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***Drimia cryptopoda*,**
a new combination in Hyacinthaceae
from Madagascar

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A b s t r a c t : The species *Hyacinthus cryptopodus* BAKER endemic to the island of Madagascar has been investigated. Based on karyological, morphological and molecular evidence, the taxon is suggested to be included within subfamily Urgineoideae and not within Hyacinthoideae, where other members of the genus *Hyacinthus* belong to. Consequently, *H. cryptopodus* has to be transferred to the genus *Drimia* as *D. cryptopoda* (BAKER) PFOSSER, WETSCHNIG & SPETA comb. nov.

K e y w o r d s : Hyacinthaceae, *Hyacinthus cryptopodus*, *Drimia*, Madagascar, cytology, *trnL-F* sequence data

Introduction

The Hyacinthaceae from Madagascar have never been in the focus of systematic research and therefore remain largely unknown until today. PERRIER DE LA BATHIE (1938) includes for the flora of Madagascar within Liliaceae-Scilleae (today circumscribed as the independent family Hyacinthaceae, SPETA 1998a, b) the six genera *Rhodocodon*, *Dipcadi*, *Hyacinthus*, *Urginea*, *Scilla* and *Ornithogalum*. The genus *Hyacinthus* in Madagascar comprises only one species *H. cryptopodus* BAKER.

The analysis of molecular data (mainly plastid DNA sequences) has corroborated the recognition of four subfamilies within Hyacinthaceae (SPETA 1998a, b; PFOSSER & SPETA 1999). The New World subfamily Oziroeoideae is restricted to South America, whereas the Old World subfamilies Urgineoideae, Ornithogaloideae and Hyacinthoideae show a more or less pronounced split between a primary center of diversity with species occurring mainly in southern Africa, Madagascar, Arabian peninsula and Indian subcontinent on the one hand and a secondary center of diversity in the Mediterranean/Eurasian region. This bimodal distribution pattern has resulted in the recognition of the tribes Dipcadiaceae and Ornithogaleae, and Massonieae and Hyacintheae for subfamilies Ornithogaloideae and Hyacinthoideae, respectively (SPETA 1998a, b; PFOSSER & SPETA 1999, 2004, WETSCHNIG & PFOSSER 2003). Later, subfamily Hyacinthoideae was further divided into a third monotypic tribe Prospereae (MANNING et al. 2004). In all molecular analyses aimed at resolving phylogenetic lineages in Hyacinthaceae, the generitype of *Hyacinthus*, *H. orientalis* is included with high support values in the northern hemisphere

tribe Hyacintheae of subfamily Hyacinthoideae (MANNING et al. 2004, PFOSSER & SPETA 1999, PFOSSER et al. 2003, WETSCHNIG & PFOSSER 2003). The phylogenetic separation into a northern hemisphere assemblage of taxa of tribe Hyacintheae and a southern hemisphere group (tribes Massonieae and Prospereae) already raised doubts on the systematic position of Madagascan *H. cryptopodus*. MANNING et al. (2004) obviously felt uneasy in keeping the generic assignment of this species and placed it into the predominantly southern hemisphere *Ledebouria* pending further evidence. They based their decision on morphological features like one or two globose seeds per locule and the loose bulb scales that produce fibres when torn. However, they also noted that the Madagascan species is distinctive in *Ledebouria* in its tubular flowers.

To contribute to the clarification of its systematic position we have re-evaluated morphological, karyological and molecular characters in *H. cryptopodus*.

Material and Methods

Taxa sampled. – This analysis is based on material of family *Hyacinthaceae* sampled throughout its distribution range from South Africa, Eurasia to East Asia. *Hyacinthus cryptopodus* was collected at the type locality in the Ankaratra massif (Madagascar). Voucher information for all plant accessions, geographic origin, and EMBL database accession numbers are provided in Appendix 1. Nomenclature follows that of SPETA (1998a, b) for subfamily Ozirocoideae, and tribe Hyacintheae of subfamily Hyacinthoideae, and that of MANNING et al. (2004) for all other taxa.

Karyological analysis. – Root tip meristems of plants grown in soil were used for studying chromosome morphology. Root tips were pretreated for 12 hours in a 0.002 M solution of 8-hydroxyquinoline at 5° C. After pretreatment, the root tips were fixed in ethanol:acetic acid 3:1. After staining with carmine acetic acid squash preparations of the meristems were performed.

DNA sequencing. – Total genomic DNA was extracted from lyophilized and powdered leaf or seed material in 700 µl CTAB buffer (2% CTAB, 100 mM Tris, 1.4 M NaCl, 20 mM EDTA, 0.2% mercaptoethanol, pH 8.0) for 30 minutes at 60° C. 500 µl chloroform/isoamylalcohol (24/1) were added and the extraction mix was incubated for 15 minutes at 4° C. After centrifugation, the DNA was precipitated with 500 µl isopropanol. The pellet was washed with 70% ethanol and dissolved in 100 µl TE buffer.

Two non-coding regions of the plastid genome were sequenced. The *trnL(UAA)* intron and the intergenic spacer (IGS) between the *trnL(UAA)*-3' exon and the *trnF(GAA)* gene were amplified together in a single PCR reaction (PFOSSER & SPETA 1999). Amplified double-stranded DNA fragments were sequenced directly on a MegaBace 500 automated sequencer (General Healthcare, USA) following the DYEnamicET cycle sequencing protocol (General Healthcare, USA). Both strands were sequenced using the nested sequencing primers as described for the *trnL-F* region (PFOSSER & SPETA 1999). On average, less than 1% of data matrix cells were scored as missing data.

All sequences have been deposited in the EMBL database (for accession numbers refer to Appendix 1). Twenty-five indels in the data matrix were coded as additional characters, and tree searches were performed using the nucleotide data together with the indel data. Phylogenetic analysis using the maximum parsimony (MP) method were

performed with the computer program PAUP* version 4.0b10 (SWOFFORD 2000). MP analyses were performed either without or with successive character weighting (rescaled consistency index) until tree lengths remained the same in two successive rounds. Most parsimonious trees were obtained by 1000 replicates of random sequence addition using tree bisection-reconnection (TBR) branch swapping under the Fitch criterion (FITCH 1971). Ten thousand fast bootstrap replicates (FELSENSTEIN 1985) were used to assess confidence limits for the resulting tree topologies.

Results and Discussion

Based on plastid DNA sequences a clear separation among the four subfamilies of Hyacinthaceae is supported by bootstrap values $\geq 75\%$ in the phylogenetic analysis (Fig. 1). Within Hyacinthoideae the classification into the monotypic South African tribe Pseudoprosperae, the north hemispheric, Eurasian and North African tribe Hyacintheae and the tribe Massonieae with species occurring south of the Sahara, the Arabian peninsula, Madagascar and India, as suggested by MANNING et al. (2004) is also supported. The generitype of *Hyacinthus*, *H. orientalis*, forms a highly supported clade (99% bootstrap support) with the north hemispheric genera *Puschkinia*, *Prospero*, *Hyacinthella*, *Fessia* and *Zagrosia* and no affinity to any of the members of Massonieae is found. Contrary to MANNING et al. (2004) who placed *Hyacinthus cryptopodus* based on morphological grounds into the genus *Ledebouria* of Massonieae, our phylogenetic analysis does not support this decision. Instead, based on plastid DNA sequences, *H. cryptopodus* clearly falls within subfamily Urgineoideae and appears to be closely related to other Madagascan members of subfamily Urgineoideae like *Drimia mascarenensis* and *D. urGINEOIDES* (84% bootstrap support).

In their generic synopsis MANNING et al. (2004) provide a key to the genera of Hyacinthaceae in sub-Saharan Africa and start with the dichotomous split of whether or not the bracts are spurred:

- 1a. Bracts (at least the lower) usually spurred, sometimes deciduous;.... (subfamily Urgineoideae) 2
 1b. Bracts never spurred..... 4

(1a) correctly leads further to the genera *Drimia*, *Igidia* and *Bowiea*, of subfamily Urgineoideae, whereas (1b) comprises all other genera including the genus *Ledebouria*. In the description of the genus they again emphasize the character state of the bracts as being not spurred. As it is obvious from Fig. 2B, the bracts of *H. cryptopodus* are clearly spurred and therefore this taxon cannot belong to *Ledebouria* but has to be placed within subfamily Urgineoideae, a position also corroborated by our molecular data.

Karyologically, *H. cryptopodus* with its diploid chromosome number of $2n=18$ also fits to subfamily Urgineoideae although the most abundant basic haploid chromosome number in this group appears to be $x=10$ (SPETA 1998a). The same basic chromosome number of $x=9$ as in *H. cryptopodus* is found, for example, in the generitype of *Drimia*, *D. elata* but also in the Rhadamanthus-group of *Drimia* sensu GOLDBLATT & MANNING 2000. This group also contains the closest relatives of *H. cryptopoda*, the Madagascan species *D. urGINEOIDES* and *D. mascarenensis* (SPETA 1998a). In *Ledebouria* sensu MANNING et al. (2004) a variety of chromosome numbers have been found from $2n=20$

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up to $2n=68$ (SPETA 1998a). The diploid chromosome number of $2n=10$ for *Ledebouria humifusa* (WETSCHNIG in WETSCHNIG & PFOSSER 2003) appears to be the only case so far where a chromosome number smaller than $2n=20$ has been reported for this group.

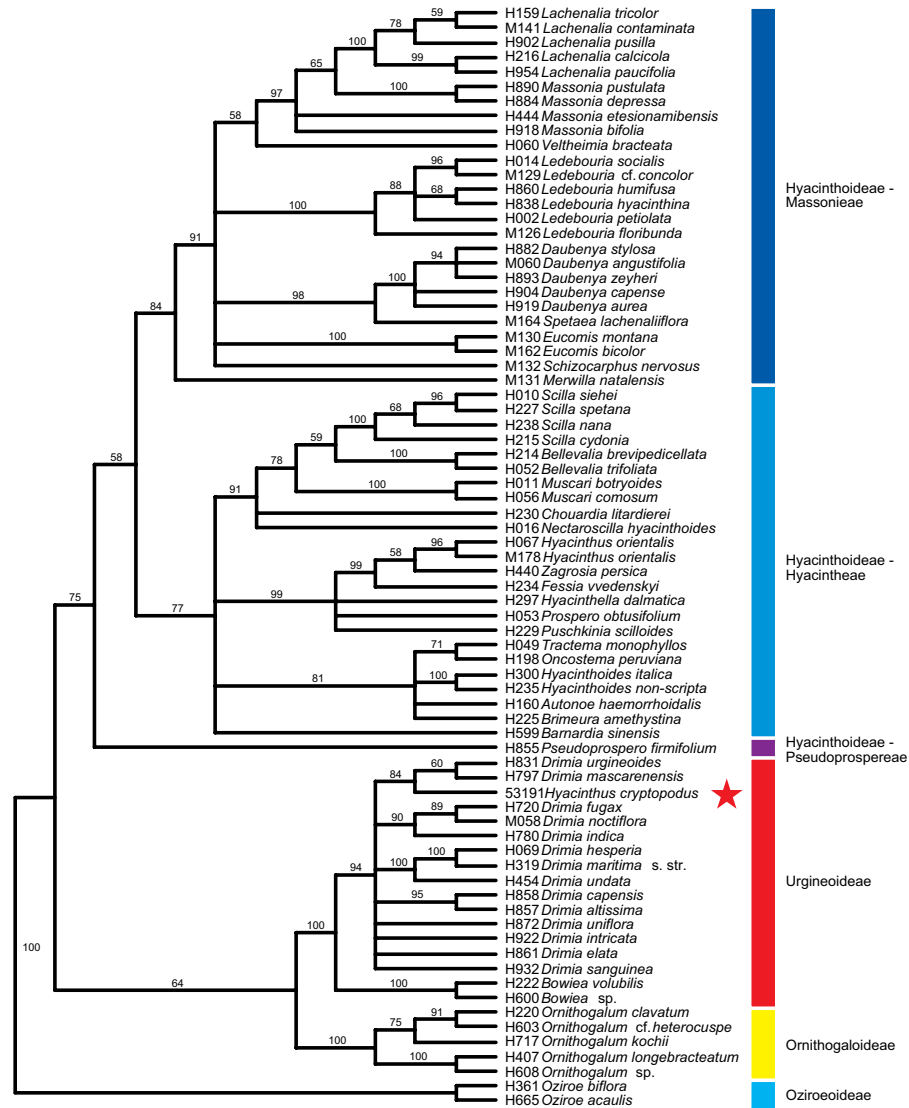


Fig. 1. Strict consensus phylogenetic tree (maximum parsimony) of family Hyacinthaceae. Bootstrap support values after character weighting are indicated above branches. The position of *Hyacinthus cryptopodus* within subfamily Urgineoideae is indicated by an asterisk.

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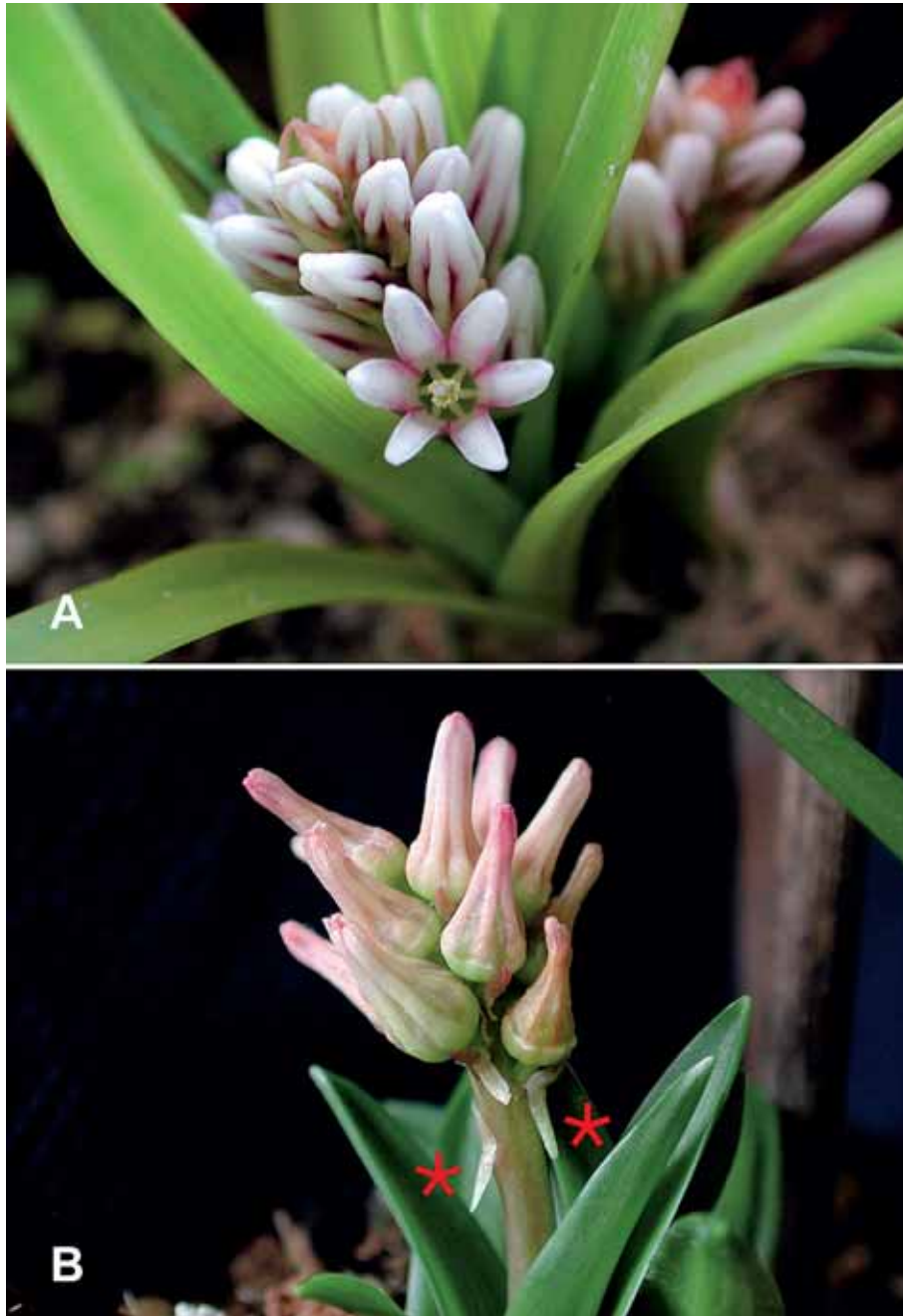


Fig. 2. *Hyacinthus cryptopodus*. The conspicuously spurred bracts are marked in B.

Based on molecular, morphological and karyological data *Hyacinthus cryptopodus* should be transferred from subfamily Hyacinthoideae to subfamily Urgineoideae. In the most recent generic synopsis (MANNING et al. 2004) subfamily Urgineoideae contains the three genera *Bowiea*, *Igidia* and *Drimia*. The genus *Bowiea* forms a clearly separated monophyletic basal clade in all analyses until now (Fig. 1, PFOSSER & SPETA 1999, 2004, WETSCHNIG & PFOSSER 2003, MANNING et al. 2004). Although *Igidia* was not available for analysis and the genus *Drimia* itself may prove to be a rather heterogeneous assemblage when analyzed more thoroughly we suggest *H. cryptopodus* to be placed best within *Drimia* sensu MANNING et al. (2004) for the time being. This decision is not intended as a final one, but should serve as a starting point for future research in an alliance still suffering from a lack of detailed systematic knowledge on the species level. The same holds true for subfamily Ornithogaloideae which lacks an adequate distribution of species into more uniform genera. To adopt a broad and comprehensive generic concept like that of MANNING et al. (2004) only shifts problems to another level, because the distribution of species into genera mainly defined by molecular data results in genera unrecognizable by morphological synapomorphies. Possibilities for further subdivisions for all subfamilies of Hyacinthaceae based on a combination of morphological, cytological, karyological and chemotaxonomical characters have already been proposed (SPETA 1998a, b, PFOSSER & SPETA 1999). Recently, *Drimiopsis* and *Resnova* have been resurrected as independent genera of their own again (LEBATHA et al. 2006), after they have been sunk into *Ledebouria* before (MANNING et al. 2004). This could serve as an example of how to arrive at a situation with more homogeneous and thus also more practically recognizable genera.

Drimia cryptopoda (BAKER) PFOSSER, WETSCHNIG & SPETA, **comb. nov.** Basionym: *Hyacinthus cryptopodus* BAKER, J. Linn. Soc., Bot. **20**: 274 (1883). Synonym: *Ledebouria cryptopoda* (BAKER) J.C. MANNING & GOLDBLATT, Edinb. J. Bot. **60**: 560 (2004).

Zusammenfassung

Die für Madagaskar endemische Art *Hyacinthus cryptopodus* BAKER wurde untersucht. Auf Grund von karyologischen, morphologischen und molekularen Daten scheint klar zu sein, dass diese Art in die Unterfamilie Urgineoideae zu stellen ist und nicht zur Unterfamilie Hyacinthoideae, zu der andere Arten der Gattung *Hyacinthus* zu rechnen sind. Konsequenterweise muss daher *Hyacinthus cryptopodus* zu *Drimia cryptopoda* (BAKER) PFOSSER, WETSCHNIG & SPETA comb. nov. neu kombiniert werden.

Acknowledgements

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Appendix 1: List of taxa investigated in this study, with vouchers, citation information and EMBL accession numbers. All vouchers are deposited in LI. Two accession numbers for the *trnL*-F locus indicate that the sequences of the *trnL* intron and the *trnL*-F spacer have been deposited separately.

Species	Voucher	Origin	EMBL acc.
<i>Autonoe haemorrhoidalis</i> (WEBB & BERTH.) SPETA	Klenner H160	Spain	AJ232518/AJ2326411
<i>Barnardia scilloides</i> LINDL.	Pfossen H599	Korea	AJ507998
<i>Bellevalia trifoliata</i> KUNTH	Speta H052	Greece	AJ232548/AJ2326711
<i>Bellevalia brevipedicellata</i> TURRILL	Jahn H214	Greece	AJ232547/AJ2326701
<i>Bowiea</i> sp.	Pfossen H600	Madagascar	AJ507922
<i>Bowiea volubilis</i> HARV. ex HOOK. f.	Pfossen H222	S Africa	AJ232454/AJ2325771
<i>Brineura amethystina</i> (L.) CHOUARD	Pfossen H225	cult. ex B.G. Tallinn	AJ232510/AJ2326331
<i>Chouardia litardierei</i> (BREISTR.) SPETA	Pfossen H230	Croatia	AJ232541/AJ2326641
<i>Daubenya angustifolia</i> (L.f.) A.M. VAN DER MERWE & J.C. MANNING	Wetschnig 1101	S Africa	AJ507960
<i>Daubenya aurea</i> LINDL.	Wetschnig 1162	S Africa	AJ507956
<i>Daubenya capense</i> SCHLTR.	Wetschnig 1129	S Africa	AJ507955
<i>Daubenya stylosa</i> (W.F. BARKER) A.M. VAN DER MERWE & J.C. MANNING	Wetschnig 1160	S Africa	AJ507957
<i>Daubenya zeyheri</i> (KUNTH) J.C. MANNING & A.M. VAN DER MERWE	Wetschnig 1153	S Africa	AJ507961
<i>Drimia altissima</i> (L. f.) KER GAWL.	Wetschnig H857	S Africa	AM410909
<i>Drimia capensis</i> (BURM.F.) D.O. WIJNANDS	Wetschnig H858	S Africa	AM410910
<i>Drimia cryptopoda</i> (BAKER) PFOSSER, WETSCHNIG & SPETA	Sieder 53191	Madagascar	AM410914
<i>Drimia elata</i> JACQ.	E. v. Jaarsveld s. n.	S Africa	AM410906
<i>Drimia fugax</i> (MORIS) STEARN	J. Sixtus H720	Tunisia	AM410913
<i>Drimia hesperia</i> (WEBB & BERTH) J.C. MANNING & GOLDBLATT	Speta H764	Spain	AJ4260882
<i>Drimia indica</i> (ROXB.) JESSOP	Jha H780	India	AM410911
<i>Drimia intricata</i> (BAKER) J.C. MANNING & GOLDBLATT	Pfossen H922	S Africa	AM410907
<i>Drimia maritima</i> s. str. (L.) STEARN	Speta H319	Spain	AJ232466/AJ232589
<i>Drimia mascarenensis</i> (BAKER) J.C. MANNING & GOLDBLATT	Pfossen H610	Madagascar	AJ507923
<i>Drimia noctiflora</i> (BATT. & TRAB.) STEARN	Pfossen M058	Morocco	AM410912
<i>Drimia uniflora</i> J.C. MANNING & GOLDBLATT	Wetschnig H872	S Africa	AM410908
<i>Drimia sanguinea</i> (SCHINZ) JESSOP	Speta H932	S Africa	AM410905
<i>Drimia undata</i> STEARN	Raus M117	Israel	AJ507924
<i>Drimia urgineoides</i> (BAKER in HOOKER) J.C. MANNING & GOLDBLATT	Pfossen H831	Madagascar	AM410915
<i>Eucomis bicolor</i> BAK.	Schnabel M162	S Africa	AJ507933
<i>Eucomis montana</i> COMPTON	Schnabel M130	S Africa	AJ507932
<i>Fessia vvedenskyi</i> (PAZIJ) SPETA	Speta H234	Uzbekistan	AJ232535/AJ2326581
<i>Hyacinthella dalmatica</i> CHOUARD	Gutermann H297	Croatia	AJ232526/AJ2326491
<i>Hyacinthoides italica</i> (L.) ROTHM.	Pfossen H300	France	AJ232519/AJ2326421
<i>Hyacinthoides non-scripta</i> (L.) CHOUARD ex ROTHM.	Pfossen H235	France	AJ232524/AJ2326471
<i>Hyacinthus orientalis</i> L.	Speta H067	Romania	AJ232539/AJ2326621
<i>Hyacinthus orientalis</i> L. var. <i>alba</i>	J. Plass M178	Syria	AJ508002
<i>Lachenalia calcicola</i> (U. & D. MÜLLER-DOBLIES) J.C. MANNING & GOLDBLATT	Müller-Doblies H216	S Africa	AJ232506/AJ2326291

Appendix 1, continued

Species	Voucher	Origin	EMBL acc.
<i>Lachenalia contaminata</i> AITON	Pfosser M141	S Africa	AJ507985
<i>Lachenalia paucifolia</i> (W.F. BARKER) J.C. MANNING & GOLDBLATT	Wetschnig 1154	S Africa	AJ507990
<i>Lachenalia pusilla</i> JACQ.	Wetschnig 1115	S Africa	AJ507986
<i>Lachenalia tricolor</i> (L. f.) ENGL.	Pfosser H159	cult. B.G. Vienna	AJ232508/AJ2326311
<i>Ledebouria</i> cf. <i>concolor</i> (BAK.) JESSOP	Wetschnig 1412	S Africa	AJ507946
<i>Ledebouria floribunda</i> (BAKER) JESSOP	Wetschnig 1433	S Africa	AJ507937
<i>Ledebouria humifusa</i> (BAKER) J.C. MANNING & GOLDBLATT	Wetschnig 1524	S Africa	AJ507942
<i>Ledebouria hyacinthina</i> ROTH.	Jha H838	India	AJ507944
<i>Ledebouria petiolata</i> (LINDLEY) J.C. MANNING & GOLDBLATT	Speta H002	cult. LI	AJ232502/AJ2326251
<i>Ledebouria socialis</i> (BAKER) JESSOP	Pfosser H014	cult. B.G. Vienna	AJ232501/AJ2326241
<i>Massonia bifolia</i> (JACQ.) J.C. MANNING & GOLDBLATT	Wetschnig 1130	S Africa	AJ507966
<i>Massonia depressa</i> HOUTT.	Wetschnig 1142	S Africa	AJ507980
<i>Massonia etesionamibensis</i> (MÜLLER-DOLBLES) J.C. MANNING & GOLDBLATT	Lavranos & Pehlemann H444	Namibia	AJ232504/AJ2326271
<i>Massonia pustulata</i> JACQ.	Wetschnig 1148	S Africa	AJ507970
<i>Mervilla natalensis</i> (PLANCHON) SPETA	Wetschnig 1534	S Africa	AJ507931
<i>Muscari botryoides</i> (L.) MILL.	Kleesadl H011	Austria	AJ232545/AJ2326681
<i>Muscari comosum</i> (L.) MILL.	Neuner H056	Italy	AJ232546/AJ2326691
<i>Nectaroscilla hyacinthoides</i> (L.) PARL.	Scheibltreiter H016	Portugal	AJ232542/AJ2326651
<i>Oncostema peruviana</i> (L.) SPETA	Pfosser H198	Portugal	AJ232516/AJ2326391
<i>Ornithogalum</i> cf. <i>heterocuspis</i> (BAKER) J.C. MANNING & GOLDBLATT	Pfosser H603	Madagascar	AJ507926
<i>Ornithogalum clavatum</i> (MASTERS) J.C. MANNING & GOLDBLATT	Speta H220	cult. B.G. Vienna	AJ232475/AJ2325981
<i>Ornithogalum kochii</i> PARL.	Speta H717	Slovenia	AJ507927
<i>Ornithogalum longibracteatum</i> JACQ.	Pfosser H407	cult. B.G. Vienna	AJ232471/AJ2325941
<i>Ornithogalum</i> sp.	Pfosser H608	Madagascar	AJ507925
<i>Oziroë acaulis</i> (BAKER) SPETA	Weigend s.n.	Peru	AJ507921
<i>Oziroë biflora</i> (RUIZ & PAVON) SPETA	MWC 793 (K)	Chile	AJ232453/AJ2325761
<i>Prospero obtusifolium</i> (POIRET) SPETA	HC H053	Morocco	AJ232529/AJ2326521
<i>Pseudoprospero firmifolium</i> (BAKER) SPETA	Wetschnig 1322-01	S Africa	AJ507928
<i>Puschkinia scilloides</i> ADAMS. var. <i>libanotica</i>	Pfosser H229	Lebanon	AJ508688
<i>Schizocarphus nervosus</i> (BURCH.) VAN DER MERWE	Saunders M132	S Africa	AJ507936
<i>Scilla cydonia</i> SPETA	Jahn et al. H215	Greece	AJ232549/AJ2326721
<i>Scilla nana</i> (J.A. & J.H. SCHULTES) Speta	Speta H238	Greece	AJ232552/AJ2326751
<i>Scilla siehei</i> (STAPF) SPETA cv. "Pink Giant"	Speta H010	cult. LI	AJ232551/AJ2326741
<i>Scilla spetana</i> KERESZTY	Speta H227	Austria	AJ232556/AJ2326791
<i>Spetaea lachenaliiflora</i> WETSCHNIG & PFOSSER	Saunders M164	S Africa	AJ507954
<i>Tractema monophylos</i> (LINK) SPETA	Raus H049	Spain	AJ232513/AJ2326361
<i>Veltheimia bracteata</i> HARV. ex BAKER	Speta H060	cult. LI	AJ232503/AJ2326261
<i>Zagrosia persica</i> (HAUSSKN.) SPETA	Leep H440	Turkey	AJ232537/AJ2326601

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