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Review Article

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DNA barcoding of orchids in India: A systemic review

Sontu Bugh

Assistant Professor in Botany
Department of Botany, Muralidhar Girls' College (Affiliated to University of Calcutta)

Abstract

Orchids comprise the second-largest family of flowering plants in the world. Their flowers are one of the most beautiful god's creations. It is estimated that 1256 species (140 genera) of orchids are found in India, with highest concentration in north-eastern India, and the most endemism in the Western Ghats. Indian orchids are rare and threatened, and listed in Appendix 1 and 2 of the Convention on International Trade in Endangered Species of Wild Fauna and Flora. So, the development of a reliable method for the identification of orchids is the need of the hour to save them.

Currently, DNA barcoding is a popular and useful technology for quickly and accurately identifying plant species. The presentreview looked for appropriate published studies using a variety of sources in accordance with the Cochrane Collaboration Guidelines for systemic reviews. The search comprised four abstracting, referencing, and indexing electronic databases libraries released between 2003 and 2023. A total of 545 relevant studies were systematically analyzed. We found seven relevant studies on the barcoding of Indian orchids. Researchers studied *rpoC1*,*rpoB*, *rbcL*, and *matK* from the chloroplast genome, *trnH-psbA*, an intergeneric spacer, and *nrITS* from the nuclear genome to find best candidate barcode for orchids. *ITS* was noted as the most effective candidate barcode for the identification of Indian orchids among single-locus barcoding genes. A combination of *ITS* with *matK* and *matK+ rpoB+ rpoC1*combination were also noted as effective candidate barcodes.

Keywords: Coelogyne, Dendrobium, ITS, matK, Paphiopedilum

Introduction

ORCHIDACEAE is the world's second-largest and cosmopolitan family of flowering plants, with over 28,000 accepted species belonging to 763 genera (Chase *et al*., 2015). Orchids are one of the largest families of higher plants in India which consist of 9% of the total flora (De, 2015). A recent study by The Botanical Survey of India (2019) concluded that there are 1256 orchid species, belonging to 140 genera in India (Singh *et al*., 2019). Orchid species concentration is comparatively high in north-eastern India, and the endemism is more in the Western Ghats (Jalal and Jayanthi 2012; Prasad *et al*., 2019). Orchids are mainly known for their beautiful, exotic and long-lasting flowers as well as their medicinal values (De, 2015). But Indian orchids are listed in Appendix 1 and 2 of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) which means they are rare and threatened in the wild so their trade is prohibited (Huxley 2013).

As all Indian orchids are rare and valuable, accurate identification of orchid species and their sustainable utilization as plant resources is critical for their conservation (Deb *et al*., 2021). Morphology-based taxonomic identification of orchid species is very difficult due to phenotypic plasticity and genotypic variability of characters. Another difficulty to identify these species is their slow growth and long maturity periods (Cuypers *et al*., 2022). Thus, DNA barcoding is the only reliable, cost-effective, and efficient tool for taxonomists, conservationists, scientists, and all others who need to create information on unknown live, damaged, or dry orchids (Hebert *et al*., 2003). DNA barcoding methodology is very simple. In this process (Fig 1), DNA is extracted from the unknown sample of orchid plant, it is amplified, sequenced and then the phylogenetic analysis is done to identify the sample orchid (Srivastavaand Manjunath, 2020).

DNA barcoding is a tool for rapid and precise identification of a plant species even from a small tissue of unknown sample of plant origin. The main objective of the DNA barcoding studies is to construct online libraries of core barcoding sequences for all economically important and endangered plant species that can serve as a standard to match any unidentified specimen (Rajphriyadharshini and Weerasena, 2020). This technology may help in identifying live, damaged, dried, or processed products of plant origin in a cost-effective manner. It will keep a check on the industry making fake and illegal herbal medicines, food items, and beauty products of plant origin (Mir *et al*., 2021). *ITS* prosperous application in the examination of illegally traded endemic and endangered plant species would be important. This can assuage several intrinsic problems faced by traditional taxonomy to identify morphologically similar species and/ or immature and damaged plants (Antil *et al*., 2022). Although it is an indispensable area of research but only a few species are explored in India.

Plant kingdom do not have a single universal DNA barcode marker for plants (China Plant BOL Group. 2011). The studies on DNA barcoding of plants have been focused mainly on the plastid genome, intergenic spacer regions and Internal transcribed spacer genes (*ITS)*. These small regions of plastid genes*, ITS* and intergenic spacer are conserved among flowering plantspecies and considered as "candidate barcode genes" (China Plant BOL Group. 2011; COBOL, 2009). A few important candidate barcode genes in plants are listed in Table 1 (Srivastava and Manjunath, 2017).

Table 1: Important barcodes used for the identification of Plants

S.N.	Barcodes	Location
A	**DNA barcodes based on plastid genes**	
1	*matK*	Maturase K gene
2	*rbcL*	large subunit of the ribulose-1,5-bisphosphate carboxylase/oxygenase
3	*accD*	β-carboxyl transferase subunit of acetyl-CoA carboxylase gene
4	*nad1*	Mitochondrial intron sequence
5	*rpoB*	encode B subunits of the plastid RNA polymerase
6	*rpoC1*	encode C1 subunits of the plastid RNA polymerase
7	*ndhF*	NADH-dehydrogenase subunit F coding gene
8	*ndhJ*	NAD(P)H-quinoneoxidoreductase subunit J coding gene
9	*ycf1*	Encodes plastid gene *ycf1*
10	*ycf5*	Encodes plastid gene *ycf5*
11	*Xdh*	Encodes Xanthine dehydrogenase
B.	**DNA barcodes based on non-coding intergenic region**	
12	*trnH-psbA*	Intergeneric space between *psbA* and *trnH*
13	*atpF-atpH*	Localized in plastid where *atpF* and *atpH* encode ATP synthase subunit *ITS*
14	*psbK-psbI*	localized between two small membrane-spanning proteins psbK and psbL of photosystem II
C	**DNA barcodes based on Internal transcribed spacer**	
15	*ITS 1*	Space between 18S and 5.8S rRNA genes
16	*ITS 2*	Space between 5.8S and 26S rRNA

Several studies have been conducted on species of medicinally and horticulturally important orchids in India. In the present review, we conducted a systemic analysis of the existing published articles that identified DNA barcodes of Indian orchids.

2. Methods

2.1 Identification of relevant studies:

We looked for appropriate published studies using a variety of sources in accordance with the Cochrane Collaboration Guidelines for systemic reviews (Higgins and Green, 2011). The search comprised four abstracting, referencing, and indexing electronic database libraries released between 2003 and 2023. Pubmed, Wiley, Science Direct, and Google Scholar databases were included. This analysis included all studies conducted to find unique DNA barcode sequences for the identification of orchids in India. Additionally, manual searches were done by looking through the reference lists of the studies that were included. The search strategy was as given in Table 2.

Table 2: Strategies to search literature

1. PubMed	1. Search "DNA barcoding of orchids in India" [Title/Abstract])
	2. Search "DNA barcoding of Indian orchids" [Title/Abstract])
	3. Search ("Identification of orchids of India through DNA barcoding" [Title/Abstract])
2. Science Direct database	1. Find articles: Keyword search ("DNA barcoding of Indian orchids")
	2. Find articles: Keyword search ("Identification of orchids of India through DNA barcoding")
3. Google Scholar database	1. 'DNA barcoding of Indian orchids'
	2. 'Identification of orchids of India through DNA barcoding'

2.2 Screening of study:

All citations had been exported to EndNote and duplicates were removed. Then citations were screened through titles as well as abstracts. The full text of all appropriate research was retrieved and assessed by two reviewers autonomously for inclusion criteria.

2.3 Inclusion strategy:

The following were considered as inclusion criteria for the present study: (1) studies that included DNA barcoding sequences in relation to orchids (2) Studies focusing on the identification of orchids through DNA barcoding(3) studies that reported data regarding DNA barcoding of orchids and (4) Related peer-reviewed full-text articles which were accessible. Exclusion criteria included were: (1) Conference articles with only abstracts, editorial comments, and recommendations. (2) Studies not focusing on DNA barcoding of Indian orchid species. (3) Studies not included candidate barcoding sequences mentioned in Table 1.

3. Results and Discussion

3.1 Identification of included studies:

Relevant databases were searched for DNA barcoding of orchids of India and 545 results appeared. Among them, 125 pieces of literature were the same, which were identified through different databases. Some (22) literatures were in other language or conference proceedings. Those duplicate and irrelevant studies were excluded and a total of 398 abstracts were screened further. 278 potentially eligible full-text research studies, which had keywords DNA barcoding and orchids were retrieved further. Only seven studies that were identified on Indian orchids and potential barcoding sequences were found suitable and included in this study based on a thorough analysis of full-text data (Figure. 1). Based on 7 included studies DNA barcoding studies on orchids of India were analyzed.

3.2 Potential candidate Barcoding locus:

Studies included in this review screened one or many loci to find their potential as effective barcodes. They were the RNA polymerase-β' subunit (*rpoC1*), RNA polymerase-β subunit (*rpoB*), Rubisco large subunit (*rbcL*) *trnH-psbA* spacer, and maturase K (*matK*) from the chloroplast genome and nuclear ribosomal internal transcribed spacer (*nrITS*) from the nuclear genome (Xu*et al*., 2015; Yao *et al*., 2010).

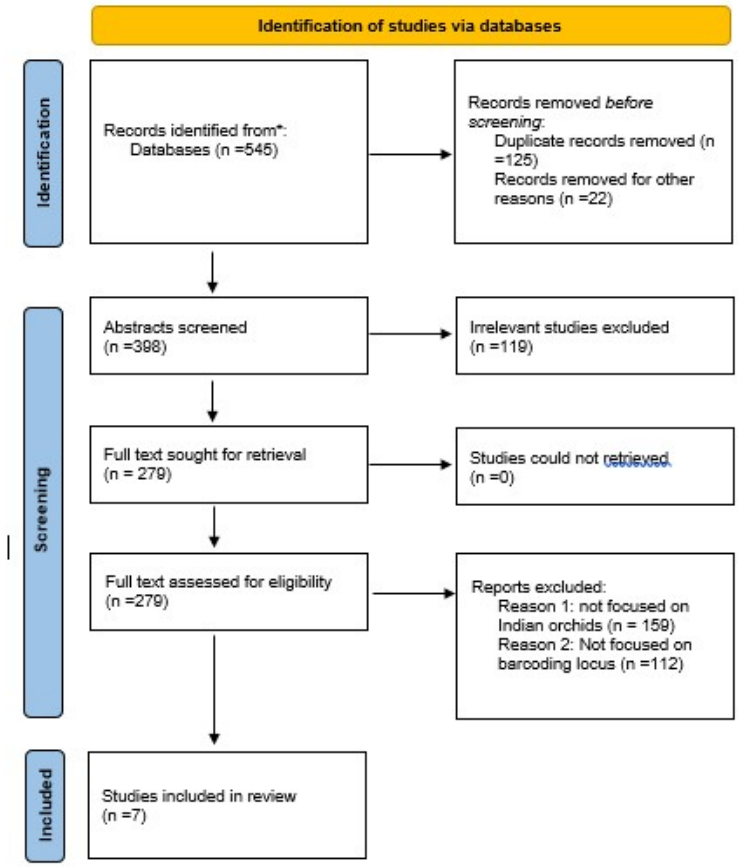

Figure 1: Literature search strategy

3.3 Methods Used for Analysis of DNA Sequence Data:

DNA barcoding process includes the collection of DNA samples from live, damaged, or dry orchid plants or flowers. DNA extraction is followed by the amplification of candidate barcoding sequences and their sequencing. All studies used Local Alignment Search Tool BLAST to identify their sequences with the GenBank nucleotide database. The presence of identified sequences in the database with lesser similarity with alike species and 100% identity to other species/genera were not considered as barcode sequences. Only unique sequences were taken for further analysis.

The studies aligned the identified sequences using CLUSTAL W, a tool for multiple sequence alignment (Larkin *et al*., 2007).They constructed the phylogenetic trees by implementing the discrete character method-Maximum Likelihood (ML) or Neighbour Joining Trees Method in MEGA tool software. The Kimura two-parameter (K2P) model was used to find the intra-specific and interspecific divergence used to discriminate species (Figure 2).

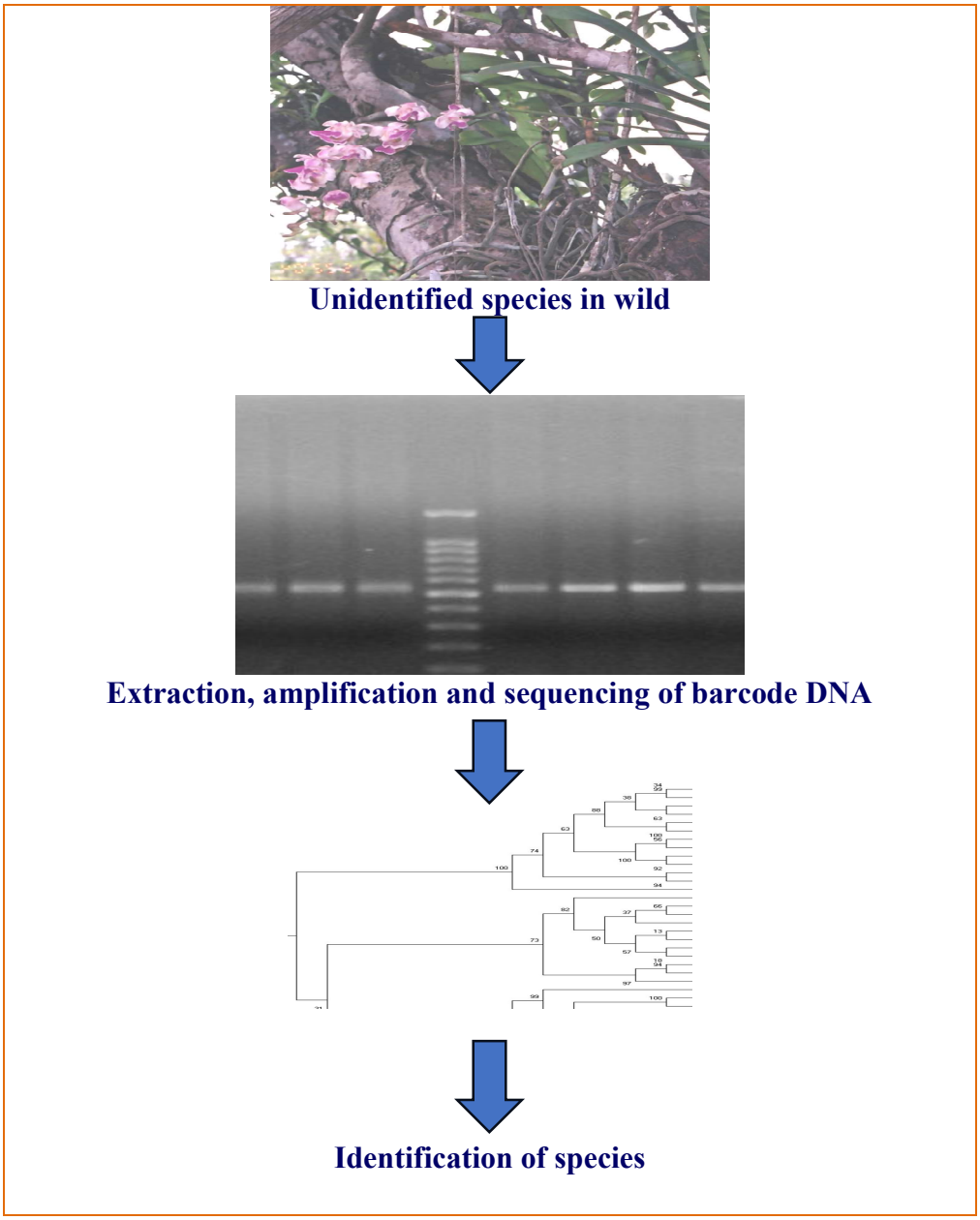

Figure 2: Process of DNA Barcoding

3.4 DNA barcoding of Indian orchid species

Results of seven relevant studies are summarised in Table 3 and explained below.

Using loci from the nuclear genome (*ITS*) and the chloroplast (*matK, rbcL, rpoC1, rpoB*), Parveen *et al*., (2012) created DNA barcodes for the Indian species of *Paphiopedilum* and their three natural hybrids. With a 100% species resolution and an average inter-specific divergence value of 0.9%, the *matK* was able to clearly discriminate all eight *Paphiopedilum* species. When a blast analysis of these sequences was performed on the NCBI, it was discovered that each *matK* sequence was unique for the species, further confirming the sequences' capacity to identify different species. Even though the average inter-specific divergence value for *nrITS* was 4.4%, it only allowed for 50% species resolution.

Using DNA barcoding, Singh *et al*., (2012) distinguished 36 *Dendrobium* species. Among the studied loci, *ITS*, which has been suggested as a potential plant barcode, identified all the species completely. Another locus, *matK*, which is also suggested as an all-purpose plant barcode, resolved 80.6% of species. *matK*+*rbcL*, a two-

locus combination, could distinguish 36 species with an accuracy of 86.11%. When more sequences from the NCBI database were analyzed, the barcode's ability to distinguish between species was lowered to 80.77%. The barcode based on three loci—*matK, rpoB*, and *rpoC1*—resolved the most species among the suggested combinations.

The nine species of *Coelogyne* orchids were assessed using DNA barcoding by Ramudu and Khasim (2016) utilizing the*rbcL* locus. For *rbcL*, the average K2P distance between various species of *coelogyne* was 0.007. Three species pairings with distance estimates of zero were the outcome of the *rbcL* locus. 44.44 was recorded for the species discrimination rate.

Table 3: Details of research on orchids of India

S.N.	Authors	Candidate Loci tested	Recommended Loci
1	Parveen *et al*., (2012)	*rpoB, rpoC1, rbcLmatK* and nr*ITS*	*matK*
2	Singh *et al*., (2012)	*matK, rbcL, rpoB, rpoC1, trnH-psbA* spacer and *ITS*	*ITS* and*matK+ rpoB+ rpoC1*
3	Ramudu and Khasim (2016)	*rbcL*	*rbcL*
4	Parveen *et al*., (2017)	*rbcL, rpoB, rpoC1, matK,* and *ITS*	*ITS+matK.*
5	Chattopadhyay*et al.,* (2017)	*rbcL*, *matK*, *trn*L-*trn*F, *ITS1, ITS2*	*ITS1-ITS2*
6	Srivastava and Manjunath (2020)	*ITS, matK, rbcL,* and*trnH-psbA*	*ITS*
7	Mahadani *et al*., (2022)	*ITS, matK, rbcL, trnH-psbA*	*ITS*

Another study by Parveen *et al*.,(2017) tested approx. 400 accessions of 94 Indian orchid species belonging to 47 genera, including one classified in Appendix I of CITES and 26 medicinal plants.*ITS* produced the greatest species discrimination rate of 94.9% with a species discrimination percentage of 94.9%. While *matK*, with a species identification rate of 85.7%, had the greatest performance among the chloroplast loci. None of the examined loci could successfully distinguish all species on their own. Therefore, they tested the capacity of multi-locus combinations of up to five loci to discriminate species. *ITS+matK* showed the highest species-specific resolution (86.7%) among two-locus combinations for the detection and identification of Indian orchids.

Chattopadhyay *et al*., (2017) screened four DNA barcoding candidate sequences *rbcL, matK, trnL-trnF*, and *ITS1-ITS2* for identification of 65 Indian orchid species. To find the best locus for addressing the phylogeny-related difficulty below the taxonomic level of the genus, they considered 31 distinct *Dendrobium* species. They concluded that *matK* and *rbcL* showed 52% and 48% of species resolving capacities respectively and cannot be considered suitable tools for taxonomic identification. Phylogeny construction found that the highest mean Kimura 2-parameter distance with the highest species resolving ability (95.23%) was shown to be *ITS1-ITS2 lo*cus.

For DNA barcoding research, 62 samples overall from 35 species and 7 genera were gathered. They produced 133 barcoding sequences, of which 46were determined to be original and new to the GenBank database. Evolutionary divergence analysis produced the best results for *ITS*. It showed a glaring barcoding gap, which was enough to reliably deduce taxonomic identities. According to a BLAST-based analysis, the *ITS*

locus was the most effective in identifying barcode sequences (94.64%), followed by the *rbcL* locus (78.69%) and the *matK* locus (51.61%). The *ITS* locus sequences were also used to create the ideal phylogenetic trees (Srivastava *et al*., 2020).

Medicinally significant *Dendrobium* species DNA was extracted from young leaves and *ITS, rbcL, matK,* and *trnH-psbA* were amplified and sequenced for species-level identification. It was simple to amplify and sequence *the ITS, rbcL*, and *trnH-psbA*. Nine of the 54 sequences were new and submitted to GenBank. With the exception of two species, *Dendrobium thyrsiflorum* and *Dendrobium densiflorum*, *ITS* was proved to be the most effective method for similarity searches to identify all *Dendrobium* species. The characters-based technique, however, was able to distinguish between *Dendrobium thyrsiflorum* and *Dendrobium densiflorum* with ease. They concluded that a combination of *ITS* sequencing data and similarity-based approaches may aid in accurate identification of *Dendrobium* species (Mahadani *et al*., 2022).

Conclusion

Studies on orchids of India highlight *ITS* as the best candidate barcode sequence for identification. *matK* in combination of *ITS* and *rpoB+ rpoC1* can also be used as a barcode sequence.

References

[1] Antil, S., Abraham, J.S., Sripoorna, S., Maurya, S., Dagar, J., Makhija, S., Bhagat, P., Gupta, R., Sood, U., Lal, R. and Toteja, R. (2022). DNA barcoding, an effective tool for species identification: a review. Molecular Biology Reports, 56:1-15.

[2] CBOL Plant Working Group. (2009). A DNA barcode for land plants. Proceedings of the National Academy of Sciences USA 106: 12794– 12797.

[3] Chase MW, Cameron KM, Freudenstein JV, Pridgeon AM, Salazar G, Berg C, Schuiteman A. (2015). An updated classification of Orchidaceae. Botanical Journal of the Linnean Society 177: 151–174.

[4] Chattopadhyay, P., Banerjee, G. and Banerjee, N. (2017). Distinguishing orchid species by DNA barcoding: Increasing the resolution of population studies in plant biology. *OMICS: A Journal of Integrative Biology*, 21:711-720.

[5] China Plant BOL Group. (2011). Comparative analysis of a large dataset indicates that internal transcribed spacer (*ITS*) should be incorporated into the core barcode for seed plants. Proceedings of the National Academy of Sciences USA 108: 19641– 19646.

[6] Cuypers, V., Reydon, T. A., & Artois, T. (2022). Deceiving insects, deceiving taxonomists? Making theoretical sense of taxonomic disagreement in the European orchid genus Ophrys. Perspectives in Plant Ecology, Evolution and Systematics, 56, 125686.

[7] De, L.C. (2015). Commercial orchids. In *Commercial Orchids*. De Gruyter Open Poland.

[8] Deb, C.R., Longchar, T.B., Kamba, J. and Jakha, H.Y. (2021). Wild orchid resources of Nagaland, India: updated status. *Pleione*, 15:113-126.

[9] Hebert, P.D., Cywinska, A., Ball, S.L. and DeWaard, J.R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 270:313-321.

[10] Higgins, J.P., Altman, D.G., Gøtzsche, P.C., Jüni, P., Moher, D., Oxman, A.D., Savović, J., Schulz, K.F., Weeks, L. and Sterne, J.A. (2011). The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. BMJ, 343.

[11] Huxley, C. (2013) November. CITES: the vision. In Endangered Species Threatened Convention. Routledge. pp23-32

[12] Jalal, J.S. and Jayanthi, J. (2012). Endemic orchids of peninsular India: a review. Journal of Threatened Taxa, 4:3415-3425.

[13] Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R. and Thompson, J.D. (2007). Clustal W and Clustal X version 2.0. *bioinformatics*, 23:2947-2948.

[14] Mir, R.A., Bhat, K.A., Rashid, G., Ebinezer, L.B., Masi, A., Rakwal, R., Shah, A.A. and Zargar, S.M. (2021). DNA barcoding: a way forward to obtain deep insights about the realistic diversity of living organisms. *The Nucleus*, 64:157-165.

[15] Parveen, I., Singh, H.K., Malik, S., Raghuvanshi, S. and Babbar, S.B. (2017). Evaluating five different loci (*rbc L, rpo B, rpo C1, mat K,* and *ITS*) for DNA barcoding of Indian orchids. Genome, 60:665-671.

[16] Parveen, I., Singh, H.K., Raghuvanshi, S., Pradhan, U.C. and Babbar, S.B. (2012). DNA barcoding of endangered Indian Paphiopedilum species. *Molecular Ecology Resources*, 12:82-90.

[17] Prasad, K., Karuppusamy, S. and Pullaiah, T., 2019. Orchids of Eastern Ghats (India). Scientific Publishers.

[18] Rajphriyadharshini, R. and Weerasena, O.V.D.S.J., 2020. DNA barcoding of medicinal plant: a systemic review. *Int J Pharm Sci Invent*, 9:06-16.

[19] Ramudu, J. and Khasim, S.M., DNA Barcoding of Some *Coelogyne, Orchid Soc. India,* 30:65-73, 2016

[20] Singh, H.K., Parveen, I., Raghuvanshi, S. and Babbar, S.B., 2012. The loci recommended as universal barcodes for plants on the basis of floristic studies may not work with congeneric species as exemplified by DNA barcoding of Dendrobium species. *BMC research notes*, 5:1-11.

[21] Singh, S.K., Agrawala, D.K., Jalal, J.S., Dash, S.S., Mao, A.A. and Singh, P., 2019. *Orchids of India: A pictorial guide*. Botanical Survey of India, Ministry of Environment, Forest and Climate Change.

[22] Srivastava D, Manjunath K. (2017)DNA barcoding of orchids in India: benefits and requirements toskar Newsletter, 4;4: 9- 12.

[23] Srivastava, D. and Manjunath, K., 2020. DNA barcoding of endemic and endangered orchids of India: A molecular method of species identification. Pharm. Mag, 16: 290-299.

[24] Xu, S., Li, D., Li, J., Xiang, X., Jin, W., Huang, W., Jin, X. and Huang, L. (2015). Evaluation of the DNA barcodes in *Dendrobium* (Orchidaceae) from mainland Asia. *PloS one*, 10:0115168.

[25] Yao, H., Song, J., Liu, C., Luo, K., Han, J., Li, Y., Pang, X., Xu, H., Zhu, Y., Xiao, P. and Chen, S. (2010). Use of ITS2 region as the universal DNA barcode for plants and animals. *PloS one*, 5:e13102.

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