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Structure and distribution of stomata and cystoliths in some species of *Ficus* L. (Moraceae) in Arunachal Pradesh, India

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Abstract

The study deals with the distribution pattern and morphology of stomata and cystoliths in the leaves of six taxa of the genus *Ficus* of the family Moraceae in Arunachal Pradesh. All the plant species studied contained stomata and cystoliths, which vary in shape and size. Actinocytic stomata type was present in only one species (*Ficus* sp. 1). Anomocytic type in three, and paracytic in four species. Stomatal frequency calculation revealed that the highest frequency was reported in *F. hispida* and the lowest in *F. rumphii*. There is also an indication that semi-coriaceous and coriaceous glabrous leaved species of *Ficus* are linked to the stomatal characters. The results have shown the presence of stalks in all the cystoliths of various sizes. Different shapes such as star-shaped, bean-shaped, solitary, round, and oblong cystoliths are recorded during the study. The largest cystoliths were found in *F. rumphii*, where its spinous surface is quite distinct from all other species and can be recognized very easily. Cystoliths are found to occur on both sides of the epidermal layer in three species (*F. religiosa, F. rumphii* and *Ficus* sp.3) whereas, it was found in the upper epidermal layer in other species. Photographic details, along with a systematic key using the cystoliths characters were also established.

Keywords: cystolith; Eastern Himalaya; *Ficus*; idioblast; stomata

Introduction

Ficus is the largest genus in the family Moraceae, which comprises about 735 species worldwide (Berg and Corner, 2005; Chaudhary *et al.*, 2012*;* Buragohain *et al.*, 2014). The genus is primarily distributed in tropical and subtropical regions, especially in Indo-Malaysia, Australia, Africa, and America, but sometimes also extends to temperate areas. While the African floristic region hosts around 105 species, its highest diversity has been recorded in the Australasian region with about 500 species (Corner, 1965; Berg and Corner, 2005). The genus is tree dominating, but shrubs and climbers are also common. They are generally hemi-epiphytic stranglers. The Arunachal Pradesh alone records 40 species of Ficus out of over 115 taxa reported from India (Chaudhary *et al.* 2012).

Cystoliths are calcium-carbonate depositions in the form of a bunch of grapes formed in leaf-epidermal cells, called idioblasts, of some higher plants (Metcalfe and Chalk, 1979, 1983; Setoguchi *et al.*, 1989) and are referred to as lithocysts (Ummu-Hani and Noraini, 2013). However, Choopan and Grote (2015) defined

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cystoliths as silicified or calcified bodies with cellulose skeletons but occasionally not encrusted. Though it occurs mainly in leaves, it is sometimes found in various other parts of plants, including xylem and phloem rays. It is found only in some angiosperm families, particularly in Cannabaceae, Moraceae, Urticaceae, and Acanthaceae (Metcalfe and Chalk, 1979, 1983; Mauseth, 1988). Cystoliths can be identified by their nature, shape, size, colour, and occurrence (Ummu-Hani and Noraini, 2013). According to Mauseth (1988), cystoliths can be found as papillate or hair-like lithocysts and occur mostly in the leaves' epidermal layer. Cystoliths are apoplastic, meaning they protrude into a single cell from beyond the plasma membrane, almost entirely filling the cell interior (Gabel *et al.*, 2021).

The exact significance and function of cystoliths are still unknown, but it is assumed that cystoliths' possible function is the sequestration of heavy metals such as Cadmium (Wu and Kuo-Huang, 1997). Cystoliths are of restricted occurrence, and that is why they are not used as diagnostic characters for any family. However, in a few families such as Moraceae, Acanthaceae, Urticaceae, Boraginaceae, etc., various forms of cystoliths can be used as a good character at least for genus and species level identification and characterization (Metcalfe and Chalk, 1950). The occurrence of cystoliths in Moraceae, including the genus *Ficus* was reported for a long (Metcalfe and Chalk 1979, 1983). The identification of different species of *Ficus* creates many difficulties due to the overlapping of characters (Buragohain *et al.*, 2014). For the recognition and classification of this genus, the cystolith characters were studied in detail.

The stomata and associated epidermal cells are known to provide an increasingly important source of taxonomic characters. Some of the stomatal parameters have been discussed in this work viz; stomatal types, stomatal count, and stomatal frequency.

The literature on the epidermal morphology of *Ficus* is relatively rare though there is proper documentation of the taxonomic value of epidermal morphology in botanical literature (Stace, 1965). It has been suggested by Stebbins and Kush (1961) that stomata with many subsidiary cells are considered to be the most primitive. Stomatal frequency, stomatal index, the position of stomata, and other epidermal features have been emphasized as of significant value not only in classification and phylogeny but also in segregating various cytotypes as evidenced by the work of Solereder (1908); Paliwal (1966); Inamdar and Patel (1970), Van Cotthem (1970), Pant and Banerji (1965) recognized 10 stomatal types, mainly based on their ontogeny. Frynsclassens and Van cotthem (1973), Cronquist (1988) and Guyot (1971) in their study reported that the primitive nature of paracytic stomata in angiosperms has been useful in several systematic reviews. Greuning *et al.* (1984) reported that anatomical characteristics of the *Ficus* leaves are important for taxonomy even at the species level.

The genus is well represented in India, including North-east India (Chaudhary *et al.*, 2012; Buragohain *et al.*, 2014). Giri *et al.* (2008) listed 51 species of *Ficus* from Arunachal Pradesh, but their identification becomes difficult just with external morphological characters. Through this research, an attempt has been made to obtain additional data on the structure of lithocysts and apply them to characterize the species under study.

Materials and Methods

Cystolith

Seven species of the genus *Ficus* (Moraceae) occurring in Arunachal Pradesh were selected for the study (Table 1). Voucher specimens were processed into mounted herbarium sheets and deposited in HAU (Herbarium of Arunachal University). Plants were identified following several literatures, including Giri *et al.* (2008), and Buragohain *et al.* (2014). For confirmation, the specimen was matched at ASSAM Herbarium.

Cystoliths were studied from the matured lamina through free-hand sections, studied under a light microscope, and photographed using a digital camera. In general, the methodology of Dilcher (1974) was followed. Small pieces of lamina were taken from the basal, middle, and upper parts avoiding major veins. These

pieces were placed in separate test tubes, heated with 90% ethanol to remove chlorophyll, and treated with 5% sodium hypochlorite solution for 4-5 hours for decolourization. These were washed in distilled water, and thin hand sections were made using blades. The sections were stained with aqueous 4% safranin and observed under a light microscope. The photographs of the cystoliths and measurements were taken using a compound light microscope. Fifteen cystoliths per leaf for a species were studied for their shape, size, distribution, and orientation.

Table 1. Different species of *Ficus* collected from Arunachal Pradesh

Taxa	Habit	Voucher	Locality
F. rumphii	Tree	M. Deori and T. Bani RGU0106	Yupia, Papum Pare
F. religiosa	Tree	M. Deori and T. Bani RGU0111	Pasighat, East Siang
F. elastica	Tree	M. Deori and T. Bani RGU0118	Potin, Lower Subansiri
Ficus sp.1	Climber	M. Deori and T. Bani RGU0128	R.G.U. campus, Papum Pare
Ficus sp.2	Tree	M. Deori and T. Bani RGU0132	Potin, Lower Subansiri
F. hederacea	Climber	M. Deori and T. Bani RGU0137	Nirjuli, Papum Pare
Ficus sp.3	Climber	M. Deori and T. Bani RGU0139	R.G.U. campus, Papum Pare

Stomata

Fresh collected leaves of different species of *Ficus* were collected and then washed properly to remove any dirt and debris. The leaves with soft texture were peeled gently, and the abaxial epidermis was mounted and observed under a microscope at 10X and 40X objectives. The stomatal count was done from the replicas prepared by applying nail polish or superglue on the abaxial surface of leaves. Staining was done as per requirement, mostly with aqueous safranin. The leaves with rough and hairy surfaces were scraped using scalpels and blades. The number of stomata was counted, and the stomatal frequency was calculated. The stomatal guard cells and the subsidiary cells were observed under the microscope, which helped in recognizing the type of stomata. Permanent slides were also prepared for future references. Photographs of stomata were taken. The stomatal frequency was calculated using the formula:

Stomatal frequency = Number of stomata/Area of the microscopic field.

Results

From the study, it was found that stomata are of different types, having different distribution frequencies. The paracytic type of stomatal complex was most frequent throughout the species, and the stomata are dispersed randomly over the entire abaxial surface. Anomocytic stomata were also observed. The types of stomata and cystoliths are depicted in Figure 1 and Figure 2 respectively.

The highest stomata frequency of 32.4 was recorded in *F. hispida*, followed by *F. pumila* (25.87) and *F. auriculata* (25.07) (Table 3). On the other hand, the lowest stomatal frequency was recorded in *F. rumphii*, i.e., 7.87, and a slightly higher value was recorded from *F. religiosa* (8.13). The remaining species showed moderate values between 12.13 (in *F. elastica*) and 21.6 (Sp.-1).

The Unidentified Sp.-2 is with a stomatal frequency of 20.8. So, the hispid-hairy group tends to moderate to a high number of stoma per unit area of the leaf. It might be a significant observation, but for confirmation, some more species need to be explored. The low stomatal frequency group viz. *F. rumphii, F. religiosa* and *F. elastica* were earlier clubbed for their similar stomatal type. So, it may indicate that semi-coriaceous and coriaceous glabrous leaved species of *Ficus* are linked to both the stomatal characters.

Cystoliths were found in all the studied taxa and are summarized in Table 2. In *F. religiosa, F. rumphii* and *Ficus* sp.3, the cystoliths were observed in both upper and lower epidermal layers. Whereas it was

confined to the upper epidermal layer in the case of *F. elastica, F. pumila, Ficus* sp. 1 and 2. The present investigation of some taxa of the genus *Ficus* reveals the presence of 4 different types of cystolith i.e., bean-shaped, star-shaped, solitary-rounded, and oblong-shaped with all of them having a distinct stalk which is almost similar to the results obtained by Ummu-Hani and Noraini (2013).

Table 2. Size, distribution and type of Cystoliths and Idioblast cells in the leaves of some species of *Ficus* in Arunachal Pradesh

Taxa	Cystolith Size			Idioblast cell size		Distribution	Type
	Stalk length (µm)	Length (µm)	Breadth (µm)	Length (µm)	Breadth (µm)		
F. rumphii	39	90-100	60-80	95-107	120-130	Upper and lower epidermal layers	Star-shaped
F. religiosa	7-12	17-55	16-47	25-70	23-65	Upper and lower epidermal layers	Bean shaped
F. elastica	8-15	8-14	7-11	65-95	25-40	Upper epidermal layer only	Solitary, rounded
Ficus sp.1	30	40-65	35-40	95-110	80-85	Upper epidermal layer only	Oblong shaped
Ficus sp.2	10-15	29-38	47-53	36-65	54-66	Upper epidermal layer only	Bean shaped
F. hederacea Roxb.	10	49-60	45-53	95-107	120-130	Upper epidermal layer only	Solitary rounded
Ficus sp.3	30	53-75	60-80	80-95	74-90	Upper and lower epidermal layers	Star-shaped

Table 3. Stomatal frequency and different types of stomata found in some species of *Ficus* in Arunachal Pradesh, India

Sl. No.	Species name	Stomatal frequency (per sq. mm)	Mean stomatal frequency (per sq. mm.)	Stomata type
1.	*Ficus rumphii*	Upper -6.4 Middle - 8 Lower -9.2	7.87	Paracytic
2.	*Ficus* sp 1.	Upper -20.4 Middle -25.2 Lower -19.2	21.6	Actinocytic
3.	*Ficus* sp 2.	Upper -19.2 Middle -22 Lower -21.2	20.8	Anomocytic
4.	*Ficus pumila* L.	Upper -24.4 Middle -26 Lower -27.2	25.87	Anomocytic
5.	*Ficus elastica*	Upper -12 Middle -12.8 Lower -11.6	12.13	Paracytic
6.	*Ficus religiosa* L.	Upper - 8.4 Middle -8.8 Lower -7.2	8.13	Paracytic
7.	*Ficus hispida* L.f.	Upper -27.6 Middle -35.6 Lower -34	32.4	Anomocytic
8.	*Ficus auriculata* Lour.	Upper -22.4 Middle -25.2 Lower -27.6	25.07	Paracytic

The star-shaped cystoliths were found in *F. rumphii* and *Ficus* sp.3, while, the bean-shaped in *F. religiosa* and *Ficus* sp.2 (unidentified). The oblong-shaped cystoliths were found in *Ficus* sp.1 (unidentified) and the solitary-rounded cystoliths in *F. elastica* and *F. pumila*. The results have shown the presence of stalks in all the cystoliths of various sizes. Cystolith, along with the stalk may have significant value, especially in providing additional data for species identification. The largest cystoliths were found in *F. rumphii*, where its spinous surface is quite distinct from all the other species and can be recognized very easily.

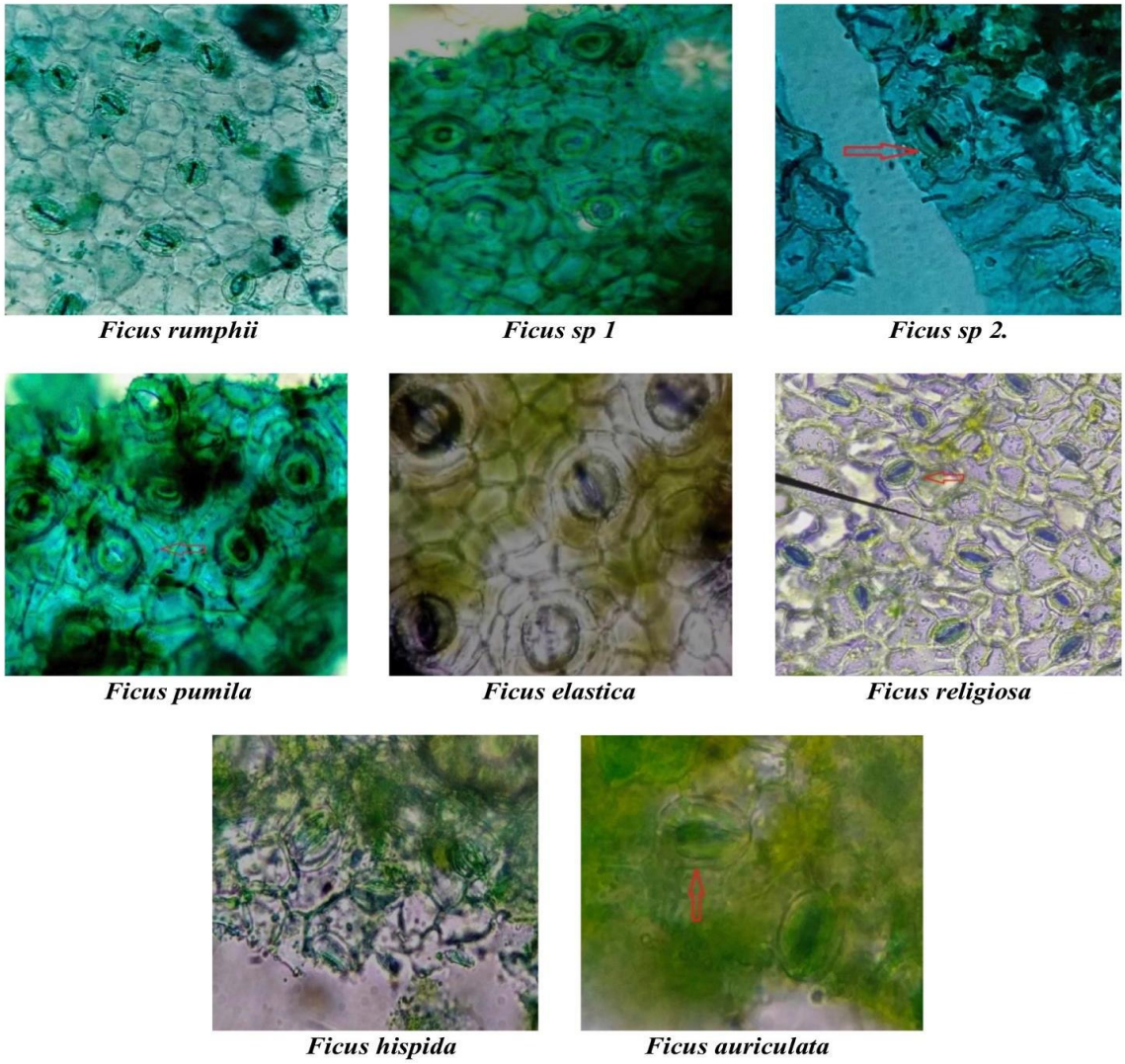

Figure 1. Different types of stomata in species of *Ficus* from Arunachal Pradesh, India

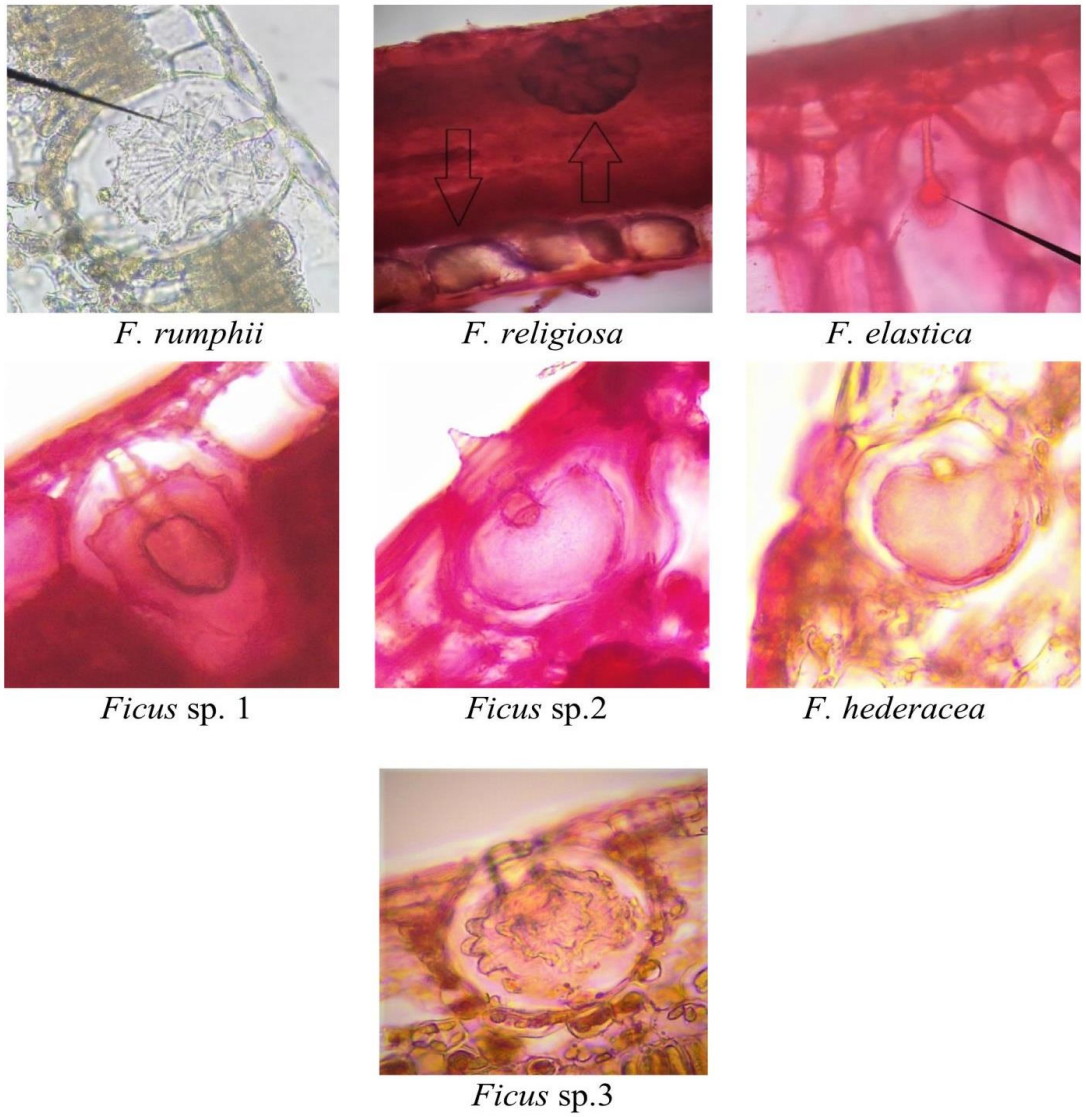

Figure 2. Different types of cystoliths present in species of *Ficus* from Arunachal Pradesh, India

Using the Cystoliths' morphological characters, an artificial dichotomous key has prepared to identify these seven species under study as follows:

1a. Cystoliths present on both the epidermises -------- 2
1b. Cystoliths present only on the upper epidermis--------- 4
2a. Large star-shaped with sharp spikes --------- ***F. rumphii***
2b. Small bean-shaped, surface tubercled ------ 3
3a. Cystoliths with elongated tubercles; ±60-80 μm wide and ± 53-75 μm long ---- Sp.-**3**
3b. Cystoliths with rounded tubercles; ±16-47 μm wide and ±17-55 μm long --- ***F. religiosa***
4a. Cystoliths very small, globose, outer layers ill-formed ------ ***F. elastica***
4b. Cystoliths well-formed -------- 5
5a. Cystoliths triangular-oblong, spear-shaped; surface tubercled ------- **Sp.-1**
5b. Cystoliths bean-shaped, surface more or less smooth ----- 6
6a. Cystoliths bean-shaped; ±47-53 μm wide and ± 29-38 μm long ------ **Sp.-2**
6b. Cystoliths very broadly bean-shaped (more or less rounded); ±45-53 μm wide and ±49-60 μm long ------- ***F. pumila***

Discussion

The leaf anatomical characters of *Ficus* were said to make it easier for the taxonomic identification of species, especially when clubbed together with morphological characters (Chantarasuwan, 2014). The present study observed actinocytic to be the rarest type of stoma among the local species of *Ficus*. The species is yet to be identified but an epiphyte turns into a tree with a glabrous lamina. The anomocytic type has been recorded from three species, all of which are having thickly hispid hairs, rough or somewhat soft. On the other hand, 50% of the species under study showed the paracytic type of stoma. These four species are with leaves either glabrous or glabrescent, and remain hairy only on the very young lamina.

The study records the linkage of the stomatal type and the hairiness of leaves. Sp.-1 appears to be not related to other species under study though it is native to Arunachal Pradesh. The three species with anomocytic stoma are thickly hairy, and in such conditions, probably, the assistance of subsidiary cells is not required for the proper functioning of the stoma. The clothing of hairs on the lamina surface protects those stomas from the loss of excessive moisture and may be linked to the xeric nature of these plants. Of these, three species, two (sp.-2 and *F. pumila*) were epiphytic climbers but establish root contact with soil and generally do not function like a strangler. *F. hispida* is a small tree but can grow as an epiphyte or muralophyte i.e., having strong tolerance to arid habitat.

The paracytic stoma, in the present study, has been recorded from four species. These are huge trees, though they can start their life as an epiphyte but soon can be independent after getting in contact with soil. Of these, two local species (*F. rumphii*, *F. religiosa*) and the introduced *F. elastica* were good stranglers. While, *F. auriculata* was not found as a strangler. However, all of them were trees with glabrous or glabrescent leaves. As the study revealed the importance of characteristics of stomatal character, there were also many other similar works done on the importance of stomata for species identification and classification. Narahayaan et al. (2022), found the comparisons between Plaintains and Horn bananas on the basis of the average value of the number of epidermal cell, a number of stomata, length, width and area of stoma, stomatal index, and stomatal density.

Cystoliths were also reported in all the studied species. Nur Fatihah *et al.* (2014) stated that most of the members of the mulberry family (Moraceae) characterized the presence of cystoliths. The present investigation of some taxa of the genus *Ficus* also reveals the presence of 4 different types of cystolith i.e., bean-shaped, star-shaped, solitary-rounded, and oblong-shaped with all of them having a distinct stalk which is almost similar to the results obtained by Ummu-Hani and Noraini (2013). Bercu and Popoviciu (2014) reported lithocysts possessing calcium oxalate deposits – solitary stalk cystoliths resembling the shape of grape cluster with 456 μm in length. The cystoliths were found to be presented adjacent to the adaxial epidermis layer and not abaxial. Pierantoni *et al.* (2020) also looked at leaves from six Ficus species with different kinds of cystolith and their locations. Cystolith, along with the stalk may have significant value, especially in providing additional data for species identification. Mohapatra and Janarthanam (2020) also clearly stated that cystolith shape, size and sculpturing pattern among Ficus species have great taxonomic significance

Conclusions

The study of morphology such as shape, size, colour, and distribution of cystoliths in the epidermal layers of the leaf lamina and the presence of stalk in cystoliths have shown to be a useful characters for the taxonomic characterization of different species in the genus. *Ficus* is a huge genus. So, to reach a reliable conclusion on the taxonomic and ecological values of such character-states linked to different stomatal characters and cystolith structures, many more species need to be studied from different distributional areas and geographical coverage. The present investigation of stomata and cystolith study can be a reliable tool for the characterization and analysis of different species.

Authors' Contributions

Conceptualization and writing of original draft by TB, Investigation by MD, Resources and Software by TW, Supervised by APD, and Validation and communication by ST.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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