Check for updates

RESEARCH ARTICLE

Phylogeny and biogeography of the carnivorous plant family Droseraceae with representative *Drosera* species from Northeast India [version 1; peer review: 1 approved, 1 not approved]

Devendra Kumar Biswal ¹, Sureni Yanthan², Ruchishree Konhar ¹,
Manish Debnath ¹, Suman Kumaria ², Pramod Tandon^{2,3}

¹Bioinformatics Centre, North-Eastern Hill University, Shillong, Meghalaya, 793022, India

²Department of Botany, North-Eastern Hill University, Shillong, Meghalaya, 793022, India

³Biotech Park, Jankipuram, Uttar Pradesh, 226001, India

V1 First published: 14 Aug 2017, 6:1454
<https://doi.org/10.12688/f1000research.12049.1>
Latest published: 14 Aug 2017, 6:1454
<https://doi.org/10.12688/f1000research.12049.1>

Abstract

Background: Botanical carnivory is spread across four major angiosperm lineages and five orders: Poales, Caryophyllales, Oxalidales, Ericales and Lamiales. The carnivorous plant family Droseraceae is well known for its wide range of representatives in the temperate zone. Taxonomically, it is regarded as one of the most problematic and unresolved carnivorous plant families. In the present study, the phylogenetic position and biogeographic analysis of the genus *Drosera* is revisited by taking two species from the genus *Drosera* (*D. burmanii* and *D. peltata*) found in Meghalaya (Northeast India).

Methods: The purposes of this study were to investigate the monophyly, reconstruct phylogenetic relationships and ancestral area of the genus *Drosera*, and to infer its origin and dispersal using molecular markers from the whole ITS (18S, 28S, ITS1, ITS2) region and ribulose biphosphate carboxylase (rbcL) sequences.

Results: The present study recovered most of the findings by previous studies. The basal position of Droseraceae within the non-carnivorous Caryophyllales indicated in the tree topologies and fossil findings strongly support a date of origin for Droseraceae during the Paleocene (55-65 mya). Within the family Droseraceae, the sister relationship between *Aldrovanda* and *Dionaea* is supported by our ITS and rbcL dataset. This information can be used for further comparative and experimental studies.

Conclusions: *Drosera* species are best suited as model systems for addressing a wide array of questions concerning evolutionary dynamics and ecological processes governing botanical carnivory.

Open Peer Review

Approval Status

	1	2
version 1 14 Aug 2017	view	view

1. **Andreas Fleischmann**, Ludwig-Maximilians-Universität München, Munich, Germany
2. **Lingaraj Sahoo**, Indian Institute of Technology Guwahati (IIT Guwahati), Guwahati, India

Any reports and responses or comments on the article can be found at the end of the article.

Keywords

Botanical carnivory, Droseraceae, Ancestral area reconstruction, Biogeography, Taxongap

This article is included in the **Phylogenetics** collection.

Corresponding authors: Devendra Kumar Biswal (devbioinfo@gmail.com), Pramod Tandon (profptandon@gmail.com)

Author roles: **Biswal DK:** Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Resources, Supervision, Validation, Writing – Original Draft Preparation, Writing – Review & Editing; **Yanthan S:** Data Curation, Formal Analysis, Methodology, Writing – Original Draft Preparation; **Konhar R:** Data Curation, Formal Analysis, Methodology, Software, Validation, Writing – Review & Editing; **Debnath M:** Data Curation, Formal Analysis, Methodology, Software, Validation, Visualization; **Kumaria S:** Methodology, Resources, Supervision, Validation; **Tandon P:** Conceptualization, Funding Acquisition, Investigation, Project Administration, Resources, Supervision, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: This work was supported by the Department of Biotechnology, Government of India (<http://btisnet.gov.in/>; grant ID BT/BI/04/035/98 sanctioned to DKB and PT) and University Grants Commission-Rajiv Gandhi National Fellowship to SY.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Copyright: © 2017 Biswal DK *et al.* This is an open access article distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: Biswal DK, Yanthan S, Konhar R *et al.* **Phylogeny and biogeography of the carnivorous plant family Droseraceae with representative *Drosera* species from Northeast India** [version 1; peer review: 1 approved, 1 not approved] F1000Research 2017, 6:1454 <https://doi.org/10.12688/f1000research.12049.1>

First published: 14 Aug 2017, 6:1454 <https://doi.org/10.12688/f1000research.12049.1>

Introduction

The carnivorous plant family Droseraceae is well known for its complex taxonomic diversity in temperate climatic regions. The family comprises nearly 200 species with two monotypic genera *Aldrovanda* and *Dionaea* and one large genus *Drosera* (popularly named as sundew) with a maximum number of species^{1,2,3}. The name *Drosera* is derived from the Greek word meaning 'dew-drops'. These types of plants usually exhibit remarkable tolerance to high-stress habitats and have acquired adequate reproductive fitness on the evolutionary ladder for their survival⁴. Specialized carnivory traps common to all *Drosera* species are in fact highly modified leaves lined with mucilaginous glandular trichomes or tentacles. *Drosera* species mostly inhabit regions of the Southern hemisphere and Southwestern Australia. In India, *Drosera* species are found in some parts of the Northeastern region, Deccan peninsular region, Southern India and along regions in West Bengal^{5,6}. Of the three known *Drosera* species (*D. burmanii* Vahl., *D. indica* L. and *D. peltata* Thund.) reported in India, two are found in Meghalaya i.e., *D. burmanii* and *D. peltata*⁷.

Drosera species can be grouped into five different habits depending on their growth forms, such as temperate sundews, pygmy sundews, subtropical sundews, tuberous sundews and the petiolaris complex. The diversity of growth forms in this genus is so vast that it comprises annual species forming hibernaculum in winter dormancy or underground tubers in extreme dry summers. The long tentacles on leaves are often brightly coloured and tipped with nectar secreting glands, adhesive compounds, as well as digestive enzymes. These tentacles start moving in to bring as many secretory glands as possible in contact with the prey upon capture. According to Darwin⁸, glandular formations present in *Drosera* leaves secrete proteolytic enzymes similar to those found in the animal stomach. It also demonstrates that the substances solubilized and decomposed by the action of enzymes are absorbed by plant foliage. In some species, (for example *D. burmannii*), the tentacle motion is quite remarkable as the glands can bend 180° in just fractions of a second.

Many *Drosera* species are best known for their valuable natural products. Secondary metabolites from sundews, such as 1,4-naphthoquinones and flavonoids, have significantly contributed to folklore medicinal practices worldwide⁹. There are reports in ancient literature describing medicinal usage of different species of *Drosera* in treating epilepsy¹⁰. Many species of *Drosera* are threatened in India due to their confined distribution and extensive usage in the herbal industry, and thus have been categorized as vulnerable by the International Union for Conservation of Nature^{6,11,12}.

Candolle proposed the first infrageneric classification of *Drosera*, with two recognized sections of the plant based upon the characteristics and morphology of their styles¹³. Later Seine and Barthlott¹⁴ described three subgenera and 11 sections based on morphological, anatomical, palynological and cytotoxicological studies. The phylogenetic study of Williams *et al.*¹⁵, based on ribulose biphosphate carboxylase (rbcL) sequences and morphological data, could identify three major lineages within *Drosera* with subgenus *regia* emerging as the first branch, followed by

subgenus *capensis*. Rivadavia *et al.*¹⁶ made an attempt to understand *Drosera* systematics based on the rbcL and 18S regions. The study highlighted *D. regia* and *D. arcturi* to be basal species for *Drosera*.

The species distribution of the genus *Drosera* ranges from both the hemispheres with about ~80 species in Australia, ~30 species in Africa, including North Africa and South Africa, ~30 species in South America, and less than 10 species in North America and Eurasia¹⁷. The phylogeography is not merely an extension of phylogenetic principles to the intraspecific level, rather it describes the population strata by utilizing the information belied in geographical patterns of ancestral lineage across the range of a species¹⁸. Understanding the process of colonization and population divergence of this species is fundamental to the study of its evolutionary diversification. Previous studies based on rbcL markers¹⁶ showed that the South American *Drosera* species arose from Australian species by dispersal, and the African species other than *D. regia* and *D. indica* arose subsequently from their ancestors in South America. Another study conducted by Rivadavia *et al.*¹⁹ on multidisciplinary studies of *D. meristocaulis*, prevalent in Neblina highlands of northern South America, proposed a long-distance dispersal from Australia to South America. It was also found that the section *Bryastrum* diversified from its ancestor about 13-12 MYA and does not agree to the Gondwanan origin for the *D. meristocaulis*^{19,20}. Rivadavia *et al.*¹⁶ vouched for South African/ Australian origin of *Drosera*. Though the outcomes of their analysis could be attributed to Croizat and Gondwanan vicariance, the origin of *Drosera* is not supported by the recent studies on Droseraceae and their evolution¹⁶. It implies more work needs to be done to fully understand the evolution of the family Droseraceae and the genus *Drosera*, in particular.

In the present study, the phylogenetic position and biogeographic study of the genus *Drosera* is revisited, by representing the two species of genus *Drosera* (*D. burmanii* and *D. peltata*) found in Meghalaya (Northeast India). The purposes of this study were (1) to investigate the monophyly of the genus *Drosera*, reconstruct phylogenetic relationships and ancestral area reconstruction of the genus *Drosera* in the family Droseraceae, (2) to infer the origin and dispersal of *Drosera*, and (3) to infer the phylogenetic relationships among *Aldrovanda*, *Dionaea*, and *Drosera*, using molecular markers from the whole ITS (18S, 28S, ITS1, ITS2) region and rbcL sequences.

Methods

Survey, collection and taxon sampling

Insectivorous plant species in the genus *Drosera* were collected from different regions of Meghalaya, according to their present availability. The collected plants included two species of *Drosera* viz. *D. peltata* and *D. burmannii* (Figure 1 and Figure 2). *Drosera burmanii* Vahl. was collected from Jarain, Jaintia Hills District, Meghalaya (N 25°36', E 92°15') and *Drosera peltata* Sm. was collected from Cherrapunjee, East Khasi Hills District, Meghalaya (N 25°07', E 91°28'). Identification of these insectivorous plants was carried out at the Botanical Survey of India (BSI), Eastern circle, Shillong, Meghalaya. Herbariums were prepared and submitted in BSI and Department of Botany, North-Eastern Hill University

Figure 1. *Drosera peltata*. (a) Plant in natural habitat, (b) close up view of leaf, (c) trapped insect, and (d) whole plant.

Figure 2. *Drosera burmanii*. (a) Plant in natural habitat, (b) close up view of leaf, (c) trapped insects (blue arrows), and (d) whole plant.

(NEHU), Shillong. Specimen voucher numbers (NEHU) and accession numbers (BSI) of *Drosera burmanii* are 11924 and 86843; and *Drosera peltata* are 11962 and 86840, respectively. We amplified the whole ITS and *rbcL* regions from all the above-mentioned plants for the proposed work. In addition, we collected GenBank data that included these markers from representative species belonging to the genus *Nepenthes*, *Drosera*, *Aldrovanda*, *Dionaea* and *Sarracenia*, along with their geographical distribution information (Table 1).

DNA extraction, PCR amplification and sequencing

For *Drosera* sp., the leaves and the stems were taken, washed thoroughly with water removing all the dirt and debris of insects, kept in 70% alcohol for a few minutes, dried, and then wrapped in aluminum foil and stored in liquid nitrogen for further use. Total genomic isolation from *Drosera* was carried out using DNeasy Plant Mini Kit (Qiagen, USA), according to the manufacturer's instructions with minor modifications (combination of a borate extraction buffer with the DNA extraction kit, and a proteinase K

Table 1. List of *rbcL* and ITS GenBank accessions for representative species belonging to Nepenthaceae, Droseraceae and Sarraceniaceae carnivorous plant families used in this study along with their geographical distribution.

Name	18s ITS1 28s ITS2	<i>rbcL</i>	Distribution
<i>Aldrovanda vesiculosa</i>	AB330991.1	AB072550.1	Europe, Asia, Africa, Australia
<i>Dionaea muscipula</i>	AB675913.1	L01904.2	North Carolina, South Carolina
<i>Drosera anglica</i>	AB355666.1	AB355692.1	Japan, Southern Europe, Hawaiian island of Kaua'i, and California, Alaska, Canadian provinces
<i>Drosera barbigera</i>	JQ712490.1	JQ712489.1	Western Australia
<i>Drosera binata</i>	HM204879.1	AB072922.1	Australia, New Zealand
<i>Drosera brevifolia</i>	JN388055.1	AB072519.1	Southeast Asia, Australia
<i>Drosera burmannii</i>	JN388056.1	KT794003	India, Southeast Asia, Australia
<i>Drosera capensis</i>	HM204880.1	AB917049.1	South Africa
<i>Drosera capillaris</i>	JN388059.1	KJ773463.1	Africa, United States, Brazil
<i>Drosera chrysolepis</i>	JN388060.1	AB072522.1	Peru, Brazil, Ecuador
<i>Drosera cistiflora</i>		AB072523.1	South Africa
<i>Drosera collinsiae</i>	JN388061.1	AB072524.1	South Africa
<i>Drosera communis</i>	JN388070.1		Brazil, Colombia, Paraguay, Venezuela
<i>Drosera cuneifolia</i>		AB072525.1	South Africa
<i>Drosera dielsiana</i>	JN388062.1		Malawi, Zimbabwe, South Africa, Mozambique
<i>Drosera falconeri</i>	HM204882.1	KP268943.1	Australia
<i>Drosera filiformis</i>	JN388063.1	KJ773464.1	United States
<i>Drosera glanduligera</i>	JN388039.1	AB072511.1	Australia
<i>Drosera graminifolia</i>	JN388064.1	AB072528.1	Brazil
<i>Drosera graomogolensis</i>	JN388065.1	AB072529.1	Brazil
<i>Drosera hamiltonii</i>	HM204884.1	AB072921.1	Australia
<i>Drosera hirtella</i>	JN388066.1	AB072531.1	Brazil
<i>Drosera indica</i>	JN388067.1		Australia, Asia, Africa
<i>Drosera intermedia</i>	JN388069.1	JN891175.1	Europe, United states, Cuba, South America
<i>Drosera madagascariensis</i>	JN388071.1	AB072533.1	Madagascar, South Africa, Botswana
<i>Drosera meristocaulis</i>	JN388038.1	JN388035.1	Venezuela, Brazil
<i>Drosera montana</i>	JN388072.1	AB072536.1	Venezuela, Peru, Bolivia, Brazil, Paraguay, Argentina
<i>Drosera natalensis</i>	JN388073.1	AB072537.1	South Africa, Mozambique, Madagascar

Name	18s ITS1 28s ITS2	rbcl	Distribution
<i>Drosera nidiformis</i>	HM204885.1		South Africa
<i>Drosera nitidula</i>	JN388040.1	JN388036.1	Australia
<i>Drosera occidentalis</i>	JN388042.1	AB072506.1	Australia
<i>Drosera ordensis</i>	JN388075.1	JN388037.1	North West Australia
<i>Drosera paleacea</i>	HM204886.1	KP268941.1	South West Australia
<i>Drosera paradoxa</i>	JN388043.1		North West Australia
<i>Drosera pauciflora</i>		AB072552.1	South Africa
<i>Drosera peltata</i>	KF016002.1	KT794002	Australia, New Zealand, India, Southeast Asia
<i>Drosera pulchella</i>	JN388076.1	KP268939.1	South West Australia
<i>Drosera pygmaea</i>		AB072505.1	Australia, New Zealand
<i>Drosera rotundifolia</i>	AB355664.1	AB355691.1	North America, Korea, Japan, New Guinea
<i>Drosera schizandra</i>		KP268953.1	North East Australia
<i>Drosera scorpioides</i>	JN388041.1	AB072509.1	South West Australia
<i>Drosera sessilifolia</i>	JN388057.1	AB072551.1	Brazil, Guyana, Venezuela
<i>Drosera sewelliae</i>		KP268951.1	South West Australia
<i>Drosera slackii</i>	HM204889.1		South Africa
<i>Drosera spathulata</i>	AB355671.1	AB355696.1	China, Taiwan, Japan, Philippines, Indonesia, Malaysia, Palau, Australia, New Zealand
<i>Drosera tokaiensis</i>	AB355687.1	AB355698.1	Japan
<i>Drosera stenopetala</i>		AB072539.1	New Zealand
<i>Drosera tomentosa</i>	JN388053.1		South America
<i>Drosera villosa</i>	JN388054.1	AB072541.1	Brazil
<i>Nepenthes khasiana</i>	KT354296.1	KT285307.1	India
<i>Sarracenia flava</i>	JX042565.1	AB917045.1	United States

treatment during extraction). The chosen markers were subjected to PCR amplification with desired forward and reverse primer pairs, as listed in [Table 2](#). Reactions were performed in PCR tubes with a final volume of 100 µl. Each reaction mixture contained 4 µl of genomic DNA (30 ng/µl), 6 µl of dNTP* (2mM), 6 µl of 10X taq buffer B*, 4 µl of MgCl₂ (25mM)*, 0.8µl of taq polymerase (3 units/µl)*, 8µl of each primer pair (10 pm) (Metabion, Germany), final volume was made by adding sterilized Millipore water (*Bangalore Genei, India). DNA amplification was performed in an Applied Biosystems Gene-Amp PCR System 2700 programmed for 35 cycles (4 min at 94°C, 30 sec at 94°C, 1 min at 56°C, 40 sec at 72°C and 10 min at 72°C). Sequencing was carried out at Macrogen, Inc, Korea.

Phylogenetic analysis

Maximum likelihood. All ITS and rbcl sequences were first aligned using [MUSCLE](#)²¹ and subsequently concatenated using [MESQUITE V3.03](#)²². Highly variable sequence regions were excluded from analyses of the extended data set. Because initial separate calculations using noncoding spacer regions and coding matK sequences, yielded congruent but incompletely resolved

topologies, respectively, both partitions were combined in all subsequent analyses. Maximum likelihood (ML) analyses were carried out using [MEGA 7](#)²³. To find the best substitution model for our analyses, ML fits of 24 different nucleotide substitution models were performed. Models with the lowest BIC scores (Bayesian Information Criterion) are considered to describe the best substitution pattern. For each model, AICc value (Akaike Information Criterion, corrected), Maximum Likelihood value (lnL), and the number of parameters (including branch lengths) were also computed. The following models were verified for this study: General Time Reversible (GTR); Hasegawa-Kishino-Yano (HKY); Tamura-Nei (TN93); Tamura 3-parameter (T92); Kimura 2-parameter (K2); Jukes-Cantor (JC).

For ITS and rbcl dataset, the evolutionary history was inferred by using the ML method based on the Tamura-Nei model²⁴. Sequence information for the aligned dataset pertaining to total number of sites (excluding sites with gaps/missing data), sites with alignment gaps or missing data, invariable (monomorphic) sites, G+C content, parsimony informative sites, number of haplotypes (h), haplotype gene diversity (Hd), Nucleotide diversity per site (Pi),

average number of nucleotide differences (k) were computed and are shown in [Table 3](#). The evolutionary history of the taxa analyzed are represented from the bootstrap consensus tree from 500 replicates of the original dataset²⁵. Branches with less than 55% bootstrap replicates were collapsed. Initial tree(s) for the heuristic search were obtained by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. Evolutionary analyses were conducted in MEGA 7. The ML tree was further used in divergence time analysis.

Bayesian inference. The concatenated dataset from the previous analysis (in nexus format) was further analyzed with [MrBayes v.3.1.2](#)²⁶. The model of best fit was (GTR + Γ + I), determined with Modeltest. Posterior probabilities were estimated by sampling trees from the posterior probability distribution using the Metropolis-coupled Markov chain-Monte Carlo approach (MCMCMC) implemented in MrBayes²⁶, using default priors. The temperature of the heated chain was set to 0.2. Three

times, four chains were run for 1 million generations. Consensus trees, clade posterior probabilities, and mean branch lengths were computed relying on the trees sampled every 10 generations after the burn-in and the tree was viewed in [FigTree v1.3.1](#). A split decomposition median network graph tree was generated in [SplitsTree4](#)²⁷, with the variable positions in the same dataset.

TaxonGap analysis: Testing of markers for barcode suitability

A visualization analysis tool, [TaxonGap 2.4.1](#)²⁸, was used to illustrate the sequence divergences within and between species of the candidate markers from the ITS, rbcL and matK regions representing the family Droseraceae.

Secondary structure prediction and analysis

Consensus structures of ITS2 regions for the three genera in the Droseraceae family were predicted using [LocARNA](#)²⁹ from Freiburg RNA tools server, which outputs a multiple alignment

Table 2. Details of primers used in this study.

Regions	Primers	Sequences (5'-3')	References
ITS 1, 5.8S, ITS 2	ITS5	GGAAGTAAAAGTCGTAACAAGG	White <i>et al.</i> , 1990
ITS 1, 5.8S, ITS 2	ITS4	TCCTCCGCTTATTGATATGC	White <i>et al.</i> , 1990
rbcL	1F	ATGTCACCACAAACAGAAAC	Fay <i>et al.</i> , 1998
rbcL	1460R	TCCTTTTAGTAAAAGATTGGCCGAG	Fay <i>et al.</i> , 1998

Table 3. Sequence information of molecular markers used in this study.

Parameters	18s ITS1 28s ITS2	rbcL
Number of sequences	44	45
Selected region	1-818	1-1227
Number of sites	818	1227
Total number of sites (excluding sites with gaps / missing data)	320	498
Sites with alignment gaps or missing data	498	729
Invariable (monomorphic) sites	68	397
Variable (polymorphic) sites	252	101
G+C content, G+C	0.62 (320.00 sites)	0.436 (498.00 sites)
Parsimony informative sites	190	57
Number of Haplotypes, h	40	37
Haplotype (gene) diversity, Hd	0.966	0.991
Nucleotide diversity (per site), Pi	0.21118	0.03658
Average number of nucleotide differences, k	67.57717	18.21818

together with a consensus structure. For the folding, a very realistic energy model for RNAs was used that features RIBOSUM-like similarity scoring and realistic gap cost. The high performance of LocARNA was mainly achieved by employing base pair probabilities during the alignment procedure.

Fossil data calibration, age estimation and ancestral area reconstruction

A Time-tree was generated using the RelTime method in MEGA 7^{23,30}. Divergence times for all branching points in the user-supplied topology for estimating phylogenetic history and divergence times of Droseraceae were calculated using the ML method based on the Tamura-Nei model²⁴. The estimated log likelihood value of the topology shown is -26876.8062. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 2.0910)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 0.0000% sites). The tree is drawn to scale, with branch lengths measured in the relative number of substitutions per site. There were a total of 530 positions in the final dataset.

Fossils of Droseraceae pollens from the Eocene (55-38 MYA)³¹, present day *Aldrovanda vesiculosa* and its ancestral species (pollen fossil records) dating to Miocene in South Ural, Eocene in Kazakhstan and Belgium and Paleocene in East Germany were considered as internal calibration points while drawing the Time-tree phylogeny. The first real *Drosera* pollens have appeared in sediments from Miocene (25-5 MYA)³¹. Based on these studies and fossil data information on Droseraceae³¹, the following constraints were applied with a normal prior distribution that spanned the full range of nodal age estimates: the most recent common ancestor (MRCA) of Droseraceae (divergence between *Drosera* and *Aldrovanda* species) was set with a minimum and maximum divergence time to 38 and 55, respectively; the MRCA of *Drosera* species was set to 22-5 MYA.

For biogeographic inference, Bayesian Binary MCMC (BBM) and Statistical Dispersal-Vicariance Analysis (S-DIVA) methods were employed in which biogeographic reconstructions were averaged over a sample of highly probable Bayesian trees³². In S-DIVA the occurrence of an ancestral range at a node was computed using all alternative reconstruction frequencies generated by the DIVA algorithm for each tree in the data set. To account for both phylogenetic and ancestral states uncertainty S-DIVA was utilized for an entire posterior distribution of trees. Different geographic areas of endemism for the carnivorous plants meant for this study consistent with the present distribution with both outgroup and in-group sampling are outlined in Table 1.

Results

Phylogenetic analysis

The rbcL gene and ITS regions were separately aligned and ML and parsimonious trees were used for the phylogenetic analyses. The nucleotide sequences were aligned without any insertions or deletions. A total of 52 accessions from the Droseraceae family, including *N. khasiana* (Nepenthaceae) and *S. flava* (Sarracenaceae) (used as out-groups), were considered for

revisiting the *Drosera* phylogeny (Table 1). A separate concatenated dataset of ITS and rbcL were taken for Bayesian phylogeny reconstruction. The consensus core secondary structures of ITS regions for *Drosera* species as per their geographical distribution were drawn and shown in Figure 3. The ML tree was further used for time divergence studies.

Major clades within *Drosera* determined via phylogenetic analysis were subjected to relative rate tests using ML estimates of substitutions per site between taxon groups according to the model of best fit (GTR + Γ + I), determined with Modeltest in MEGA 7²³. Relative rates were intended to provide evidence of whether certain longer branches within *Drosera* were the result of rate acceleration of individual species. Models with the lowest BIC scores were considered suitable for the analysis. For each model, AICc value, ML value and the number of parameters (including branch lengths) were also presented (Table 4). Evolutionary rates among sites and their non-uniformity were modeled by using a discrete Gamma distribution (+G) with 5 rate categories. Estimates of gamma shape parameter and/or the estimated fraction of invariant sites are shown (Table 4). For each model assumed or estimated values of transition/transversion bias (R) are shown and are followed by nucleotide frequencies (f) and rates of base substitutions (r) for each nucleotide pair. Sum of r-values is made equal to 1 for each model.

For estimating ML values, a user-specified topology was used. The analysis involved 52 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 530 positions in the final dataset. All analyses supported the monophyly of Droseraceae. *Aldrovanda* and *Dionaea* species cladded well, and emerged as a sister branch to the genus *Drosera*. *N. khasiana* and *S. flava* emerged as outgroups. The Bayesian tree and the split tree also congrued with the results of ML tree, though there were slight changes in the overall topology of *Drosera* species, with very high support (100 percent bootstrap support [BS] values; 1.0 Bayesian posterior probability). *Drosera* species (*D. burmannii* and *D. peltata*) from Meghalaya grouped separately and emerged to be evolutionarily primitive (Figure 4 and Figure 5).

TaxonGap analysis

A DNA barcode marker is judged by its resolving power to discriminate species at generic and infrageneric levels. The intra- and inter-specific sequence divergence amongst the candidate markers chosen for the present study showed a comparative pictorial barcode gap in form of taxon-plots for the marker candidates (ITS, matK, rbcL) for species representing the family Droseraceae. The results are summarized in Figure 6. For each species, sequence similarity of the same gene within the same species was high; therefore, the relevant intra-specific variation (shown as dark grey bars) was low. TaxonGap plots have the discriminatory power to gauge better marker barcodes when phylogenetic trees for multiple genes need to be compared. Moreover, it uses the same scaling for depicting distance values based on individual biomarkers, thus making it straightforward to evaluate multiple genes rather than the need for comparing separate gene trees drawn for each of the taxonomic units. In the present study, it emerged that a combination of

Figure 3. Maximum likelihood (ITS + rbcL) tree of family Droseraceae. *Nepenthes khasiana* and *Sarracenia flava* were taken as out groups. Substitutions per site between taxon groups according to the model of best fit (GTR + Γ + I) determined with Modeltest in MEGA 7. Consensus ITS2 core secondary structures drawn in LOCARNA for representative *Drosera* species based on geographical distribution are shown next to their respective phylogenetic groupings.

Table 4. Maximum likelihood fits of 24 different nucleotide substitution models.

Model	#Param	BIC	AICc	InL	Invariant	Gamma	R	Freq A	Freq T	Freq C	Freq G	A=>T	A=>C	A=>G	T=>A	T=>C	T=>G	C=>A	C=>T	C=>G	G=>A	G=>T	G=>C
GTR+G	110	70032.93	69129.17	-34454.14	n/a	0.48	0.02	0.22	0.21	0.27	0.30	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.51	0.00	0.00	0.47
GTR+G+I	111	70043.16	69131.19	-34454.14	0.00	0.48	0.02	0.22	0.21	0.27	0.30	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.51	0.00	0.00	0.47
TN93+G	107	70251.26	69372.12	-34578.64	n/a	0.33	0.56	0.22	0.21	0.27	0.30	0.07	0.09	0.10	0.07	0.11	0.10	0.07	0.08	0.10	0.07	0.07	0.09
TN93+G+I	108	70261.48	69374.14	-34578.64	0.00	0.33	0.56	0.22	0.21	0.27	0.30	0.07	0.09	0.10	0.07	0.11	0.10	0.07	0.08	0.10	0.07	0.07	0.09
T92+G	104	70810.23	69955.72	-34873.46	n/a	0.36	0.70	0.21	0.21	0.29	0.29	0.06	0.08	0.12	0.06	0.12	0.08	0.06	0.09	0.08	0.09	0.06	0.08
HKY+G	106	70811.20	69940.27	-34863.72	n/a	0.36	0.69	0.22	0.21	0.27	0.30	0.06	0.08	0.12	0.06	0.11	0.09	0.06	0.09	0.09	0.09	0.06	0.08
T92+G+I	105	70820.46	69957.74	-34873.46	0.00	0.36	0.70	0.21	0.21	0.29	0.29	0.06	0.08	0.12	0.06	0.12	0.08	0.06	0.09	0.08	0.09	0.06	0.08
HKY+G+I	107	70821.42	69942.28	-34863.72	0.00	0.36	0.69	0.22	0.21	0.27	0.30	0.06	0.08	0.12	0.06	0.11	0.09	0.06	0.09	0.09	0.09	0.06	0.08
JC+G	102	72156.99	71318.90	-35557.07	n/a	0.66	0.50	0.25	0.25	0.25	0.25	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
JC+G+I	103	72167.22	71320.91	-35557.07	0.00	0.66	0.50	0.25	0.25	0.25	0.25	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
TN93	106	72315.44	71444.51	-35615.84	n/a	n/a	0.49	0.22	0.21	0.27	0.30	0.07	0.09	0.09	0.07	0.10	0.10	0.07	0.08	0.10	0.07	0.07	0.09
K2	102	72497.07	71658.97	-35727.10	n/a	n/a	0.44	0.25	0.25	0.25	0.25	0.09	0.09	0.08	0.09	0.08	0.09	0.09	0.08	0.09	0.08	0.09	0.09
TN93+I	107	72531.16	71652.03	-35718.59	0.00	n/a	0.50	0.22	0.21	0.27	0.30	0.07	0.09	0.09	0.07	0.10	0.10	0.07	0.08	0.10	0.07	0.07	0.09
T92+I	104	72557.75	71703.23	-35747.22	0.00	n/a	0.51	0.21	0.21	0.29	0.29	0.07	0.09	0.10	0.07	0.10	0.09	0.07	0.07	0.09	0.07	0.07	0.09
HKY+I	106	72586.49	71715.56	-35751.37	0.00	n/a	0.50	0.22	0.21	0.27	0.30	0.07	0.09	0.10	0.07	0.09	0.10	0.07	0.07	0.10	0.07	0.07	0.09
JC	101	72603.43	71773.54	-35785.40	n/a	n/a	0.50	0.25	0.25	0.25	0.25	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
K2+G	103	72682.03	71835.72	-35814.47	n/a	3.87	0.48	0.25	0.25	0.25	0.25	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
K2+G+I	104	72692.25	71837.74	-35814.47	0.00	3.87	0.48	0.25	0.25	0.25	0.25	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
K2+I	103	72762.70	71916.39	-35854.81	0.00	n/a	0.54	0.25	0.25	0.25	0.25	0.08	0.08	0.09	0.08	0.09	0.08	0.08	0.09	0.08	0.09	0.08	0.08
GTR	109	73184.72	72289.17	-36035.15	n/a	n/a	0.50	0.22	0.21	0.27	0.30	0.05	0.08	0.09	0.05	0.11	0.10	0.06	0.08	0.13	0.06	0.07	0.12
T92	103	73249.40	72403.09	-36098.16	n/a	n/a	0.49	0.21	0.21	0.29	0.29	0.07	0.10	0.10	0.07	0.10	0.10	0.07	0.07	0.10	0.07	0.07	0.10
HKY	105	73254.86	72392.14	-36090.66	n/a	n/a	0.48	0.22	0.21	0.27	0.30	0.07	0.09	0.10	0.07	0.09	0.10	0.07	0.07	0.10	0.07	0.07	0.09
GTR+I	110	73409.95	72506.18	-36142.65	0.00	n/a	0.48	0.22	0.21	0.27	0.30	0.05	0.07	0.09	0.05	0.10	0.10	0.06	0.08	0.13	0.07	0.07	0.12
JC+I	102	74271.05	73432.96	-36614.10	0.00	n/a	0.50	0.25	0.25	0.25	0.25	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08

Figure 4. Bayesian phylogenetic tree of family Droseraceae based on concatenated whole ITS and rbcL markers. Colour codes represent phylogenetic nodes with percent probability scores. Bayesian phylogeny reconstruction obtained by posterior probabilities for the nodes in the ML tree. GTR evolutionary model was implemented in MrBayes 3.2 with four chains during 50,000 generations and trees were sampled every 100 generations.

Figure 5. Split tree graph using Neighbourjoin NET in SplitTree for the family Droseraceae based on concatenated whole ITS and rbcL markers.

Figure 6. Comparisons of intra and inter-specific variations among ITS, matK and rbcL genes of the carnivorous plant family Droseraceae. The grey and black bars represent the intra- and inter-specific variations, respectively. The thin, black lines denote the smallest inter-specific variation. Names appearing next to the dark bars denote the closest species to that listed on the left.

different markers (rbcL+ITS+matK) would render them better discriminatory power for identifying species in the carnivorous plant diversity.

Molecular divergence time estimates

Several genera of Droseraceae have been blessed with fossil pollens. A single record called Fischeripollis from European Mid Miocene has been assigned to *Dionaea*³³. Fossil seed information and even leaves have contributed to the understanding of *Drosera* origin on the geological timescale. *Drosera* pollens have been recorded since Lower Miocene from New Zealand³⁴. Several findings of tertiary pollen in the Mid Miocene from Europe have

been assigned to either *Drosera* (*Droserapollis*) or *Nepenthes* (*Droseridites*)³⁵. The molecular data calibration with cue from previous studies is in congruence with the fossil record information of Droseraceae pollen, thus testifying a wide distribution of the progenitors of *Aldrovanda* in the Droseraceae family since Late Cretaceous (Figure 7).

Ancestral area reconstruction

The RASP tree (Figure 8) indicated the phylogenetic roots of *Dionaea* and *Aldrovanda* to originate in the northern hemisphere, while *Drosera* species would have most probably had an Australasian origin. Apparently all palaeoendemics

Figure 7. Rel TimeTree chronogram for the combined dataset of rbcL and ITS showing divergence time estimation of Droseraceae. Divergence times for all branching points in the user-supplied calibrated topology were calculated using the ML method based on the Tamura-Nei model. A discrete Gamma distribution was used to model evolutionary rate differences among sites [5 categories (+G, parameter = 2.0910)]. Numbers at nodes are median ages in million of years (Ma) with two internal calibration points. Evolutionary analyses were conducted in MEGA7.

Figure 8. Ancestral area reconstruction and world map distribution of species belonging to the family Droseraceae using RASP (S-DIVA and BBM). Alternative ancestral ranges of nodes (with frequency of occurrence) are shown in pie chart form. Bootstrap support values/Bayesian credibility values posterior probabilities (50% and higher) are indicated near the pie chart in the Bayesian tree. Colour key to possible ancestral ranges at different nodes represent other ancestral ranges. *Nepenthes khasiana* and *Sarracenia flava* were taken as out-groups.

(*D. meristocaulis*, *D. burmannii*, *D. arcturi*) are scattered throughout the southern hemisphere and also in tropical America. In this respect, the extant fossil record, i.e. European Miocene fossils, are somewhat noteworthy. A very old age (Cretaceous) can therefore be hypothesized for the whole family, dating back to stages of tectonic development when South America, Africa, and Australia were in closer proximity compared to present day geographical barriers. From the recent studies, it emerges that Australia is perhaps the secondary center of diversity of the genus *Drosera*, and most of the *Drosera* descendants can be assumed to have originated here.

Discussion

The family Droseraceae exhibited a monophyletic nature with representative species from *Drosera*, *Dionaea*, and *Aldrovanda* (Figure 3 and Figure 4), confirming that these highly diverse plants merit further investigation with a higher number of markers from different genomic regions. Though this study targeted some popular markers from the nuclear and extra chromosomal regions, similar markers, other than *rbcL*, could not be found in the public repositories for other species in the Droseraceae family to substantiate our research findings. The carnivorous plants are excellent evolutionary models and despite their dramatic journey in the course of evolu-

tion, which is no less than an interesting Gothic novel, botanical carnivory is a severely understudied area. In this study, family Droseraceae was revisited with present day investigative DNA marker tools from the chloroplast and nuclear regions trying to comprehend some of the bewildering scientific stories, which these meat-loving plants had to offer. Phylogenetic graphs based on the concatenated rbcL and ITS markers from the rDNA datasets exhibited 100% bootstrap support in most of the clades. The ML tree for the combined data set also showed that *Dionaea* and *Aldrovanda* form a sister group with 100% bootstrap values (Figure 3). Though *Drosera* differs markedly from that of the snap trap system of *Dionaea* and *Aldrovanda*, some structures still have strong resemblance at molecular level reflecting homology between them. A strong similarity is seen in the cellular architecture of stalked glands of *Drosera* and trigger hairs of *Aldrovanda*, whose origin can be traced to adhesive glands seen in the Plumbaginaceae and other families that are out-groups to the family Droseraceae. This study hints at a common evolutionary origin of trapping mechanisms in *Drosera*, *Dionaea* and *Aldrovanda*. All these findings attest to the sister relationship of *Dionaea* and *Aldrovanda*, indicating a single evolutionary origin of an elaborate snap trap system in carnivorous plants.

The clade from *D. barbigera* to *D. glanduligera* in Figure 3 covers a wide range of species spread across Australasia. Species in this clade are well adapted to dry environments and have tubers and stout roots. Subspecies with each adaptive trait forms a different clade. Except *D. pygmaea*, other species in section *Bryastrum* have pentamerous flowers and are endemic to southwestern Australia. *D. pygmaea* has been placed in a different section owing to its unique distributary features and tetramerous flowers,^{36,37}. This implies that tetramerous flowers are an autapomorphic character and would have evolved from the pentamerous flowers shared by other pygmy sundews. More work needs to be done for understanding the different sections of *Drosera* for systematic revision of these plants.

For the species *D. burmannii* and *D. sessilifolia* of section *Thelocalyx*, plesiomorphic pollen features are quite apparent with simple cohesion similar to *Aldrovanda* and *Dionaea*, instead of cross wall cohesion as observed in other *Drosera* species, except *D. glanduligera*. These two species share a common ancestor with 100 bootstrap values in the Bayesian phylogeny (Figure 4). The overall topology of the Droseraceae family though monophyletic, the genus *Drosera* showed a polyphyletic nature with so many subclades within the tree. *D. uniflora* and *D. stenopetala* formed a sister group, which is also supported by their similar morphological characters. The clades from *D. capillaris* to *D. hamiltonii* (Figure 3 and Figure 4) encompass species distributed in Eurasia and America. *D. rotundifolia* and *D. anglica* are widely distributed in both Eurasia and North America, while the other species studied are native to North and South America. While the clades from *D. graminifolia* to *D. hirtella* are distributed in South America, mainly in central and eastern Brazil, the clade from *D. collinsiae* to *D. slackii* is composed of African species. The concatenated dataset of rbcL and ITS tree supports the geographical classification of

Drosera species grouped into separate clades. Further, TaxonGap analysis speculates the combinatorial use of ITS and rbcL markers to design smart barcodes in delineating and discriminating species with high-resolution power (Figure 6).

Though the phylogenetic reconstruction approach could reveal some clades to be in sync with morphological characters and geographic distribution of *Drosera* species, it becomes imperative to advance the phylogeny research with a genome to phenome approach by targeting more species and new markers from the genomes of this highly diverse and interesting group of plants.

Biogeographic hypotheses

It is widely believed that *Drosera* has colonized itself in both the hemispheres³⁷ and Australia happens to be the center of diversity of *Drosera* species, where more than 80 species thrive^{38–40}. Over 30 species are distributed in Northern Africa and half of the species are distributed in South Africa. South America also has about 30 species, some of which have migrated into North America. Eurasia and North America harbor nearly 10 species although some of these species are cosmopolitan. *Aldrovanda* is widely distributed in both the hemispheres, including Australia and Africa, while *Dionaea* is restricted to North America.

The different phylogenetic trees (Figures 3–5 and 7) corroborate some of the previous hypotheses on the origin and dispersal of *Drosera* species. Australia to South America dispersal could be seen in the clade that includes *D. burmannii* and *D. sessilifolia*. *D. stenopetala* has disjunct distributions in South America and New Zealand. New Zealand and South American *Drosera* species have been reported to share close relationships⁴¹, and there might be some unknown mechanism for long-distance dispersal between these two continents. There are reports on dispersal events to have occurred in *D. burmannii*, *D. indica*, and *D. peltata* from Australia to Asia via Southeast Asia without any proper explanation for such events. A large number of *Drosera* species are spread across the Southern hemisphere compared to the Northern hemisphere, which implies that the species in the Northern hemisphere (*D. indica*, *D. capillaris*, *D. burmannii*, *D. anglica*, *D. brevifolia*, *D. filiformis*, *D. peltata* and *D. rotundifolia*) would have expanded their distributions to the Southern Hemisphere. Further analyses with more taxa would be required to confirm this inference.

Conclusions

The combinatorial use of different markers along with different computational tools, ideally the use of NeighborNet algorithm⁴², takes a different approach to inferring species relationships. A relationship network is drawn rather than restricting the data into a stubborn single line tree structure by incorporating MP trees onto a ML tree. The present study corroborates most of the findings by previous studies. The basal position of Droseraceae within the non-carnivorous Caryophyllales, indicated in the tree topologies and fossil findings, strongly support a date of origin for Droseraceae during the Paleocene (55–65 MYA). Contrary to this hypothesis, which makes the family more ancient, Rivadavia *et al.*¹⁶ argue

that the Droseraceae are located close to the tip of the angiosperm phylogenetic tree. Within Droseraceae, the sister relationship between *Aldrovanda* and *Dionaea* is supported by various rDNA marker [ITS (18s, ITS1, 5.8s, ITS2, 28s) + rbcL] dataset. Our studies would further help in comparative and experimental studies using carnivorous taxa with similar strong selective pressures. *Drosera* species are thus genuine plant model systems for addressing a wide array of questions concerning evolutionary and ecological studies governing botanical carnivory.

Data availability

Sequence data have been submitted to GenBank: accession numbers KR081966 – KR081968; KF015998, KF015996; KR081983 - KR081985; KT794003, KT794002, KT285307.1.

The remaining sequences from previous studies were downloaded from GenBank at NCBI and are outlined in [Table 1](#).

Competing interests

No competing interests were disclosed.

Grant information

This work was supported by the Department of Biotechnology, Government of India (<http://btisnet.gov.in/>; grant ID BT/BI/04/035/98 sanctioned to DKB and PT) and University Grants Commission-Rajiv Gandhi National Fellowship to SY.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgements

We acknowledge the support received from the DBT-sponsored Bioinformatics Centre at North-Eastern Hill University, Shillong for carrying out the research work.

References

- Juniper BE, Robins RJ, Joel DM: **The carnivorous plants**. Academic Press, New York; 1989.
[Reference Source](#)
- Król E, Plachno BJ, Adamec L, *et al.*: **Quite a few reasons for calling carnivores 'the most wonderful plants in the world'**. *Ann Bot*. 2012; **109**(1): 47–64.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- McPherson S: **Carnivorous plants and their habitats**. Redfern Natural History Productions, Poole, Dorset, England; 2010; 2.
[Reference Source](#)
- Ellison AM, Gotelli NJ: **Energetics and the evolution of carnivorous plants--Darwin's 'most wonderful plants in the world'**. *J Exp Bot*. 2009; **60**(1): 19–42.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Jayaram K, Prasad MN: ***Drosera indica* L. and *D. burmanii* Vahl., medicinally important insectivorous plants in Andhra Pradesh-regional threats and conservation**. *Curr Sci*. 2006; **91**(7): 943–947.
[Reference Source](#)
- Majumdar K, Datta BK, Shankar U: **Community structure and population status of *Drosera burmanii* Vahl. with new distributional record in Tripura, India**. *J Ecol Nat Environ*. 2011; **3**(13): 410–414.
[Reference Source](#)
- Joseph J, Joseph KM: **Insectivorous Plants of Khasi and Jaintia Hills, Meghalaya, India: A Preliminary Survey**. Calcutta: Botanical Survey of India; 1986.
[Reference Source](#)
- Darwin C: **Insectivorous Plants**. New York: D Appleton and Company; 1875.
[Reference Source](#)
- Zhang Q, Su J, Zeng L: **[The isolation and identification of flavonoids from *Drosera burmannii*]**. *Zhong Yao Cai*. 1998; **21**(8): 401–403.
[PubMed Abstract](#)
- Clarke JH: **A dictionary of practical Materia Medica**. Health Science Press; 1982.
[Reference Source](#)
- Ravikumar K, Ved DK: **Hundred red listed medicinal plants of conservation concern in Southern India**. Bangalore, India: Foundation for Revitalization of Local Health Traditions Publisher limited; 2000.
[Reference Source](#)
- Zhuang X: ***Drosera burmannii***. The IUCN Red List of Threatened Species. 2011; e.T169038A6566220.
[Publisher Full Text](#)
- Candolle AD: **Droseraceae**. In: *Prodromus systematis naturalis regni vegetabilis*. Treuttel and Wutz, Paris; 1824; 1.
- Seine R, Barthlott W: **Some proposals on the infrageneric classification of *Drosera* L.** *Taxon*. 1994; **43**(4): 583–589.
[Publisher Full Text](#)
- Williams SE, Albert VA, Chase MW: **Relationships of Droseraceae: a cladistic analysis of rbcL sequence and morphological data**. *Am J Bot*. 1994; **81**(8): 1027–1037.
[Publisher Full Text](#)
- Rivadavia F, Kondo K, Kato M, *et al.*: **Phylogeny of the sundews, *Drosera* (Droseraceae), based on chloroplast rbcL and nuclear 18S ribosomal DNA sequences**. *Am J Bot*. 2003; **90**(1): 123–130.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Lowrie A: **Carnivorous plants of Australia**. Western Australia, Australia: University of Western Australia Press; 1998; 3.
[Reference Source](#)
- Avise JC: **Molecular markers, natural history and evolution**. Chapman and Hall, J Evol Biol. 1994; **7**(6): 766–767.
[Publisher Full Text](#)
- Rivadavia F, de Miranda VF, Hoogenstrid G, *et al.*: **Is *Drosera meristocaulis* a pygmy sundew? Evidence of a long-distance dispersal between Western Australia and northern South America**. *Ann Bot*. 2012; **110**(1): 11–21.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Yesson C, Culham A: **Phyloclimatic modeling: combining phylogenetics and bioclimatic modeling**. *Syst Biol*. 2006; **55**(5): 785–802.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Edgar RC: **MUSCLE: a multiple sequence alignment method with reduced time and space complexity**. *BMC Bioinformatics*. 2004; **5**: 113.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Maddison WP, Maddison DR: **Mesquite: a modular system for evolutionary analysis**. 2016; Version 2.75.
[Reference Source](#)
- Kumar S, Stecher G, Tamura K: **MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets**. *Mol Biol Evol*. 2016; **33**(7): 1870–4.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Tamura K, Nei M: **Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees**. *Mol Biol Evol*. 1993; **10**: 512–526.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Felsenstein J: **Confidence limits on phylogenies: An approach using the bootstrap**. *Evolution*. 1985; **39**: 783–791.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Ronquist F, Huelsenbeck JP: **MrBayes 3: Bayesian phylogenetic inference under mixed models**. *Bioinformatics*. 2003; **19**(12): 1572–1574.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Huson DH, Bryant D: **Application of phylogenetic networks in evolutionary studies**. *Mol Biol Evol*. 2006; **23**(2): 254–267.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Slabbinck B, Dawyndt P, Martens M, *et al.*: **TaxonGap: a visualization tool for intra- and inter-species variation among individual biomarkers**. *Bioinformatics*. 2008; **24**(6): 866–867.
[PubMed Abstract](#) | [Publisher Full Text](#)

29. Will S, Joshi T, Hofacker IL, *et al.*: **LocARNA-P: accurate boundary prediction and improved detection of structural RNAs.** *RNA*. 2012; **18**(5): 900–14.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
30. Tamura K, Battistuzzi FU, Billings-Ross P, *et al.*: **Estimating divergence times in large molecular phylogenies.** *Proc Natl Acad Sci U S A*. 2012; **109**(47): 19333–19338.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
31. Degreef JD: **Early history *Drosera* and *Drosophyllum*.** *Carnivorous Plant Newsletter*. 1989; **18**(3): 86–89.
[Reference Source](#)
32. Yu Y, Harris AJ, Blair C, *et al.*: **RASP (Reconstruct Ancestral State in Phylogenies): a tool for historical biogeography.** *Mol Phylogenet Evol*. 2015; **87**: 46–49.
[PubMed Abstract](#) | [Publisher Full Text](#)
33. Krulzsch W: **Zur Kenntnis fossiler disperser tetraden pollen.** *Palaontologische Abhandlungen*. Abt. B. Palobotanik Berlin. 1970; **3**(3–4): 399–433.
34. Mildenhall DC: **New Zealand late Cretaceous and cenozoic plant biogeography: A contribution.** *Palaeogeogr Palaeoclimatol Palaeoecol*. 1980; **31**: 197–233.
[Publisher Full Text](#)
35. Krutzsch W: **Über Nepenthes-Pollen im europäischen Tertiär.** *Gleditschia*. 1985; **13**: 89–93, 36.
36. Planchon JE: **Sur la famille des Droséracées.** *Annales des Sciences Naturelles Botanique*. 1848; **3**(9): 79–98, 185–207, 285–309.
[Reference Source](#)
37. Schlauer J: **A dichotomous key to the genus *Drosera* L. (Droseraceae).** *Carnivorous Plant Newsletter*. 1996; **25**: 67–88.
[Reference Source](#)
38. Lowrie A: **Carnivorous plants of Australia.** University of Western Australia Press, Western Australia, Australia, 1987; **1**.
[Reference Source](#)
39. Lowrie A: **Carnivorous plants of Australia.** University of Western Australia Press, Western Australia, Australia, 1989; **2**.
[Reference Source](#)
40. Lowrie A: **Carnivorous plants of Australia.** University of Western Australia Press, Western Australia, Australia, 1998; **3**.
[Reference Source](#)
41. Yokoyama J, Suzuki M, Iwatsuki K, *et al.*: **Molecular phylogeny of *Coriaria*, with special emphasis on the disjunct distribution.** *Mol Phylogenet Evol*. 2000; **14**(1): 11–19.
[PubMed Abstract](#) | [Publisher Full Text](#)
42. Bryant D, Moulton V: **Neighbor-net: an agglomerative method for the construction of phylogenetic networks.** *Mol Biol Evol*. 2004; **21**(2): 255–65.
[PubMed Abstract](#) | [Publisher Full Text](#)

Open Peer Review

Current Peer Review Status:

Version 1

Reviewer Report 28 September 2017

<https://doi.org/10.5256/f1000research.13035.r25929>

© 2017 Sahoo L. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Lingaraj Sahoo

Department of Biosciences and Bioengineering , Indian Institute of Technology Guwahati (IIT Guwahati) , Guwahati, Assam, India

The manuscript has attempted to place the phylogenetic relationship and ancestral origin of two species of *Drosera* found in the Meghalya, to that of other species of world using nuclear and chloroplastic markers. The evidences given are fair enough to support the claims.

At places, the authors have used strong ornamental words such as “meat-eater plants”, “bewildering scientific stories” etc. must be replaced with scientific words.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 20 September 2017

<https://doi.org/10.5256/f1000research.13035.r25549>

© 2017 Fleischmann A. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Andreas Fleischmann

Botanische Staatssammlung München and GeoBio-Centre, Ludwig-Maximilians-Universität München, Munich, Germany

The article was reviewed for a different journal by myself previously, and rejected there. Most critics raised there were not improved in the present submission. The article still claims to present a global phylogeny of *Droseraceae* based on taxa sampled from India. However only 5 taxa of a genus with globally ca. 250 taxa occur in India. And only sequences from those taxa occurring in India were generated *de novo*, ALL other sequence data was taken from those published in Rivadavia *et al.* (2003) and Rivadavia *et al.* (2012). Interestingly, those previously published phylogenies did also include the 5 respective Indian taxa newly sampled here, thus none of the data presented here is actually new. The paper repeats the results of above-mentioned two articles, without adding any substantial new information.

Additionally, some of the literature consulted is cited in wrong context, e.g. when stating "Apparently all palaeoendemics (*D. meristocaulis*, *D. burmannii*, *D. arcturi*) are scattered throughout the southern hemisphere and also in tropical America." Rivadavia *et al.* (2003) CLEARLY show evidence that *D. burmannii* (and its sister *D. sessilifolia*) is NOT a palaeoendemic, and Rivadavia *et al.* (2012) do so for *D. meristocaulis* – both are cases of recent LDD, not old vicariance, thus cannot be palaeoendemics. ONLY *D. arcturi* can be considered a palaeoendemic of the list of species presented here (in addition to *D. regia* from South Africa). In contrast, *Dionaea* and *Aldrovanda*, which can be considered palaeoendemic lineages, occur in the Northern Hemisphere.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

The benefits of publishing with F1000Research:

- Your article is published within days, with no editorial bias
- You can publish traditional articles, null/negative results, case reports, data notes and more
- The peer review process is transparent and collaborative
- Your article is indexed in PubMed after passing peer review
- Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com

F1000Research