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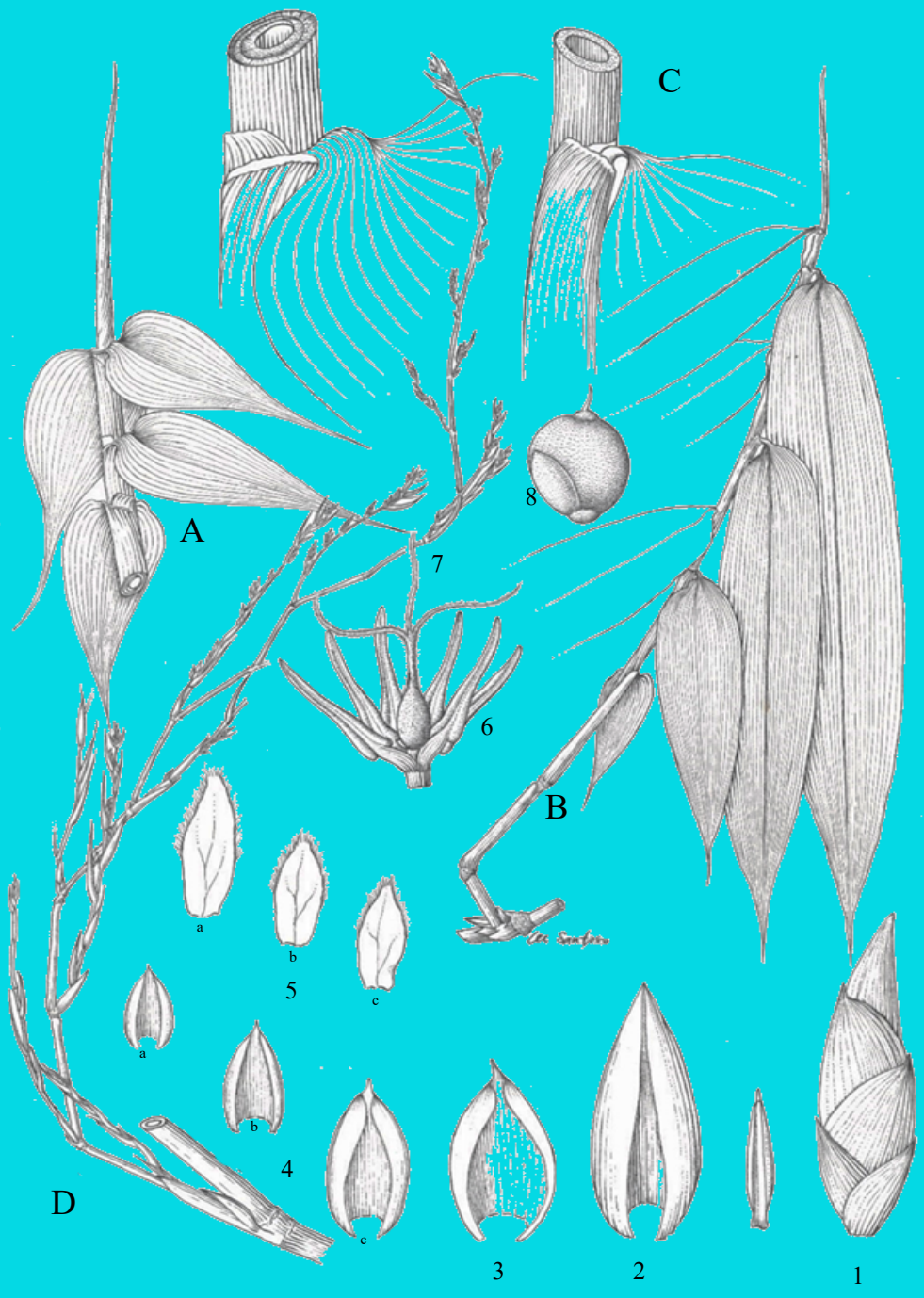

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REDISCOVERY OF *ALDROVANDA VESICULOSA* L. (DROSERACEAE), AN ENDANGERED PLANT, FROM MANIPUR IN INDIA AFTER SIX DECADES, WITH STUDIES ON MICROMORPHOLOGY AND PHYSICO-CHEMISTRY OF WATER

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ABSTRACT
NGANGBAM, R. D., DEVI, N. P., DEVI, M. H. & SINGH, P. K. 2019. Rediscovery of *Aldrovanda vesiculosa* L. (Droseraceae), an endangered plant, from Manipur in India after six decades, with studies on micromorphology and physico-chemistry of water. *Reinwardtia* 18(2): 71–80. — A small population of the aquatic insectivorous plant *Aldrovanda vesiculosa* L. of Droseraceae family was found growing in *Yena pat*, a small lake having an area of about 0.939 km^2 in Bishnupur district, Manipur state, India. This marks its rediscovery in India after a long gap of 64 years. The present status of the species in the updated IUCN Red List of Threatened Species version 2018.2 is endangered and considered extinct from India. A general description and micromorpho-taxonomic studies are included. As the species is a bioindicator, physico-chemical studies of the water were also investigated so as to provide aid in both in-situ and ex-situ conservation strategies. Immediate conservation initiatives are warranted to maintain the species after its rediscovery.

Key words: *Aldrovanda vesiculosa*, ecology, India, Manipur, rediscovery, taxonomy.

ABSTRAK
NGANGBAM, R. D., DEVI, N. P., DEVI, M. H. & SINGH, P. K. 2019. Penemuan kembali *Aldrovanda vesiculosa* L. (Droseraceae), tumbuhan terancam punah, dari Manipur, India setelah enam dekade, telah dipelajari mikromorfologi dan sifat fisika kimia airnya. *Reinwardtia* 18(2): 71–80. — Suatu populasi kecil tumbuhan insektivora akuatik *Aldrovanda vesiculosa* L. suku Droseraceae ditemukan tumbuh di *Yena pat*, sebuah danau kecil ±0.939 km^2 di Bishnupur District, Manipur State, India. Hal ini menandai penemuan kembali jenis ini di India setelah 64 tahun. Status terkini didalam 'IUCN Red List of Threatened Species' versi terbaru 2018.2 terancam punah dan diperkirakan akan punah dari India. Dipelajari pula deskripsi umum dan mikromorfo-taksonomi. Jenis ini juga dapat digunakan sebagai indikator biologi, oleh karena itu dipelajari pula sifat fisika kimia air untuk menunjang konservasi strategis secara *in-situ* and *ex-situ*. Inisiatif konservasi sesegera mungkin akan menjamin keberlangsungan jenis ini setelah penemuan kembali.

Kata kunci: *Aldrovanda vesiculosa*, ekologi, India, Manipur, penemuan kembali, taksonomi.

INTRODUCTION

Aldrovanda vesiculosa L. (Droseraceae), the Waterwheel or common Aldrovanda, is an aquatic free-floating and rootless insectivorous plant. It is the sole living member of the genus and represents a monotypic taxon. Through the perusal of literature, it has been confirmed that the species, though first established by Carolus Linnaeus in 1753, had its origin in India through Plukenet's documented collection in 1696 as *Lenticula palustris Indica* (Breckpot, 1997; Zaman *et al.*, 2011; Cross, 2012). The species was widely distributed in at least 43 countries of Africa, Asia, Australia and Europe but due to rapid decline in the population, it has been assessed in the IUCN

Red list category & criteria as Endangered B2ab (iii,v) ver. 3.1, and extinct from many regions of the world including India (Adamec, 1995; Cross, 2012).

As far as the occurrence of *Aldrovanda vesiculosa* in India is concerned, it has been reported only from two regions *i.e*., marshes and salt lakes of Calcutta and some lakes of Manipur (Biswas & Calder, 1936). There is no other record of this species from any region of India except Deb's collection in 1953, again from Manipur (Deb, 1957). At present, there are four herbarium specimens of *Aldrovanda vesiculosa* at CAL, Central National Herbarium, Howrah, India, with its last specimen deposit record in 1955 (Panda, 2014). Reinvestigation of the species over three decades in the wetlands of West Bengal and north-east and India failed to reveal the existence of even a single plant (Panda *et al.*, 2009).

During a recent floristic and limnological exploration in *Yena pat*, a small lake in the north-eastern state Manipur, India, the occurrence of *A. vesiculosa* was noticed. It was found floating in small clumps in association with free-floating and submerged macrophytes such as *Ceratophyllum demersum* L., *Hydrocharis morsus-ranae* L., *Ricciocarpos natans* (L.) Corda, *Salvinia cucullata* Roxb., *Utricularia aurea* Lour., etc. in areas slightly shaded by loose stands of reeds and sedges like *Phragmites australis* (Cav.) Trin. ex Steud. and *Zizania latifolia* (Griseb.) Turcz. ex Stapf. Earlier, the floristic composition of macrophytes of *Yena pat* was studied in detail but it did not include the presence of this plant (Devi & Sharma, 2007). Since the species was not collected from anywhere in India with its last record in 1953, and being considered extinct, the present collection of *A. vesiculosa* from *Yena pat* is a remarkable rediscovery after 64 years.

Aldrovanda vesiculosa is a stenotopic species that prefers to grow in a microhabitat of shallow water bodies having mesotrophic and dystrophic conditions with warm temperature, high CO_2 concentration and abundant zooplankton as a food source. It is strongly sensitive to eutrophic conditions and is considered ecologically as a potential bioindicator (Adamec, 1995). The plant is native to the Old World continents but, at present, it is facing a potent threat in its natural habitat due to environmental factors such as eutrophication and alterations in climate, as well as the limiting physiological needs of the plant. It has been the intention of many conservationists to raise this rare and endangered carnivorous plant. As a part of conservation efforts at global scale, some plants of Japanese origin have been introduced to North America and are now naturalizing at a phenomenal rate (Lamont *et al.*, 2013).

MATERIALS AND METHODS

Study Area. The present study was carried out during March to May 2017 in Manipur. Manipur is one of the north-eastern states of India which is located between 92°59'-94°46'E longitude and 23°59'-25°47'N latitude covering a total geographic area of 22,327 km^2. It is surrounded by Assam to the west, Mizoram to the south, Nagaland to the north and shares the international boundary with Myanmar to the east. The state is comprised of 16 districts, namely Bishnupur, Chandel, Churachandpur, Imphal East, Imphal West, Jiribam, Kakching, Kamjong, Kangpokpi, None, Pherzawl, Senapati, Tamenglong, Tengnoupal, Thoubal and Ukhrul. The study site is a small lake called "*Yena pat*" located at Ngaikhong Khulen village which lies in the northern part of Bishnupur district. With an area of 0.939 km^2, the lake extends within the longitude between 93°47'27.6" and 93°48'10.8"E and latitude between 24°38'16.8" and 24°39'0.0"N. The lake was divided into four sites (site I, site II, site III and site IV) to serve our purpose. Interestingly, the species was restricted to only site I and collected from three different locations (Fig. 1).

Sample Collection. As a part of the phytosociological survey and limnological exploration in the study area, the plant was collected from the marginal shallow region of site I by using a hand net. Photographs were taken while fresh by using Nikon P900. Morphological and anatomical studies were carried out using a Phase-Contrast Microscope MOTIC BA210. The species was identified by consulting relevant literature (Darwin, 1876; Shu & Zao, 2001; Zaman *et al.*, 2011). It was preserved as wet specimens in 10:1:1:8 (96% ethanol: formaldehyde: glycerol: water) solution formulated by Muñoz (Glime *et al.*, 2013). The wet as well as herbarium specimens were deposited in the Manipur University Museum of Plants (MUMP), Department of Life Sciences, Manipur University, Canchipur.

Water Analysis. Water samples were collected in triplicate each from the four sites of the study area during the morning hours between 8:00 am to 11:00 am. Physico-chemical analyses of water were carried out in accordance with the techniques of Trivedi and Goel (1984) and APHA (2012). Dissolved O_2 (Winkler's Iodometric Method), free CO_2 (titrated against 0.05 N NaOH), pH and temperature (waterproof pen-type pH meter with temperature display of model Erma pH-035), transparency (Secchi disc) and depth (measuring tape attached to a long stick) were determined on spot. Quantification of nitrate, phosphate, chlorophyll-a (Systronics WVS Spectrophotometer

117) and total alkalinity (carbonate hardness titrated against 0.1 N HCl) were carried out in the laboratory. In accordance to Carlson (1977), only three physico-chemical parameters viz., transparency, total phosphorous and chlorophyll-a were used in determining the trophic status of a lake. The simplified equations of Carlson's formulae in terms of Trophic State Index (TSI) was adopted and are given below.

I. Secchi depth transparency: TSI (SD) = 60-14.41 ln (SD)
II. Total phosphorus: TSI (TP) = 14.42 ln (TP) + 4.15
III. Chlorophyll-a: TSI (chl-a) = 9.81 ln (chl-a) +30.6
IV. Carlson's Trophic State Index (CTSI) = [TSI (SD) + TSI (TP) + TSI (chl-a)]/3

where ln is the natural logarithm, total phosphorus and chlorophyll-a are in microgram per litre and secchi depth transparency in meters.

RESULTS

TAXONOMIC TREATMENT

ALDROVANDA VESICULOSA L. Sp. Pl. 1:281. (1753); Roxb., Fl. Ind. 2: 112 (1832); C.B. Clarke in Hook. f., Fl. Br. Ind. 2: 425 (1878); Deb in Bull. Bot. Surv. India 3: 327 (1961); Singh *et al*., Fl. Manipur 1: 373 (2000) (Droseraceae).
Synonym: *Aldrovanda verticillata* Roxb., *Fl. Ind*. 2: 112 (1832).

Aquatic perennial herb, wheel-like, yellowish green to green, free-floating, rootless; *stem* cylindrical, slender, 2.5–19 cm long, glabrous, branched or unbranched with distinct nodes and internodes, articulated with a whorl of 7–9 equal leaves connate at base like radial spokes of a wheel, *whorl* 1–2 cm diameter; *internode* 3–7 mm long, 0.6–0.8 mm diameter; *leaf* spatulate, *petiole* resembles handle and lamina bowl; *petiole* flattened, 5–7 mm long, 1.5–2 mm wide, narrow at the nodal region, becoming broader towards leaf base, apex bears 4–5 pointed spinous tentacles, tentacles 5–6 mm long, projected beyond lamina, cross section of the petiole oblong, abaxial surface flattened, adaxial surface slightly convex, lacuna present, spherical to triangular; *lamina* modified into bladder; *turions* as vegetative propagules, turions rhomboid, 5–6 × 3–5 cm, show permanent apical and lateral growth and fragmentation.

Micromorphology of bladder. *Bladder* consists of two semicircular lobes joined at midrib, *lobes* kidney-shaped when opened, each lobe 3–4 mm long, 2–3 mm wide, filled with many hyaline or purplish pink oil bodies, differentiated into inner thick concave and outer thin flat region; *inner concave region* bears numerous digestive glands, absorptive glands and sensitive filamentous trichomes; digestive glands globular, 36–38 µm across multicellular, colorless, sessile or stalked, arranged in a single row on midrib and scattered towards periphery; absorptive glands pillow shaped, 48 × 15 µm, two-celled, stalked, filled with oil bodies, appressed on both sides of the midrib along the margin; trichomes 500–550 µm, multicellular, arranged in two rows of cells being articulated at the basal and medial regions, growing outwards from the midrib and also on the surface of the inner region itself; *outer flat region* extended with slightly curved periphery, presence of many two-celled bifid (V-shaped) and four-celled quadrifid (X-shaped) processes of absorptive nature, quadrifid process 55 × 25 µm; *margin of the bladder* spinous, spines 40–80 µm long; *midrib* 2.7–3.0 mm long, projecting beyond lamina lobes, pointed towards apex, tip needle-like (Figs. 2 and 3).

Habitat and Ecology. The plant grows well among sparsely distributed macrophytes in shallow water bodies. Abundant prey species (zooplankton) are also available in the region. Small changes in the environment will cause a decline in the plant's population.

Distribution. Africa: Botswana, South Africa (Cross, 2012); Asia: India, Australia (Cross, 2012); Europe: Bulgaria, Greece, Hungary, Lithuania, Poland, Romania, Russia, Serbia, Ukraine, Republic of Macedonia (Cross, 2012; Jovanovska *et al.*, 2017); North America (introduced): New Jersey, New York, Virginia (Lamont *et al.*, 2013).

Specimen examined. India: Manipur, District Bishnupur district, *Yena pat*, 760 m asl, 24° 38'38.66"N and 93°47'38.29"E, found freely floating in shallow littoral region of the lake, May 14, 2017, *Romita Devi Ngangbam & Naorem Premita Devi, 003498* (MUMP).

PHYSICO-CHEMICAL ANALYSIS OF WATER

At the time of collection of the species, certain physico-chemical parameters of the lake correlated with the growth of the plant were measured and are given in Table 1. The lake is found to have the following ranges of these parameters: dissolved O_2 1.2–6.5 mg L^{-1}, free CO_2 15.4–44.0 mg L^{-1}, total alkalinity 80–85 mg L^{-1}, temperature 27.8–29.3°C, pH 6.5–7.1, depth 1.10–1.41 m, secchi depth transparency 0.435-0.860 m, total nitrogen 44.296–49.268 µg L^{-1}, total phosphorus 5.868-6.520 µg L^{-1}

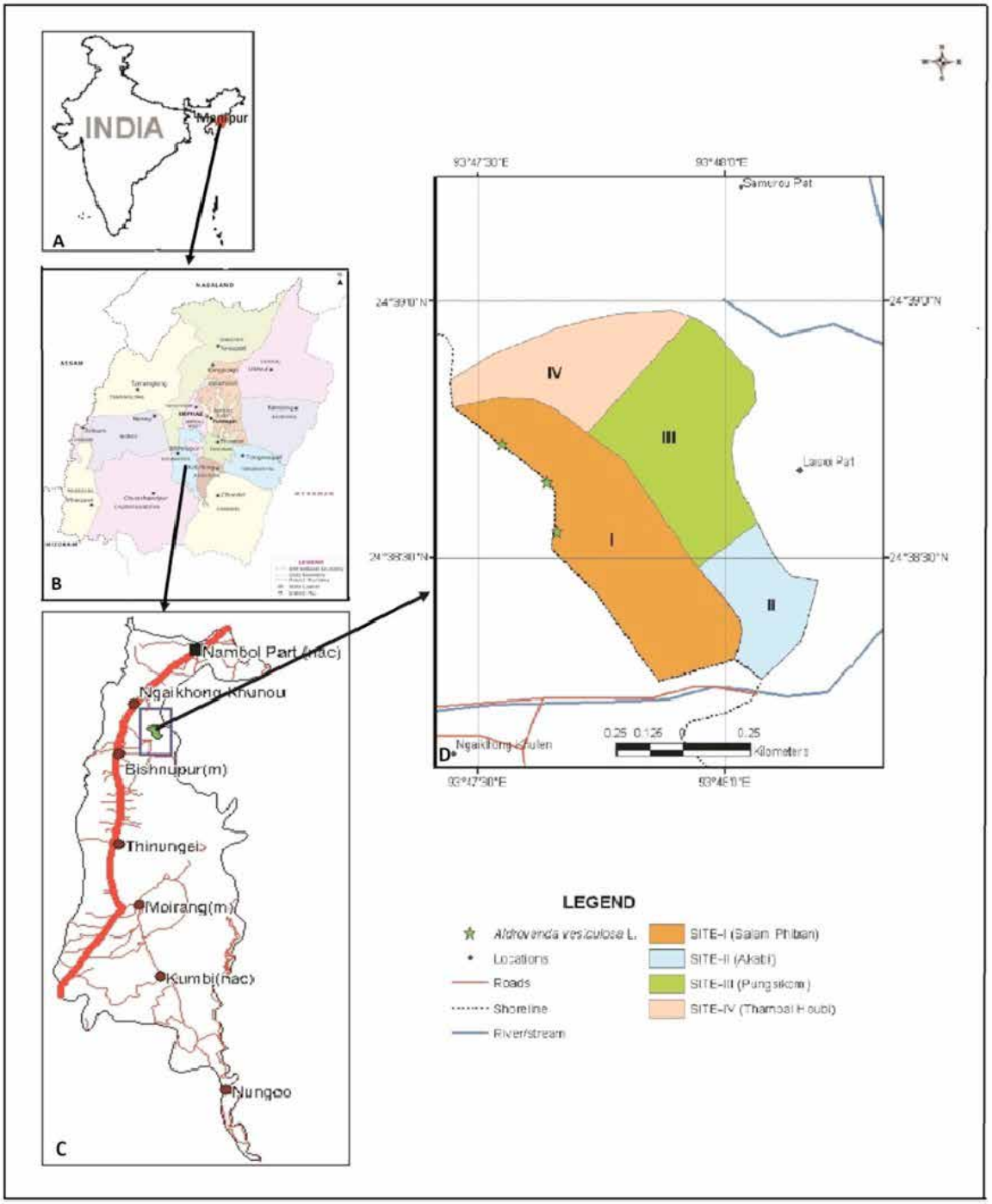

Fig. 1. Study area. A. Map of India showing the location of Manipur; B. Map of Manipur showing districts; C. Map of Bishnupur district showing the location of *Yena pat*. D. Map of *Yena pat* showing the locations where *Aldrovanda vesiculosa* L. was collected.

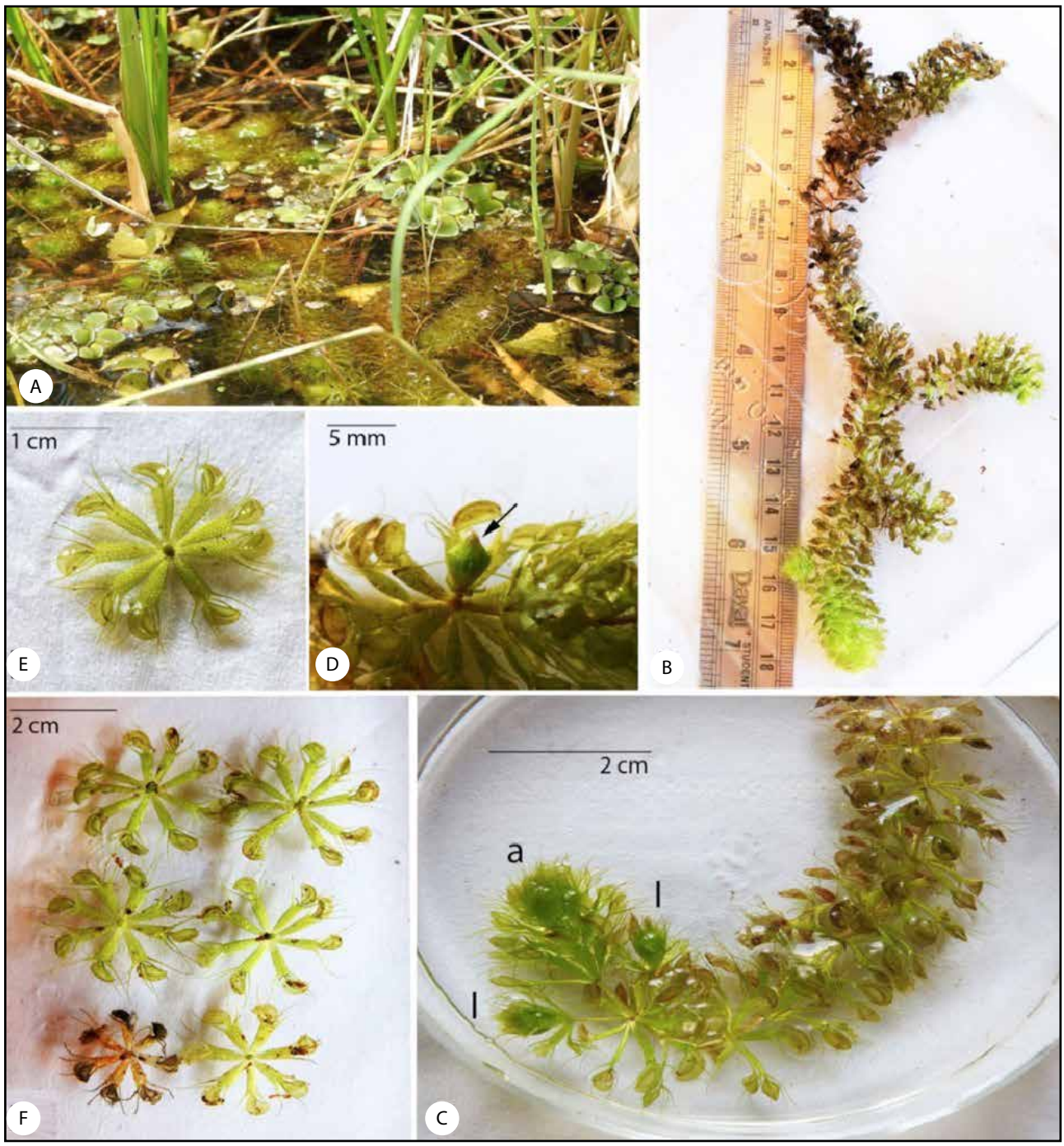

Fig. 2. Morphological features of *Aldrovanda vesiculosa* L. A. Habit. B. A whole single plant with branches. C. Apical (a) and lateral (l) turions. D. Single turion showing its rhomboidal shape (arrow). E. Single leaf whorl showing leaves bearing 4 to 5 tentacles. F. Leaf whorls with 7 to 9 leaves.

and chlorophyll-a 2.944–3.297 µg L^{-1}.

For determining the trophic status of the lake, Carlson's Trophic State Index (CTSI) values of the requisite parameters of each study sites are calculated and shown in Table 2. According to Carlson's Trophic State Index, the correlation between trophic categories and CTSI values are given as, Oligotrophic < 40; Mesotrophic 40–50; Eutrophic > 50 (Carlson & Simpson, 1996). The CTSI value of each of the four sites I, II, III and IV of the lake are 48.248, 44.905, 45.035 and 45.135 respectively and the average CTSI value of the lake is 45.831. Thus, the overall trophic status of *Yena pat* is mesotrophic in nature during the study period.

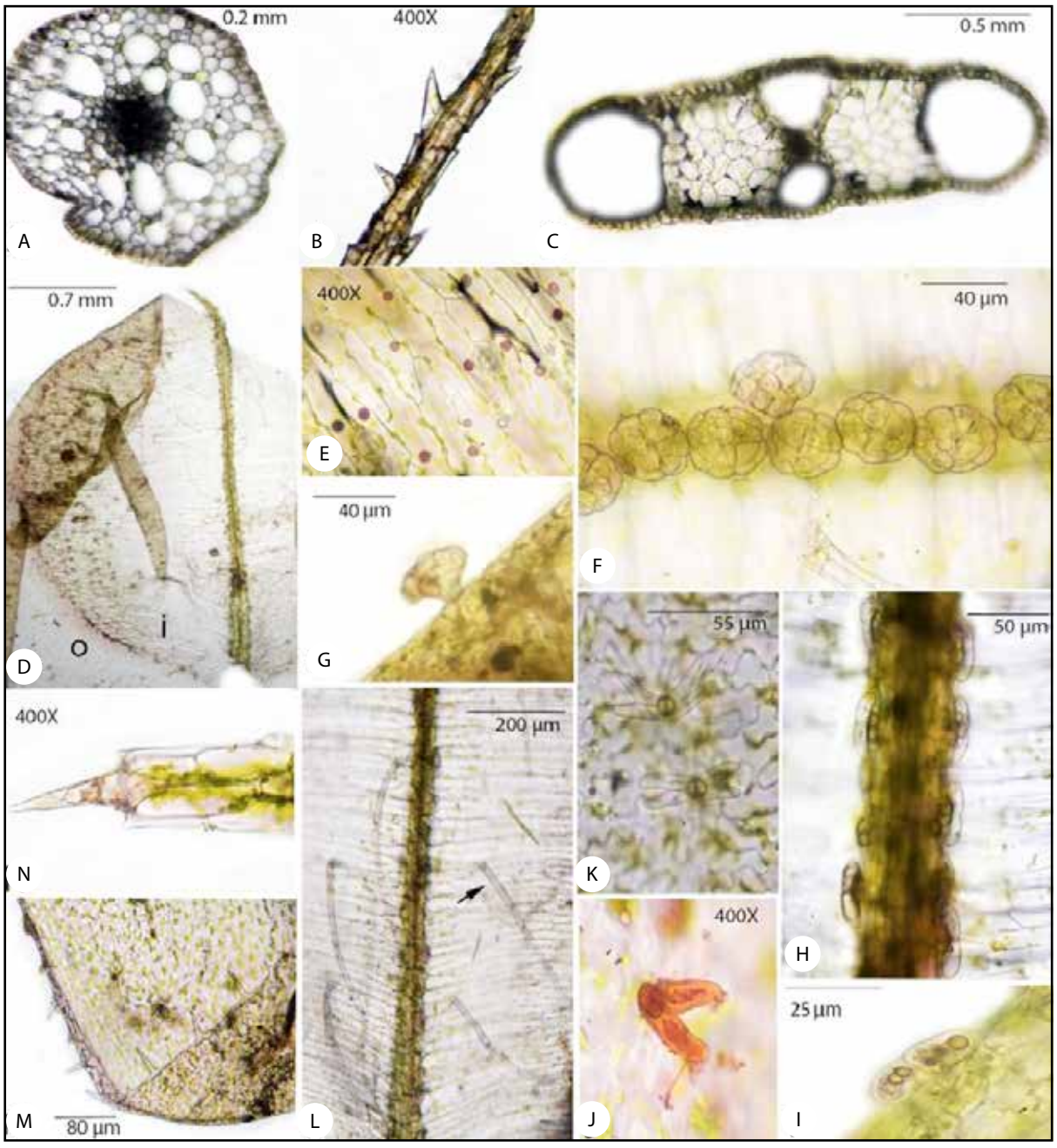

Fig. 3. Anatomical features of *Aldrovanda vesiculosa* L. A. T.S. stem; B. A tentacle showing spines; C. T.S. petiole; D. Opened leaf blade (half) showing inner curved and outer flat regions; E. Oil bodies; F. Digestive glands in single row on midrib; G. Stalked digestive gland; H. Absorptive glands (two-celled) on margin of midrib in two rows; I. Absorptive gland with oil bodies inside; J-K. Bifid and quadrifid processes; L. Sensitive trichome (arrow); M. Bladder margin showing spines; N. Apical portion of midrib with needle like tip. Note: T.S. = Transverse section

Table 1. Physico-chemical analysis of water of *Yena pat* based on monitoring from March to May 2017.

Sites	DO ($mg\ L^{-1}$)	Free CO_2 ($mg\ L^{-1}$)	TA ($mg\ L^{-1}$)	Temp. (°C)	pH	Depth (m)	SD (m)	TN ($\mu g\ L^{-1}$)	TP ($\mu g\ L^{-1}$)	Chl-a ($\mu g\ L^{-1}$)
Site I	5.9	19.8	80.0	28.1	6.8	1.10	0.435	49.268	6.194	3.297
Site II	1.2	44.0	85.0	29.3	6.5	1.39	0.780	46.104	5.868	3.028
Site III	3.4	24.2	80.0	29.1	6.7	1.41	0.860	46.104	6.520	3.115
Site IV	6.5	15.4	80.0	27.8	7.1	1.20	0.770	44.296	6.194	2.944

*DO - Dissolved O_2; TA - Total alkalinity; Temp. - Temperature; SD - Secchi depth transparency; TN - Total nitrogen; TP - Total phosphorus; Chl-a - Chlorophyll-a.

Table 2. Values of TSI (SD), TSI (TP), and TSI (Chl-a) and CTSI of *Yena pat*.

Sites	TSI (SD)	TSI (TP)	TSI (Chl -a)	CTSI
Site I	71.995	30.446	42.303	48.248
Site II	63.580	29.666	41.469	44.905
Site III	62.173	31.186	41.746	45.035
Site IV	63.766	30.446	41.193	45.135
Average	**65.379**	**30.436**	**41.678**	**45.831**

*TSI - Trophic State Index; SD - Secchi depth transparency; TP - Total phosphorus; Chl-a - Chlorophyll-a; CTSI - Carlson's Trophic State Index.

DISCUSSION

As per the assessment of *Aldrovanda vesiculosa* in IUCN Red List of Threatened Species in 2012, the species was reported as endangered worldwide and as extinct from India (Cross, 2012). The present study indicates an interesting rediscovery of *A. vesiculosa* in India after 64 years from *Yena pat* of Manipur which is one of the north-eastern states of India. Analysis of the physico-chemical parameters of the water in which the species grows reveals its ecological requirements. Adamec (1995) & Adamec & Kovářová (2006) studied the physico-chemistry of water for growth conditions of *A. vesiculosa* in detail and the optimum ranges of the studied parameters given were dissolved O_2 4.9–11.4 mg L^{-1}, free CO_2 22–88 mg L^{-1} with minimum concentration above 4.4 mg L^{-1}, total alkalinity 3–150 mg L^{-1}, temperature 23–26°C, pH 6.93–7.10 and depth 0.2–0.4 m. It also mentioned that water of *Aldrovanda* stand should be relatively transparent but no specific value was given. According to him, the ideal growth of the plant occurs in the mesotrophic condition. In the present study, the ranges of the parameters obtained in the *Yena pat* are dissolved O_2 1.2–6.5 mg L^{-1}, free CO_2 15.4–44.0 mg L^{-1}, total alkalinity 80–85 mg L^{-1}, temperature 27.8–29.3°C, pH 6.5–7.1, depth 1.10–1.41 m, secchi depth transparency 0.435–0.860 m and the trophic status is mesotrophic (CTSI 45.831). Thus, it has clearly shown that the parameters, dissolved O_2, free CO_2, total alkalinity, temperature, pH and trophic status are near to the optimal ranges except for the depth. However, an interesting result is the restricted occurrence of *A. vesiculosa* only in the littoral region of site I (Fig 1D). This can be attributed by the following factors.

The normal range of depth required by *A. vesiculosa* is 0.1–0.6 (optimum 0.2–0.4) (Adamec, 1995). In our investigation, it is observed that the depth of the *Yena pat* ranges 1.10–1.41 m and of the site I is 1.10 m, both values are deeper than the normal range. But, the littoral region of site I of the lake is shallowest and having a depth of 0.40–0.70 m. This region can provide a substratum for the growth of *A. vesiculosa* along with other macrophytes. Although the other sites (II, III and IV) fulfilled the optimum conditions in physico-chemistry of water, they show a great depth which could not favor the growth of the plant. It should be noted that site I is the only site with a marginal region whereas other sites are in open-water region and connect to water bodies of the neighboring lakes. Therefore, depth

can be considered as a crucial factor for the growth of this plant in this lake. Absence of the plant in the remaining three sites and the deeper region of the site I other than its shallow littoral region suggests that it cannot grow in open water beyond the depth of 1 m.

It is also observed that the vegetational composition affects the growth of *A. vesiculosa* in site I. It harbors the maximum number of emergent, submerged and free-floating macrophytes. This is supported by the fact that there is a sharp increase in the value of CTSI (48.248, mesotrophic) and total nitrogen (49.268) and reduced transparency (0.435) when compared to other sites as shown in Table 1. The emergent macrophytes provide a slightly shaded microhabitat for the plant to grow as well as a favorable habitat for zooplankton that serve as prey. As the plant shows true carnivory, presence of zooplankton is a must besides having appropriate nutrients in the stand. Thus, the important factors that greatly support the growth of *A. vesiculosa* in site I of study area are depth (shallow, 0.40–0.70 m), trophic status (mesotrophic, CTSI 45.831), transparency (0.429–0.440 m), high total nitrogen content of water (47.391–51.145), slightly shaded microhabitat and presence of prey.

In the remote past, the plant was collected from some lakes of Manipur and College Reserve Tank, Imphal by different workers but the exact locations were not specified (Biswas & Calder, 1936; Deb, 1957). Since then, a lot of phytosociological studies have been carried out in Manipur but none of the workers have mentioned the occurrence of this carnivorous plant from anywhere inside the state, except in the present case. The present rediscovery is a very fascinating one which can be viewed from different angles. While studying the floristic composition of macrophytes of *Yena pat*, the lake was in eutrophic condition and *A. vesiculosa* was not in the list (Devi & Sharma, 2007). It is possible that *Yena pat* may be one of those lakes mentioned by Biswas & Calder (1936) but with passage of time, the lake becomes eutrophic and might have wiped out the growth of the plant as it is sensitive to eutrophic. The present mesotrophic condition of the lake might again favor the germination of long-dormant seeds embedded inside it long years back and results into the reappearance of the plant. In another way, the plants might have arisen in some other lakes nearby and vegetative fragments and/or turions might have been carried on legs of migratory birds from those lakes to *Yena pat,* where they started propagating since the current ecological condition of the lake supports the physiological needs of the plant.

CONCLUSION

Rediscovery reports of *A. vesiculosa* from across the world (Serbia, Greece, Republic of Macedonia) are credited to legislative protection and management strategies adopted for restoration of lake ecosystems (Tomović *et al.*, 2009; Cross, 2012; Jovanovska *et al.*, 2017). Likewise, it is worth mentioning that the mesotrophic condition of *Yena pat* and the recent flourishing of *A. vesiculosa* might be due to the application of conservation and management strategies in connection with *Loktak lake*, a wetland of international importance designated as a Ramsar site in 1990 (The Ramsar Convention on Wetlands, 2019). As a part of this conservation strategy, all the *Phumdis*, floating heterogenous masses of vegetation of *Loktak* and its associated lakes were cleared in 2010-2011. *Yena pat* is one of the associated lakes as it is situated very close to the northern part of *Loktak*. At present, there is little intrusion by local people for livelihood activities like catching fish and collection of vegetables but not at an overexploited level. Physico-chemical analysis reveals that water of the lake fits in the optimal ranges required for the growth of *A. vesiculosa* but only a small portion of the site I in its littoral region could meet the suitable habitat requirements for the luxuriant growth of this rare plant as discussed above. Since *A. vesiculosa* is a stenotopic species with very low competitive ability, slight alterations in its habitat characteristics will cause decline of the population, resulting to the loss of the species. So, we should avoid all those activities such as drainage and land encroachment for agricultural and fish farming activities which will bring about degradation of the lake, so that *A. vesiculosa* can thrive well. “Now” is the right time to prioritize joint conservation efforts from both government and non-government organizations, conservationists and researchers, to protect its natural habitat. There is also the potential for *ex-situ* conservation and application of tissue culture techniques to enable the survival of the species after its remarkable rediscovery.

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