Morpho-anatomical observations on some Indian species of *Loxogramme* (Loxogrammaceae)

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Detailed studies on the morphology, anatomy and palynology of seven species of *Loxogramme* (Bl.) Presl, namely – *L. assimilis* Ching, *L. chinensis* Ching, *L. carinata* Pric, *L. ducloxii* Christ, *L. elevata* Ching, *L. involuta* (D. Don) Presl, *L. lankokiensis* (Rosenst.) C. Chr., have been made. The different species of *Loxogramme* differ in size, stomatal morphotypes, in the presence or absence of hairs and paraphyses and in the stipe and the rhizome anatomy. A key has been prepared for delineating these members. The genus, possess some characters, which have similarities with Grammitaceae and Polypodiceae, and justify its placement between these two families.

Key-words – Indian Loxogrammaceae, Morphology, Anatomy, Palynology

INTRODUCTION

BLUME (1828) considered *Loxogramme* as a subgenus of *Antrophyum*, however, generic name *Loxogramme* dates from Presl (1836). As this genus has some similarities with the members of Grammitaceae and Polypodiaceae, it was considered either as grammitoid (Christensen 1938; Copeland 1947) or as polypodioid fern (Holttum 1949; Hennipman *et al.* 1990). Pichi Sermolli (1974) placed this genus in the family Loxogrammaceae following Ching (1940). Considering the earlier reports, Dixit (1984) listed seven species of *Loxogramme* from India. Nineteen more species were added afterwards by different workers (Singh & Panigrahi 1985; Price 1990; Dixit & Das 1993, 1994; Nampy & Madhusoodanan 1995) either as new species or as new reports in India. All these studies are mainly based on morphological descriptions. Anatomical work is practically absent except the rhizome anatomy of *L. involuta* made by Bir and Trikha (1980) and the root anatomy by Schneider (1996).

Nayar (1963) reported both monolete and trilete spores in *L. lanceolata* in addition to some intergrading forms. Chang (1963) and Pichi Sermolli (1977) considered *Loxogramme* related to Grammitaceae than Polypodiaceae. Dixit and Das (1977) and Bhandari and Mukhoapdhyay (2001) correlated the plant size with the spore forms. The larger plants bear monolete spores while the smaller plants contain trilete spores. Nayar (1963) and Verma and Khullar (1976), however, suggested that it is not possible to say with certainty that whether the bilateral spore form is primitive or advanced.

Present study was undertaken to elucidate the detailed anatomical and morphological differences along with spore morphologies.

MATERIAL AND METHOD

Different epiphytic species of *Loxogramme* were collected from Sikkim, Assam and Darjeeling during the month of September and October. Microtome sections of rhizome, leaf and root, fixed in formalin-acetic acid-alcohol, were made following routine procedure (Johansen 1940). Epidermal studies were made from cleared leaf materials. Cleaning was made by 5% NaOH solution placed in an oven at 55°C. The specimens were washed thoroughly, bleached in sodium hypocloride solution and stained in 1% safranin, dehydrated and made permanent for microscopic observations.

Light microscopic study on spore morphology is based on acetolysed preparations of spores following Erdtman (1952). Measurements are made from unacetolysed spores.

Provenances of studied specimens are as follows:

1. *Loxogramme assimilis* Ching, SR Ghosh & B Ghosh 59599 (CAL), Wipong, 1985.

   JB Bhandari 20040 (BURD), Wipong, 1999.
2. *L. carinata* Price, JB Bhandari 2009 (BURD), Gangtok, 1999.
3. *L. chinensis* Ching, J Joseph Kamag 40323 (CAL), NEFA, 1964.
4. *L. ducloxii* Christ, SR Ghosh 55778 (CAL), Sikkim, 1984.

   G Gangly GG-53 (BURD), Sikkim, 2003
5. *L. elevata* Ching, Chhana 2622 (CAL), Sikkim, 1983.
6. *L. involuta* (D. Don) Presl, B Ghosh 16498 (CAL), Manipur, 1984.

   G Ganguly GG-37 (BURD), Sikkim, 2002.
7. *L. lankokiensis* (Rosenst) C. Chr., J Joseph 48934 (CAL), NEFA, 1970. JB Bhandari 20013 (BURD), Assam, 1999.

   JB Bhandari 20013 (BURD), Assam, 1999.

OBSERVATIONS

Seven species of *Loxogramme* mentioned above were worked out and the morphological and anatomical features are given in Table 1, 2 respectively.

Key to studied species of *Loxogramme* :

1a. Spores only monolete 2
b. Spores are monolete and or trilete.....4
2a. Paraphyses absent in sori.....1. *L. ducloxii*
b. Paraphyses present in son.....3
3a. Lamina lanceolate, 15-25cm long, sori confined to upper 3/4 or more of the lamina, margin involute, stomata anomocytic.....2. *L. involuta*
b. Lamina oblanceolate, 25-45cm long, sori confined to upper 1/.2 or less of the lamina, margin not involute, stomata polocytic.....3. *L. carinata*
4a. Fronds dimorphic.....4. *L. lankokiensis*
b. Fronds monomorphic.....5
5a. Soral paraphyses present .....5. *L. chinensis*
b. Soral paraphyses absent.....6
6a. Vascular trace 3 at base of the stipe, rhizome scale is ovate lanceolate. 6. .....*L. assimilis*
b. Vascular trace 2 at base of the stipe, rhizome scale is linear lanceolate. 7. *L. elevata*

DISCUSSION

Out of the seven species studied, four species, viz. *L. assimilis, L. chinensis, L. elevata* and *L. lankokiensis* are smaller, being less than 20 cm in size. While, *Loxogramme carinata, L. ducloxii and L. involuta* are distinctly larger and well above 20cm. Previously, Bhandari and Mukhopadhyay (2001) correlated plant size with the spore forms. Larger plants were found associated with monolete spores while, the smaller plants were associated with trilete spores. This study confirms the presence of monolete spore in all the above mentioned larger sized species. But, occurrence of both monolete and trilete spores forms are noted in three smaller species, viz. *L. assimilis, L. chinensis* and *L. elevata* earlier only trilete spores were noted. Only *L. lankokiensis,* which is the smallest species, bears trilete spores.

Fig. 1 A-G. Habit sketch of *Loxogramme* spp.; A. *Loxogramme lankokiensis* x 1; B. *L. ducloxii* x 0.25; C. *L. invoulta* x 0.5; D. *L. carinata* x 0.3; E. *L. assimilis* 0.65; F. *L. chinensis* x 0.5; G. *L. elevata* x 0.6

Table 1. Comparative Morphology of seven spp. of *Loxogramme*

Characters	*Lssimilis*	*L. carinata*	*L. chinensis*	*L. ducloxii*	*L. elevata*	*L. involuta*	*L. lankokiensis*
Plant size	12-17cm (Fig. 1E)	24-45 cm (Fig. 1D)	7-12 cm (Fig. 1F)	40-42cm (Fig. 1B)	12-18 cm (Fig. 1G)	15-25 cm (Fig. 1C)	3-4cm (Fig. 1A)
Frond Type	Monomorphic, non-articulate, linear, 5-9 mm broad, acute	Monomorphic non-articulate, oblanceolate, 3-5cm broad, acuminate.	Monomorphic, non-articulate, linear, 5-7mm broad, acuminate.	Monomorphic non-articulate, ovate-lanceolate, 2.5-2.7cm broad, acuminate.	Monomorphic, non-articulate, linear-lanceolate 5-8mm broad, acute.	Monomorphic, non-articulate, linear-lanceo-late, 1.8-2.2cm broad, acute	Dimorphic, smaller leaf ovate-rounded, 6-10mm broad; larger leaf linear-obovate 3-4 mm broad, obtuse.
Midvein	Prominent throughout the leaf. (Fig. 2Q)	Prominent throughout the leaf.	Prominent throughout the leaf.	Prominent only in the basal portion.	Prominent throughout the leaf.	Prominent throughout the leaf.	Preent only in the basal portion (Fig. 2L)
Stomatal Type	Anomocytic (Fig. 2F)	Polocytic (Fig. 2C)	Polocytic & Anomocytic (Fig. 2CF)	Polocytic & Anomocytic (Fig. 2 CF)	Anomocytic (Fig. 2F)	Anomocytic (Fig. 2F)	Anomocytic (Fig.2F)
Trichome or Hair	Absent	Absent	Absent	Absent	Present (Fig. 2D)	Absent	Present (Fig. 2D)
Soral Paraphyses	Absent	Present (Fig. 2K)	Present (Fig. 2P)	Absent	Absent	Present (Fig. 2P)	Absent
Spore							
i. Type	Monolete & Trilete	Monolete	Monolete & Trilete	Monolete	Monolete & Trilete	Monolete	Trilete only
ii. Size	55-64 x 46-49µm. (Fig. 2 G-H)	95-102 x 45-57 µm	50-60 x 54-55 µm (Fig. 2M-N)	43-52 x 61-66 µm.	47-51 x 53-61 µm. (Fig. 1I-J)	42-60 x 62-90 µm	30-35 x 40-44 µm
Rhizome							
Scale							
i. Shape	Ovate-lanceolate	Ovate-lanceolate	Ovate-lanceolate	Lanceolate	Linear lanceolate	Ovate-lanceolate	Lanceolate
ii. Apex	Acute, non-glandulate	Acute, glandular	Acute, glandular	Acuminate, glandular	Acute-non-glandular	Acute, non-glandular	Acute, glandular
iii. Size	3-4 x 0.5 mm	3-4 x 0.6 mm	4.5 x1-1.5 mm	3-4 x 0.5 mm	4.5 x 0.5-1mm	5-8 x 0.5-1 mm	1-2 x 0.5mm

Table 2. Comparative Anatomy

Characters	*Lassimilis*	*L. Carinata*	*L. chinensis*	*L. ducloxii*	*L. elevata*	*L. involuta*	*L. lankokiensis*
T.S. of Root							
i. Epidermis	Single layered with root hair.	Single layered with root hair.	Single layered with root hair.	Single layered with root hair.	Single layered with root hair.	Single layered with root hair.	Single layered with root hair.
ii. Cortex	Consists of two zones inner and outer cortex. Inner cortex sclerenchymatous, consists of passage cells outer parenchymatous.	Consists of two zones inner and outer cortex. Inner cortex sclerenchymatous, consists of passage cells outer parenchymatous	Consists of two zones inner and outer cortex. Inner cortex continuously sclerenchymatous, outer parenchymatous.	Consists of two zones inner and outer cortex. Inner cortex sclerenchymatous, consists of passage cells outer parenchymatous	Consists of two zones inner an outer cortex. Inner cortex continuously sclerenchymatous, outer parenchymatous	Consists of two zones inner and outer cortex. Inner cortex continuously sclerenchymatous, outer parenchymatous	Consists of two zones inner and outer cortex. Inner cortex continuously sclerenchymatous, outer parenchymatous
iii. Stele	Protostelic endodermis single layered. (Fig. 3B)	Protostelic endodermis single layered. (Fig. 3B)	Protostelic endodermis single layered. (Fig. 3A)	Protostelic endodermis single layered. (Fig. 3B)	Protostelic endodermis single layered. (Fig. 3A)	Protostelic endodermis single layered. (Fig. 3A)	Protostelic endodermis single layered. (Fig.3A)
T.S. of Rhizome							
i. Epidermis	Single layered, cuticularised	Single layered, cuticularised	Single layered, cuticularised	Single layered, cuticularised	Single layered, cuticularised	Single layered, cuticularised	Single layered, cuticularised
ii. Cortex	Two layered, outer parenchymatous, inner sclerenchymatous	Two layered, outer parenchymatous, inner sclerenchymtous.	Two layered, outer parenchmatous, inner sclera-nchymatous.	Two layered, outer parenchymatous, inner sclerenchymatous	Two layered, outer parenchymatous, innr sclerenchymatous.	Two layered, outer parenchymtous, inner sclerenchymatous.	Single layered, parenchymatous, parenchyma cells have sinuate walls.
iii. Stele	Dictyostelic, endodermis single layered	Dictyostelic, endodermis single layered	Dictyostelic, endodermis single layered.	Dictyostelic, endodermis single layered.	Dictyostelic, endodermis single layered.	Dictyostelic, endodermis single layered.	Solenostelic, endodermis single layered.
iv. No. of Meristem	6 (Fig. 3FD)	10 (Fig. 3ED)	5 (Fig. 3KD)	6 (Fig. 3M%D)	6 (Fig. 3HD)	3 (Fig. 3PD)	2 (Fig. 3 GC)
T.S. of petiole							
i. Shape	Broadly winged	Not winged	Broadly winged	Not winged	Broadly winged	Not winged	Not winged
ii. Epidermis	Single layered.	Single layered.	Single layered.	Single layered.	Single layered.	Single layered.	Single layered.
iii. Cortex	Two layered, outer parenchymatous, inner sclerenchymatous	Two layered, outer parenchymatous, inner sclerenchymatous	Two layered, outer parenchymatous, inner sclerenchymatous	Two layered, outer parenchymatous, inner sclerenchymatous	Two layered, outer parenchymatous, inner sclerenchymatous	Two layered, outer parenchymatous, inner sclerenchymatous	Two layered, outer parenchymatous, inner sclerenchymatous
iv No. of bundles at the base	3 (Fig. 3 OS)	6 (Fig. 3 S)	2 (Fig. 3 LS)	7 (Fig.3. QS)	2 (Fig. 3NS)	8 (Fig.3 IS)	1 (Fig.3 RS)

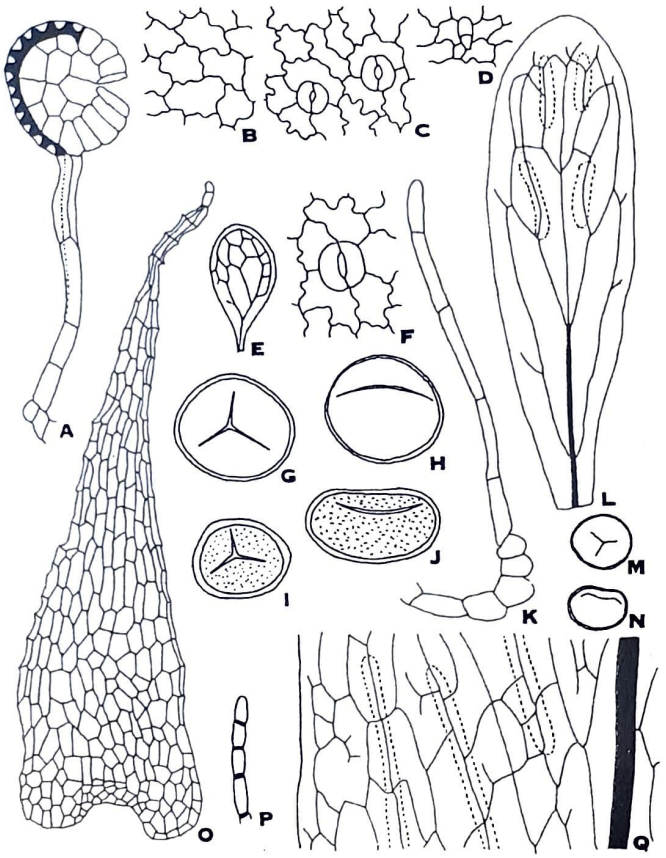

Fig. 2 A-Q. A. Sporangium of *Loxogramme elevata* x 105; B. Upper leaf epidermis of *L. elevata* x 120; C. Lower leaf epidermis bearing polocytic stomata of *L. ducloxii* x 105; D. Hair on lower leaf epidermis of *L. elevate* x 105; E. Sterilie leaf of *L. lankokiensis* x 3; F. Lower epidermis bearing anomycytic stomata of *L. assimilis* x 140; G-H. Spores of *L. assimilis* x 420; I-J. Spores of *L. elevata* x 420; K. Paraphysis of *L. carinata* x 109; L. Fertile leaf of *L. lankokiensis* x 12; M-N. Spores of *L. chinensis* x 230; O. Rhizome scale of *L. chinensis* x 50; P. Paraphysis of *L. involuta* x 150; Q. Venation pattern and soral positions in leaf of *L. carinata* x 6.

Leaves in all these Loxogrammes are non-articulate and venation pattern is essentially reticulate. Branched or unbranched included veinlets occuring frequently in the larger species but they are rare in the small sized species. Bi-celled hairs are present on the leaves of only *L. lankokiensis and L. elevata.* Scales are present on the rhizome and stipe base in all these species. Morphologically they are similar, but differing in size and in the presence of distal glands. Mature stomata are polocytic (*L. carinata*) to polo- and anomocytic (*L. ducloxii, L. chinensis*) to only anomocytic (*L. assimilis, L. elevata, L. involuta* and *L. lankokiensis*). Paraphyses are not present uniformly in all these species. *L. carinata, L. chinensis* and *L. involuta* bear paraphyses.

Anatomically, the root is protostelic and diarch and the stele is surrounded by a sclerotic sheath in all these species. However, passage cells within the sclerotic sheath are noted in *L. assimilis, L. carinata* and *L. ducloxii.* Occurrence of passage cells in the inner cortex of roots of Grammitaceae and Polypodiaceae have been noted by Schneider (1996). Rhizome is dictyostelic in all these species except in *L. lankokiensis.* Number of meristeles in rhizome varies from three to eight. In *L. lankokiensis* the stele is a solenostele. The cortex of *L. lankokiensis* also differs from other species in its lobed nature of the

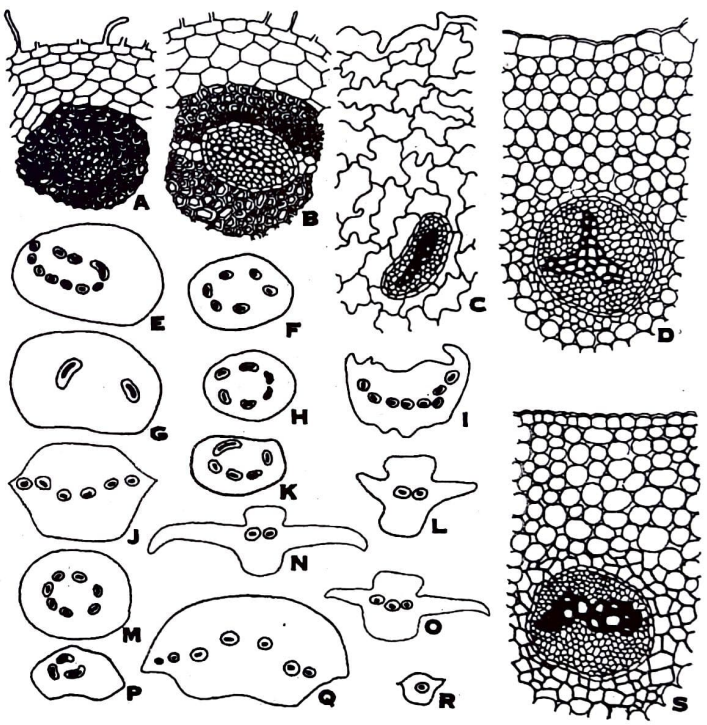

Fig. 3 A-S.
A. T.S. of root of *L. chinensis* showing cellular details x 120; B. T.S. of root of *L. ducloxii* showing passage cells and cellular details x 120; C. T.S. of rhizome of *L. lankokiensis* x 56; D. T.S. or rhizome of *L. ducloxii* x 125; E-R. Diagrammatic representation of transaction of : E. Rhizome of *L. carinata* x 14; F. Rhizome of *L. assimilis* x 14; G. Rhizome of *L. lankokiensis* x 14; H. Rhizome of *L. elevata* x 14; I. Petiole of *L. involuta* x 15; J. Petiole of *L. carinata* x 16; K. Rhizome of *L. chinensis* x 14; L. Petiole of *L. chinensis* x 14; M. Rhizome of *L. ducloxii* x 15; N. Petiole of *L. elevata* x 16; O. Petiole of *L. assimilis* x 15; P. Rhizome of *L. involuta* x 15; Q. Petiole of *L. ducloxii* x 14; R. Petiole of *L. lankokiensis* x 14; S. Cellular structure of petiole of *L. carinata* showing vascular details in T.S. x 125.

composing cells and in the absence of sclerotic sheath around the stele.

From the above, it is clear that Loxogrammes differ among themselves morphologically, anatomically and palynologically. Loxogrammes have similarities with the members of Grammitaceae in the non-articulate nature of leaves, in having two kinds of spore forms and in the presence of both anomocytic and polocytic stomata. While, the reticulate venation, more than one row of sporangial stalk cells, only polocytic stomata, presence of paraphyses and monolete spores in some species justify their relation with the Polypodiaceae. So, placement of *Loxogramme* in the family Loxogrammaceae between Grammitaceae and Polypodiaceae is justified (Pichi Sermolli (1977). *L. lankokiensis* may be considered primitive due to the presence of solenostelic rhizome and trilete spore forms.

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