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Molecular Phylogenetic Evidence and Biogeographic History of Indian Endemic *Portulaca* L. (Portulacaceae) Species

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Abstract: The genus *Portulaca* L. belongs to the monogeneric family Portulacaceae and consists of about 157 species worldwide. In India, it is represented by 11 taxa; among them, *Portulaca badamica*, *Portulaca lakshminarasimhaniana*, *Portulaca oleracea* var. *linearifolia*, and *Portulaca laljii* are endemic. So far, the phylogenetic positions of these species have not yet been analyzed. We have reconstructed the Bayesian and maximum likelihood phylogenies based on a combined chloroplast and nuclear DNA sequence dataset to reveal phylogenetic placements of Indian *Portulaca*. Phylogenetic analyses indicate that all the sampled Indian *Portulaca* species (except *Portulaca wightiana*) are placed in the AL clade, which contains most of the known species of the family Portulacaceae. We used reconstructed phylogeny to study the historical biogeography of Indian endemic species by employing S-DIVA analysis. S-DIVA analysis suggested *P. lakshminarasimhaniana* has origin in India, it may be the result of in situ speciation in India, and *P. badamica* was dispersed from Africa to India. We have also discussed the systematic placements of endemic species and their morphological relationships with closely allied species. In addition, this study also provides taxonomic treatment for endemic species.

Keywords: biogeography; ITS; ndhA; ndhF; Portulaca; phylogeny; trnT-psbD

1. Introduction

The genus *Portulaca* L. includes about 157 taxa, which are annual and perennial species [1–5]. *Portulaca* species are distributed worldwide in the tropical and subtropical areas with centers of diversity in South America and Africa [1]. Ocampo and Columbus [1] performed comprehensive phylogeny of the genus *Portulaca*; in which they discussed the phylogenetic analysis and historical biogeography of the genus. Their phylogenetic analyses reveal two major lineages: the OL clade (opposite-leaved species) and the AL clade. Species from the OL clade are mainly distributed in Africa, Asia, and Australia, whereas species from the AL clade originated in the New World [1]. AL clade comprises three strongly supported groups: the Oleracea, Umbratica, and Pilosa clades.

Sivarajan [6] made the first attempt to revise the genus *Portulaca* from India and recognized four distinct species and three infra-specific taxa. He treated *Portulaca grandiflora* Hook. as a subspecies of *Portulaca pilosa* L. and *Portulaca tuberosa* Roxb. was considered a variety of *P. pilosa* subsp. *pilosa*. Singh and Sanjappa [7] reported six species of *Portulaca*...
from India, viz. *P. grandiflora* Hook., *P. oleracea* L., *P. pilosa*, *P. quadrifida* L., *P. tuberosa*, and *P. wightiana* Wall. ex Wight and Arn. and one variety, i.e., *P. oleracea* var. *linearifolia* Sivarajan and Manilal. Dalvi et al. [3] described two new species from India: *Portulaca badamica* S.R.Yadav and Dalavi and *Portulaca lakshminarasimhaniana* S.R.Yadav and Dalavi. Recently, Sivaramakrishna and Yungandhar [8] described *Portulaca laljii* from the Eastern Ghats of India. *Portulaca umbraticola* Kunth. is a highly polymorphic species cultivated throughout India for ornamental foliage and flowers [9]. It is differentiated from the rest of the Indian species by means of winged pyxis [9]. Therefore, up to date, there are total of 11 taxa of *Portulaca* reported from India. Among these, *P. badamica*, *P. lakshminarasimhaniana*, *P. oleracea* var. *linearifolia*, and *P. laljii* are endemic to India, and their phylogenetic positions have yet not been assessed. Details of species found in India, their geographical distribution, and their representation in molecular studies are listed in Table 1. Ocampo and Columbus [1] provided comprehensive phylogenetic analyses based on a combined dataset of three plastid regions (*ndhA* intron, *ndhF*, and *trnT-psbD*) and the nuclear ITS region. They studied phylogeny based on the historical biogeography of the genus and hypothesized that the ancestral distribution area of *Portulaca* included southern hemisphere continents and Asia.

Table 1. List of *Portulaca* species that occur in India, their distribution, and representation in molecular studies. Taxa that occur in India and have never been included in a molecular study are marked with a cross in bold.

| Taxa Name | Geographical Distribution | Taxa Included in Previous Molecular Phylogenies [1,10,11] | Indian Accessions Included in This Study |
|-----------|---------------------------|----------------------------------------------------------|-----------------------------------------|
| *Portulaca badamica* S.R.Yadav and Dalavi | Endemic to India (Badami hills of Bagalkot District, Karnataka) | - | X |
| *Portulaca grandiflora* Hook. | South America, North America, Africa, and Asia | X | X |
| *Portulaca lakshminarasimhaniana* S.R.Yadav and Dalavi | Endemic to India (Badami, Karnataka and Mahbubnagar, Andhra Pradesh) | - | X |
| *Portulaca laljii* Sivaramakrishna and Yungandhar | Endemic to India (few localities of Prakasam District, Andhra Pradesh) | - | - |
| *Portulaca oleracea* L. var. *oleracea* | Africa and Asia | X | X |
| *Portulaca oleracea* var. *linearifolia* Sivarajan and Manilal | Endemic to India (Punjab, Uttar Pradesh, Bihar, West Bengal, Assam, Orissa, Gujarat, Maharashtra, Tamil Nadu, and Badami) | - | X |
| *Portulaca pilosa* L. | South America, North America, Africa, Asia, and Australia | X | X |
| *Portulaca quadrifida* L. | Africa and Asia | X | - |
| *Portulaca tuberosa* Roxb. | Asia and Australia | X | X |
| *Portulaca umbraticola* Kunth | South America, North America, and Asia | X | X |
| *Portulaca wightiana* Wall. ex Wight and Arn. | Africa and Asia | X | X |

This study presents phylogenetic analyses of *Portulaca* species based on four different loci (*ndhA*, *ndhF*, *trnT-psbD*, and ITS). It addresses the following goals: (i) to determine phylogenetic placements of Indian endemic *Portulaca* species, (ii) the use of reconstructed phylogeny to study the biogeographic history of endemic species based on statistical dispersal-vicariance analysis (S-DIVA), and (iii) to provide taxonomic treatment for endemic species and a distribution map.

2. Materials and Methods
2.1. Taxon Sampling and DNA Extraction

We have sampled nine out of eleven Indian *Portulaca* taxa; three are endemic to India. Collected plant samples processed for herbarium preparation and voucher specimens deposited at the Department of Botany, Shivaji University, Kolhapur, India. Herbarium
preparation of all the species of *Portulaca* was performed by the standard protocol adopted from Jain and Rao [12]. To confirm the identity and distribution of every species of *Portulaca* from India we explored all the available literature such as Dalavi et al. [3,4], Ocampo [2], and Sivarajan [6]. Details of collected plant species are provided in Table 2. Total genomic DNA was extracted from young and fresh leaves of collected plant species using the modified CTAB method [13] with some modifications mentioned in Tamboli et al. [14]. We amplified and sequenced the *ndhA*, *ndhF*, *trnT-psbD*, and ITS region from them. We constructed a dataset of 63 *Portulaca* species in 11 infraspecific taxa, which covers sampling from all its nine major distribution areas and is used for further phylogenetic and biogeographic analyses. We created a dataset of 89 accessions of *Portulaca*, which contains sequencing data of 80 accessions of *Portulaca* taken from previous studies [1,10,11] and sequencing data of 9 Indian *Portulaca* accessions generated in this study (Table 2). Single representative species from three families, namely *Talinopsis frutescens* A. Gray (Anacampserotaceae), *Pereskia aculeata* Mill. (Cactaceae), and *Talinum paniculatum* (Jacq.) Gaertn. (Talinaceae) selected as an outgroup as Anacampserotaceae, Cactaceae, and Talinaceae are the closest relatives to Portulacaceae [1]. Details of the DNA sequence retrieved from GenBank are provided in Supplementary Material S1.

**Table 2.** Details of Indian *Portulaca* species studied: Area of collection Voucher ID and GenBank accession numbers for ITS, *ndhF*, *trnT-psbD*, and *ndhA* intron.

| Taxa Name | Area of Collection | Voucher ID | ITS Accession | *ndhF* Accession | *trnT-psbD* Accession | *ndhA* Intron Accession |
|-----------|--------------------|------------|---------------|------------------|-----------------------|------------------------|
| *P. badamica* S.R.Yadav and Dalavi | Badami, Karnataka, India | JVD-1261 | OM111215 | OM160989 | MZ394024 | MZ394019 |
| *P. grandiflora* Hook. | Kolhapur, Maharashtra, India | JVD-1264 | MZ382894 | ON058265 | - | ON058268 |
| *P. lakshminarasimhasiana* S.R.Yadav and Dalavi | Badami, Karnataka, India | JVD-1260 | MZ382893 | OM160990 | MZ394026 | MZ394020 |
| *P. oleracea* L. var. *oleracea* | Badami, Karnataka, India | JVD-1457 | - | OM160991 | MZ394028 | MZ394022 |
| *P. oleracea* var. *linearifolia* | Badami, Karnataka, India | JVD-1263 | - | OM160992 | - | MZ394023 |
| *P. pilosa* L. | Badami, Karnataka, India | JVD-1259 | MZ382892 | OM160993 | MZ394025 | - |
| *P. tuberosa* Roxb. | Badami, Karnataka, India | JVD-1681 | MZ382895 | ON058266 | - | - |
| *P. umbraticola* Kunth | Badami, Karnataka, India | JVD-988 | - | OM160994 | OM160997 | OM160996 |
| *P. wightiana* Wall. ex Wight and Arn. | Badami, Karnataka, India | JVD-1257 | - | ON058267 | - | MZ394021 |

2.2. **PCR and DNA Sequencing**

A list of primers and PCR conditions is listed in Table 3. In this study, we generated 26 sequences out of that 10 were amplified and sequenced at the Department of Biochemistry, Shivaji University, Kolhapur, India. The other 16 were sequenced at Macrogen Corporation (Seoul, Korea). The procedure of PCR amplification and DNA sequencing was done at the Department of Biochemistry, Shivaji University, India, as follows: PCR reactions were performed in 25 µL of medium containing 12.5 µL AmpliTaq Gold® 360 Master mix (Applied Biosystem, Foster City, CA, USA), 8.5 µL nuclease-free water, 0.5 µL GC enhancer (Applied Biosystem, Foster City, CA, USA), 0.5 µL each forward and reverse primer (15 µM), and 2.5 µL of DNA. PCR reactions were carried out in a thermal cycler (BIO-RAD Laboratories, Hercules, CA, USA). Amplified PCR products were then purified using the GenElute™ PCR Clean-Up kit (SIGMA-ALDRICH, St. Louis, MO, USA). Sequencing of purified PCR products was done using BigDye® Terminator v 3.1 cycle sequencing kit (ThermoFisher Scientific, Waltham, MA, USA) on 3500 Genetic Analyzer (Applied Biosystems, Foster City, USA). The protocol for PCR amplifications carried out at at Plant Systematics Laboratory, Department of Biology, Kyungpook National University, Daegu, South Korea was as follows: PCR reactions were performed in 30 µL medium containing 15 µL nuclease-free water, 10 µL EmeraldAmp GT PCR Master Mix (TaKaRa, Kusatsu, Shiga, Japan), and 1 µL each forward and reverse primer (10 µM). PCR reactions were carried out in a thermal cycler (TaKaRa, Kusatsu, Shiga, Japan). Amplified PCR
product was further purified and sequenced at Macrogen Corporation (Seoul, South Korea). Sequences of *ndhA* intron, *ndhF*, *trnT-psbD*, and ITS region were submitted to the GenBank NCBI database. GenBank accession numbers are provided in Table 2.

### Table 3. List of primers used and PCR conditions.

| Marker          | Primers       | Sequence (5’ to 3’)                       | PCR Conditions                                                                 |
|-----------------|---------------|-------------------------------------------|--------------------------------------------------------------------------------|
| ITS             | ITS1          | TCCGTAGGTGAACCTGCGG                       | 94 °C for 4 min, 35 cycles (94 °C for 30 s, 55 °C for 1 min, and 72 °C for 1 min), and final extension at 72 °C for 10 min |
|                 | ITS5          | GGAAGTAAAGCTGTAACAGG                      |                                                                                  |
|                 | ITSLeu1       | GTCCACTGAACCTTATCATTTAG                   |                                                                                  |
|                 | ITS4          | TCCTCCGCTTTATIGGATIGC                     |                                                                                  |
| *ndhA*          | *ndhA*×1      | GCYCAATCWATTAGTTATGAAATACC                | 94 °C for 4 min, 35 cycles (94 °C for 30 s, 55 °C for 1 min, and 72 °C for 1 min), and final extension at 72 °C for 10 min |
|                 | *ndhA*×2      | GGTTGACGCCAMARATCACA                     |                                                                                  |
| *ndhF*          | *ndhF* _mF   | ACTTGTACCTTGCTC                              | 94 °C for 4 min, 35 cycles (94 °C for 30 s, 50 °C for 1 min, and 72 °C for 2.5 min), and final extension at 72 °C for 7 min |
|                 | *ndhF* _R      | CTCACAAGTAAGTAAATA                       |                                                                                  |
| *trnT-psbD*     | trnT          | CCTTTAATAGTCATCGTAG                          | 94 °C for 4 min, 35 cycles (94 °C for 30 s, 55 °C for 1 min, and 72 °C for 1 min), and final extension at 72 °C for 10 min |
|                 | psbD          | CTCCGTAACCTGCCATCACATA                    |                                                                                  |

2.3. Sequence Alignment and Phylogenetic Analyses

Output files containing raw DNA sequences generated after the sequencing run were checked and edited using Sequencher v. 5.1 (Gene Codes Corporation, Ann Arbor, MI, USA) [15]. Multiple sequence alignment of each gene was carried out using MUSCLE [16] implemented in MEGA 7 [17] and further refined using GBlocks v. 0.91b (Information Génomique et Structurale, France) [18]. For checking incongruence among four sequence datasets, we performed the incongruence length difference (ILD) test [19] in PAUP 4.0a152 [20] as well as assessed the combinability of the different four loci by constructing phylogeny based on each gene and comparing topologies. The ILD test was performed with 1000 heuristic searches, by keeping simple taxon addition and TBR as branch swapping algorithm. Results of individual analyses revealed no significant topological conflicts among four different markers (Figures S1–S4) and the ILD test also revealed no significant incongruence between the chloroplast and nrITS dataset (*p*-value = 0.0100); thus, all subsequent analyses were performed on a combined data set (Supplementary Material S2). The *ndhA*, *ndhF*, *trnT-psbD*, and ITS sequence datasets were concatenated into a single matrix (Supplementary Material S2) using SequenceMatrix [21]. jModelTest 2 [22] on XSEDE on the CIPRES science gateway (http://www.phylo.org/ (accessed on 23 March 2022)) [23] was used to find out the best-fit nucleotide substitution model for the combined dataset under the Akaike Information Criterion (AIC). The best-fit nucleotide model for the combined dataset was GTR + I + G.

The Bayesian and Maximum likelihood methods were performed for phylogenetic analyses. Bayesian phylogenetic (BI) analysis was performed using MrBayes v.3.2.6 [24] on XSEDE via the CIPRES portal [25]. Markov chain Monte Carlo (MCMC) was conducted using the best-fit nucleotide model and consisted of four independent runs of 50,000,000 generations each with one cold chain and three heated chains with a tree sampled every 1000 generations. The first 10% trees were discarded as burn-in, and the remaining were used to obtain a 50% majority-rule consensus Bayesian tree having clades supported by posterior probabilities (PPs). At the end of the run, the average standard deviation of split frequencies was below 0.0100. Tracer 1.7.1 [25] was used to check effective sample size (ESS); the ESS presented values were well above 400 for all statistics, which ensured enough selected settings for the sampling [26]. Maximum likelihood analysis (ML) was performed using RaxML-HPC v.8.0 on XSEDE [27] via the CIPRES portal [23] using the GTR + G model with 1000 rapid bootstrap replicates.
2.4. Biogeographic Analysis

The distribution range of *Portulaca* was divided into nine areas: (A) South America, (B) North America, (C) Caribbean, (D) Central America, (E) Africa, (F) Asia, (G) Australia, (H) Islands of Pacific Ocean, and (I) Europe. We used S-DIVA analysis implemented in RASP v 3.2 (http://mnh.scu.edu.cn/soft/blog/RASP (accessed on 2 May 2022)) [28] to reconstruct the possible ancestral ranges of the genus *Portulaca* on the Bayesian phylogenetic trees. To account for uncertainties in phylogeny, we used 811 Binary trees and ran S-DIVA on all of them. The maximum number of areas at each node was kept as 4. S-DIVA analyses were performed on the Bayesian tree with all compatible groups (Figure S5) as it does not accept polytomies in the target tree. The possible ancestral ranges at each node on a selected tree were obtained. The RASP program output of S-DIVA analysis for important nodes discussed in this study are provided in Supplementary Material S3.

3. Results

3.1. Phylogenetic Analyses

Bayesian and maximum likelihood phylogenies strongly supports the recognition of two main clades in family Portulacaceae, namely the OL clade (PP = 1 and ML BS = 100) and the AL clade (PP = 1 and ML BS = 100) (Figure 1). The AL clade is divided into four strongly supported subclades: the Cryptopetala clade (PP = 1 and ML BS = 95), Oleracea clade (PP = 1 and ML BS = 100), Umbraticola clade (PP = 1 and ML BS = 100), and Pilosa clade (PP = 1 and ML BS = 100) (Figure 1). The maximum likelihood tree with the highest log-likelihood value of −22,064.18 is shown in Figure S6.

In the Bayesian phylogeny presented, Indian *Portulaca* accessions *P. wightiana* (JVD-1257) grouped with other accessions of it: *P. wightiana* (Burgoyne 3613) (PP = 1 and ML BS = 100), *P. oleracea* var. *oleracea* (JVD-1457), and *P. oleracea* var. *linearifolia* (JVD-1263) were placed in the Oleracea clade. *P. umbraticola* (JVD-988) was grouped with its subspecies and was placed in the Umbraticola clade with PP = 1 and ML BS = 100 support. *P. badamica* (JVD-1261), *P. tuberosa* (JVD-1681), *P. grandiflora* (JVD-1264), *P. lakshminarasimhaniana* (JVD-1260), and *P. pilosa* (JVD-1259) have their phylogenetic placements in the Pilosa clade with strong BI PP value and ML BS support (Figure 1).

3.2. Historical Biogeography Reconstructions

S-DIVA Analysis

The reconstruction is shown in a maximal S-DIVA value of 5058.717 (Figure 2). Node 180 indicates there are three possibilities; South America (A), Asia (F), and Australia (G) may have the place of origin for comment ancestor of the OL and AL clades (Figure 2). The Indian endemic species *P. oleracea* var. *linearifolia*, which is placed in the Oleracea clade, was dispersed from either North America (B) or Africa (E) to India (Asia (F)) (evidence from Node 114). Recently described Indian endemic species, *P. lakshminarasimhaniana* has its origin in India (Asia) (evidence from Node 162) and Node 136 suggests a dispersal event with 82.50% probability, which indicates that the dispersal of *P. badamica* from Africa (E) to India (Asia (F)) (Figure 2).
Figure 1. 50% majority-rule consensus Bayesian phylogenetic tree based on combined (ITS + ndhA + ndhF + trnT-tpsbD) data. Bayesian posterior probability values and maximum likelihood bootstrap values (BPP/ML BS) are provided above branches. Asterisk (*) indicates ML BS values < 50%. The herbarium number of each taxon is in the bracket after the taxa name. The species sampled in this study are in red color.
Figure 2. Graphical output from S-DIVA analysis (exported from RASP). The reconstruction is shown in a maximal S-DIVA value of 5058.717 based on the Bayesian tree with all compatible groups. After the tip of the tree, the taxa name with its voucher number is provided, and the distribution codes of the taxa are mentioned in brackets. Biogeographical regions: A: South America; B: North America; C: Caribbean; D: Central America; E: Africa; F: Asia; G: Australia; H: Islands of Pacific Ocean; I: Europe. The species sampled in this study are in red color.
4. Discussion

Previous studies on Portulaca demonstrated phylogenetic analysis based on a combined data matrix that retrieved highly supported phylogeny, which can be used as a framework to show phylogenetic placements of species [1,10,11]. Our reconstructed BI and ML phylogenies resulted in the recovery of all major clades observed in previous phylogenies [1,10,11]. Those are the OL clade and AL clade with four subclades: the Cryptopetala clade, Oleracea clade, Umbraticola, and Pilosa clade with substantial Bayesian PP value Maximum likelihood bootstrap support (Figure 1).

4.1. Systematic Positions and Morphological Relationships of Indian Endemic Species with Their Closely Related Species

Out of 11 species of Indian Portulaca, P. badamica, P. lakshminarsimhaniana, P. oleracea var. linearifolia, and P. laljii are endemic to India. P. badamica and P. lakshminarsimhaniana were placed in the Pilosa clade (Figure 1). Species belonging to this clade have alternate leaves with conspicuous hairs in the axils, and flowers are generally arranged in heads; in some cases, the capitulum is reduced to very few flowers [1]. Portulaca badamica (JVD-1261) shows a sister relationship to P. massaica and P. foliosa with Bayesian PP value = 1 and ML BS = 98 (Figure 1). Portulaca badamica is morphologically closer to P. pilosa and P. oleracea var. linearifolia [3]. Portulaca badamica differs from P. pilosa by its annual, slender, sparsely branched, erect, habit (compared to perennial, robust, much-branched, spreading habit), usually cleistogamous yellow flowers (compared to chasmogamous, pink flowers), 3–4-fid style (compared to 5–7-fid style), 8–12 stamens (compared to 20–25 stamens), and bluish seeds with stellulate flat cells without central elevations (compared to bluish seeds with stellulate flat cells with central elevations) [3]. It is also similar to Portulaca oleracea var. linearifolia but differs in having hairs in axils of leaves (compared to completely glabrous habit), 1–6-flowered capitulum (compared to 1–10-flowered capitulum), 3–4-fid style (compared to 5 fид style), very delicate, transparent sepals (compared to thick green sepals covering capsules), and bluish seeds having stellulate flat cells without central elevations (compared to black seeds with circular central elevations in cells) [3].

P. lakshminarsimhaniana (JVD-1260) is grouped with our added Indian accession of P. pilosa (JVD-1259) and P. grandiflora (JVD-1264) with PP = 1 and ML BS = 68 (Figure 1). P. lakshminarsimhaniana is morphologically closer to P. suffrutescens [3], and both of these species are placed in the Pilosa clade (Figure 1). Portulaca lakshminarsimhaniana differs from P. suffrutescens by its non-tuberous root (compared to tuberous root), stem scabrous with scaly bark (compared to a smooth stem without scaly bark), dark pink to red flowers (compared to magenta to orange flowers), 8–15 stamens (compared to more than 40 stamens), strictly 4 fид style (compared to 5–6-fid style) and oblong capsules (compared to sub-globose capsules) [3]. P. oleracea var. linearifolia (JVD-1263) was placed in the Oleracea clade and grouped with the other members of the Oleracea group (Figure 1). Unfortunately, we could not sample recently described P. laljii in this study, which is flagged for future molecular studies. P. laljii is closely related to P. lakshminarsimhaniana but differs in its tuberous root (compared to non-tuberous root), stem herbaceous smooth without scaly bark (compared to woody, scabrous with scaly bark), pinkish-orange flowers with the deep pinkish-orange throat (compared to pink to red flowers with dark red throat), 6–7 stamens (compared to 8–15 stamens), strictly 3 fид style (compared to 4 fид style), and prolate capsule (compared to oblong capsule) [8]. If the phylogenetic placement of P. laljii is studied in the future, we hypothesize that it would fall in the Pilosa clade, based on morphological similarities with P. lakshminarsimhaniana.

4.2. Biogeographic History of Indian Endemic Species

Phylogeny-based historical biogeographical reconstructions are now essential to illuminate the organism’s evolutionary history [29]. A previous historical biogeography of the genus was studied using the S-DIVA method [1] and in this study, we used that method to determine the historical biogeography of Indian endemic species. We have enriched
the sampling of Ocampo and Columbus [1] by the addition of nine Indian Portulaca taxa (among which three are endemic to the region) and recently described two new species, namely P. juliomartinezii G. Ocampo [10] and P. almeviae G. Ocampo [11]. Phylogeny-based historical biogeographic reconstruction of Ocampo and Columbus [1] proposed three of the four possibilities in the southern hemisphere (Africa, Asia, Australia, and South America) as the place of origin of Portulaca. Our S-DIVA analysis also revealed that South America (A), Asia (F), and Australia (G) might have the place of origin for the genus (Figure 2). The place of origin of Portulaca is uncertain, and several hypotheses about an ancestral area of the genus were previously proposed [30–32]. The greatest biodiversity of Portulaca species is in the southern hemisphere, and it would be originated there [30,32]. This is the possible reason behind having the origin of the genus in any of the continents of the southern hemisphere.

S-DIVA suggested two possibilities for the dispersal of P. oleracea var. linearifolia, from either North America (B) or Africa (E) to India. The molecular phylogeny of Ocampo and Columbus [1] and phylogeny presented in this study show that P. oleracea is not monophyletic and subspecies of oleracea is now distributed worldwide as a weed. A previous study [1] and this study show that there are a number of subspecific species of P. oleracea from the Oleracea clade inferred with the highest probability to have originated in North America (Node 111, Figure 2). The addition of more sampling from the P. oleracea clade complex will be helpful to gain more knowledge about the geographic origin, although it is very difficult to estimate ancestral area for taxa having widespread distribution as shown in other groups within Cactineae [33,34]. P. badamica was dispersed from Africa to India (evident from Node 136, Figure 2). It could be very well that there were African populations of Portulaca that were the source of dispersal to India. Although, there is no obvious mechanism of species dispersal to such great distances. Ridley [35] suggested birds can disperse the seeds up to a long distance, water also can act as a dispersal agent; including intercontinental (by sea) and intracontinental dispersal, it is demonstrated that seeds can remain viable after floating for a number of weeks in sea water [35,36]. Another dispersal agent is tropical storms (a wind dispersal mechanism), it has been proposed to distribute seeds from the Caribbean to the southeastern USA [37]. Humans are also effective dispersal agents [35] and have widely propagated species (intentionally or inadvertently), especially in urban areas, agricultural fields, along roads, and at ports. P. lakshminarasimhaniana is a sister to the widespread cosmopolitan P. pilosa species and it may have originated in India. It could very well be that P. lakshminarasimhaniana diverged from the P. Pilosa Indian populations within India, i.e., the result of in situ speciation in India. There are more population sampling and population genetic approaches that are necessary to investigate this further.

4.3. Taxonomic Treatment for Indian Endemic PORTULACA Species

Portulaca badamica S.R.Yadav and Dalavi in Phytotaxa 376 (1): 068; Dalavi et al. Phytomorphology 69 (1 and 2) 2019: 17 (Figure 3).

Type: INDIA, Karnataka, Bagalkot District, Badami, 613 m.s.l., 15.918394 N, 75.703487 E, 14 June 2018, S.R. Yadav, J.V. Dalavi and P.V. Deshmukh, JVD-1250 (CAL!).

Morphological description: seasonal, slightly branched, erect, slender herbs, 5–15 cm in height with fibrous roots. Stem: erect, slender, sparsely pilose in earlier stages, glabrous when old. Leaves: 0.8–1.5 × 0.1–0.3 cm, simple, alternate, linear terete, subsessile, glaucous, veins not distinct; petiole with a small tuft of hairs at nodal portion. Flowers: 1–6, sessile in terminal capitulum surrounding by 4–7 involucral bracts and a ring of white hairs. Flowers 1 cm across, usually cleistogamous, rarely chasmogamous, sessile, yellow. Sepals: 2, 4–6 × 2–3 mm, ovate, connate at base, membranous, 4–6-veined, pale yellow in color, glabrous. Petals: 4–5, 5–6 × 2–3 mm, connate at the base, ovate to obovate, glabrous, entire, pale yellow to bright yellow. Stamens: 8–12, free, filaments connate at the base forming ring and adnate to petals, unequal in length; anthers 0.7–0.9 mm, bithecous, yellowish in color, dehiscing via longitudinal slits; filaments 1.0–2.5 mm long, glabrous. Gynoecium:
4–5 carpellary, syncarpous; ovary 2–3 × 1–2 mm, sub-globose, lower portion sunk into the base of the calyx tube, glabrous. Style: 3–4 mm long, glabrous; 3–4 fid, pale yellow; stigma 1–2 mm, papillate, yellow. Fruits: circum-scissile pyxis, 4–6 × 2–3 mm, sub-globose, basal disc, and upper operculum nearly equal in length. Seeds: 40–60 per capsule, sub-reniform, 0.6–0.7 mm in diameter, bluish black with stellulate flat cells arranged in 3–4 circular rings without central elevations.

**Habitat:** Plant shows a very discrete population in moist sandy plains.

**Flowering and fruiting:** May to August.

**Distribution:** India (Only known from Badami hills of Bagalkot District, Karnataka) [3,4].

**Status:** The species is not yet accessed for IUCN categorization and is kept as DD. It is endemic to south peninsular India.
Specimens examined: INDIA, Karnataka, Bagalkot District, Badami, 613 m a.s.l., 15.918394 N, 75.703487 E, 14 June 2018, S.R. Yadav, J.V. Dalavi and P.V. Deshmukh, JVD-1250 (Holotype CAL!; isotypes BSI, K, MH).

Portulaca lakshminarasimhaniana S.R. Yadav and Dalavi in Phytotaxa 376 (1): 068; Dalavi et al. Phytomorphology 69 (1 and 2) 2019: 17. (Figure 4).

Figure 4. Portulaca lakshminarasimhaniana S.R. Yadav and Dalavi. (A) Habit; (B) leaves; (C, D) flowers; (E) sepals; (F) petals; (G) gynoecium with staminal column; (H) stigma; (I) pyxis; (J) seeds.
Type: INDIA, Karnataka, Bagalkot District, Badami, 611 m a.s.l., 15.918955 E, 75.696699 N, 14 June 2018, Yadav, Dalavi and Deshmukh, JVD-1251 (holotype CAL!).

Morphological description: Perennial woody subshrubs, 20–80 cm in height. Tap root: woody, 10–20 $\times$ 0.5–1.0 cm, brownish, hard. Stem: 20–70 $\times$ 0.5–1.0 cm, much branched, woody, and scabrous with scaly bark. Leaves: 1–2 $\times$ 0.1–0.3 cm, terete, fleshy, tapering at both ends, glabrous, green; petiole short; with a tuft of hairs in the leaf axil. Inflorescence: 2–4-flowered, flowers in terminal sessile clusters surrounded by 3–4 involucral bracts. Flowers: c. 1.0–1.5 cm across, actinomorphic, hermaphrodite, sessile, pink to red with a dark red throat. Sepals: 2, 3–4 $\times$ 1–2 mm, ovate, connate at base, acuminate at apex, membranous, pale pink to yellow, glabrous. Petals: 4–5, obovate, 4–6 $\times$ 2.0–3.5 mm, acuminate at apex, thin delicate, transparent. Stamens: 8–16, free, adnate to the base of petals forming a ring around the ovary; anthers bicolous, 1 mm long, yellow, dehiscing by longitudinal slits; filaments 2–3 mm long, free, basally connate, glabrous. Ovary: 3–4 carpellate, syncarpous; 2–3 $\times$ 1.0–1.5 mm, globose, glabrous; style 3–4 mm, 4-fid, glabrous; stigmas slightly coiled, papillate. Fruits: a circum-scissile capsule, 4–5 $\times$ 1–2 mm, oblong, glabrous, the upper dome is two times longer than the basal disc. Seeds: 20–30 per capsule, 0.7–0.9 mm in diameter, ovoid with unique shiny golden luster and stellate cells throughout the surface.

Habitat: Rocky crevices under the shelter of spiny bushes or in sandy plains of open areas.

Flowering and fruiting: June to October.

Distribution: Badami (Bagalkot Dist., Karnataka), Mahbubnagar (Andhra Pradesh) [3,38].

Status: The species is yet not accessed for IUCN categorization due to a lack of statistical data on all populations.

Specimen examined: INDIA, Karnataka, Bagalkot District, Badami, 611 m a.s.l., 15.918955 E, 75.696699 N, 14 June 2018, S.R. Yadav, J.V. Dalavi and P.V. Deshmukh, JVD-1251 (holotype CAL!), isotypes BSI!, K!, SUK!).

Portulaca oleracea var. linearifolia Sivar. and Manilal New Botanist, Int. Quart. J. Pl. Sci. Res. 4: 30.1977; Sivarajan, J. Bomb. Nat. Hist. Soc., 78. 258. 1981; Rao in Fl. Ind. 3: 4. 1994; Dalavi et al. Phytomorphology. 69. 15–24. 2019 (Figure 5).

Type: INDIA, Uttar Pradesh, Saharanpur. Chandra-42790.

Morphological description: annual, decumbent succulent, herbs, 10–25 cm high. Stem: 0.2–0.5 cm in diam., glabrous, pink tinged. Leaves 0.3–3 cm, elliptic to oblanceolate, thick fleshy, sessile without axillary hairs, cuneate at base, rounded at apex, dark green rarely pink tinged in stress conditions. Inflorescence: 2–5 flowered capitulum. Flowers: 0.8–1.2 cm across, sessile, pentamerous, bright yellow; sepals: 2, 0.3–0.6 $\times$ 0.2–0.4 cm, ovate, thick, fleshy, persistent; petals: 5, 0.3–0.6 $\times$ 0.2–0.4 cm, obovate, obtuse, glabrous, bright yellow; stamens: 10–20, polyandrous, filaments connate at the base forming ring around gynoecium; gynoecium 3–5 mm, pentacarpellary, syncarpus, ovary superior, ovules numerous on basal placentas. Fruits: 2–4 $\times$ 1.5–2.5 mm, oblong, circum-scissile pyxis, with 55–75 seeds on basal placentas; seeds: 55–75 per capsule, periclinial walls convex, nearly flat to low-convex; the cells on the peripheral face of the seed par dome shaped.

Habitat: Common in open sandy areas and cultivated fields.

Flowering and fruiting: May to September.

Distribution: Endemic to India; Punjab, Uttar Pradesh, Bihar, West Bengal, Assam, Orissa, Gujarat, Maharashtra, Tamil Nadu, and Badami (Karnataka) [4,6].

Status: The variety is endemic to India [4,6].

Specimens examined: INDIA, Karnataka, Bagalkot Dist., Badami, 29/07/2018 Rupali Chougule and Jagdish Dalavi JVD-1263 (SUK!).

Portulaca liljii P.Sivaramakrishna and P. Yugandhar.

We were unable to sample this species in this study, the full morphological description of this species is provided in Sivaramakrishna and Yugandhar [8].
Inflorescence: 2–4-flowered, flowers in terminal sessile clusters surrounded by 3–4 involucral bracts. Flowers: c. 1.0–1.5 cm across, actinomorphic, hermaphrodite, sessile, pink to red with a dark red throat. Sepals: 2, 3–4 × 1–2 mm, ovate, connate at base, acuminate at apex, membranous, pale pink to yellow, glabrous. Petals: 4–5, obovate, 4–6 × 2.0–3.5 mm, acuminate at apex, thin delicate, transparent. Stamens: 8–16, free, adnate to the base of petals forming a ring around the ovary; anthers bithecous, 1 mm long, yellow, dehiscing by longitudinal slits; filaments 2–3 mm long, free, basally connate, glabrous. Ovary: 3–4 carpellate, syncarpous; 2–3 × 1.0–1.5 mm, globose, glabrous; style 3–4 mm, 4-fid, glabrous; stigmas slightly coiled, papillate. Fruits: a circum-scissile capsule, 4–5 × 1–2 mm, oblong, glabrous, the upper dome is two times longer than the basal disc. Seeds: 20–30 per capsule, 0.7–0.9 mm in diameter, ovoid with unique shiny golden luster and stellate cells throughout the surface.

Habitat: Rocky crevices under the shelter of spiny bushes or in sandy plains of open areas.

Flowering and fruiting: June to October.

Distribution: Badami (Bagalkot Dist., Karnataka), Mahbubnagar (Andhra Pradesh) [3,38].

Status: The species is yet not accessed for IUCN categorization due to a lack of statistical data on all populations.

Specimen examined: INDIA, Karnataka, Bagalkot District, Badami, 611 m a.s.l., 15.918955 E, 75.696699 N, 14 June 2018, S.R. Yadav, J.V. Dalavi and P.V. Deshmukh, JVD-1251 (holotype CAL!), isotypes BSI!, K!, SUK!).

Portulaca oleracea var. linearifolia Sivar. and Manilal New Botanist, Int. Quart. J. Pl. Sci. Res. 4: 30.1977; Sivarajan, J. Bomb. Nat. Hist. Soc., 78. 258. 1981; Rao in Fl. Ind. 3: 4. 1994; Dalavi et al. Phytomorphology. 69. 15–24. 2019 (Figure 5).

5. Conclusions

The present study confirms phylogenetic placements of Indian endemic Portulaca species, namely P. badamica, P. lakshminarsimhanina, and P. oleracea var. linearifolia. Morphological similarities and differences between endemic and closely related species were also addressed. Reconstructed phylogeny reveals that all the sampled Indian Portulaca species (except P. wightiana) are phylogenetically placed in the AL clade. This clade includes the best-known species of the family Portulacaceae and is characterized by alternating to sub-opposite leaves [1]. In addition to this, the present study provides taxonomic treatment for endemic species and a distribution map (Figure 6). S-DIVA analysis suggested that Indian endemic species P. badamica were dispersed from Africa to India and P. lakshminarsimhanina may be diverged from the Indian P. Pilosa populations and the result of in situ speciation in India.
Figure 6. Distribution map of Indian endemic Portulaca species: Portulaca badamica, Portulaca lakshminarasimhaniana, Portulaca oleracea var. linearifolia and Portulaca laljii.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d14060443/s1, Supplementary Material S1: Details of Portulaca accessions included in phylogenetic analyses: herbarium number and GenBank accession numbers for ITS, ndhF, trnT-PSB-D and ndhA intron. Supplementary Material S2: Aligned combined sequence data (ITS + ndhA + ndhF + trnT-PSB-D) matrix. Supplementary Material S3: RASP program results of S-DIVA analysis for important nodes discussed in this study. Figure S1: Maximum likelihood tree based on ITS data. Figure S2: Maximum likelihood tree based on ndhA data. Figure S3: Maximum likelihood tree based on ndhF data. Figure S4: Maximum likelihood tree based on trnT-psbD data. Figure S5: Bayesian phylogenetic analysis on the combined dataset with all compatible groups. Figure S6: Maximum likelihood tree based on combined (ITS + ndhA + ndhF + trnT-psbD) dataset with the highest log-likelihood value (−22,064.18).
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