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Journal of Threatened Taxa

The international journal of conservation and taxonomy

www.threatenedtaxa.org

ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

ARTICLE

REDISCOVERY, SYSTEMATICS AND PROPOSED RED LIST STATUS OF LEDEBOURIA JUNNARENSIS S.S. RAHANGDALE AND S.R. RAHANGDALE NOM. NOV. (ASPARAGACEAE) - AN ENDEMIC SPECIES FROM THE WESTERN GHATS, MAHARASHTRA, INDIA

Savita Sanjaykumar Rahangdale & Sanjaykumar Ramlal Rahangdale

26 February 2016 | Vol. 8 | No. 2 | Pp. 8421–8433 10.11609/jott.2167.8.2.8421-8433



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ISSN 0974-7907 (Online) ISSN 0974-7893 (Print)

ODEN 400500





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Abstract: A new name in the genus *Ledebouria* Roth is validated for *Scilla viridis* Blatter & Hallberg [non *Scilla viridis* (L.) Salisbury]. It is rediscovered after about 85 years of its first and only report. It is also redescribed on the basis of morphology, anatomy, cytology and assigned the name *Ledebouria junnarensis* S.S. Rahangdale & S.R. Rahangdale belonging to the family Asparagaceae, subfamily Hyacinthoideae. As this is a species endemic to the Western Ghats, Maharashtra, India, it is studied for threat status as per IUCN criteria & guidelines and assigned the status Critically Endangered B1ab(iii)+2ab(iii). Identification keys for the genera and species of subfamily Hyacinthoideae reported from India are prepared on the basis of reported and observed characters.

Keywords: Asparagaceae, cytology, Critically Endangered, endemic, Hyacinthoideae, India, IUCN Red List assessment, Maharashtra, *Nomen novum*, systematics, Western Ghats.

Marathi Abstract: शतावरी कुळातील लेडिबोरिया प्रजातीमधील 'उन्मादकंद' किंवा 'पुष्पायतिकंद' म्हणजे सिला हिरिडीस ब्लॅटर व हालवर्ग ह्या जातीसाठी नविन वानसशास्त्रीय नाव अधिकृत करण्यात आले आहे . ही वनस्पती तिच्या प्रथम व एकमेव नोंदीनंतर ८५ पेक्षा जास्त वर्षांनी पुनर्शोधित करण्यात आली आहे . सोवतच तिच्या वाह्यांग, अंतरंग, प्रजनन, तसेच कोशिकीय वानसशास्त्रीय अभ्यासानंतर पुनर्विणंत करून लेडिबोरिया जुन्नरेंसिस एस एस . एस . एहां एडाले व एस . आर . एहां एडाले असे नवनामांकरण केले आहे . ही वनस्पती महाराष्ट्रातील उत्तर पश्चिम घाटामध्ये स्थानवद्ध असल्यामुळे तिचा आय . यु. सी. एन . ची आंतरराष्ट्रीय मानके व मार्गदर्शक तत्वांनुसार सखोल अभ्यास करून ह्या वनस्पतीस 'अतीभयग्रस्त' जाती म्हणून प्रमाणित करण्यात येत आहे . सोवतच ह्या कुळातील भारतात आढळत असलेल्या लेडिबोरिया (खाजकंद) सारख्या इतर प्रजाती व त्यातील जातींची ओळख पटविण्यासाठी ओळख किल्ली सदर शोधनिवंधात देण्यात आली आहे .

DOI: http://dx.doi.org/10.11609/jott.2167.8.2.8421-8433

Editor: N.P. Balakrishnan, Retd. Joint Director, BSI, Coimbatore, India.

Date of publication: 26 February 2016 (online & print)

Manuscript details: Ms # 2167 | Received 20 July 2015 | Final received 03 February 2016 | Finally accepted 05 February 2016

Citation: Rahangdale, S.S. & S.R. Rahangdale (2016). Rediscovery, systematics and proposed Red List status of *Ledebouria junnarensis* S.S. Rahangdale and S.R. Rahangdale nom. nov. (Asparagaceae) - an endemic species from the Western Ghats, Maharashtra, India. *Journal of Threatened Taxa* 8(2): 8421–8433; http://dx.doi.org/10.11609/jott.2167.8.2.8421-8433

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Funding: None.

Conflict of Interest: The authors declare no competing interests.

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Author Contribution: SSR contributed in the taxonomic part of the study materials, while SRR dealt with cytology, anatomy and comparisons with the other species. The paper was written jointly by both the authors with equal contribution.

Acknowledgements: Authors sincerely thank Dr. Brita Stedje, University of Oslo, Norway and Dr. Bohumil Trávniček, Palacky University, Czech Republic for providing valuable literature and suggestions. Critical suggestions and support from Dr. Aparna Watve of Tata Institute of Social Sciences, Tuljapur Campus are also gratefully acknowledged. The thanks are due to Hon. Editor Dr. N.P. Balakrishnan for his valuable suggestions about nomenclature and Dr. Sanjay Molur for the help in IUCN analysis. The help extended from Botanical Survey of India, Western Circle, Pune; Agharkar Research Institute, Pune and Blatter Herbarium, St Xavier's College, Mumbai is also being acknowledged with sincere thanks. We also thank the Department of Forests, Government of Maharashtra for support during the field work and the Management authorities and Principals of DGM's B.J. Arts, Commerce and Science College, Ale and PDEA's A.W. College, Otur for facilities.





INTRODUCTION

The genus Ledebouria Roth of the family Asperagaceae, subfamily Hyacinthoideae is distributed in tropical Africa and Asia including India. The genus was described on the basis of the type species L. hyacinthina Roth from India in 1821 (Venter 2008). The species was later placed in the genus Scilla L. under the section Ledebouria involving many species of genus Scilla by Baker (1870). After the study of South African bulbous Liliaceae collections, Jessop (1970) reinstated the genus Ledebouria, on the basis of bulb with deciduous leaves, erect/flaccid inflorescences, pair of basal ovules in each locule and stipitate ovary. A revision of the genus Ledebouria for South Africa and sub-Saharan collections was done by Venter (2008), comprising more than 60 species. Based on his observations, he stated that, there may be more than one species of this genus in India.

Speta (1998a) revised the family Hyacinthaceae and on the basis of morphological, anatomical, developmental, and biochemical characters delimited a total of 65 genera of the family under five subfamilies, viz.: Chlorogaloideae Oziroeoideae Speta, Urgineoideae Speta, Ornithogloideae Speta and Hyacinthoideae Link. According to her the genus Ledebouria comprising about 50 species and Fessia Speta comprising about 10 species are represented by 1 or 2 species in India, while the genus Scilla L. (sensu stricto) comprising about 30 species is naturally distributed in European, south-west Asian and Mediterranean regions but does not occur in India as well as south of the Sahara. Speta (1998a) also commented that sexual reproduction is predominant; besides this, agamospermy and vegetative reproduction by bulbils may also occur in some taxa. Lenski (1958) reported that, some species of Ledebouria produce bulbils on the leaf tips. The phylogenetic linkages of the taxa in family Hyacinthaceae were studied by Pfosser & Speta (1999) on the basis of plastid DNA sequences. They placed the genus Scilla in Scilla clade, Fessia with two species complexes: (1) the S. hohenackeri Fischer & Meyers group and (2) the S. bisotunensis Speta group in Fessia clade and Ledebouria in Massonieae clade. On the basis of phylogenetic and molecular characters it has been shown that the genus Scilla is not monophyletic and divided into several smaller genera, viz.: Zagrosia Speta, Prospero Salisbury, Othocallis Salisbury, Nectaroscilla Parlatore, Fessia and Scilla (Pfosser & Speta 1999). Further, Stedje (2001) emphasized the use of qualitative morphological characters for generic delimitation of Hyacinthaceae and the molecular or quantitative data as supportive information. Thus, on the basis of the above facts it is clear that the Indian taxa treated under the genus *Scilla* need critical observations and revision to resolve the taxonomic ambiguity.

In older Indian floras (Hooker 1892; Cooke 1958) genus Scilla L. has been treated under the family Liliaceae. In India, only three species of this genus were recorded: S. hohenackeri Fischer & Meyers, S. hyacinthina (Roth) J.F. McBride, and S. viridis Blatter & Hallberg (Deb & Dasgupta 1981; Karthikeyan et al. 1989). The Indian species traditionally placed in the genus Scilla L., are now segregated into three distinct clades (i.e., the Massonieae clade, Fessia clade and Scilla clade) and taxonomically treated as Ledebouria revoluta (L.f.) Jessop (Syn. Ledebouria hyacinthina Roth, Scilla hyacinthina (Roth) J.F. McBride, S. indica Baker) (Lebatha et al. 2006; Venter 2008; Govaerts 2013); Fessia hohenackeri (Fisch. & C.A. Mey.) Speta (Syn. Scilla hohenackeri Fischer & Meyers) (Govaerts 2013); and Scilla viridis Blatter & Hallberg. Recently, a new species, Ledebouria hyderabadensis Ramana, Prasanna & Venu with erect leaves, without blotches on leaves, penicillate stigma and 1 or 2 lobed capsules, was described from Hyderabad and surrounding areas having a close affinity to L. revoluta and was added to the Indian flora (Ramana et al. 2012).

Of these, S. viridis Blatt. & Hallb. is endemic to Maharashtra (India) and considered to be 'possibly extinct' (Mishra & Singh 2001). The species was collected for the first time from 'Khandala' [Centre of 'Khandala' Village 18°0'N & 73°23'E (as per Santapau 1958)] by McCann in vegetative state in September 1918. It was planted in St. Xaviers' College, Mumbai, where it bloomed in the month of March 1919 and based on this flowering it was described as a new species with the Holotype-14500 (BLAT!) by Blatter & Hallberg (1921). The original protologue of the species comprises only description in the Latin and type specimen, without any figure, geographic coordinates, elevation or ecological notes. Santapau (1958) did not record the species from 'Khandala' in his 'The Flora of Khandala on the Western Ghats of India'. Subsequent attempts by different taxonomists were also unfruitful in collection of the species from Khandala, the type location or elsewhere (Mishra & Singh 2001). Only the Holotype - 14500 (BLAT!) is the proof for its occurrence in India (Blatter & Hallberg 1921; Santapau 1958). Therefore, Nayar & Sastry (1987) included it in the Red Data Book under Endangered category and also by Sharma et al. (1996). While doing a reassessment of threatened flowering plants of Maharashtra, Mishra & Singh (2001) were also unable to re-collect the species and it was declared

'probably extinct'. The Plant List placed it in the category of 'unresolved taxa names' because of deficiency of information on the taxon and also non-availability of the authentic specimens (The Plant List 2015).

In the year 2005 during the month of June, the present authors collected interesting specimens, which were distinct from commonly occurring *Ledebouria revoluta*, from Durgawadi-Pimparwadi area of Taluk Junnar, District Pune, Maharashtra State. The vegetative and reproductive characters of the specimens resulted in their identification as *S. viridis* Blatt. & Hallb. (Rahangdale 2008). Moreover, as there are eco-morphs and cytological variations in *L. revoluta*, and *S. viridis* was considered to be possibly extinct, it was decided that, the specimens needed further confirmation on the basis of critical observations, comparison with specimens of other species and detailed study.

The homonymy in the species remained unnoticed for a long time and Karthikeyan et al. (1989), Sharma et al. (1996), and Mishra & Singh (2001) considered the taxa, S. viridis and S. hohenackeri as accepted names in their works. In the Flora of Maharashtra (Almeida 2009), S. viridis was transferred to the genus Ledebouria Roth as a new combination Ledebouria viridis (Blatt. & Hallb.) S. Datta and P. Harvey based on the holotype McCann, Blatter 14500, with description in English on the basis of the original Latin protologue and Scilla viridis Blatt. & Hallb. as a synonym. Interestingly, in the original protologue and the description in the Flora of Maharashtra (Almeida 2009) there is no comment on the leaf characters. Until the year 2015, the species was placed as an 'unresolved taxon' on the databases of The Plant list (2015) and IPNI (2015) (both accessed on 22 June 2015); while S. hohenackeri as a synonym of Fessia hohenackeri (The Plant list 2015). On 14 August 2015 the status of the taxon under study, at IPNI was revised by Prof. K.N. Gandhi and named Ledebouria viridis S.R. Datta & P. Harvey ex Almeida with the remark, 'The protologue shows Ledebouria viridis as a new combination' (IPNI 2015; assessed on 03 January 2016).

On searching the literature, it was found that, the name *Scilla viridis* Blatter & Hallberg is a later homonym of *Scilla viridis* (L.) Salisbury, [Prodr. Stirp. Chap. Allerton 243. 1796; with the synonyms *Lachenalia viridis* Soland. in Ait. Hort. Kew, v.1, p: 462 and *Hyacinthus viridis* L. Sp. Pl. ed. 2, p: 554]. The types are also different for both these homonyms. Therefore, according to ICBN Article 53.1 (McNeil et al. 2012) *Scilla viridis* Blatt. & Hallb. becomes an illegitimate name. Later, the species was transferred to the genus *Ledebouria* Roth as *Ledebouria viridis* (Blatt. & Hallb.) S. Datta & P. Harvey

and validly published as per ICBN Article 41.8. It is a new combination based on the illegitimate name *Scilla viridis* Blatt. & Hallb. and its only type. Thus, the new combination also becomes illegitimate as per ICBN Article 58.1 (Ex.1.) and Article 11.4 (For any taxon below the rank of genus, the correct name is the combination of the final epithet of the earliest legitimate name of the taxon in the same rank with the correct name of the genus to which it is assigned). Therefore, a new name is required for the taxon.

As the species has been re-collected after about 85 years since its first and only collection, its IUCN categorization was also needed. Hence, after a critical study during the period 2005 to 2014 with respect to morphology, phenology, anatomy, cytology and reproduction, it was assigned a new name. The IUCN status has also been assigned and the identification keys for the genera and species found in India are prepared and presented in this paper.

MATERIALS AND METHODS

Plant Materials

Specimens of the species under study were collected from Durgawadi Pimparwadi area, Taluk Junnar, district Pune, and for comparison the specimens of *Ledebouria* were also collected from different locations in Maharashtra state comprising the Western Ghats, Konkan, Pune, Marathwada and Vidarbha regions in subsequent years (Table 1). The plant specimens were observed in their natural habitat as well as under cultivation till date for phenology of the specimens, fruit set, and other characters. The herbarium specimens at Blatter Herbarium (BLAT!), St. Xavier's College, Mumbai; Agharkar Herbarium Maharashtra Association for Cultivation of Sciences (AHMA!), Agharkar Research Institute, Pune; and Herbarium of Botanical Survey of India, Western Regional Centre (BSI!), Pune were

Table 1. Plant specimens collected for the present study.

	Species	Locations of collection	Year of collection
1	L. junnarensis nom. nov.	Durgawadi area, Pune	2005, 2012
2	L. revoluta	Ratnagiri	2008
3	L. revoluta	Junnar, Pune	2005, 2008
4	L. revoluta	Kas, Satara	2009
5	L. revoluta	Deogiri, Aurangabad	2012
6	L. revoluta	Gondia	2014

referred to for comparison and confirmation of identity of the collected specimens.

Methods

The cytological observations were made using root tips treated with an aqueous solution of Colchicine (0.02%) for one hour to arrest the metaphase in dividing root tip cells and in the separation of chromosomes. The root tips were then fixed using Carnoy's Fluid II and then squashed and stained with aceto-carmine. The observations were made under a microscope using 100×10 objective to eyepiece magnification (Dnyansagar 1986).

The observations of anatomical characters were made using Lawrence & Mayo Stereo-zoom microscope for micro-morphological characters and Research microscope attached with micro-photographic set up with a Nikon Coolpix 6000 digital camera.

The identification keys for genera and species recorded from India are prepared on the basis of literature, observed characters of specimens and the online herbarium specimens of the Royal Botanic Gardens Kew, England available on the website www. kew.org/science-conservation/collections/herbarium at apps.kew.org/ herbcat/navigator.do. (RBG Kew 2015).

For threat status assessment, the number of individuals was recorded at each visit to the location and the geo-coordinates were obtained using hand-held GPS (Garmin etrex vista HCX). The primary and secondary information required as per IUCN Red List Categories and Criteria version 6.2 were collected, following the guidelines for assigning the criteria (Standards and Petitions Working Group IUCN 2006).

RESULTS

The present study leads to the identification of the specimens as *S. viridis* Blatt. & Hallb. *nom. illeg.*, which could not be used. As per the current taxonomy, the species fits in the genus *Ledebouria* Roth and should be named accordingly. The epithet 'viridis' also could not be used as per ICBN Article 11.4, therefore, the species is renamed as:

Ledebouria junnarensis S.S. Rahangdale & S.R. Rahangdale, nom. nov.

Replaced synonyms

Scilla viridis Blatt. & Hallb. in J. Indian Bot. 2: 52 (1921), nom. illeg.;

Ledebouria viridis (Blatt. & Hall.) S. Dutta & P. Harvey in Almeida, Fl. Mah. 5A: 187 (2009) nom. illeg.;

non. Scilla viridis (L.) Salisbury in Prodr. Stirp. Chap. Allerton 243 (1796). Deb & Dasgupta in Bull. Bot. Surv. India 14: 45. (1975) 1978 & Fasc. Fl. India 7: 15 (1981); Karthikeyan et al. in Florae Ind. Enum. Monocot. Flora India ser. 4: 101. (1989); Sharma et al., in Fl. Mah. State, Monocot. 40-41. (1996); Mishra & Singh in End. Threat. Fl. Pl. Mah. 240-241. (2001).

Type

Holotype: 14500 (BLAT!), September 1918, Khandala, Pune District, Maharashtra, India, 18°0'N & 73°23'E, elevation 536m, coll. McCann.

Paratypes: 22190, 22191 (AHMA!), 18.vi.2005, Durgawadi, Junnar Taluk, Pune District, Maharashtra, India, 19°11'42.10"N & 73°43'43.69"E, 990m, coll. S.B. Nagarkar (now S.S. Rahangdale); 235, 236, 237, 238 (Herbarium of B.J. College, Ale), 25.vi.2012, Inglun Ghat (Durgawadi), Junnar Taluk, 19°11'42.10"N & 73°43'43.69"E, 1068m, coll. S.S. Rahangdale.

Etymology of the epithet

The epithet 'junnarensis' is derived from the locality of the present specimens, falling under the tehsil Junnar in Pune District, Maharashtra State, India. The re-collection of specimens from the original location Khandala was unfruitful and considered to be extirpated from there

Description

Perennial bulbous scapigerous herbs. Bulb without bulblets, tunicate, tunic brown, lustrous on inner side, ca. 4.2 × 2.5 cm, ovoid, solid; roots arising from the basal disc of bulb, roots thick, branched. Bulb scales of three vegetation periods with one scape per year, imbricate, 2 in one ring. Leaves radical, thickened in the middle, without conspicuous veins, 3–4 in number in one vegetative period, hysteranthous, ca. 14–45×1.5–2.1 cm, length increases after flowering, linear; canaliculate towards tip, apex acute with blunt, fleshy, pink tip; leaves pinkish-green when young, fully develop after flowering, spots on lamina vinaceous, disappear at maturity; the leaf tip always produces one or rarely two bulbils maturing into a plantlet at the completion of the

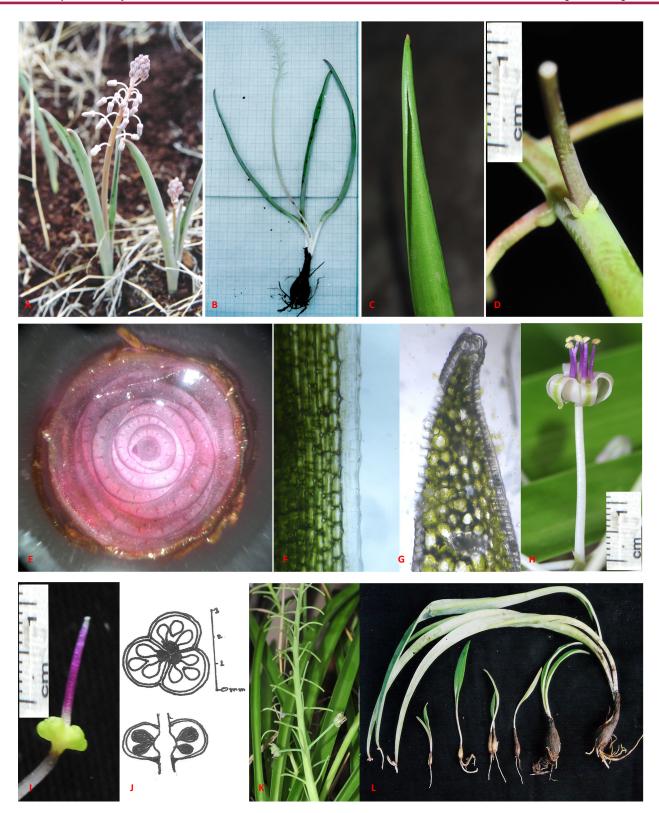


Image 1. Ledebouria junnarensis S.S. Rahangdale & S.R. Rahangdale.

A - Habit; B - Single plant at full growth on graph paper; C - Canaliculate leaf with fleshy tip; D - Bracts; E - Cross section of bulb showing scale leaves of 2–3 vegetation period with single scape per vegetation period; F - Leaf margin; G - Cross section of leaf margin; H - Flower; I - Carpel; J - TS & VS of ovary; K - Fruits with persistent perianth; L - Multiplication through bulbils produced at leaf tips (series of developmental stages). (Photographs and diagrams by S.R. Rahangdale)

vegetation period (Image 1). Scape single from each bulb, ca. 18-40cm long, pinkish-purple when young, erect, glabrous. Inflorescence lax raceme, from upper half of scape, 15–30 flowered. Flowers purplish-green, 0.8-1.2 cm in diameter, stellate, on 1.0-1.5 cm long pedicels with two unequal bracts of ca. 0.1-0.2 cm long, glabrous. Perianth reflexed, tepals 6, purplish, ca. 0.5cm long each with a single prominent green vein, perigone forming very short tube at base. Stamens 6, epitepalous, outer 3 longer than inner; filaments dark pink to purple, glabrous, anthers purple, small, oblong, dorsifixed, pollen grains yellow. Ovary tricarpellary, stipitate, forming a dome, trilocular, subtrigonous, green, style ± equal to the length of filament, purple, ovules 2 or 3 in each locule on axile placenta, ovules not side by side. Fruit set rarely. Perianth persistent at fruiting. Seeds ellipsoidal, dark brown, shiny with polygonal reticulation on testa.

Phenology

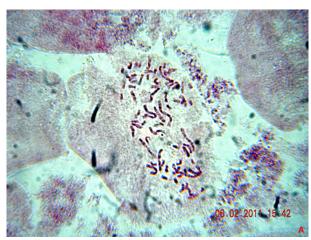
Plants emerge after the pre-monsoon showers as inflorescence and 2 or 3 leaves in the month of May–June. The leaves grow very fast after the flowering and attain a length of 30–45 cm. Fruits develop and mature in June-July, but the number of mature fruits per plant is very low (about 1–3 fruits). The same flowering period has been observed for the specimens under cultivation. The plants remain dormant from September to mid May in the form of underground bulbs.

Ecology

The population occurs on strong slopes of well-aerated deep soil rich in humus accumulated from grass biomass. The habitat is shared by other ephemerals, viz.: Chlorophytum tuberosum Baker, Crinum latifolium L. var. latifolium, Cyanotis tuberosa (Roxb.) J.A. & J.H. Schult., Euphorbia fusiformis Buch-Ham. ex D. Don, Habenaria grandifloriformis Blatt. & McCann, and Iphigenia magnifica Ansari & Rolla Rao and Iphigenia pallida Baker. The average annual rainfall is about 200–250 cm with a cool climate from June to October, and misty climate with saturation of relative humidity from July to September. The annual air temperature ranges between 15°C and 36°C.

Cytology

The mitotic metaphase cell plates of root tip squash revealed that the cells contain 2n=70 chromosomes. This chromosome number is the highest and not yet reported from any of the *Ledebouria* species (Image 2).



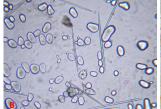




Image 2. *Ledebouria junnarensis* S.S. Rahangdale & S.R. Rahangdale A - Somatic metaphase chromosomes in root tip cell 2n=70;

- B Starch grains with concentric striations and Silica crystals;
- C Stoma on leaf epidermis. (Photographs by S.R. Rahangdale)

Leaf Anatomy

The leaf anatomy revealed that the margin of the leaf is single cell in cross section. Epidermal cells are rectangular on both the surfaces. Leaves are amphistomatic; stomata paracytic with two small reniform subsidiary cells parallel to both the guard cells (Image 2). Cataphyll cells contain acicular silica crystals and ellipsoidal starch grains with concentric striations.

Diagnostic characters

Bulb tunicate, scales imbricate, scales of three vegetation period, silica crystals in cells of scale leaves acicular, starch grains ellipsoidal with concentric striations. Leaves hysteranthous, linear, canaliculate towards tip, margin ending in single cell thickness. Leaves amphistomatic, stomata paracytic with two distinct subsidiary cells. The scape is a single per vegetation period, inflorescence lax raceme, ca. 30 flowered, pedicels relatively longer than that in *L. revoluta*, bracts two minute, deltoid, flowers pinkpurple, perianth purplish with green midrib, reflexed, ovary stipitate, green, trilocular, 3 lobed, 2 or 3 ovules per locule, on axile placenta, bulbils at the leaf tip fleshy, producing plantlets.

Identification keys

For the identification of genera and species found in India the genus and species keys are prepared using the literature and characters of specimens studied.

DISCUSSION

The species is confirmed on the basis of morphological, anatomical, and cytological characters. A comparison in the characteristics of this species with other three species reported from India is given in Table 2. The leaves in *Ledebouria junnarensis* nom. nov. are 2 or 3 per vegetation period, linear, canaliculate with fleshy tip always producing bulbils and plantlets, upto 45cm long, scape single, inflorescence lax raceme, bracts two, ovary forming a dome, placenta axile, 2 or 3 ovules in each locule, chromosomes 2n=70, smaller silica crystals and ellipsoidal starch grains with concentric striations. L. revoluta has larger silica crystals and irregularly shaped large starch grains without concentric striations. The epidermal cells and stomata are larger than that of L. revoluta, therefore, stomatal density is relatively less in the species under study. The stomatal index is also less. Floral parts, viz., scale, pedicel, perianth and stamens are pink/purple in *L. junnarensis*, whereas they are pale greenish-pink in *L. revoluta*. The flowers are smaller in later species.

Cytology

2.

The mitotic metaphase cell plates of root tip squash revealed that the plants contain 2n=70 chromosomes. This chromosome number is the highest and not yet reported from any of the *Ledebouria* species. Rao (1956) had identified three cytological variables - diploids, triploids and tetraploids of *L. revoluta* (*S. indica*) populations into two natural morphological groups.

One morphological group comprises diploids (2n=30) and tetraploids (2n=60); while the other morphological group of triploids (2n=45). Unfortunately, detailed morphological characterization is not clear for triploid as well as the tetraploid forms. More importantly, the triploid form studied by him was collected from the Coromandel Coast from Machilipatanam (in Andhra Pradesh) to Chennai (Madras) while, prevalence of diploids and tetraploid forms is in Maharashtra, particularly in the Mumbai area (Rao 1956). Though the triploid form of L. revoluta, which has linear leaves and without or very rarely with seed set, is different in chromosome number, i.e., 2n=45 when compared to L. junnarensis nom. nov. A specimen at Kew - K000802739 collected by Wight in October 1835 from Tamil Nadu, India (RBG Kew 2015) had long linear leaves without scape and bulbils which might be a triploid form of L. revoluta. Comparison of the cytological evidences and present observations of the cytology strongly suggest that the specimen under study is a distinct species and not the triploid form of *L. revoluta*. The chromosomes recorded here are 2n=70; are first observations and the highest known 2n chromosome number in the genus. Speta (1998a) reported that the genus Ledebouria has species with chromosome numbers 2n=20, 24, 26, 28, 30, 32, 34, 36, 44, 45, 46, 54, 58, 60, and 66. The observations of the present study also confirm a larger number of metaphase chromosomes in the genus, further supporting the distinctiveness of the taxon. L. junnarensis also shows affinities with F. hohenackeri which has linear leaves and axile placentation; however they differ in having a dome shaped, stipitate, green ovary and stellate non-blue flowers with cup shaped perigone and the chromosome number is also different; 2n=10 in later species (Rao 1954).

Key for the genera of the subfamily Hyacinthoideae considering the species reported from India.

	Tepals distinctly reflexed, perigone with a short tube; ovary green, stipitate, gugelhupf (dome/torus) shaped; anthers and style at least partially vinaceous; capsule wall papery; seeds without elaiosomesLedebouria
1.	Tepals not reflexed, perigone free; ovary otherwise, not stipitate, globose or bottle shaped; anthers and style otherwise; capsule wall succulent; seeds with/without elaiosomes2
2.	Ovary bottle shaped, locules many ovulate; seeds with elaiosomes
2.	Ovary globose/oblong, locules 2-7 ovulate; seeds without elaiosomes Fessia
	Key for the species of <i>Ledebouria</i> in India.
1.	Bracts 2, leaves hysteranthous; placenta axile
1. 1.	Bracts 2 , leaves hysteranthous; placenta axile

Table 2. Comparison of the characters of four taxa of Hyacinthoidae reported from India.

Characters	Ledebouria junnarensis nom. nov.	Ledebouria revoluta	Ledebouria hyderabadensis	Fessia hohenackeri
Distribution	Restricted in one location in Sahyadri, northern Western Ghats, Maharashtra at 990–1095 m.	Widely distributed in India in low elevation plains below 800m.	Hyderabad and surroundings Andhra Pradesh, India at 533m.	Drier western parts of India from Gujarat, Kashmir, Western Himalaya, Pakistan, Afghanistan around 660–1500 m.
Habitat	Grassy hill slopes with gravelly soil rich in humus	Grassy plains and open fallow lands on varied soil types	Open fallow land on gravelly soil and rock boulders	Hills on rocky sides or crevices as well as grassy fallow fields on hill side
Morphology				
Bulb	Tunicate, brown, bulb leaves imbricate, of 3 vegetation period, with single scape / year, scales compactly arranged	Tunicate, brown, bulb leaves, imbricate, of 3 vegetation period, 2 or 3 scapes / year, scales relatively loosely arranged	Tunicate, dark brown, single scape/year	Imbricate, white, more than one scape/ year
Leaves	Leaves 3 or 4 per vegetation period, hysteranthous, linear, canaliculate, tip fleshy, always producing single (rarely two) bulbil(s), vinacious spots present when young, vanish towards maturity, 30–45 cm long	3 or more, synanthous, variable from ovate to spathulate, not canaliculate, without fleshy tip, some forms may produce bulbils but not always, 10–20 cm long	2–6, synanthous, linear, without spots, not canaliculate, acute at tip, without bulbils, 5–10 cm long	4–6, linear - lorate, synanthous, without spots, not canaliculate, without bulbils, 10–32 cm long
Scape	Single, terminal, up to 40cm long, lax raceme with upto 30 flowers,	Two to three, axillary and terminal, dense raceme up to 100 flowers.	Single, 8–14 cm, 10–25 flowered raceme.	Single, lateral, scape 15–45 cm with few flowered lax raceme.
Bract	Two, minute, entire, deltoid, caducous	One, bifid, with serrate margin	One, lunate, entire, with semicircular notch	Two, entire, lanceolate
Perianth	Purplish, reflexed with green midrib	Greenish-pink, reflexed	Pinkish-green, reflexed	Blue, not reflexed
Ovary	Stipitate, trilocular, subtrigonus, forming a dome	Stipitate, ovoid, trilocular, 6 lobed forming a shallow cup	Stipitate, trilocular, globose, distinctly 3 lobed	Globose - oblong, deeply trilobed
Stigma	Simple, with club shaped papillae	Simple	Triquetrous, penicillate	Simple
Placenta	Axile	Basal	Basal	Axile
No. of ovules	2 or 3, not side by side	2, side by side	Single	3–6
Seed	Rare, single seed / locule, dark brown with reticulation on shiny testa	1 or 2 seeds / locule, obovoid, brown	Single seed / locule, obovoid, brownish, smooth	3 or 4 seeds / locule. Obovoid, brownish-black, dull, maturing differently in different locules
Multiplication	Rarely by seeds, and mostly through terminal single bulbil per leaf, essentially forming a plantlet	Seeds	Seeds only, bulbils absent	Seeds only, bulbils absent
Anatomy				
Leaf margin	Tapering to single cell	Extended & thickened with at least two cells	NA	NA
Epidermal cells	Rectangular, av. size $23\mu \times 249\mu$ (5727 μ^2)	Rectangular, av. size 17.5μ × 278μ (4865μ²)	NA	NA
Stomata	Amphi-stomatic, Paracytic	Amphi-stomatic, Paracytic	NA	NA
Stomatal Index	Upper surface = 14.29 Lower Surface = 22.43	Upper surface = 15.92 Lower Surface = 25.14	NA	NA
Stomatal Density/mm²	Upper surface = 52.99 Lower Surface = 97.83	Upper surface = 93.76 Lower Surface = 187.52	NA	NA
Cytology				
2n Chromosome No. (root tips mitosis)	70	Variable: Diploids - 30, Triploids - 45 Tetraploids - 60 Aneuploids - 44, 46, 58	NA	10
Starch grains (in bulb scale cells)	Mostly ellipsoidal & spherical with concentric striations, ca. 2.5–15μ × 5–25μ with average diameter of 14.6μ.	Irregular shaped (oval, triangular, ellipsoid irregular, semicircular, lanceolate) without concentric striations, ca. 5–20µ × 7.5–45µ with average size of 15 × 24µ.	NA	NA
Silica crystals (in bulb scale cells)	Acicular, pointed to both tips, present in bundles in cells, ca $2.5\mu \times 60$ – 95μ with average length of 32μ .	Acicular, pointed to both tips, present in bundles in cells, ca 5μ × 75–170μ with average length of 56μ.	NA	NA

NA - Data not available

Phenology

During the present study, the flowering is observed from May to June and later fruiting has been recorded. The same flowering & fruiting period has been observed for the specimens under cultivation. The flowering period reported in the literature as March (Blatter & Hallberg 1921; Santapau 1958; Deb & Dasgupta 1975, 1981) and from September to March (Sharma et al. 1996; Mishra & Singh 2001) is found to be dubious as later workers referred to the type specimen, which was reported to flower in March. Moreover, the plants are monsoon ephemeral and become dormant by September. The flowering in the month of March reported for the type specimen may be due to drying up after collection and then warm weather with watering can induce flowering due to breaking of the dormancy of the bulb. A similar phenomenon was also observed in a plant under cultivation.

IUCN ASSESSMENT

The present location is on the hill slopes of the Sahyadri in the northern Western Ghats, in Pune District. The Pimparwadi-Inglun Ghat (Durgawadi), is the location of the single present population of the species. The geographic position of the location is 19º11'42.10"N & 73°43'43.69"E, with an elevation range of 990–1095 m. The present population is of about 680 individuals (in 2013) in a very small area (AOO) of 0.52km² (Image 3) along the grassy hill slope between 990-1095 m in association with Chlorophytum tuberosum Baker, Crinum latifolium L. var. latifolium, Cyanotis tuberosa (Roxb.) J.A. & J.H. Schult., Euphorbia fusiformis Buch-Ham. ex D. Don, Habenaria grandifloriformis Blatt. & McCann, Iphigenia magnifica Ansari & Rolla Rao and Iphigenia pallida Baker. The species has been recorded for the first time from the region. Extensive surveys by the authors from 2005 to 2014 comprising Maharashtra State including the Western Ghats, especially Pune District and type Locality 'Khandala' did not result in the observation of specimens similar to the present one. The population is more or less stable and maintained by the plantlet formation by bulbils and rarely by seeds.

The present locality is a steep slope and surrounded by privately owned lands on the upper plateau as well as the lower slope. Landslides due to tree felling, and heavy grazing are the major threats. The major population is along the cut edges of the road approaching Durgawadi Plateau, and road widening is a major threat to the population. Thus, deterioration of the habitat is a critical

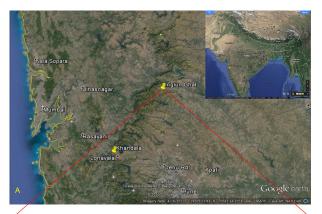




Image 3. Distribution of *Ledebouria junnarensis* S.S. Rahangdale & S.R. Rahangdale in Western Ghats of Maharashtra, India.

problem to the present population of the species.

On communication with local people, it came to light that the plant is not presently being used for any purpose. As locals do not find any application, they are not interested in conservation of the plant at present. As the plants are amidst the grasses, grazing by goats and cattle cause elimination of terminal bulbils, which are a major means of multiplication. Therefore, grazing is also one of the most potent causes of destruction of the population.

The type locality 'Khandala' around 18°0'N & 73°23'E and with an elevation of about 536m is situated about 60km away towards the southwest of the present location. Repeated attempts by taxonomists in the past and by the present authors to re-collect the species from the type locality have been unsuccessful. While working for about 12 years from 1940 to 1951 on the Flora of Khandala, Santapau was unable to collect any specimen of the species and reported it only on the authority of first description (Santapau 1958), and the same was followed by subsequent researchers (Deb & Dasgupta 1981; Nayar & Sastry 1987; Karthikeyan et al. 1989; Sharma et al. 1996). Based on these reports the taxon was declared as 'possibly extinct'. Thus, it is clear that,

one location is lost forever. The AOO of this location is not known.

An important reason behind the elimination of plants from their type location may be rapid anthropogenic developmental activities that took place at the hill station 'Khandala'. To understand this, one must consider the following facts: (i) the year of reporting of the specimen is 1921; (ii) the first attempt to re-collect the specimen by Santapau, from 1940 to 1951 was about 30 years after the first description; (iii) another 30 or more years are taken for subsequent unfruitful attempts to recollect the specimen by other taxonomists; and (iv) very rapid developmental activities and construction of farm houses and residential complexes. The present situation is that there is hardly any space for natural populations of plants in the hill town and the slopes of the valleys are almost unapproachable.

Information collected during this study indicates that *L. junnarensis* nom. nov. fulfils the necessary criteria to place it in the 'Critically Endangered' category (IUCN 2015) as it faces a high risk of extinction. The EOO is approximately 60km² considering its type locality and the new locality under present study. It is very important that, specimens could not be found at the type locality, thus the original location is lost for the taxon. This fact has reduced the EOO to almost 50% or more. Even if both the locations are considered for EOO, it is less than 100km² and the species satisfies the condition B1.

The AOO of the taxon at the present location is about 0.52km², which is far less than 10km². Considering the lost population at type location there is sure reduction in the AOO also. Therefore it satisfies condition B2a.

The EOO and AOO have declined and the quality of habitat is showing a continuous decline. These facts satisfy the criteria for the categorization of the taxon as Critical Endangered (CR) under criterion B of IUCN.

It is evident from the literature that the species was spread over a larger area but now reduced to only one location. This location also has a small population of <1000 individuals. The emphasis should also be given to the mode of reproduction through bulbils at the tip of each leaf. A single plantlet results from each bulbil. Considering the average leaves per vegetation period 3–4 (av. 3.5) each plant can produce 3.5 plantlets per vegetation period. But this is not actually observed; instead the population shows more or less a stable number of individuals. The main reason behind this phenomenon is the grazing by cattle, which eliminate leaf bulbils while grazing and the plantlets could not be produced.

The present population of the species is subjected

to heavy grazing, trampling by grazers and cattle. The threat of road widening and landslides are added ones. The lack of dispersal of plantlets due to the mode of reproduction is very important and also a threat to the species. The locals are unaware about the species and so are not interested in conserving it in the absence of any usage value to it. It is also noted by the authors that the visitors from neighbouring areas are increasing yearly for pilgrimage and tourist activities at Durgawadi Plateau. The transport facilities are likely to be improved in the very near future, which is primarily in the form of road widening. Thus, the fate of the species is unknown.

The above measured and quantified facts and observations clearly indicate that the species fulfils both the conditions B1 & B2 of Criterion B to qualify for CR category. Therefore, the status of *Ledebouria junnarensis* S.S. Rahangdale & S.R. Rahangdale is assigned as Critically Endangered B1ab(iii)+2ab(iii) with justification (Appendix 1).

CONCLUSION

The taxon which was declared as 'possibly extinct' after unfruitful attempts to re-collect it from its type location and other locations by several taxonomists has now been rediscovered after about 85 years. The taxon has been studied with respect to vegetative and floral anatomy, cytology and the identity has been confirmed. The taxon is assigned to the new name in the present systematics framework as, *Ledebouria junnarensis* S.S. Rahangdale & S.R. Rahangdale nom. nov.

It is also evaluated for threat status as per IUCN criteria and guidelines Version 6.2 and categorized as 'Critically Endangered' vide condition B1ab(iii)+2ab(iii). The population at the type location is lost due to anthropogenic activities and the present location is supporting the only surviving population of the species. This location is also under threat of grazing, habitat degradation, landslide and road widening, beside the propagation restriction of formation of plantlets from the leaf tip bulbil and very rarely through seeds. Therefore, in situ, as well as ex situ conservation measures should be prioritized. It can be introduced in other similar and safer habitats in the Western Ghats after evaluation as per the guidelines of the IUCN Reintroduction Specialist Group. It is evident from the experience of authors that the plant thrives well under pot/bed cultivation and also multiplies. It is a beautiful ephemeral whose purple/ pink flowers and blotched leaves have an ornamental potential. Therefore, ex situ conservation is also possible.

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Appendix 1. IUCN Red List Assessment: Ledebouria junnarensis nom. nov.

Kingdom: Plantae Division: Tracheophyta Class: Monocots Order: Asparagales Family: Asparagaceae Genus: *Ledebouria* Species: *junnarensis*

Authority: S.S. Rahangdale & S.R. Rahangdale

Common name: Unmad kand, Pushpayati kand (The names are based on Sanskrit meaning youthful blooming, which relates to the sudden and short lived blooming of the plants after 1 or 2 first pre-monsoon showers).

Taxonomic notes: The species was described by Blatter & Hallberg (1921) from Khandala in the Western Ghats, in Pune District, Maharashtra with holotype *McCann* 14500 deposited in BLAT!. It is rediscovered from another location in Pune District by Rahangdale & Rahangdale and assigned the new name *Ledebouria junnarensis* S.S. Rahangdale & S.R. Rahangdale.

ASSESSMENT INFORMATION

Red List Category and Criteria (Version 6.2): Critically Endangered B1ab(iii)+2ab(iii).

Justification: *Ledebouria junnarensis* is assessed as Critically Endangered as it is restricted to only one location with the extent of occurrence less than 100km^2 and the area of occupancy less than 1km^2 . The population size is also very small comprising of about 680 mature individuals. The species has not been re-collected from its type locality even after many efforts by different taxonomists since 1918 and is confirmed to be locally extirpated. Vegetative propagation from bulbils on leaf tips and low seed settings severely limit the population. The external threats for the species are high, including heavy grazing, loss and degradation of habitat due to landslides, road widening and growing tourism. Thus, the species is likely to be lost in the near future if adequate measures are not taken to protect and conserve it.

GEOGRAPHIC RANGE / DISTRIBUTION INFORMATION

Range description: The species is restricted to the northern Western Ghats in Pune District of Maharashtra, India (Image 3).

Countries of occurrence: Native to India (Maharashtra State).

Extent of Occurrence (EOO): EOO is approximately 60km² considering the present population at Inglun Ghat Hill slope and the extinct population at type locality 'Khandala' of the species. The present population is at about 60km in aerial distance away from the type locality on the Western Ghats in north-east direction.

Area of Occupancy (AOO): AOO is estimated to be less than 1km² (0.52km²) considering the extant population at Inglun ghat. The AOO of extirpated population is not known.

Number of locations: The species is currently restricted to only one location. The species is extirpated from its type locality and no other populations have been observed since the type description.

Range map: Distribution of the taxon is given in Image 3.

POPULATION INFORMATION

Population: The species is estimated to have less than 1000 mature individuals, of which 680 individuals are restricted to Inglun ghat.

Population trend: The population appears to be more or less stable at present. Over the last 10 years landslide and road widening has caused some damage to the population. Populations in one of the two locations have been extirpated.

HABITAT AND ECOLOGICAL INFORMATION

Habitat and ecology: The population occurs on the steep slope with well aerated, deep soil, rich in humus accumulated from grass biomass. The habitat is shared by other ephemerals viz., *Habenaria grandifloriformis* Blatt. & McCann, *Crinum latifolium* L. var. *latifolium, Chlorophytum tuberosum* Baker and *Euphorbia fusiformis* Buch-Ham. ex D. Don. The average annual rainfall is about 250cm with a cool climate from June to October, and misty climate with saturation of relative humidity from July to September. The average annual air temperature ranges between 15°C and 36°C. The altitudinal range is 992–1095 m. Though there are similar habitats in the neighbouring areas it is not found elsewhere.

The plants flower from May-June after at least two showers of rain of sufficient quantity. The same flowering period has been observed for the specimens under cultivation. The leaves grow very fast after the flowering attaining a length of 30–45 cm. Fruiting is observed rarely, producing dark brown seeds.

INFORMATION ON THREAT

Threats: The main threats to the remaining population are from heavy grazing by cattle, trampling by grazers, livestock and developmental activity. The grazing eliminates many of the leaf tip bulbils through which the species multiplies. The present available population is on the hill slopes and the road widening may cause severe damage to the population. The trees are cut down by locals for firewood and other usage which increases the chances of landslides.

Additional threats: An increased number of tourists and pilgrims to the Durgawadi plateau need wider roads and the road widening activities will surely eliminate a major chunk of the population. A road to the plateau and associated development activities in the near future will increase the threat to the population.

USE AND TRADE INFORMATION

Use: Local people do not collect the species as there is no usage value for the plant.

Livelihoods and sustenance: Communities are not dependent on this species for their livelihoods or sustenance.

Trend in off take from the wild: Not yet observed.

Trend in off take from cultivation: It is not cultivated, but has potential as an ornamental ephemeral.

Commercial value: The species has no known local, domestic, national or international commercial value.

INFORMATION ON CONSERVATION ACTIONS

Conservation actions: The species is not in any systematic conservation programme. The site of the present population on Inglun Ghat is a forest area which generally does not imply conservation of the species. The species does not occur in any formal protected area. An urgent conservation action is needed to protect the species from anthropogenic threats as well as site protection.

Research in place: There is no systematic research in place other than casual surveys.

Research needed: Systematic surveys, monitoring, propagation studies, effects of threats on population, and introduction of specimens in similar habitats in nature and development of cultivation practices are some of the much needed research actions on the species.

Monitoring in place: There is no monitoring of the species, population or habitat in place.

Monitoring needed: Population and site monitoring is essential and must be implemented at the earliest.

Education in place: No formal or informal education about the species is in place.

Education needed: Outreach programmes about the species to local communities and forest department are crucial.





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ISSN 0974-7907 (Online); ISSN 0974-7893 (Print)

February 2016 | Vol. 8 | No. 2 | Pages: 8421–8540

Date of Publication: 26 February 2016 (Online & Print)

DOI: 10.11609/jott.2016.8.2.8421–8540

www.threatenedtaxa.org

Article

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