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Isolation of *Acrodontium crateriforme* as a pitcher trap inquiline

Prabhugaonkar A and Pratibha J

Botanical Survey of India, Eastern Regional Centre, Shillong-793003.

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Abstract

Acrodontium crateriforme was isolated from pitcher trap liquid of *Nepenthes khasiana* in Khasi hills of Meghalaya. Molecular phylogenetic analysis of culture obtained from plating of pitcher trap liquid confirmed the identity of the species. The association of fungus is reported in this manuscript. Pitcher plant trap biota is matter of interest to many biologists, as it is known to trap insects and provide nourishment to plants, but it also provides habitat for numerous species of inquilines ranging from bacteria to vertebrates which survive in pitcher trap liquid. This report of presence of *A. crateriforme* is important record of fungal association in unique habitat provided by pitcher trap.

Key words – Anamorphic fungus – Khasi hills – Taxonomy

Introduction

Nepenthes khasiana, the pitcher plant is endemic to Khasi hills of Meghalaya. The species is becoming rare in the wild. In our recent study carried out for fungal associations with native plants of the region, we observed an unusual fungus usually associated with pitcher trap liquid. Upon culturing and molecular phylogenetic analysis the taxon was identified as *Acrodontium crateriforme* (J.F.H. Beyma) de Hoog. *Acrodontium crateriforme* is recorded on various hosts including from insect excretions (De Hoog, 1972). Pitcher plant trap biota is matter of interest to many biologists, as it is known to trap insects and provide nourishment to plants, it also provides habitat for numerous species of inquilines ranging from bacteria to vertebrates which survive in pitcher trap liquid (Adlassnig et al. 2011). This report of presence of *A. crateriforme* is important record of fungal association in unique habitat provided by pitcher trap.

Materials & Methods

Freshly gathered pitcher plant trap samples from various places of Khasi hills, Meghalaya were taken to the laboratory in polythene bags and examined under a stereomicroscope. Serial dilution and plating of pitcher trap liquid was carried out to obtain various fungal cultures. *A. crateriforme* often isolated from pitcher trap liquid was selected for further study. Molecular sequencing was done at Rajiv Gandhi Centre for Biotechnology, Thiruvananthpuram, Kerala, India.

DNA isolation and PCR Analysis

Fresh fungal mycelia (20 mg), scraped from the growing culture incubated at 28°C for 7 days. DNA isolation and PCR amplification was done according to Prabhugaonkar & Bhat (2011). The

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Corresponding Author: Ashish Prabhugaonkar – e-mail – ashishprabhugaonkar@gmail.com

internal transcribed spacers (ITS), large subunits of the nuclear ribosomal RNA genes (LSU) gene regions were amplified and sequenced using the primer pairs ITS5/ITS4 (White et al. 1990) and LROR/LR5 (Vilgalys & Hester, 1990), respectively. The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out using Geneious Pro v5.1 (Drummond et al. 2010).

Sequence alignment and phylogenetic analysis

The sequences were blasted in GenBank with Blastn. Based on the blasts analysis and related literature (Videira et al. 2016) further related sequences were assembled. ITS and LSU sequence data sets were generated (Table 1). The combined data matrix was aligned using MAFFT v.7 (http://mafft.cbrc.jp/alignment/software) and manually adjusted using MEGA 7 to allow maximum alignment and maximum sequence similarity. A phylogenetic analysis was conducted using maximum likelihood (ML) in MEGA 7 (Kumar et al. 2008) with 1000 bootstrap replicates. The most suitable substitution models was selected by using MEGA 7. Kimura 2-parameter model with Gamma distributed with Invariant sites (G+I) was used in analysis. Gaps were treated as a pairwise deletion and trees were viewed with MEGA 7. Newly generated sequences used in this study are deposited in GenBank.

Results

Acrodontium crateriforme (J.F.H. Beyma) de Hoog. Fig. 1

Colonies on MEA upto 3 cm in 8 days, greeish brown, cottony, with aerial mycelial cords. Mycelium branched, septate, hyaline, smooth-walled, 1–2 µm, forming thick cords. Asexual morph: Conidiophores macronematous, mononematous, septate, unbranched, rarely branched. Conidiogenous cells arising from the tip of a subtending cell, hyaline, tapering towards the tip, sympodial, denticulate, 25–75 × 1–2 µm. Conidia hyaline, smooth, ellipsoidal, guttuliform, 2–3 × 1–2 µm. Sexual morph: Undetermined.

Material examined – India, Meghalaya, Khasi hills, Umiam, Barapani, Experimental botanical garden in traps of *Nepenthes khasiana*, 17 December 2016, coll. A. Prabhugaonkar, ASSAM-AVP 113, ex-type culture AVPC 113. India, Meghalaya, Khasi hills, Mawsynram, Near Mawlongbna in traps of *Nepenthes khasiana*, 19 February 2017, coll. A. Prabhugaonkar. Ex-type culture AVPC 131. India, Meghalaya, Khasi hills, Shillong, BSI Campus, Laitumkhrah 10 August 2016, coll. A. Prabhugaonkar.

Discussion

Acrodontium seems to be polyphyletic with type species *Acrodontium crateriforme* observed to be placed in the family Teratosphaeriaceae (Videira et al. 2016). Phylogenetic analysis of current isolate also showed similar results (Fig. 2). However Videira et al. 2016 also observed that LSU sequences of isolates belonging to *A. antarcticum, A. abietis, A. griseum, A. hydnicola, A. salmoneum, A. simplex* and *A. virelum* are placed in different orders of Sordariomycetes and Leotiomycetes. Maharachchimbura et al. 2016 observed that *Acrodotium* like anamorphs occur in ascomycetes *Amplistroma* sp. which is placed in Amplistromataceae, Amplistromatales, Sordariomycetes, orders incertae sedis.

Acrodontium crateriforme is previously isolated from sputum of man, various insect exudates, plant materials, from rusts and slime molds (De Hoog, 1972, Seifert et al. 2011). Though fungus is cosmopolitan very less information is available about very complex host associations of this fungus. The current study further adds to this list of complex host associations. Further studies can surely provide interesting incites in lifestyle and biological activity of this organism.

Richards in 2001 observed with example of *Utricularia purpurea* that mature bladders have living communities of algae, zooplankton, and associated debris. Association is mutualism rather than a predator–prey interaction. Often benefit to the plants from bladders is derived from this community as the community helps release nutrients from insect cadavers and other debris.

Research carried out on North American pitcher trap biota *Sarracenia alata* (Koopman et al. 2010, Miller et al. 2002) has shown similar results and pitcher trap microcosm is been studied by biologist as model microcosm with different kind of interactions such as mutualism and parasitism. Chan et al. (2016) studied 18 bacterial isolates from pitcher trap liquid and observed that most of the bacterial isolates possess chitinolytic, proteolytic, amylolytic, cellulolytic and xylanolytic activities. However there is less research on other organisms found in pitcher traps. This report of presence of *A. crateriforme* is an important record of fungal association in a unique habitat provided by pitcher plant *Nepenthes khasiana,* an endemic to Khasi hills of India. Further research on associated biota and their interactions is required as these rare plants are fast losing their natural habitat and plant animal and microbial interactions may not be same in cultivated condition as that found in natural environment.

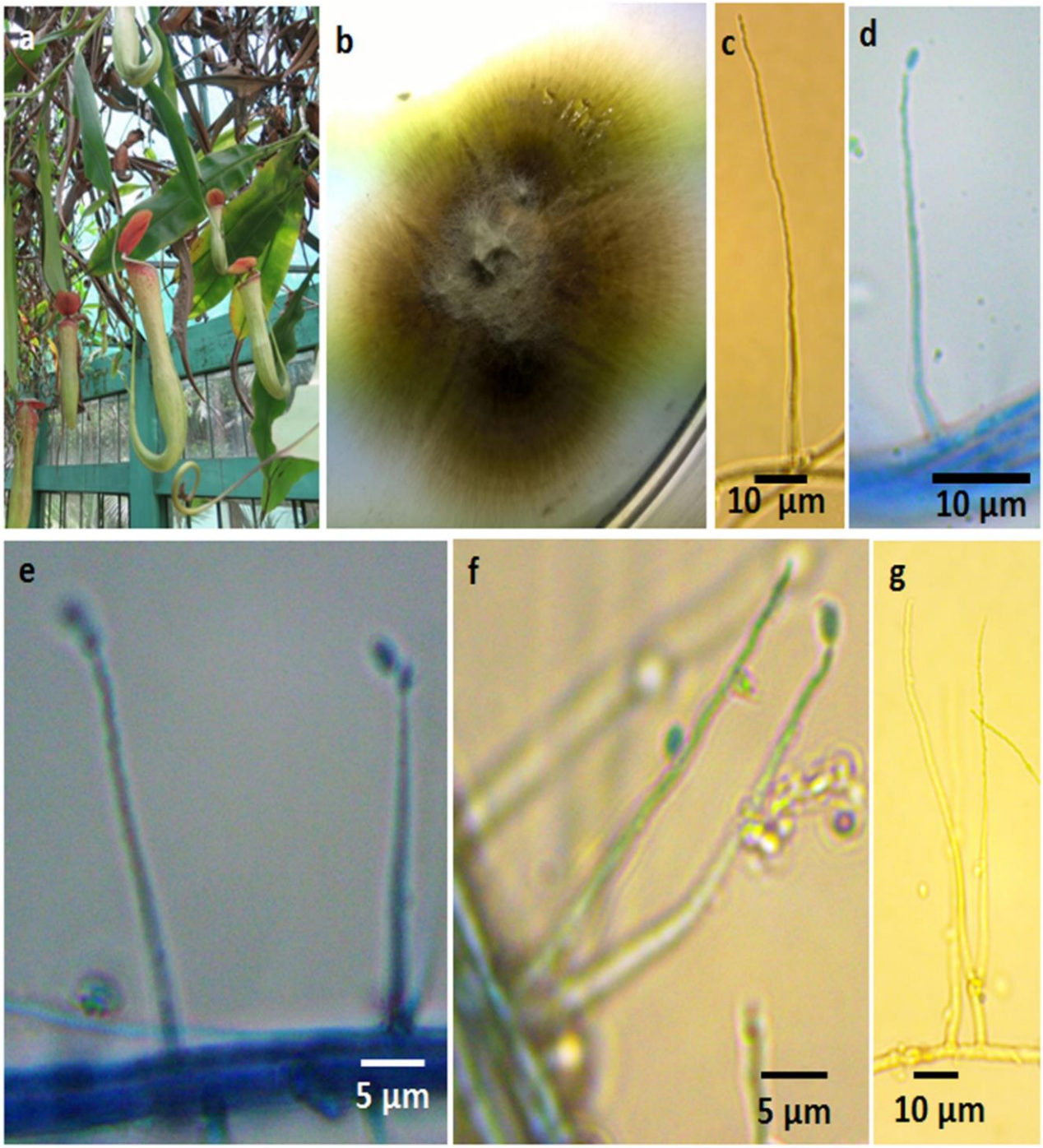

Fig. 1 – a. *Nepenthes khasiana*, Host plant. b-g. *Acrodontium crateriforme* b. Culture. c-g Conidiophores and formation of conidia.

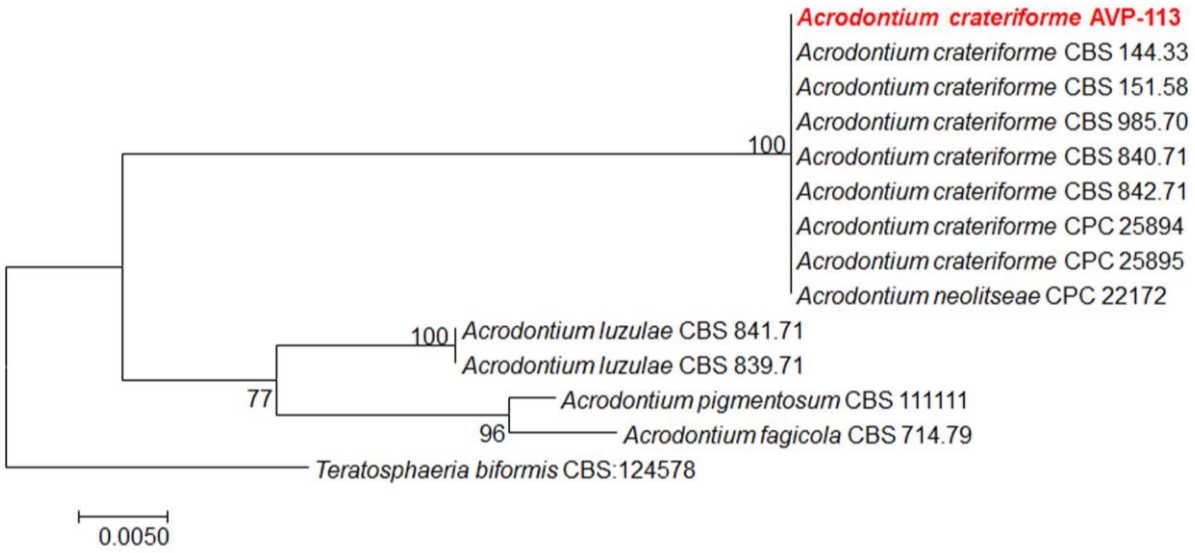

Fig. 2 – Maximum likelihood (ML) tree inferred from ITS and LSU sequence data confirming identity of *Acrodontium crateriforme* AVP 113.

Table 1 Sequence data used in combined ITS and LSU analyses. Newly deposited sequences are in bold

Taxon	Accession no.	ITS	LSU
Acrodontium crateriforme	**AVPC-113**	**MF613646**	**MF613959**
Acrodontium crateriforme	CBS 144.33	FN666565	KX286952
Acrodontium crateriforme	CBS 151.58	KX287266	KX286953
Acrodontium crateriforme	CBS 985.70	KX287267	KX286954
Acrodontium crateriforme	CBS 840.71	KX287268	KX286955
Acrodontium crateriforme	CBS 842.71	KX287269	KX286956
Acrodontium crateriforme	CPC 25894	KX287271	KX286958
Acrodontium crateriforme	CPC 25895	KX287272	KX286959
Acrodontium neolitseae	CPC 22172	KJ869127	KJ869184
Acrodontium luzulae	CBS 841.71	KX287273	KX286961
Acrodontium luzulae	CBS 839.71	KX287274	KX286962
Acrodontium pigmentosum	CBS 111111	KX287275	KX286963
Acrodontium fagicola	CBS 714.79	-	KX286960
Teratosphaeria biformis	CBS 124578	KF901564	KF901887

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