Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Seed macro-micromorphology and character evolution in Indian species of Jatropha L. (Euphorbiaceae)

Seema Yadav
University of Madras
Varnika Bhatia
University of Delhi
Cordilea Hannah
University of Madras
Senthilkumar Umapathy
senthilumapathy@mcc.edu.in

University of Madras

Research Article

Keywords: Ancestral states reconstruction, Anticlinal boundaries, Periclinal walls, Scanning electron microscopy, Seed morphology

Posted Date: November 10th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11456737/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Seed macro-micromorphology and character evolution in Indian species of *Jatropha* L. (Euphorbiaceae)

Seema Yadav[1,2] Varnika Bhatia[2] Cordilea Hannah[3] Senthilkumar Umapathy[1]*

[1]Molecular Systematics and DNA Barcoding Laboratory, Department of Botany, Madras Christian College (Autonomous), University of Madras, East Tambaram, Chennai- 600 059, India

[2]Department of Botany, Deen Dayal Upadhyaya College, University of Delhi, New Delhi-110 078, India

[3]Department of Plant Biology and Plant Biotechnology, Women's Christian College (Autonomous), University of Madras, Nungambakkam, Chennai- 600 005, India

phd.seema.21bot2111@ddu.du.ac.in; https://orcid.org/0009-0000-3819-825X

varnikabhatia@ddu.du.ac.in; https://orcid.org/0000-0002-7107-503X

cordilea09.wcc@gmail.com; https://orcid.org/0000-0003-3311-5819

*senthilumapathy@mcc.edu.in; https://orcid.org/0000-0001-9385-4117

Abstract

Seed morphology serves as a crucial source of taxonomic and evolutionary information in plants. The ornamentation, sculpturing, and architecture of the seed coat, in particular, hold significant phylogenetic importance. We investigated the seed morphology of 14 Indian *Jatropha* species using stereomicroscopy and scanning electron microscopy. Macro- and micromorphological characters, such as seed shape, size, color, caruncle morphology, epidermal cell shape, anticlinal boundaries, and periclinal wall ornamentation, were examined. Two seed surface types (reticulate and reticulate-rugose), two primary epidermal cell shapes (polygonal and isodiametrical), three anticlinal boundary types (raised, sunken, and indistinct), and four periclinal wall patterns (flat, concave, convex, and flat to concave) were recognized. Ancestral state reconstruction based on molecular phylogenetic data revealed varying evolutionary trajectories among seed characters, particularly the nature of caruncle and testa showing the strongest phylogenetic signal. These findings demonstrate the taxonomic importance of seed morphological traits and provide insights to understand the character evolution in Indian *Jatropha* species.

Keywords Ancestral states reconstruction, Anticlinal boundaries, Periclinal walls, Scanning electron microscopy, Seed morphology

Introduction

The genus *Jatropha* L. belongs to the family Euphorbiaceae (subfamily Crotonoideae, tribe Jatropheae*)* and comprises approximately 175 species distributed primarily throughout tropical regions of the Americas, Africa, and Asia (Kumar and Sharma, 2008; POWO, 2026). In India, it is represented by 15 species, of which 9 species (*J. benghalensis* (C.H.Rahaman & S.Mondal) Seema, U.Senthilkumar & B.Varnika, *J. heynei* N.P.Balakr., *J. livingstonii* Seema, U.Senthilkumar & B.Varnika, *J. maheshwarii* Subram. & M.P.Nayar, *J. nana* Dalzell & A.Gibson, *J. narasimhanii* Seema, U.Senthilkumar & B.Varnika, *J. tanjorensis* J.L.Ellis & Saroja, *J. umashaankerii* Seema, U.Senthilkumar & B.Varnika and *J. villosa* Wight) are endemic (Balakrishnan et al. 2012; Seema et al. 2026b).

Seed morphological characters are widely used in plant taxonomy because they often provide stable and diagnostic features for species delimitation. The application of scanning electron microscopy (SEM) has further enhanced seed studies by enabling detailed examination of seed coat micromorphology and surface sculpturing (Barthlott 1981; Hassan et al. 2005). However, seed morphometric studies in *Jatropha*, particularly among Indian taxa, remain poorly explored (Balakrishnan et al. 2012).

Species identification within *Jatropha* can be challenging, especially among geophytic taxa such as *J. benghalensis*, *J. heynei*, *J. livingstonii*, *J. nana*, *J. narasimhanii*, and *J. umashaankerii* which exhibit overlapping morphological characters and cryptic nature (Seema et al. 2026a,b). Seed morphology (macro- and micromorphology) may provide additional taxonomically informative characters for distinguishing such closely related species and also for the circumscription of species boundaries (Özbek and Uzunhisarcıklı 2023).

Therefore, the present study investigates the macro- and micromorphological seed characters of Indian *Jatropha* species using stereomicroscopy and SEM, evaluates their taxonomic

significance, and examines the evolutionary patterns of selected seed characters through ancestral states reconstruction.

Materials and methods

Collection of samples

Fresh young leaves and seeds were collected during several field expeditions conducted across India between August 2022 and June 2025 for molecular and seed morphometric analyses. The information on voucher specimens for all studied taxa is provided in (see Supplementary Electronica Material).

Seed morphology

For each of the 14 studied *Jatropha* species, five to ten fully mature seeds from at least three different specimens were selected for macro- and micromorphological analyses. Seed color, shape, length, width, and caruncle characteristics were measured using a stereomicroscope equipped with a digital camera (Seema et al. 2026a).

For scanning electron microscopy (SEM), seeds were mounted directly onto stubs using double-sided adhesive tape and coated with a thin layer of gold using a sputter coater (Goldstein et al. 2017). Observations were conducted at different magnifications using a Field Emission Scanning Electron Microscope (FE-SEM; GEMINI 1, SIGMA 300 VP, ZEISS). The terminology used to describe seed coat surface sculpturing follows Ehler (1976), Barthlott (1981), Park (2000), Stearn (2004), and Özbek and Uzunhisarcıklı (2023).

To estimate testa cell density, cells were counted within a 10,000 μm^2 area at five randomly selected locations on the seed surface of each species, and the average value was recorded.

DNA extraction, amplification, and sequencing

Genomic DNA extraction, purification, PCR amplification, and sequencing protocols for the *ITS2*, *psb*A-*trn*H, and *trn*L-*trn*F markers followed the methods based on Seema et al. (2026). DNA sequencing was carried out at Biokart Pvt. Ltd. (Bangalore, India). The GenBank accession numbers for newly generated and previously published sequences used in this study are provided in Supplementary Electronic Material.

Sequence alignment and phylogenetic analysis

Multiple sequence alignment was performed using the MUSCLE algorithm (Edgar 2004) implemented in MEGA11 (Tamura et al. 2021). The three markers were subsequently concatenated into a combined dataset. *Croton bonplandianus* Baill., *C. caudatus* Geiseler, and *C. glandulosus* L. were selected as outgroups based on the close phylogenetic relationship between *Croton* and *Jatropha* (Tokuoka and Tobe 2006).

The best-fitting nucleotide substitution model was selected using the Bayesian Information Criterion (BIC) in jModelTest (Darriba et al. 2012). Bayesian phylogenetic analysis was performed on the concatenated dataset using MrBayes v.3.2 (Ronquist et al. 2012).

Monte Carlo Markov Chain (MCMC) analyses were run for five million generations under the mixed-model setting (using the command '*lset nst=mixed'*) in MrBayes. The trees were sampled throughout the analysis, and a 50% majority-rule consensus tree was generated after discarding the initial 25% of sampled trees as burn-in. The final tree was visualized using FigTree v.1.4.4 (Rambaut 2018), and the posterior probability values were summarized to yield the branch support.

Ancestral state reconstruction of seed characters

Ancestral states were reconstructed for six seed characters commonly used in infrageneric classification: caruncle attachment, caruncle shape, number of testa cells, epidermal cell shape, anticlinal boundaries, and periclinal wall patterns.

Ancestral character states were inferred using parsimony methods implemented in Mesquite (Maddison and Maddison 2018). To account for phylogenetic uncertainty, the 'Trace over Trees' option was applied, and 1,000 trees randomly selected from the stabilized portion of the Bayesian MCMC analysis of the 46-taxon dataset (comprising cpDNA and nrDNA partitions) were included in the analysis. The resulting ancestral character reconstructions were mapped onto the 50% majority-rule consensus tree obtained from the Bayesian analysis.

A categorical data matrix comprising seed morphological characters was used as the input dataset and linked to the tree generated in MrBayes using the 'Taxa/Trees' option in Mesquite. The tree file and character matrix were analyzed simultaneously to reconstruct ancestral character states under the parsimony criterion. Character-state transformations were treated as unordered for all characters. The 'Trace over Trees' option summarizes, for each node, the proportion of reconstructions supporting a particular ancestral state and estimates the relative likelihood of alternative character states within a given clade.

Results

Seed macro-morphology

Stereomicroscopic images of seeds from the dorsal and ventral sides of the studied species are presented in Fig. 1, and the corresponding measurements are summarized in Table 1. Seed color in most species' ranges from brown to grey and rarely approaches black (Fig. 1). *Jatropha integerrima* Jacq. is distinct in possessing light-brown seeds with black and white patches (Fig. 1f), whereas *J. curcas* L. is characterized by uniformly black seeds (Fig. 1b).

Seed shape varies among the studied taxa and includes elliptic, ovoid, oblong, sub-globose, and nearly spherical forms. Oblong seeds are the most common among the studied taxa (Fig. 1). The smallest seeds were observed in *J. gossypiifolia* L. (7–8 x 4–5 mm; Fig. 1d), whereas *J. multifida* L. possesses the largest seeds, measuring approximately 18–19 x 16–17 mm. (Fig.

1h). Similarly, all studied taxa possess a caruncle that varies in shape, size, and color, except *J. multifida*, in which the caruncle is absent (Fig. 1h).

Seed micromorphology

The SEM micrographs of the seed surfaces are presented in Fig. 2, and the observed characters are summarized in Table 1. Epidermal cells are predominantly polygonal (pentagonal to hexagonal) in most species, including the outgroups, whereas isodiametric cells occur only in *J. nana* and *J. narasimhanii*.

Four types of periclinal wall patterns were observed: flat, convex, concave, and flat to concave (Table 1). Anticlinal boundaries are predominantly sunken in the studied species, whereas raised boundaries occur in *J. curcas*, *J. gossypiifolia, J. heynei*, *J. livingstonii*, and *J. umashaankerii* (Fig. 2). In *J. nana*, the anticlinal boundaries are indistinct (Fig. 2j).

The seed surface is primarily reticulate in all examined taxa; however, *J. curcus* and *J. umashaankerii* features an ornamentation of the reticulate-rugose type (Fig. 2b, m). *Jatropha multifida* lacks conspicuous surface sculpturing, mostly smooth across (Fig. 2i), while some of the other taxa show pronounced sculpturing.

The number of testa cells per 10,000 μm^2 area is presented in Table 1. Values range from 37–43 cells in *J. curcas* to 155–165 cells in *J. maheshwarii*. Although *J. maheshwarii* has seeds of intermediate size, it exhibits the highest testa cell density among the studied taxa.

Character evolution based on ancestral state reconstruction

Ancestral states reconstruction was performed for 12 seed characters, of which six showed significances in character evolution: caruncle shape, caruncle attachment, number of testa cells, epidermal cell shape, anticlinal boundaries, and periclinal wall patterns. Character-state

reconstruction for *J. tanjorensis* was equivocal due to the absence of seed material, resulting in missing data, and it was indicated in red color in all figures.

Caruncle shape: Caruncle shape exhibited a highly polyphyletic distribution across the selected phylogenetic dataset, with the character states capped, hooded, and crowned dispersed throughout the phylogenetic topology (Fig. 3). The capped state (sky blue) was observed in clades C3, C5 and C7, whereas hooded (green) occurred in all the clades except clades 1, 3, and 5 (C1, C3, C5). In contrast, crowned (black) occurred only in clades C1, C4, C5, and C6.

Caruncle attachment: Caruncle attachment showed a highly polyphyletic distribution across the selected phylogenetic dataset, with the character states ecarunculate, erect, and sessile dispersed throughout the phylogenetic topology (Fig. 4). The erect state (green) occurred in all clades except clades 3, and 5 (C3, C5), whereas the sessile state (black) was observed in clades C3, C5, and C7. In contrast, the state, ecarunculate was monophyletic and restricted to clade 1 (C1).

Number of testa cells: The number of testa cells exhibited polyphyly across the selected phylogenetic dataset, with the categorized character states 0–50, 51–100, 101–150, and 151–200 showing varying distributions across the phylogenetic topology (Fig. 5). The character state 0–50 (white) was observed in clades C1, C2, and C7, whereas the state 51–100 (sky blue) occurred in all clades except clades 2 and 3 (C2, C3). In contrast, the state 101–150 (green) was restricted to clades C3, C6, and C7 while 151–200 (black) was restricted to clade 4 (C4) (Fig. 5).

Epidermal cell shape: Epidermal cell shape exhibited a largely monophyletic distribution across the selected phylogenetic dataset, with the character states polygonal and isodiametrical (Fig. 6). The polygonal state (white) was observed in all clades except clade C3 and C7,

whereas the isodiametrical state (black) was restricted to clades 3 and 7 (C3, C7) and therefore formed the monophyletic lineages independently at the clades C3 and C7.

Anticlinal boundaries: Anticlinal boundaries exhibited a moderately polyphyletic distribution across the selected phylogenetic dataset, with the character states sunken, raised, and indistinct dispersed throughout the phylogenetic topology (Fig. 7). The sunken state (white) was observed in all clades except clade 2 (C2), whereas the raised state (green) occurred in clades C2, C6, and C7 (Fig. 7). In contrast, the indistinct state (black) was restricted to clade 7 (C7).

Periclinal wall patterns: Periclinal wall patterns exhibited a roughly polyphyletic distribution across the selected phylogenetic dataset, with the character states flat, concave, convex, and flat to concave dispersed throughout the phylogenetic topology (Fig. 8). The flat state (white) was observed in clades C5 and C6, while the concave state (sky blue) was found in clades C1, C2, and C6. Additionally, the convex state (green) was polyphyletic and has been found in clades C1, C4, and C7. In contrast, the flat to concave state (black) was restricted to clade C3, C4, and C7 (Fig. 8).

Discussion

The present study offers a detailed assessment of seed morphology and surface micromorphic structure for 14 Indian *Jatropha* species, including eight species for which the information is reported for the first time (Table 1; Seema et al. 2026b). Seed morphology has long been recognized as a valuable source of taxonomic information in Euphorbiaceae, and our findings further underscore the utility for species identification and its characterization. Bahadur et al. (2013) highlighted seed characters as important diagnostic traits, emphasizing their considerable diversity and taxonomic significance within the genus. Our results corroborate this observation, demonstrating that seed features, particularly, shape, color, size, and the

surface ornamentation of seed coat are among the most informative characters for identifying and delimiting *Jatropha* species in India.

The examined species exhibit considerable variation in seed shape and size. Oblong seeds are the most common and occur in *J. gossypiifolia*, *J. glandulifera* Roxb., *J. maheshwarii*, *J. podagrica* Hook. and *J. villosa*, whereas *J. multifida,* which possesses the largest seeds, was characterized by a nearly spherical shape. Seed length ranges from approximately 8 mm in *J. gossypiifolia* to about 18 mm in *J. curcas* and *J. multifida*.

Seed color and surface texture also vary among the studied species. Most taxa possess seeds in different shades of brown, whereas *J. curcas* is distinguished by its black seeds and *J. integerrima* by its light-brown seeds with black and white patches. In addition, the seed surfaces of *J. glandulifera*, *J. integerrima*, *J. maheshwarii*, *J. nana*, *J. podagrica*, and *J. villosa* appear smoother compared to other species.

Caruncle morphology provides additional diagnostic characters for species identification (Bahadur et al. 2013). Considerable variations were observed in caruncle shape, size, color, and attachment. Although brown is the predominant caruncle color, *J. integerrima* has a yellow while *J. maheshwarii, J. nana* and *J. villosa* are characterized by a golden color. An erect caruncle occurs in *J. curcas*, *J. glandulifera*, *J. gossypiifolia*, *J. integerrima*, *J. maheshwarii*, *J. nana*, *J. podagrica*, and *J. villosa*, whereas the remaining species possess sessile caruncles. Surprisingly, the caruncle is absent in *J. multifida*.

Seed micromorphological observations indicate that the seed surface is predominantly reticulate, although it differs in the degree of sculpturing. *Jatropha benghalensis*, *J. curcas*, *J. glandulifera*, *J. gossypiifolia*, *J. heynei*, *J. integerrima*, *J. maheshwarii*, *J. nana*, *J. podagrica, J. umashaankerii* and *J. villosa* exhibit pronounced surface sculpturing, whereas *J. livingstonii*

shows minimal sculpturing. In contrast, *J. multifida* and *J. narasimhanii* lack any surface sculpturing.

According to our results, there is no correlation between seed size and the number of testa cells. For example, *Jatropha maheshwarii* and *J. villosa* have almost similar seed sizes; however, considerable differences are observed in the number of testa cells per 10,000 μm^2. The highest testa cell densities were recorded in *J. maheshwarii* (155–165), followed by *J. umashaankerii* (108–120), *J. gossypiifolia* (105–115) and *J. narasimhanii* (100–110) (Table 1).

Ancestral state reconstruction showed varying degrees of phylogenetic signal among the studied seed characters, despite the dataset's limitation in the number of species from other phytogeographical regions. The shape of the caruncle has demonstrated a character evolution of the state, specifically the hooded form, which is present across all clades in the phylogenetic reconstruction. However, the states known as crowned and capped exhibit a significant polyphyletic lineage in the Indian dataset, indicating that the character evolution of these states was convergent and would have evolved independently. Additionally, the caruncle is absent in the Central American species, particularly in *J. multifida*. Despite being introduced, this species has demonstrated a monophyletic lineage with geographical disjunction.

Similarly, the caruncle attachment has shown a character evolution of the state, specifically the erect form, which is present across all clades in the phylogenetic reconstruction. In contrast, the state, sessile, has revealed a significant polyphyletic lineage, indicating the homoplasy. This state has been found in the geophytic endemics of the Deccan Plateau, specifically in the species such as *J. benghalensis*, *J. heynei*, *J. livingstonii*, *J. narasimhanii* and *J. umashaankerii*.

The number of testa cells was not directly proportional to seed size, where the size of the *J. multifida* was larger, but the number of cells per 10,000 μm^2 was less. Ancestral states reconstruction of this quantitative character unraveled predominantly a polarized tree with the

states collectively showing the less cell density (0–50 cells & 51–100 cells) throughout. In contrast, one of the categorical states, the state with 101–150 cells have demonstrated the monophyletic evolution of a seed in *J. gossypiifolia, J. narasimhanii,* and *J. umashaankerii*. Additionally, the state with 151–200 cells revealed a significant synapomorphic evolution in the endemic species *J. maheshwarii. Jatropha maheshwarii* is distributed only in the drier and coastal regions of Tamil Nadu. In Euphorbiaceae, specifically the density of the cells that helps the dormancy of the seed (Tavecchio et al. 2018) and also the dispersal efficiency (Coen and Magnani 2018). The number of testa cells and their size indeed determine the desiccation of seeds in harsh environments. Thus, the ancestral states reconstruction for this trait has shown that species growing in high arid regions have a greater number of cells than those occurring in tropical dry evergreen or deciduous forest fringes.

Epidermal cell shape has emerged as the most informative character for phylogenetic analysis, exhibiting a predominantly monophyletic distribution. The polygonal shape of epidermal cells was widespread and likely represents the ancestral (plesiomorphic) condition. In contrast, the state isodiametric cell shape, indicative of synapomorphic evolution, is exclusively found in *J. nana* and *J. narasimhanii*, a steno-endemic species of Deccan Intertrappean region of peninsular India.

The character, anticlinal boundaries of the seed coat, reflects the degree of cell interlocking mechanisms which facilitate the seed dispersal and conservation of the seed at the soil seed bank against desiccation, microbial attack and the fluctuating seasonal conditions (Radchuk and Borisjuk 2014). Ancestral state reconstruction suggests that the raised state represents a plesiomorphic trait that appears polyphyletically among widely distributed Indian species, including *J. curcas, J. gossypiifolia, J. heynei.* The widespread nature of the species mentioned above may have influenced the development of a raised pattern in the seed coat reinforcement, which could enhance seed germination and post-dispersal success across various landscapes

and seasonal conditions. The presence of this trait in endemic species like *J. livingstonii* and *J. umashaankerii* is questionable; however, it may suggest that they represent relict forms of the ancestral lineages of arid-zone geophytes. The state sunken was predominant in the dataset and was largely found in geophytes and succulent *Jatrophas*. This suggests characteristics that protect the seed coat from uncontrolled water entry and enhance seed survival during prolonged drought conditions. The synapomorphic evolution of an indistinct anticlinal boundary in *J. nana* highlights the plant's seed adaptation and survival strategies within grassland vegetation.

The evolution of character traits in the periclinal wall patterns facilitates selective adaptation of the species in seed coat architecture (Patterson et al. 2024). In Indian *Jatropha*, concave sculpturing has been identified as a plesiomorphic trait. Concave-sculpturing provides the adhesion for seed dispersal (da Silva et al. 2016) and also the flexible juncture with an adjacent cell, which facilitates the friction with soil particles (Moïse et al. 2005; Yusupov et al. 2022). Subsequently, the flat state has emerged as a transitional character state, which exhibits a nearly smooth nature. The state, convex (verrucate), was highly polyphyletic, and this surface ornamentation has shaped the seeds to avoid physical abrasion during dispersal and protects them from microbial infestations (Radchuk and Borisjuk 2014). The state, flat to concave, was not truly distinctive, but it showed the transitional evolution in its ontogeny of a few endemic species such as *J. heynei, J. maheshwarii, J. narasimhanii, J. umashaankerii*, and *J. villosa* and indicated its synapomorphy significantly.

Nevertheless, several characters, including the number of testa cells, epidermal cell shape, anticlinal boundaries, and periclinal wall patterns, may still provide useful diagnostic information when interpreted in conjunction with molecular phylogenetic evidence (Pahlevani et al. 2015). The absence of seed material for *J. tanjorensis* resulted in equivocal ancestral state reconstruction, limiting its inclusion in the character evolution framework. Overall, these

findings underscore the taxonomic relevance of seed micromorphological traits in *Jatropha* and their utility in supporting phylogenetic interpretations.

Funding

This work was supported by the Anusandhan National Research Foundation (previously the Science and Engineering Research Board) for the financial support (TAR/2021/000320) and the Council of Scientific and Industrial Research for funding a Senior Research Fellowship (08/1307(15130)/2022-EMR-I).

Acknowledgements

We are grateful to Dr. P. Wilson, Principal and Secretary, Madras Christian College (Autonomous), Chennai, for his encouragement and facilitation. We are also grateful to Dr. E. Joyce Sudandara Priya, Head, Department of Botany, Madras Christian College (Autonomous), Chennai, for her constant support. We also thank Prof. Hem Chand Jain, Principal, Deen Dayal Upadhyaya College, University of Delhi, Delhi, for his continuous help and support. We thank Ms. M. Nivetha, Research Scholar, for her help in taking the stereoscopic pictures.

Information on Electronical Supplementary Material

Online Resource 1: GenBank accession details, locality, and voucher numbers of both *Jatropha* (ingroup) and *Croton* (outgroup) used in this study.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

Bahadur B, Pullaiah T, Murthy GV (2013) Systematics of Indian Jatrophas. In: Bahadur B, Sujatha M, Carels N (eds) *Jatropha*, challenges for a new energy crop, Springer, New York, pp 153–186. https://doi.org/10.1007/978-1-4614-4915-7_10

Balakrishnan NP, Chakrabarty T, Sanjappa M, Lakshminarsimhan P, Singh, P (2012) Flora of India. Botanical Survey of India, Kolkata

Barthlott W (1981) Epidermal and seed surface characters of plants: systematic applicability and some evolutionary aspects. Nordic J Bot 1:345–355. https://doi.org/10.1111/j.1756-1051.1981.tb00704.x

Berry PE, Hipp AL, Wurdack KJ, Van Ee B, Riina R (2005) Molecular phylogenetics of the giant genus *Croton* and tribe Crotoneae (Euphorbiaceae *sensu stricto*) using *ITS* and *trn*L-*trn*F DNA sequence data. Amer J Bot 92:1520–1534. https://doi.org/10.3732/ajb.92.9.1520

Caruzo MB, Van Ee BW, Cordeiro I, Berry PE, Riina R (2011) Molecular phylogenetics and character evolution of the "sacaca" clade: novel relationships of *Croton* section *Cleodora* (Euphorbiaceae). Molec Phylogen Evol 60:193–206. https://doi.org/10.1016/j.ympev.2011.04.013

Coen O, Magnani E (2018) Seed coat thickness in the evolution of angiosperms. Cell Molec Life Sci 75:2509–2518. https://doi.org/10.1007/s00018-018-2816-x

da Silva OL, Cordeiro I, Caruzo MB (2016) Seed morphology in *Euphorbia* and its taxonomic applications: a case study in São Paulo, Brazil. Brazil J Bot 39:349–358. https://doi.org/10.1007/s40415-015-0228-9

Darriba D, Taboada GL, Doallo R (2012) jModelTest 2: more models, new heuristics and high-performance computing. Nat Methods 9:772. https://doi.org/10.1038/nmeth.2109

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32:1792–1797. https://doi.org/10.1093/nar/gkh340

Ehler N (1976) Mikromorphologie der samenoberflächen der Gattung *Euphorbia*. Pl Syst Evol 126:189–207. https://doi.org/10.1007/BF00981670

Goldstein JI, Newbury DE, Michael JR, Ritchie NW, Scott JH, Joy DC (2017) Scanning electron microscopy and X-ray microanalysis, 4th edn. Springer, New York

Guo GY, Wang SH, Xu Y, Tang L, Yu MQ, Chen F (2016) Molecular phylogenetic analysis of key *Jatropha* species inferred from nrDNA *ITS* and chloroplast (*trn*L-F and *rbc*L) sequences. Genes Genomics 38:557–566. https://doi.org/10.1007/s13258-016-0406-6

Hassan NM, Meve U, Liede-Schumann S (2005) Seed coat morphology of Aizoaceae–Sesuvioideae, Gisekiaceae and Molluginaceae and its systematic significance. Bot J Linn Soc 148:189–206. https://doi.org/10.1111/j.1095-8339.2005.00407.x

Kumar A, Sharma S (2008) An evaluation of multipurpose oilseed crop for industrial uses (*Jatropha curcas* L.): a review. Ind Crops Prod 28:1–10. https://doi.org/10.1016/j.indcrop.2008.01.001

Maddison WP, Maddison DR (2018) Mesquite: a modular system for evolutionary analysis. Version 3.6. Available at: http://mesquiteproject.org

Moïse JA, Han S, Gudynaitę-Savitch L, Johnson DA, Miki BL (2005) Seed coats: structure, development, composition, and biotechnology. In Vitro Cell Developmental Biol Pl 41:620–644. https://doi.org/10.1079/IVP2005686

Özbek F, Uzunhisarcıklı ME (2023) Taxonomic significance of seed macro-micromorphology of Turkish *Alcea* L. (Malvaceae) through light microscopy and scanning electron microscopy. Microscopy Res Techn 86:1551–1567. https://doi.org/10.1002/jemt.24385

Pahlevani AH, Liede-Schumann S, Akhani H (2015) Seed and capsule morphology of Iranian perennial species of *Euphorbia* (Euphorbiaceae) and its phylogenetic application. Bot J Linn Soc 177:335–377. https://doi.org/10.1111/boj.12245

Park KR (2000) Seed morphology of *Euphorbia* section *Tithymalopsis* (Euphorbiaceae) and related species. J Pl Biol 43:76–81. https://doi.org/10.1007/BF03030498

Patterson A, Karaismailoğlu MC, Küçüker O (2024) Seed morphology of 31 *Euphorbia* L. species (Euphorbiaceae) in Turkey and their taxonomic significance. Microscopy Res Techn 87:2654–2665. https://doi.org/10.1002/jemt.24637

POWO (2026) Plants of the world online. Royal Botanic Gardens, Kew. Available at: http://www.plantsoftheworldonline.org/. Accessed 19 Feb 2026

Radchuk V, Borisjuk L (2014) Physical, metabolic and developmental functions of the seed coat. Front Plant Sci 5:510. https://doi.org/10.3389/fpls.2014.00510

Rambaut A (2018) Figtree ver 1.4.4. Institute of Evolutionary Biology, University of Edinburgh, Edinburgh. Available at: https://www.tree.bio.ed.ac.uk/software/figtree/. Accessed 28 Feb 2026

Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol 61:539–542. https://doi.org/10.1093/sysbio/sys029

Seema, Bhatia V, Umapathy S (2026a) Unraveling cryptic lineages in *Jatropha nana*: phylogenetic evidence supports species status for *J. benghalensis* (Euphorbiaceae). Nordic J Bot e05048. https://doi.org/10.1002/njb.05048

Seema, Bhatia V, Umapathy S (2026b) Four in One: phylogeny unravels the cryptic lineages in *Jatropha heynei*, an endemic tuberous geophyte from India. Feddes Repert. https://doi.org/10.1002/fedr.70150

Stearn WT (2004) Botanical Latin, 4th edn. Timber Press, Portland

Tamura K, Stecher G, Kumar S (2021) MEGA11: molecular evolutionary genetics analysis version 11. Molec Biol Evol 38:3022–3027. https://doi.org/10.1093/molbev/msab120

Tavecchio NE, Vigliocco AE, Terenti OA, Wassner D, Reinoso HE, Pedranzani HE, Tavecchio M (2018) *Jatropha curcas* L. and *J. macrocarpa* Griseb: Seed morphology, viability, dormancy, germination and growth of seedlings. Amer J Pl Sci 9:1835–1854. https://doi.org/10.4236/ajps.2018.99134

Tokuoka T, Tobe H (2006) Phylogenetic analyses of Malpighiales using plastid and nuclear DNA sequences, with particular reference to the embryology of Euphorbiaceae *sens. str.* J Pl Res 119:599–616. https://doi.org/10.1007/s10265-006-0025-4

van Ee BW, Berry PE (2009) A phylogenetic and taxonomic review of *Croton* (Euphorbiaceae ss) on Jamaica including the description of *Croton jamaicensis*, a new species of section *Eluteria*. Syst Bot 34:129–140. https://doi.org/10.1600/036364409787602203

Yusupov Z, Ergashov I, Volis S, Makhmudjanov D, Dekhkonov D, Khassanov F, Tojibaev K, Deng T, Sun H (2022) Seed macro-and micromorphology in *Allium* (Amaryllidaceae) and its phylogenetic significance. Ann Bot 129:869–911. https://doi.org/10.1093/aob/mcac067

Legends

Fig. 1 Light microscopic images of seeds, **a** *Jatropha benghalensis*; **b** *J. curcas*; **c** *J. glandulifera*; **d** *J. gossypiifolia*; **e** *J. heynei*; **f** *J. integerrima*; **g** *J. livingstonii*; **h** *J. maheshwarii*;

i *J. multifida*; **j** *J. nana*; **k** *J. narasimhanii*; **l** *J. podagrica*; **m** *J. umashaankerii*; **n** *J. villosa*. Scale bars: **a–n**=2 mm. (Partially adapted from Seema et al. 2026b)

Fig. 2 Scanning electron microscopic photographs of seeds, **a** *Jatropha benghalensis*; **b** *J. curcas*; **c** *J. glandulifera*; **d** *J. gossypiifolia*; **e** *J. heynei*; **f** *J. integerrima*; **g** *J. livingstonii*; **h** *J. maheshwarii*; **i** *J. multifida*; **j** *J. nana*; **k** *J. narasimhanii*; **l** *J. podagrica*; **m** *J. umashaankerii*; **n** *J. villosa*. Scale bars: **a–n**=10 µm. (Partially adapted from Seema et al. 2026b)

Fig. 3 Reconstruction of ancestral character states in *Jatropha*. Optimization of seed character and character states: Caruncle shape; caruncle absent (0), capped (1), hooded (2), and crowned (3)

Fig. 4 Reconstruction of ancestral character states in *Jatropha*. Optimization of seed character and character states: Caruncle attachment; ecarunculate (0), erect (1), and sessile (2)

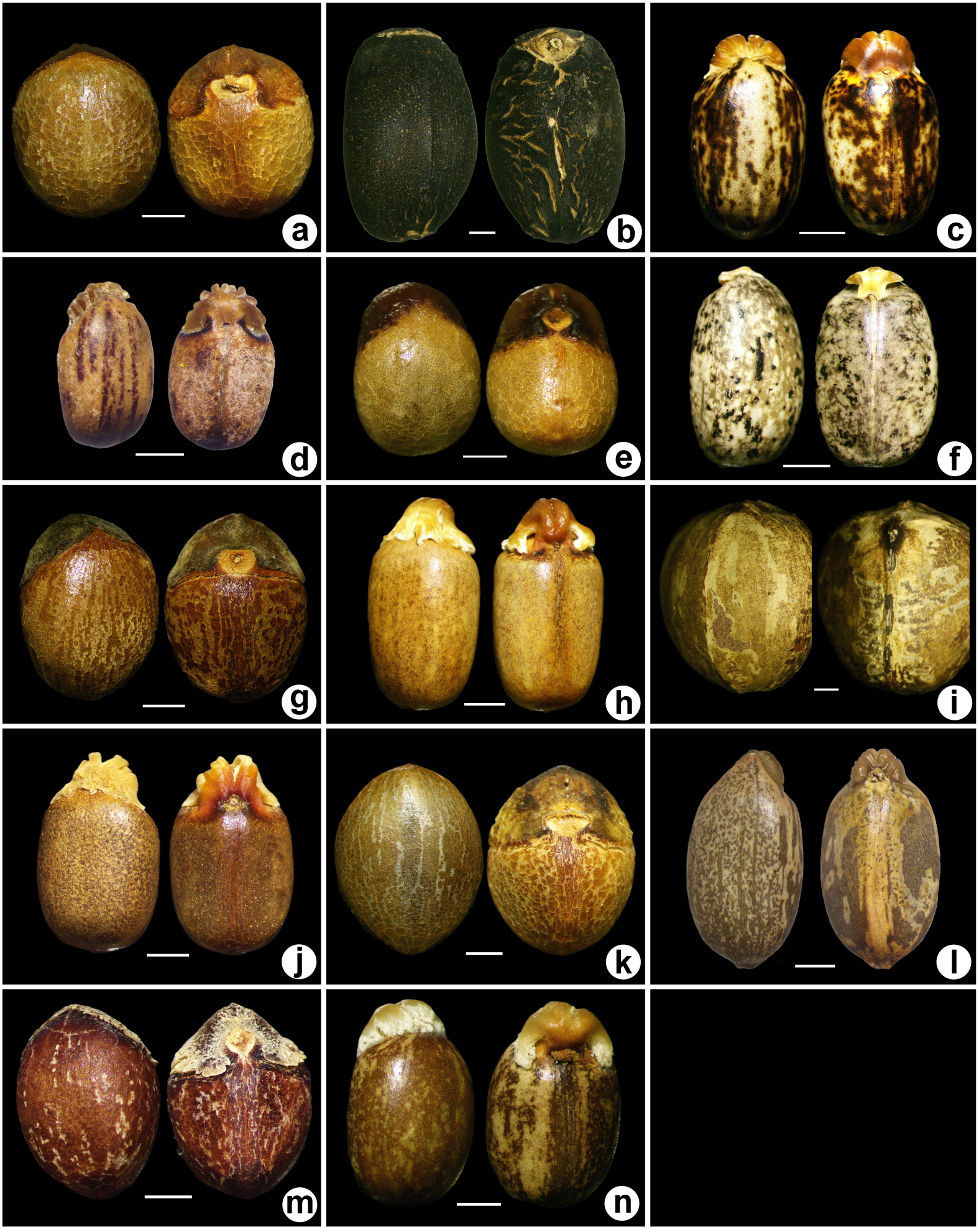
Fig. 5 Reconstruction of ancestral character states in *Jatropha*. Optimization of seed character and character states: Number of testa cells; 0–50 (0), 51–100 (1), 101–150 (2), and 151–200 (3)

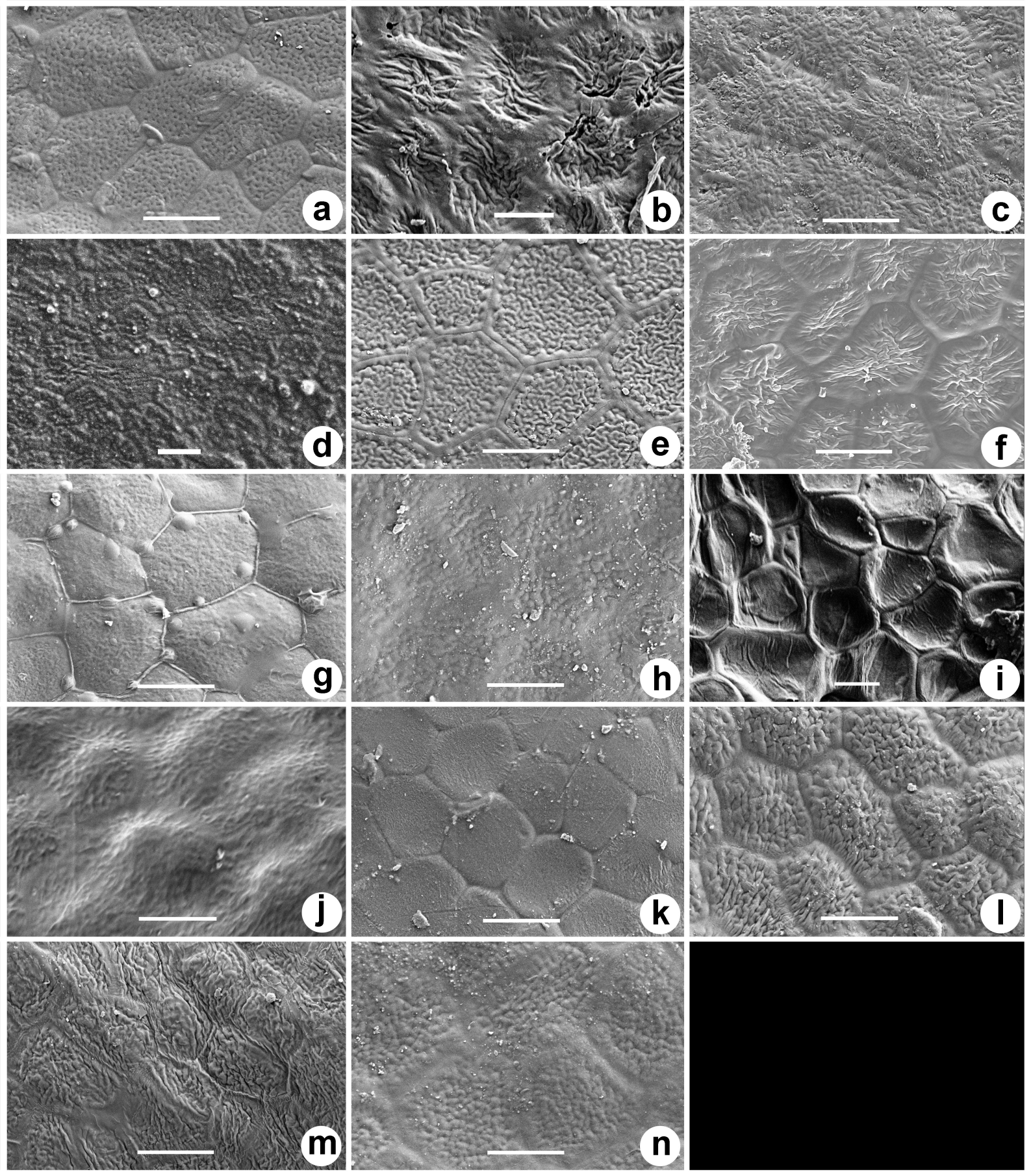
Fig. 6 Reconstruction of ancestral character states in *Jatropha*. Optimization of seed character and character states: Epidermal cell shape; polygonal (0) and isodiametrical (1)

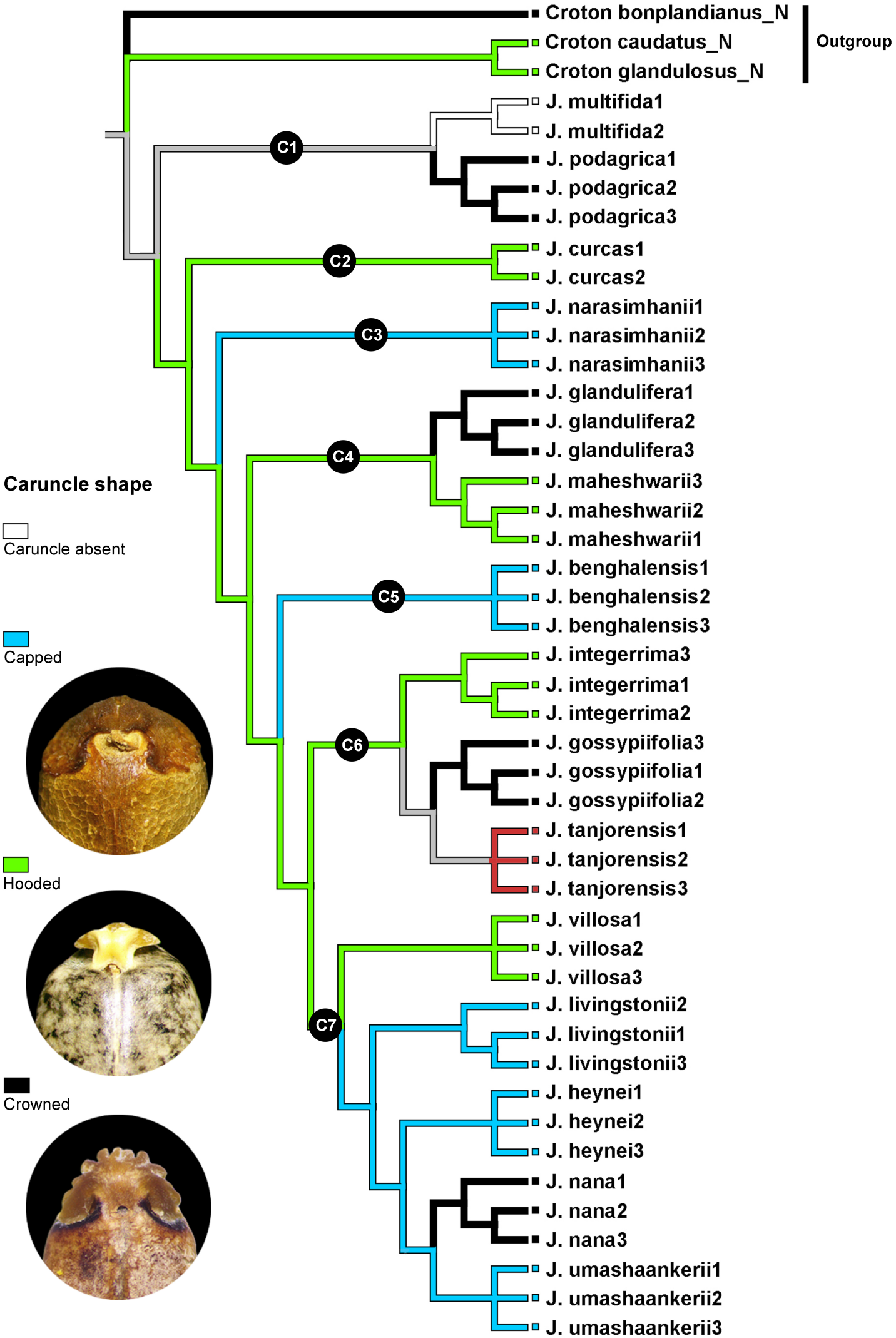
Fig. 7 Reconstruction of ancestral character states in *Jatropha*. Optimization of seed character and character states: Anticlinal boundaries; sunken (0), raised (1), and indistinct (2)

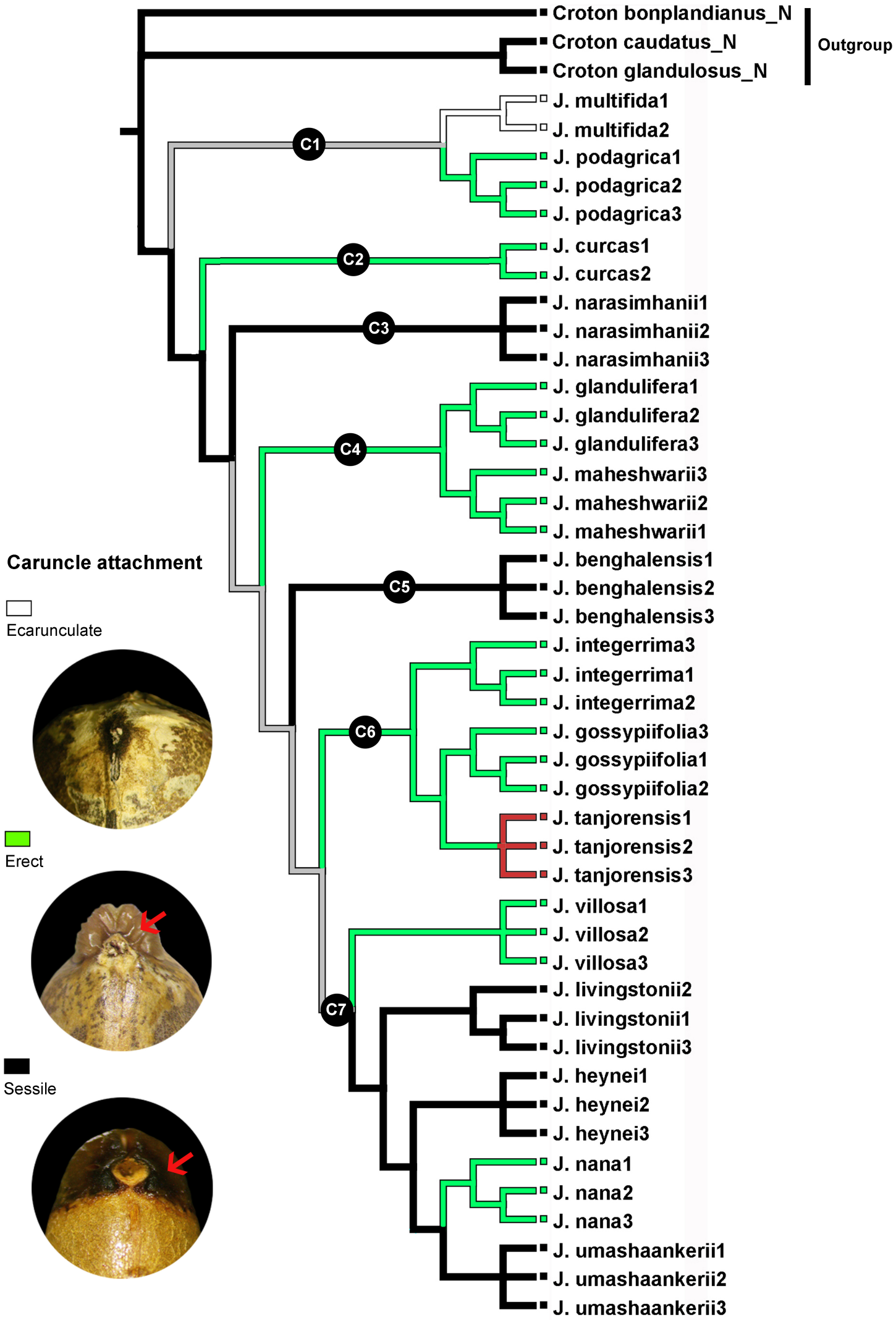
Fig. 8. Reconstruction of ancestral character states in *Jatropha*. Optimization of seed character and character states: Periclinal wall patterns; flat (0), concave (1), convex (2), and flat to concave (3).

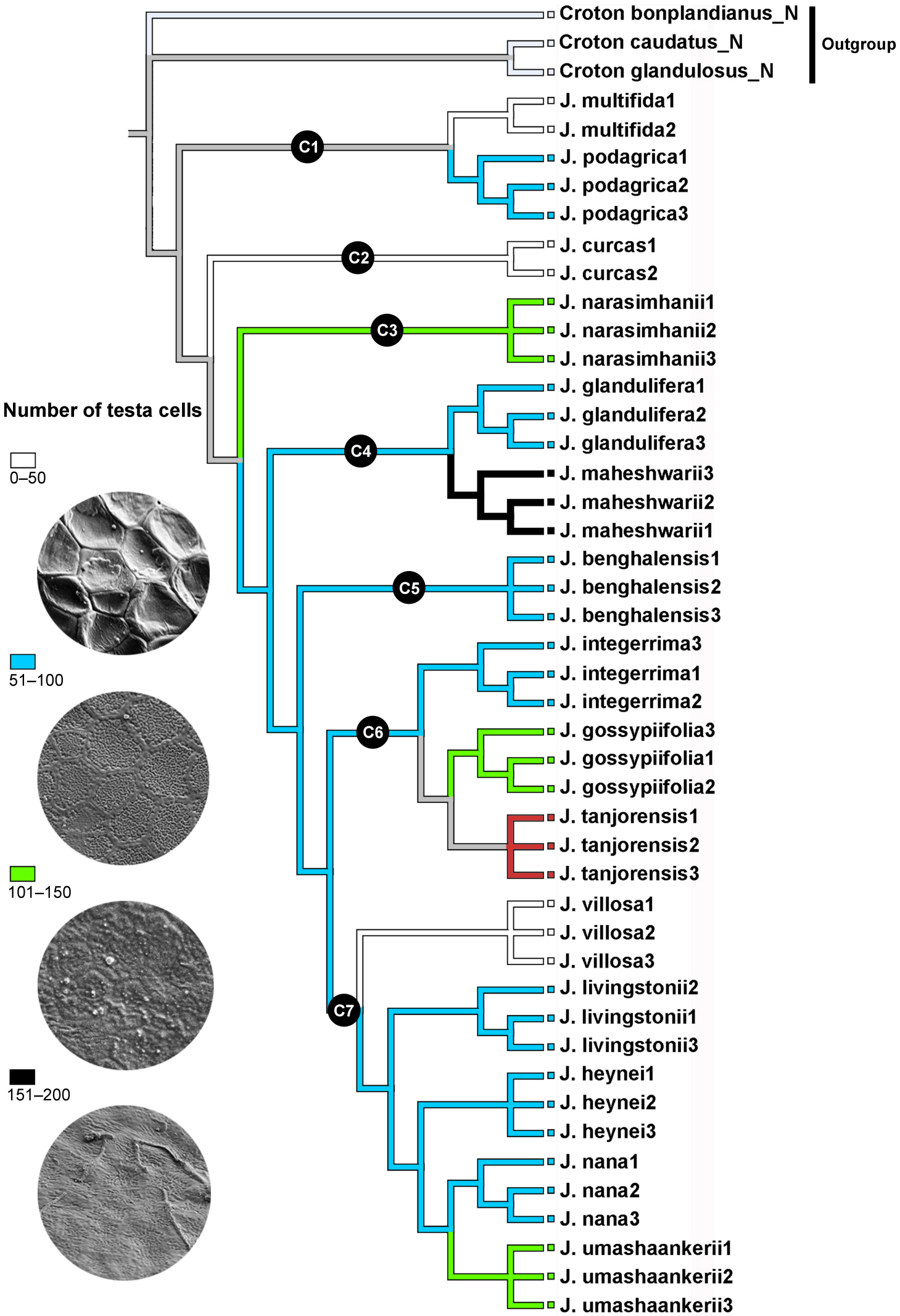
Table 1 Details of seed morphometric analysis of Indian *Jatropha* from this study. NTC indicates number of testa cells per 10,000 μm^2 and hyphen indicates the absence of the character.

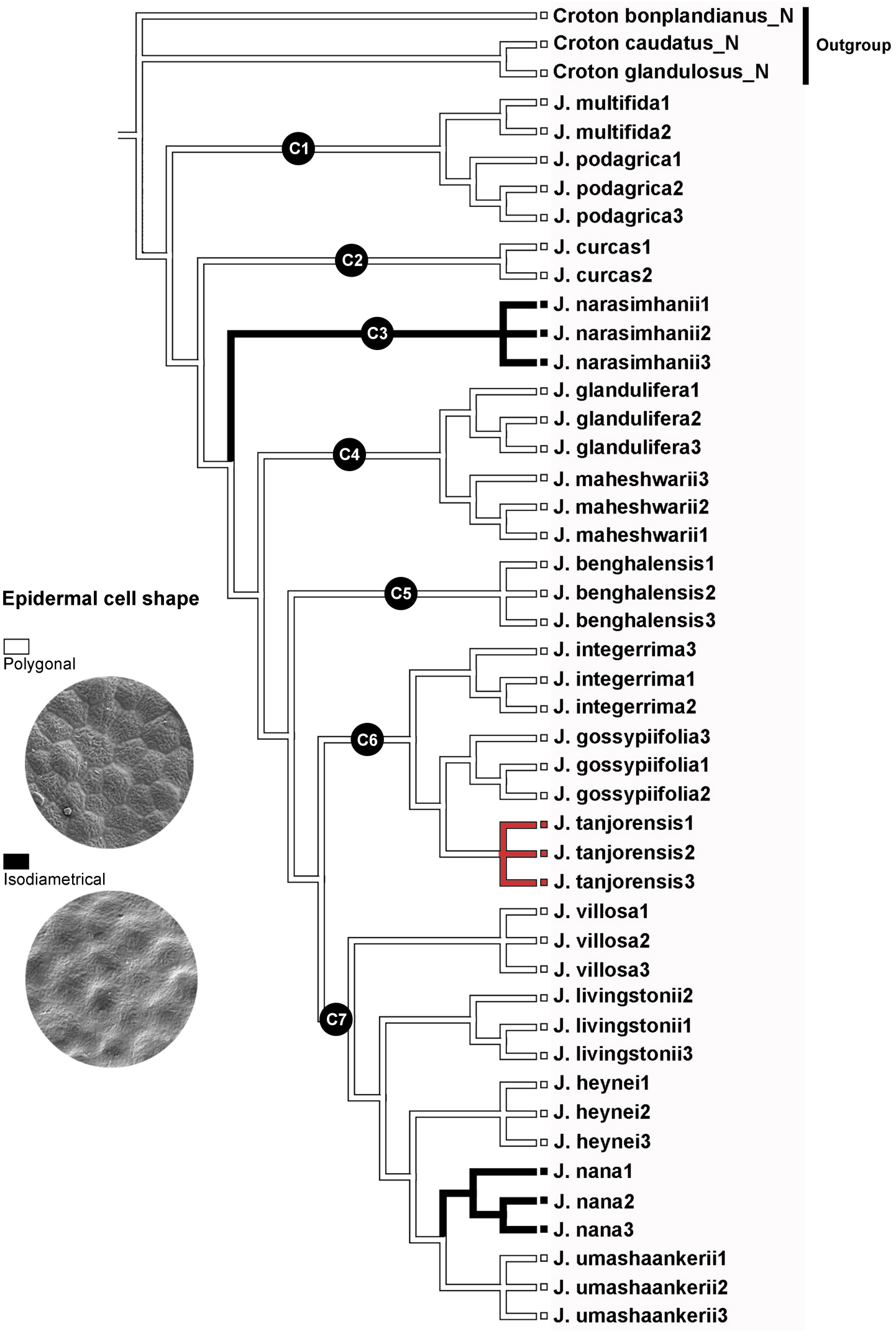

a
b
c
d
e
f
g
h
i
j
k
l
m
n

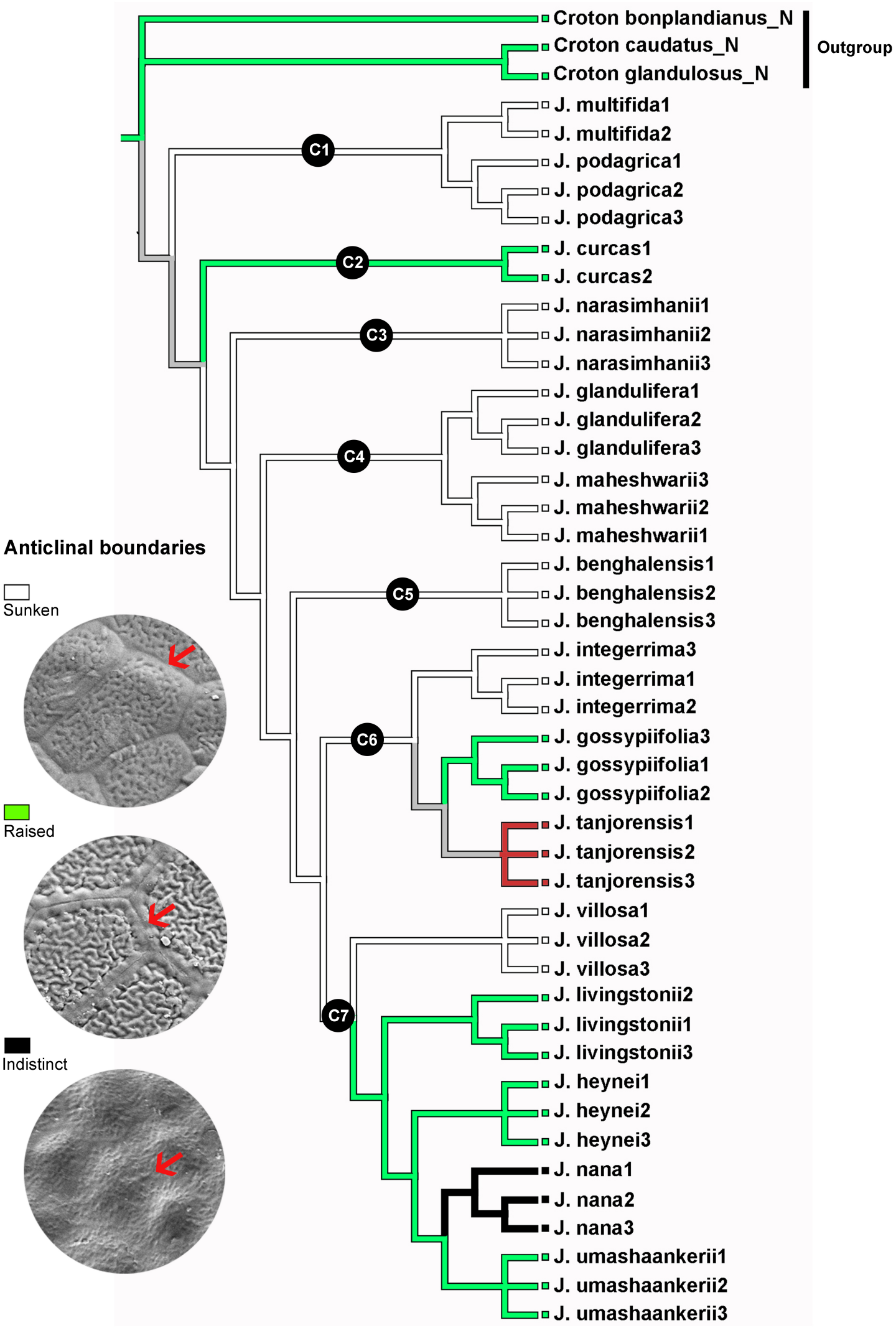

a
b
c
d
e
f
g
h
i
j
k
l
m
n


Croton bonplandianus_N
Croton caudatus_N
Croton glandulosus_N
Outgroup
J. multifida1
J. multifida2
J. podagrica1
J. podagrica2
J. podagrica3
J. curcas1
J. curcas2
J. narasimhanii1
J. narasimhanii2
J. narasimhanii3
J. glandulifera1
J. glandulifera2
J. glandulifera3
J. maheshwarii3
J. maheshwarii2
J. maheshwarii1
J. benghalensis1
J. benghalensis2
J. benghalensis3
J. integerrima3
J. integerrima1
J. integerrima2
J. gossypiifolia3
J. gossypiifolia1
J. gossypiifolia2
J. tanjorensis1
J. tanjorensis2
J. tanjorensis3
J. villosa1
J. villosa2
J. villosa3
J. livingstonii2
J. livingstonii1
J. livingstonii3
J. heynei1
J. heynei2
J. heynei3
J. nana1
J. nana2
J. nana3
J. umashaankerii1
J. umashaankerii2
J. umashaankerii3
C1
C2
C3
C4
C5
C6
C7
Caruncle shape
Caruncle absent
Capped
Hooded
Crowned


Croton bonplandianus_N
Croton caudatus_N
Croton glandulosus_N
Outgroup
J. multifida1
J. multifida2
J. podagrica1
J. podagrica2
J. podagrica3
J. curcas1
J. curcas2
J. narasimhanii1
J. narasimhanii2
J. narasimhanii3
J. glandulifera1
J. glandulifera2
J. glandulifera3
J. maheshwarii3
J. maheshwarii2
J. maheshwarii1
J. benghalensis1
J. benghalensis2
J. benghalensis3
J. integerrima3
J. integerrima1
J. integerrima2
J. gossypiifolia3
J. gossypiifolia1
J. gossypiifolia2
J. tanjorensis1
J. tanjorensis2
J. tanjorensis3
J. villosa1
J. villosa2
J. villosa3
J. livingstonii2
J. livingstonii1
J. livingstonii3
J. heynei1
J. heynei2
J. heynei3
J. nana1
J. nana2
J. nana3
J. umashaankerii1
J. umashaankerii2
J. umashaankerii3
C1
C2
C3
C4
C5
C6
C7
Caruncle attachment
Ecarunculate
Erect
Sessile


Croton bonplandianus_N
Croton caudatus_N
Croton glandulosus_N
Outgroup
J. multifida1
J. multifida2
J. podagrica1
J. podagrica2
J. podagrica3
J. curcas1
J. curcas2
J. narasimhanii1
J. narasimhanii2
J. narasimhanii3
J. glandulifera1
J. glandulifera2
J. glandulifera3
J. maheshwarii3
J. maheshwarii2
J. maheshwarii1
J. benghalensis1
J. benghalensis2
J. benghalensis3
J. integerrima3
J. integerrima1
J. integerrima2
J. gossypiifolia3
J. gossypiifolia1
J. gossypiifolia2
J. tanjorensis1
J. tanjorensis2
J. tanjorensis3
J. villosa1
J. villosa2
J. villosa3
J. livingstonii2
J. livingstonii1
J. livingstonii3
J. heynei1
J. heynei2
J. heynei3
J. nana1
J. nana2
J. nana3
J. umashaankerii1
J. umashaankerii2
J. umashaankerii3
C1
C2
C3
C4
C5
C6
C7
Number of testa cells
0–50
51–100
101–150
151–200


Croton bonplandianus_N
Croton caudatus_N
Croton glandulosus_N
Outgroup
J. multifida1
J. multifida2
J. podagrica1
J. podagrica2
J. podagrica3
J. curcas1
J. curcas2
J. narasimhanii1
J. narasimhanii2
J. narasimhanii3
J. glandulifera1
J. glandulifera2
J. glandulifera3
J. maheshwarii3
J. maheshwarii2
J. maheshwarii1
J. benghalensis1
J. benghalensis2
J. benghalensis3
J. integerrima3
J. integerrima1
J. integerrima2
J. gossypiifolia3
J. gossypiifolia1
J. gossypiifolia2
J. tanjorensis1
J. tanjorensis2
J. tanjorensis3
J. villosa1
J. villosa2
J. villosa3
J. livingstonii2
J. livingstonii1
J. livingstonii3
J. heynei1
J. heynei2
J. heynei3
J. nana1
J. nana2
J. nana3
J. umashaankerii1
J. umashaankerii2
J. umashaankerii3
C1
C2
C3
C4
C5
C6
C7
Epidermal cell shape
Polygonal
Isodiametrical


Croton bonplandianus_N
Croton caudatus_N
Croton glandulosus_N
Outgroup
J. multifida1
J. multifida2
J. podagrica1
J. podagrica2
J. podagrica3
J. curcas1
J. curcas2
J. narasimhanii1
J. narasimhanii2
J. narasimhanii3
J. glandulifera1
J. glandulifera2
J. glandulifera3
J. maheshwarii3
J. maheshwarii2
J. maheshwarii1
J. benghalensis1
J. benghalensis2
J. benghalensis3
J. integerrima3
J. integerrima1
J. integerrima2
J. gossypiifolia3
J. gossypiifolia1
J. gossypiifolia2
J. tanjorensis1
J. tanjorensis2
J. tanjorensis3
J. villosa1
J. villosa2
J. villosa3
J. livingstonii2
J. livingstonii1
J. livingstonii3
J. heynei1
J. heynei2
J. heynei3
J. nana1
J. nana2
J. nana3
J. umashaankerii1
J. umashaankerii2
J. umashaankerii3
C1
C2
C3
C4
C5
C6
C7
Anticlinal boundaries
Sunken
Raised
Indistinct

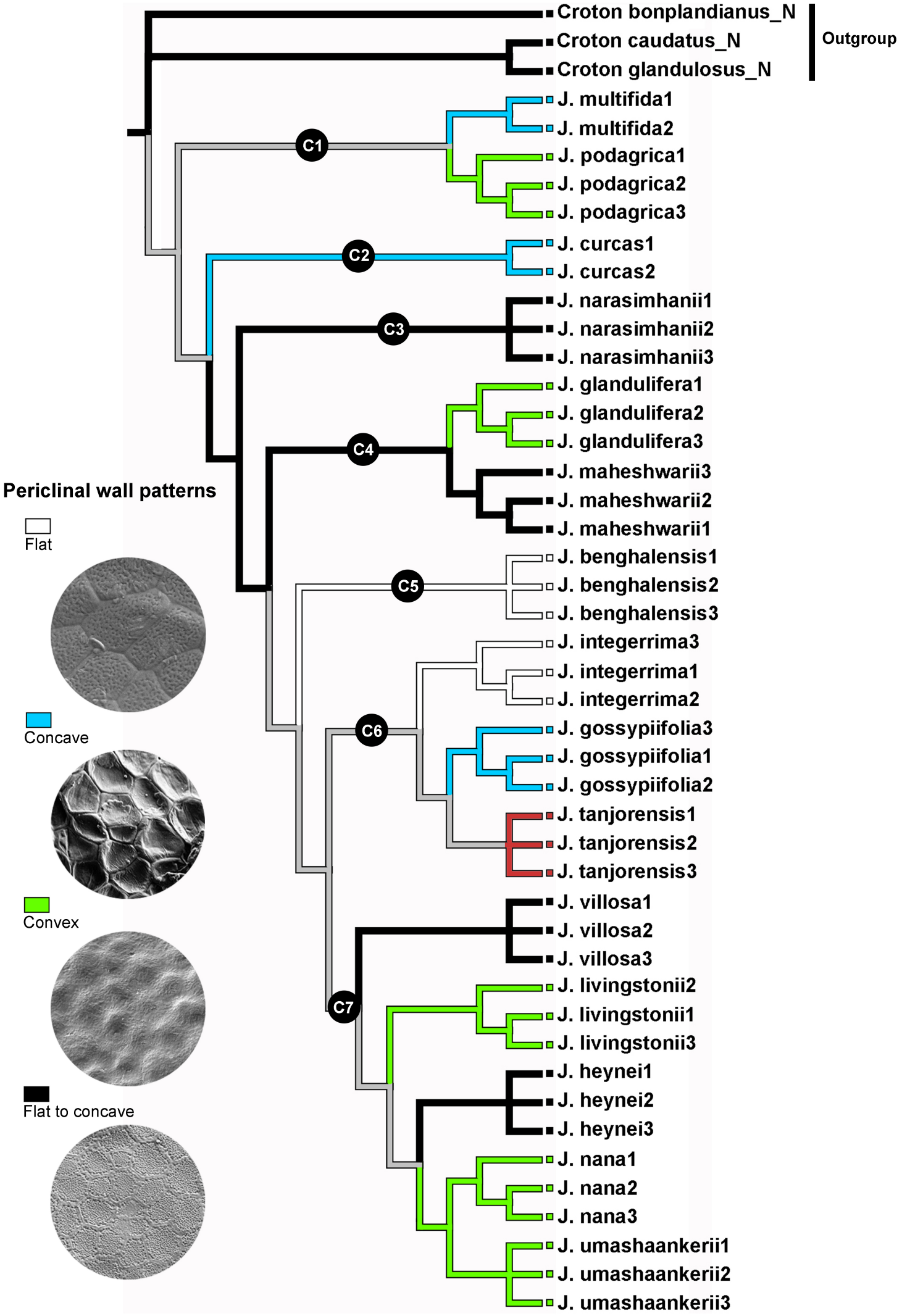

Croton bonplandianus_N
Croton caudatus_N
Croton glandulosus_N
Outgroup
J. multifida1
J. multifida2
J. podagrica1
J. podagrica2
J. podagrica3
J. curcas1
J. curcas2
J. narasimhanii1
J. narasimhanii2
J. narasimhanii3
J. glandulifera1
J. glandulifera2
J. glandulifera3
J. maheshwarii3
J. maheshwarii2
J. maheshwarii1
J. benghalensis1
J. benghalensis2
J. benghalensis3
J. integerrima3
J. integerrima1
J. integerrima2
J. gossypiifolia3
J. gossypiifolia1
J. gossypiifolia2
J. tanjorensis1
J. tanjorensis2
J. tanjorensis3
J. villosa1
J. villosa2
J. villosa3
J. livingstonii2
J. livingstonii1
J. livingstonii3
J. heynei1
J. heynei2
J. heynei3
J. nana1
J. nana2
J. nana3
J. umashaankerii1
J. umashaankerii2
J. umashaankerii3
C1
C2
C3
C4
C5
C6
C7
Periclinal wall patterns
Flat
Concave
Convex
Flat to concave

Table 2 Details of seed morphometric analysis of Indian *Jatropha* from this study. NTC indicates number of testa cells per 10,000 μm^2 and hyphen indicates the absence of the character

Taxa	**Seed morphological characters**										
	Seed size (mm)	**Seed shape**	**Caruncle attachment**	**Caruncle shape**	**Caruncle colour**	**Seed colour**	**Surface type**	**Epidermal cell shape**	**Anticlinal boundaries**	**Periclinal wall**	**NTC (min.–max.)**
Jatropha benghalensis	10–12 × 8–10	oval	sessile	capped	brown-like	brown-like	reticulate	polygonal	sunken	flat	82–87
J. curcas	16–18 × 10–12	elliptic	erect	hooded	brown-like	black	reticulate-rugose	polygonal	raised	concave	37–43
J. glandulifera	8–9 × 5–6	oblong	erect	crowned	brown-like	brown-like	reticulate	polygonal	sunken	convex	68–73
J. gossypiifolia	6–8 × 4–5	oblong	erect	crowned	brown-like	brown-like	reticulate	polygonal	raised	concave	105–115
J. heynei	7-8 × 5-6	oval	sessile	capped	brown-like	brown-like	reticulate	polygonal	raised	flat to concave	59–63
J. livingstonii	8–9 × 6–7	oval	sessile	capped	brown-like	reddish-brown	reticulate	polygonal	raised	convex	92–97
J. integerrima	8-10 × 5-6	elliptic	erect	hooded	yellow	brown-like	reticulate	polygonal	sunken	flat	65–70
J. maheshwarii	10-12 × 6-7	oblong	erect	hooded	golden	brown-like	reticulate	polygonal	sunken	flat to concave	155–165
J. multifida	18-19 × 16-17	spherical	ecarunculate	-	-	brown-like	reticulate	polygonal	sunken	concave	45–50
J. nana	10-12 × 5-6	elliptic	erect	crowned	golden	brown-like	reticulate	isodiametrical	indistinct	convex	60–65
J. narasimhanii	9–11 × 7–8	oval	sessile	capped	brown-like	brown-like	reticulate	isodiametrical	sunken	flat to concave	100–110
J. podagrica	11-12 × 7-8	oblong	erect	crowned	brown-like	brown-like	reticulate	polygonal	sunken	convex	98–105

J. umashaankerii	7–9 × 5–6	elliptic	sessile	capped	brown-like	reddish-brown	reticulate-rugose	polygonal	raised	convex	108–120
J. villosa	9-11 × 6-7	oblong	erect	hooded	golden	brown-like	reticulate	polygonal	sunken	flat to concave	46–50

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryElectronicMaterialGenBankaccessiondetails.docx