

**DIVERSITY AND MYCORRHIZAL SPECIFICITY OF VANDA
AND CYMBIDIUM GENUS (ORCHIDACEAE) OF WESTERN
AND EASTERN GHATS USING DNA BARCODING**

FINAL PROJECT REPORT

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Submitted by

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EXECUTIVE SUMMARY OF THE PROJECT

The Genus of *Vanda* and *Cymbidium* from different locations of Eastern and Western Ghats was collected. Mycorrhizal fungi are successfully isolated from the roots *Vanda* and *Cymbidium*. The cultured fungi are identified using morphology and it found to be endophytic mycorrhizae. From the cultured fungi DNA was isolated, amplified and sequenced using ITS and LSU region. The identified species are *Ceratobasidium sp.*, *Tulasnella sp.*, and *Rhizoctonia-like species*. DNA barcoding for the parallel Host (Genus *Vanda* and *Cymbidium*) was done through rbcL and trnH-psbA spacer regions. In vitro symbiotic seed germination studies showed that the specific fungi will germinate only the specific plant species. The phlogenetic tree obtained by MEGA 6.0 suggested that the fungi isolated from different accession has Genus specific to the host rather than diverse among the environment and are closely related.

Table:1 Location and number of accessions of the collected plant samples (*Cymbidium aloifolium* (L.) Sw. and *Cymbidium bicolor* Lindl.)

Name of the plant	Location	Number of Accessions	Altitude (mts)	Latitude	Longitude
<i>Cymbidium aloifolium</i> (L.) Sw.	Iduki	3	940	17°20'33"N	64°26'35"E
	Kodaikannal	2	760	11°55'8"N	56°43'21"E
	Palani hills	-	-	-	-
	Shevaroy hills	1	650	13°20'33"N	75°26'30"E
	Kolli hills	-	-	-	-
	Pachammalai	-	-	-	-
	Brahmagiri	2	845	19°78'89" N	85°61'26"E
<i>Cymbidium bicolor</i> Lindl.	Iduki	-	-	-	-
	Kodaikannal	2	625	10°19'9"N	75°34'37"E
	Palani	-	-	-	-
	Shevaroy hills	2	738	11°77'58"N	78°20'93"E
	Kolli hills	1	647	11°24'85"N	78°33'87"E
	Pachamalai	-	-	-	-
	Brahmagiri	3	743	19°78'89" N	85°61'26" E

Table:2 Location and number of accessions of the collected plant samples (*Vanda spathulata* (L.) Spreng., *V. testacea* (Lindl.) Rchb.f. and *V. tessellata*)

Name of the plant	Location	Number of Accessions	Altitude (mts)	Latitude	Longitude
<i>Vanda spathulata</i> (L.) Spreng.	Iduki	-	-	-	-
	Kodaikannal	1	674	10 ⁰ 23'81''N	77 ⁰ 48'92''E
	Palani hills	1	578	10 ⁰ 44'89''N	77 ⁰ 52'09''E
	Shevaroy hills	-	-	-	-
	Kolli hills	1	475	13 ⁰ 30'38''N	78 ⁰ 45'28''E
	Pachamalai	-	-	-	-
	Brahmagiri	-	-	-	-
<i>V. testacea</i> (Lindl.) Rchb.f.	Iduki	-	-	-	-
	Kodaikannal	-	-	-	-
	Palani hills	-	-	-	-
	Shevaroy hills	4	495	11 ⁰ 74'53''N	78 ⁰ 20'93''E
	Kolli hills	3	750	13 ⁰ 26'42''N	75 ⁰ 41'11''E
	Pachamalai	-	-	-	-
	Brahmagiri	-	-	-	-
<i>V. tessellata</i> (Roxb.) Hook. ex G.Don	Iduki	-	-	-	-
	Kodaikannal	-	-	-	-
	Palani hills	-	-	-	-
	Shevaroy hills	2	568	11 ⁰ 77'53''N	76 ⁰ 73'85''E
	Kolli hills	-	-	-	-
	Pachamali	1	431	10 ⁰ 47'89''N	77 ⁰ 44'62''E
	Brahmagiri	-	-	-	-

Table.3: Total Number of fungi isolated from the Genus *Cymbidium* from different accessions

Name of the plant	Location	Number of Accessions	Number of Mychorrizal Fungi Isolated	Number of Non-Mychorrizal Fungi Isolated	Total No. of Fungi Isolated
<i>Cymbidium aloifolium</i> (L.) Sw.	Iduki	3	2	4	6
	Kodaikannal	2	3	2	5
	Palani hills	-	-	-	-
	Shevaroy hills	1	3	2	5
	Kolli hills	-	-	-	-
	Pachamalai	-	-	-	-
	Brahmagiri	2	3	4	7
<i>Cymbidium bicolour</i> Lindl.	Iduki	-	-	-	-
	Kodaikannal	2	4	5	9
	Palani	-	-	-	-
	Shevaroy hills	2	2	3	5
	Kolli hills	1	1	2	3
	Pachamalai	-	-	-	-
	Brahmagiri	3	2	2	4

Table. 4: Total Number of fungi isolated from the Genus Vanda from different accessions

Name of the plant	Location	Number of Accessions	Number of Mychorrizal Fungi Isolated	Number of Non-Mychorrizal Fungi Isolated	Total No. of Fungi Isolated
<i>Vanda spathulata</i> (L.) Spreng.	Iduki	-	-	-	-
	Kodaikannal	1	-	-	-
	Palani hills	1	-	-	-
	Shevaroy hills	-	-	-	-
	Kolli hills	1	1	-	1
	Pachamalai	-	-	-	-
	Brahmagiri	-	-	-	-
<i>V. testacea</i> (Lindl.) Rchb.f.	Iduki	-	-	-	-
	Kodaikannal	-	-	-	-
	Palani hills	-	-	-	-
	Shevaroy hills	4	6	7	13
	Kolli hills	3	1	1	2
	Pachamalai	-	-	-	-
	Brahmagiri	-	-	-	-
<i>V. testellata</i>	Iduki	-	-	-	-
	Kodaikannal	-	-	-	-
	Palani hills	-	-	-	-
	Shevaroy hills	2	2	3	5
	Kolli hills	-	-	-	-
	Pachamalai	1	1	2	3
	Brahmagiri	-	-	-	-

Sequence Submission in NCBI

Table.5 Genbank accession numbers for fungi sequences

Fungi	Genbank accession number
<i>Ceratobasidium cereal</i>	MG002431
<i>Ceratobasidium species</i>	MG002432
<i>Ceratobasidium cereal</i>	MG002433
<i>Ceratobasidium ramicola</i>	MG002434
<i>Ceratobasidium species</i>	KY945486
<i>Ceratobasidium species</i>	KY945488
<i>Tulasnella species</i>	MH468786
<i>Tulasnella species</i>	MH479401
<i>Tulasnella species</i>	MH479402
<i>Tulasnella species</i>	MH479403
<i>Tulasnella species</i>	MH479404
<i>Tulasnella species</i>	MH479405

Table.6 Genbank accession numbers for plant sequences

Plant	Genbank accession number
<i>Vanda testacea</i>	MF446877
<i>Vanda testacea</i>	MF446878
<i>Vanda testellata</i>	MF446879
<i>Vanda testellata</i>	MF446880
<i>Vanda spathulata</i>	MF446881
<i>Cymbidium aloifolium</i>	MF446882
<i>Cymbidium bicolor</i>	MF446883
<i>Vanda testellata</i>	MF446884

Table.7: Overview of the study

No. of plant accessions collected	No. of Fungi Isolated	No. of Fungi Sequenced and submitted in Genbank	No. of Plant Sequenced and submitted in Genbank	No. of sequences yet to get accession numbers	Total No. of sequences
29	67 (31 - mycorrhizal fungi 36- non-mycorrhizal fungi)	13	12	10	35

NO. OF PUBLICATIONS OUT OF THE PROJECT

S.No.	Paper Title	Name of the Journal	Status
1.	SPECIFICITY OF MYCORRHIZAL FUNGI ISOLATED FROM THE ROOTS OF VANDA TESTACEA FROM FIVE DIFFERENT REGIONS USING DNA BARCODING	Journal of Emerging Technologies and Innovative Research	July 2018
2.	MOLECULAR CHARACTERIZATION OF MYCORRHIZAL FUNGI ISOLATED FROM THE ROOTS OF CYMBIDIUM SPECIES	World Journal of Pharmaceutical Research	May 2018
3.	DNA Barcoding of Vanda Species from the Regions of Shevaroy and Kolli Hills using rbcL gene	Biotechnological Research	October 2017
4.	Morphotypes of Mycorrhizal Fungi of Cymbidium Species	International Journal of Pure and Applied Biosciences	August 2016
5.	Morphotypes of Mycorrhizal Fungi of Vanda Species	International Journal of Applied Sciences	June 2016
6.	DNA Sequencing of Cymbidium Bicolor Lindl. Using trnL-F Intergenic Spacer	International Journal of Innovative Research in Science, Engineering and Technology	April 2016
7.	DNA SEQUENCING OF AERIDES MACULOSA LINDL USING trnL-F INTERGENIC SPACER-A NOVEL APPROACH	International Multidisciplinary e-Journal	March 2016