) and *V. deserticola* (1, 2 and 3) formed a strongly supported monophyletic clade (PP = 0.95/PB = 53; Figs. 1 & 2 clade H).

***Verbascum hema-figranum* and *V. medinecum*. —** Al-Hemaid (2001) described *Verbascum hema-figranum* Hemaid and *V. medinecum* Hemaid, both of which are endemic to Jabal Al-Figrah in Medina Province of western Saudi Arabia. However, morphological investigations conducted by Alzahrani & al. (submitted) determined that both species share the same morphological characteristics and geographic distributions. The combination of *V. hema-figranum* (1 and 2) and *V. medinecum* (1, 2 and 3) was supported by the Bayesian analysis (PP = 0.87; Figs. 1 & 2 clade D) but not by MP.

***Verbascum longibracteatum, V. luntii* and *V. nubicum*. —** Baker (1894) described *V. luntii* Baker from Alrail in Hadhramaut, Yemen; subsequently, Deflers (1896) described *V. longibracteatum* Deflers from Jabal Areys in Abyan, Yemen; they share similar morphological characteristics and have habitats that are found in close proximity to each other. Due to their similarity, Murbeck (1933) suggested that *V. luntii* should be considered a synonym of *V. longibracteatum*, which is consistent with the conclusion reached by Alzahrani & al. (submitted). Additionally, Collenette (1985) documented *V. longibracteatum* in southwestern Saudi Arabia, along with the related species *V. nubicum* Murb., which was earlier described by Murbeck (1933) from Nubia, Sudan. Some authors considered *V. nubicum* a synonym of *V. sinaiticum* Benth. or an unaccepted species due to its poor collections for morphological comparison. Nevertheless, Alzahrani & al. (submitted) regarded *V. nubicum* from Saudi Arabia as the early growth form of *V. longibracteatum* and treated it as a synonym of the latter due to their similar morphological characteristics and geographical distributions. *Verbascum longibracteatum* is a complex and highly variable species found in the southwestern region of Saudi Arabia and the southern region of Yemen. The combined analysis included only *V. longibracteatum* (1, 2, 3 and 4) and *V. nubicum* (1, 2 and 3), yielding limited support that can be interpreted as a failure to differentiate between the species (PP = 074; Figs. 1 & 2 clade B). No samples of *V. luntii* were included in this analysis.

***Verbascum melhanense*. —** *Verbascum melhanense* (Murb.) Hub.-Mor. is endemic to the southwestern Arabian Peninsula. It is easy to confuse this species with *Rhabdotosperma bottae* (Deflers) Hartl due to their similar morphological characteristics and habitats. However, it can be distinguished by its two anterior glabrous filaments and transversally elongated seeds, whereas *R. bottae* has two anterior glabrous filaments near the apex and longitudinally furrowed seeds (Alzahrani & al., submitted). The combined analysis placed *V. melhanense* (1, 2 and 3) in a strongly supported monophyletic clade (PP = 1/PB = 100; Figs. 1 & 2 subclade J2).

***Verbascum omanense* and *V. sinaiticum*. —** *Verbascum omanense* Hub.-Mor. is a species endemic to the foothills of the Hajar Mountains in Oman and the U.A.E., and it is a highly variable species, frequently misidentified as *V. sinaiticum* or *V. cedreti* Boiss. (Mandaville & Bovey, 1978; Ghazanfar, 1992, 2015; Jongbloed & al., 2003). The combined analyses revealed that samples of *V. omanense* (*V. omanense* 1, 2 and 3; *V. sinaiticum* 3) from Oman and the U.A.E. formed a strongly supported monophyletic clade (PP = 1/PB = 99; Figs. 1 & 2 clade I), which is consistent with the findings reported by Alzahrani & al. (submitted). Therefore, *V. sinaiticum* is only known from Saudi Arabia and Yemen on the Arabian Peninsula (*V. sinaiticum* 1, 2). This species can be distinguished by its dense tomentose indumentum with stellate hairs and panicle inflorescence with clustered flowers. The phylogenetic tree also showed that samples of *V. sinaiticum* (1 and 2) from Saudi Arabia and Yemen belonged to a distinct clade (PP = 1/PB = 60; Figs. 1 & 2 clade F), which includes an additional species, referred to as *V. yemense* 4, which Alzahrani & al. (submitted) recognise as a new species distinct from *V. sinaiticum* and *V. yemense* and which is intended to be published as "*V. sarawaticum*" as it is found in the Sarawat Mountains in southeast Saudi Arabia.

***Verbascum tabukum* and *V. schimperianum*. —** *Verbascum tabukum* Hemaid was treated as a distinct species

and described by Al-Hemaid (2001), based on a single specimen, without comparing it with species from neighbouring countries. However, morphological studies (Alzahrani & al., submitted) considered this species to be a synonym of *V. eremobium* Murb. due to their similar morphological characteristics and geographical distributions. The combined chloroplast and ITS analysis included *V. eremobium* specimens (which is referred to as *V. tabukum* 1, 2, and 3; *V. schimperianum* 1 and 2) and placed them in a monophyletic clade that was strongly supported by the Bayesian analysis (PP = 0.98; Figs. 1 & 2 clade G p.p.) but weakly supported by MP. In addition, *V. schimperianum* could be confounded with *V. eremobium* due to their comparable geographical distributions and shared morphological characteristics. The phylogenetic tree showed *V. schimperianum* 3 as sister to a clade that contained *V. eremobium* (PP = 0.99; Figs. 1 & 2 clade G), although the MP analysis provided weaker support.

***Verbascum transjordanicum*. —** *Verbascum transjordanicum* Murb. is a species endemic to Jordan and northern Saudi Arabia. It is distinguishable from other Arabian *Verbascum* species by its solitary flower with five stamens, dense glandular hairs with sparse simple and forked hairs above, and dense tomentose with stellate hairs below. The phylogenetic tree revealed *V. transjordanicum* (1 and 2) as sister to the clades that contained *V. schimperianum* and *V. eremobium* (PP = 1/PB = 62; Figs. 1 & 2 clade G).

***Rhabdotosperma* group (= *Verbascum*). —** Hartl (1977) separated the genus *Rhabdotosperma* from *Verbascum* on account of its seed morphology. In comparison to tropical Africa, the Arabian Peninsula is home to only two species of *Rhabdotosperma* namely, *R. bottae* and *R. saudiarabicum* A.Alzahrani (Hartl, 1977; Huber-Morath, 1984; Wood, 1997; Alzahrani & al., 2022). However, the combined analysis of *V. bottae* (*R. bottae* 1 and 2) and *V. saudiarabicum* (*R. saudiarabicum* 1 and 2) placed them in a strongly supported monophyletic clade, nested within *Verbascum* (PP = 1/PB = 100; Figs. 1 & 2 subclade J1). Therefore, the phylogenetic analysis did not support this separation, and it must be reinstated into *Verbascum*, which is consistent with the recommendation of Dong & al. (2022); thus, Alzahrani & al. (submitted) regarded these *Rhabdotosperma* species as *Verbascum* species on the Arabian Peninsula.

■ AUTHOR CONTRIBUTIONS

All authors contributed to the conception and design of the research study. AMA collected and analysed data, and wrote the draft of the manuscript. All authors reviewed, read, and approved the final manuscript.

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Appendix 1. Species and GenBank/NCBI accession numbers used in this study.

For each accession, the following voucher information is provided: species name, locality, country, collector, collection number, herbarium code, and ITS, *matK*, *rbcL*, *trnL* GenBank accession numbers. ! and * indicate unsuccessful data and newly generated DNA sequences, respectively.

Rhabdotosperma bottae (Deflers) Hartl **1***, Bait Albeshari, Al Mahwit, Yemen, *J.R.I. Wood 3108* (K), OR196975, OR232416, OR232475, OR232354; ***Rhabdotosperma bottae*** (Deflers) Hartl **2***, Jabal Taqar, Ibb, Yemen, *J.R.I. Wood 1707* (K), OR196976, OR232417, OR232476, OR232355; ***Rhabdotosperma bottae*** (Deflers) Hartl **3!**, Jabal Sabir, Taizz, Yemen, *K.J. Gordon 1* (E [E00066923]); ***Rhabdotosperma saudiarabicum*** A.Alzahrani **1***, Al-Soudah, Abha, Saudi Arabia, *L. Boulos & A.S. Ads 14165* (K), OR196977, OR232418, OR232477, OR232356; ***Rhabdotosperma saudiarabicum*** A.Alzahrani **2***, Jabal Al-Soudah, Abha, Saudi Arabia, *I.S. Collenette 3316* (K), OR196978, OR232419, OR232478, OR232357; ***Rhabdotosperma saudiarabicum*** A.Alzahrani **3!**, Jabal Al-Soudah, Abha, Saudi Arabia, *I.S. Collenette 5368* (E [E00066943]); ***Verbascum abyadicum*** Hemaid **1***, Harrat Khaybar, Khaybar, Saudi Arabia, *A. Alzahrani 151* (MUZ), OR196979, OR232420, OR232479, OR232358; ***Verbascum abyadicum*** Hemaid **2***, Harrat Khaybar, Khaybar, Saudi Arabia, *I.S. Collenette 3757* (E [E00066949]), OR196980, OR232421, OR232480, OR232359; ***Verbascum abyadicum*** Hemaid **3***, Harrat Khaybar, Khaybar, Saudi Arabia, *A. Alzahrani 150* (MUZ), OR196981, OR232422, OR232481, OR232360; ***Verbascum akdarense*** (Murb.) Hub.-Mor. **1***, Jabal Akhdar, Ad Dakhiliyah, Oman, *A. Radcliffe-Smith 3980* (E [E00066951]), OR196989, OR232430, OR232489, OR232361; ***Verbascum akdarense*** (Murb.) Hub.-Mor. **2***, Ar Ruhbah, Ad Dakhiliyah, Oman, *A. Alzahrani 189* (MUZ), OR196990, OR232431, OR232490, OR232362; ***Verbascum akdarense*** (Murb.) Hub.-Mor. **3***, Wadi Asahban, Al Batinah North, Oman, *A. Alzahrani 192* (MUZ), OR196991, OR232432, OR232491, OR232363; ***Verbascum asiricum*** Hemaid **1***, Tamniah village, Abha, Saudi Arabia, *A. Alzahrani 175* (MUZ), OR196992, OR232433, OR232492, OR232364; ***Verbascum asiricum*** Hemaid **2***, Dalagan, Abha, Saudi Arabia, *I.S. Collenette 9347* (E [E00095077]), OR196993, OR232434, OR232493, OR232365; ***Verbascum asiricum*** Hemaid **3***, Abha, Saudi Arabia, *I.S. Collenette 2091* (K), OR196994, OR232435, OR232494, OR232366; ***Verbascum chaudharyanum*** Hemaid **1***, Bilhamr, Abha, Saudi Arabia, *A. Alzahrani 178* (MUZ), OR196995, OR232436, OR232495, OR232367; ***Verbascum chaudharyanum*** Hemaid **2***, Jabal Mna'a, Abha, Saudi Arabia, *A. Alzahrani 179* (MUZ), OR196996, OR232437, OR232496, OR232368; ***Verbascum chaudharyanum*** Hemaid **3***, Bilasmar and Bilhamr road, Abha, Saudi Arabia, *A. Alzahrani 108* (MUZ), OR196997, OR232438, OR232497, OR232369; ***Verbascum decaisneanum*** Kuntze **1***, Jabal Dabbagh, Tabuk, Saudi Arabia, *I.S. Collenette 5260* (E [E00066909]), OR196998, OR232439, OR232498, OR232370; ***Verbascum decaisneanum*** Kuntze **2***, Jabal Dabbagh, Tabuk, Saudi Arabia, *I.S. Collenette 717* (K), OR196999, OR232440, OR232499, OR232371; ***Verbascum deserticola*** (Vatke ex Murb.) Hub.-Mor. **1***, Duba and Shigry road, Tabuk, Saudi Arabia, *A. Alzahrani 147* (MUZ), OR197000, OR232441, OR232500, OR232372; ***Verbascum deserticola*** (Vatke ex Murb.) Hub.-Mor. **2***, Jabal Al-Figrah road, Medina, Saudi Arabia, *A. Alzahrani 152* (MUZ), OR197001, OR232442, OR232501, OR232373; ***Verbascum deserticola*** (Vatke ex Murb.) Hub.-Mor. **3***, Wadi Buwat, Medina, Saudi Arabia, *I.S. Collenette 8215* (K), OR197002, OR232443, OR232502, OR232374; ***Verbascum hema-figranum*** Hemaid **1***, Jabal Al-Figrah, Medina, Saudi Arabia, *A. Alzahrani 103* (MUZ), OR197003, OR232444, OR232503, OR232375; ***Verbascum hema-figranum***

Appendix 1. Continued.

Hemaid **2***, Jabal Al-Figrah, Medina, Saudi Arabia, *I.S. Collenette 6977* (E [E00066970]), OR197004, OR232445, OR232504, OR232376; ***Verbascum hemafigranum*** Hemaid **3!**, Jabal Radwa, Medina, Saudi Arabia, *I.S. Collenette 5889* (E [E00066948]); ***Verbascum longibracteatum*** Deflers **1***, Baljurashi, Al-Baha, Saudi Arabia, *I.S. Collenette 4330* (K), OR196985, OR232426, OR232485, OR232377; ***Verbascum longibracteatum*** Deflers **2***, Heznah road, Al-Baha, Saudi Arabia, *A. Alzahrani 170* (MUZ), OR196986, OR232427, OR232486, OR232378; ***Verbascum longibracteatum*** Deflers **3***, Baljurashi, Al-Baha, Saudi Arabia, *A. Alzahrani 167* (MUZ), OR196987, OR232428, OR232487, OR232379; ***Verbascum longibracteatum*** Deflers **4***, Al-Abna Road, Al-Baha, Saudi Arabia, *A. Alzahrani 171* (MUZ), OR196988, OR232429, OR232488, OR232380; ***Verbascum longibracteatum*** Deflers **5!**, Jabal Al-Qahar, Jazan, Saudi Arabia, *A. Alzahrani 144* (MUZ); ***Verbascum medinecum*** Hemaid **1***, Jabal Al-Figrah, Medina, Saudi Arabia, *I.S. Collenette 7116* (E [E00066952]), OR197005, OR232446, OR232505, OR232381; ***Verbascum medinecum*** Hemaid **2***, Jabal Al-Figrah, Medina, Saudi Arabia, *A. Alzahrani 153* (MUZ), OR197006, OR232447, OR232506, OR232382; ***Verbascum medinecum*** Hemaid **3***, Jabal Odks, Medina, Saudi Arabia, *A. Alzahrani 182* (MUZ), OR197007, OR232448, OR232507, OR232383; ***Verbascum melhanense*** (Murb.) Hub.-Mor. **1***, King Khalid road, Al-Baha, Saudi Arabia, *A. Alzahrani 164* (MUZ), OR197008, OR232449, OR232508, OR232384; ***Verbascum melhanense*** (Murb.) Hub.-Mor. **2***, Jabal Melhan, Al-Mahwit, Yemen, *J.R.I. Wood 2864* (K), OR197009, OR232450, OR232509, OR232385; ***Verbascum melhanense*** (Murb.) Hub.-Mor. **3***, Jabal Mna'a, Abha, Saudi Arabia, *A. Alzahrani 109* (MUZ), OR197010, OR232451, OR232510, OR232386; ***Verbascum nubicum*** Murb. **1***, Tanomah, Abha, Saudi Arabia, *I.S. Collenette 7170* (K), OR196982, OR232423, OR232482, OR232387; ***Verbascum nubicum*** Murb. **2***, Baidhan, Al-Baha, Saudi Arabia, *A. Alzahrani 165* (MUZ), OR196983, OR232424, OR232483, OR232388; ***Verbascum nubicum*** Murb. **3***, King Abdulaziz Road, Al-Mandaq, Al-Baha, Saudi Arabia, *A. Alzahrani 168* (MUZ), OR196984, OR232425, OR232484, OR232389; ***Verbascum nubicum*** Murb. **4!**, Al-Hada, Taif, Saudi Arabia, *I.S. Collenette 1090* (K); ***Verbascum nubicum*** Murb. **5!**, Wadi Masal, Ash Shafa, Taif, Saudi Arabia, *A. Alzahrani 157* (MUZ); ***Verbascum omanense*** Hub.-Mor. **1***, Hibra, Al Batinah South, Oman, *R.P. Whitcombe 472* (E [E00219515]), OR197011, OR232452, OR232511, OR232390; ***Verbascum omanense*** Hub.-Mor. **2***, Wadi Jeema, Hatta Town, U.A.E., *J.N.B. Brown 905* (E [E00066936]), OR197012, OR232453, OR232512, OR232391; ***Verbascum omanense*** Hub.-Mor. **3***, Jabal Al Halla, Ad Dakhiliyah, Oman, *J.R. Edmondson 3399* (E [E00066931]), OR197013, OR232454, OR232513, OR232392; ***Verbascum schimperianum*** Boiss. **1***, Jabal Al-Lawz, Tabuk, Saudi Arabia, *I.S. Collenette 7048* (E [E00066928]), OR197014, OR232455, OR232514, OR232393; ***Verbascum schimperianum*** Boiss. **2***, Jabal Al-Lawz, Tabuk, Saudi Arabia, *I.S. Collenette 7227* (E [E00066930]), OR197015, OR232456, OR232515, OR232394; ***Verbascum schimperianum*** Boiss. **3***, Wadi Sawawin, Tabuk, Saudi Arabia, *I.S. Collenette 527* (K), OR197016, OR232457, OR232516, OR232395; ***Verbascum sheilae*** Hemaid **1***, Wadi Al-Disah, Tabuk, Saudi Arabia, *A. Alzahrani 85* (MUZ), OR197017, OR232458, OR232517, OR232396; ***Verbascum sheilae*** Hemaid **2***, Wadi Al-Disah, Tabuk, Saudi Arabia, *I.S. Collenette 9072* (K), OR197018, OR232459, OR232518, OR232397; ***Verbascum shiqricum*** Hemaid **1***, Bir Al-Qurr, Al-Ula, Medina, Saudi Arabia, *A. Alzahrani 149* (MUZ), OR197019, OR232460, OR232519, OR232398; ***Verbascum shiqricum*** Hemaid **2***, Shigry, Tabuk, Saudi Arabia, *A. Alzahrani 180* (MUZ), OR197020, OR232461, OR232520, OR232399; ***Verbascum shiqricum*** Hemaid **3***, Alaqan, Tabuk, Saudi Arabia, *A. Alzahrani 146* (MUZ), OR197021, OR232462, OR232521, OR232400; ***Verbascum sinaiticum*** Benth. **1***, Jabal Raymah, Raymah, Yemen, *A.G. Miller & R.A. King 5327* (E [E00066966]), OR197022, OR232463, OR232522, OR232401; ***Verbascum sinaiticum*** Benth. **2***, Ad Delil, Ibb, Yemen, *J.R.I. Wood 75/108* (E [E00066959]), OR197023, OR232464, OR232523, OR232402; ***Verbascum sinaiticum*** Benth. **3***, Wadi Mahil, Ad Dakhiliyah, Oman, *A. Radcliffe-Smith 3766* (K), OR197024, OR232465, OR232524, OR232403; ***Verbascum sinaiticum*** Benth. **4!**, Jabal Al-Lawz, Tabuk, Saudi Arabia, *A. Alzahrani 181* (MUZ); ***Verbascum* sp. 1!**, Al-Safiha road, Taif, Saudi Arabia, *A. Alzahrani 163* (MUZ); ***Verbascum* sp. 2!**, Al-Abna Road, Al-Baha, Saudi Arabia, *I.S. Collenette 9015* (E [E00092215]); ***Verbascum tabukum*** Hemaid **1***, Duba road, Tabuk, Saudi Arabia, *I.S. Collenette 9115* (E [E00092230]), OR197025, OR232466, OR232525, OR232404; ***Verbascum tabukum*** Hemaid **2***, Shigry, Tabuk, Saudi Arabia, *I.S. Collenette 4347* (E [E00066929]), OR197026, OR232467, OR232526, OR232405; ***Verbascum tabukum*** Hemaid **3***, Duba road, Tabuk, Saudi Arabia, *I.S. Collenette 9115* (K), OR197027, OR232468, OR232527, OR232406; ***Verbascum transjordanicum*** Murb. **1***, Harrat Al-Harrat, Turaif, Saudi Arabia, *A. Alzahrani 148* (MUZ), OR197028, OR232469, OR232528, OR232407; ***Verbascum transjordanicum*** Murb. **2***, Turaif, Saudi Arabia, *I.S. Collenette 9092* (E [E00092227]), OR197029, OR232470, OR232529, OR232408; ***Verbascum yemense*** Deflers **1***, Jabal An Nabi Shu'ayb, Sana'a, Yemen, *A. Miller 143* (E [E00066954]), OR197030, OR232471, OR232530, OR232409; ***Verbascum yemense*** Deflers **2***, Jabal An Nabi Shu'ayb, Sana'a, Yemen, *J.R.I. Wood 3097* (E [E00066956]), OR197031, OR232472, OR232531, OR232410; ***Verbascum yemense*** Deflers **3***, Jabal Al-Aswad, Jazan, Saudi Arabia, *A. Alzahrani 145* (MUZ), OR197032, OR232473, OR232532, OR232411; ***Verbascum yemense*** Deflers **4***, Al-Hada palm, Al-Hada, Taif, Saudi Arabia, *A. Alzahrani 155* (MUZ), OR197033, OR232474, OR232533, OR232412; ***Verbascum yemense*** Deflers **5!**, Al-Sahab park, Al-Soudah, Abha, Saudi Arabia, *A. Alzahrani 177* (MUZ). OUTGROUP: ***Scrophularia kiriloviana*** Schischk., DPC202-21; DPC202-21; DPC202-21; MW657274; ***Scrophularia multicaulis*** Turcz., ENDEM031-16, ENDEM031-16, ENDEM031-16, KY067910; ***Teedia lucida*** (Aiton) Rudolphi, AF375148, AF375187, AM235150, AJ608561.