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FRUIT AND SEED DISCOVERIES IN *STICHONEURON MEMBRANACEUM* HOOK. F. (STEMONACEAE): AN ENDEMIC TO INDO-MYANMAR

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Abstract. *Stichoneuron membranaceum* Hook. f. is an endemic species of Indo-Myanmar hotspot whose fruit and seed remained unknown to science since 1850, until they were collected from Tripura, Northeast India. Based on these gatherings, this study is the first report about the development and morphological features of fruit and seed. Earlier historical collections of this species were discussed. Its preferred habitat, possible pollinating agents and seed dispersal mechanism were also investigated.

Key words: *Stichoneuron membranaceum*, morphology, fruit, seed, hermaphroditism

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Introduction

Stemonaceae is a very important monocotyledon family, since it is the only source of the stemona alkaloids (Ye *et al.* 1994; Pilli & Ferreira 2000). The extracts from tuberous roots of Stemonaceae are popular to be used as insecticides and several other traditional medicines (Valkenburg & Bunyapraphatsara 2002; Inthachub *et al.* 2009). There are c. 3 genera (*Croomia* Torr., *Stemona* Lour. and *Stichoneuron* Hook. *f.*) and c. 25 species belonging to this family, mostly distributed in Southeast Asia but spreading also to tropical Australia and with even one species to Southeast United States (Duyfjes 1993).

There are 2 genera of the family Stemonaceae having 5 species in India viz. *Stemona tuberosa* Lour., *S. minor* Hook. *f.*, *S. curtisii* Hook. *f.*, *S. griffithiana* Kurz and *Stichoneuron membranaceum* Hook. *f.* (Hooker 1892; Karthikeyan *et al.* 1989; Mabberley 2008). Genus *Stichoneuron* comprises 5 species, distributed from Northeast India to Thailand and Malay peninsula (Heywood 1978; Duyfjes 1992; Kubitzki 1998). *S. membranaceum* distributed in Meghalaya and Tripura of Northeast India (Hooker 1892; Deb 1983), Sylhet of Bangladesh (Barbhuiya & Gogoi 2010) and Northern Burma (Tanaka *et al.* 2007; Inthachub *et al.* 2009). Whereas, species *S. bognerianum* Duyfjes, *S. calcicola* Inthachub, *S. caudatum* Ridl. and *S. halabalensis* Inthachub are mainly distributed in Peninsular Thailand and Malesia (Inthachub *et al.* 2009). Fruit and seed formation and their characteristics were well described for above mentioned four Peninsular-Malesian species; where fruit usually elongate, apex acute or beaked, seed broad-ellipsoid, longitudinally ridged, wide and hollow aril with a usually long funicle (Inthachub 2008; Inthachub *et al.* 2009).

The botanical collection of *S. membranaceum* was firstly made by J.D. Hooker probably during 1850 from Khashi Hills of Meghalaya and afterward several other collections were also done from Indo-Maynmar hotspot. In the year 1941, R.N. De and K. Biswas collected *S. membranaceum* from Shylet of Bangladesh and Jampui Hills of Tripura respectively. During 1952 Koelz collected this species from Khasia Hills of Meghalaya where functionally male flowers were recorded. Subsequently its functional female flower was also collected by D.B. Deb in the year 1962 from Chailengta of

Tripura, North-east India; where he described anther cells as short and diverging, ovary half inferior with numerous ovules hanging from the top of the locule. After a long gap, Murata et al. gathered this species in 2007 from Northern Burma where functionally male flower was reported (Tanaka *et al.* 2007). Since then, no other specimen of *S. membranaceum* had ever been collected from this region, so that its fruit and seed still remained undescribed, even in the most recent revision of the genus *Stichoneuron* (Inthachub *et al.* 2009). However such data were urgently needed for understanding its taxonomy, reproductive biology and several aspects of conservation status and ecology. In present communication we summarized our observations on a sampling of fruits and seeds, very recently recognized on the field. In addition, we discuss the possible pollinating agents, seed dispersal mechanism and preferred habitat of *S. membranaceum.*

Material and methods

In course of our floristic inventory in Tripura (Majumdar *et al.* 2011), we collected its male flower from Visam (24° 07' 13.10" N and 92° 16' 34.10" E; alt. 2351 ft amsl), North Tripura and most recently the functionally female flower including fruits were collected from Srirampur (24° 07' 33.10" N and 91° 46' 00.7" E; alt. – 351 ft amsl), Kamalpur of Tripura. The semi-evergreen patches were observed in the Visam, of North Tripura; whereas the vegetation of Srirampur in Atharamura Hills range was dominated by deciduous trees with densely mixed bamboos. We were able to identify and collect only the functional male flowers in Visam, of North Tripura during May, 2010 and June, 2012. We did not observe any hermaphroditic flower or any trace of fruit in that area. During February, 2012 both male and hermaphroditic flowers were collected from Srirampur in Atharamura Hills range of West Tripura district. And during June, 2012 we were able to collect both mature fruit and seed from Srirampur area. Observations on fruits and flower traits were carried out directly on fresh specimens living in their natural habitats and also counts or measures were

Table 1. An account of the historical collections of *Stichoneuron membranaceum* by different workers from Indo-Myanmar hotspot.

Sl. No.	Collection number	Collection Year	Collectors name	Herbarium Location	Distribution				Flower Type	Source
					Locality	State	Region	Country		
1	-	1850	J.D. Hooker	ASSAM	Khasia Hills	Meghalaya	Northeast	India	Female	Hooker (1888)
2	20284	1941	R. N. De	ASSAM	Rampur plantation	-	Sylhet	Bangladesh	-	Barbhuiya and Gogoi (2010)
3	5073	1941	K. Biswas	CAL	Hmonpui, Jampui Hills	Tripura	Northeast	India	-	Deb (1983)
4	5835	1952	Koelz	ASSAM	Cherrapunjee, Khasia Hills	Meghalaya	Northeast	India	Male	Inthachub et al. (2009)
5	2689	1962?	D.B. Deb	CAL	Chailengta	Tripura	Northeast	India	Female	Deb (1983)
6	-	2007	Murata et al.	MBK & TI	Northern Burma	-	Northern	Burma	Male	Tanaka et al. (2007)
7	1033	2010	K. Majumdar	TUH	Visam, North Tripura	Tripura	Northeast	India	Male / Female	Present study
8	1192	2012	K. Majumdar	TUH	Srirampur, Kamalpur	Tripura	Northeast	India	Male / Female	Present study

done at the laboratory using Opta-Tech zoom microscope. Other ecological characters were documented in the field. The world history of *S. membranaceum* collections from Indo-Myanmar hotspot are chronologically arranged in Tab. 1.

Corresponding herbarium sheets (*K. Majumdar 1033* and *1192*) were deposited at TUH (Tripura University Herbarium).

Results and discussion

Male flowers (Fig. 1 c, e, f) c. 3.3 mm diam., pedicel 2.4-6.33 mm long; tepals 4, subtriangular, recurved, pale green, 0.95-1.55 mm × 0.34-0.72 mm, hairy (c. 0.11 mm) outside; stamens 4, filament 0.81-0.92 mm long, dark purple papillose (c. 0.07 mm), anther bright orange, 2 dorsifixed thecae, c. 0.34 mm × 0.31 mm.

Hermaphrodite flowers (Fig. 1 d) c. 4.8 mm diam., pedicel 11.46 -12.29 mm long; tepals 4, subtriangular, recurved, pale green, 1.21-1.93 mm × 0.42-0.97 mm, glabrous inside, hairy (c. 0.14 mm) outside; stamens 4, stout, filament base adnate to tepals, filaments 0.75-0.86 mm long, violet-dark purple papillose, anther diverging into three small yellow thecae on the top of the filament, c. 0.26 mm long, two white glands, dorsifixed. *Ovary* (Fig. 1 i) minute, green, smooth, ovoid, semi-inferior, 0.33-0.62 mm × 0.47-0.56 mm, style absent, stigma inconspicuous, ovule basal.

Fruits (Fig. 1 j, m, n) capsular, glabrous, narrowly ovoid with 2-valved, 8.11-20.44 mm × 4.20-7.19 mm, apex beaked 1.5-2 mm long, dark green (immature) to pale green (mature), pedicel 5-10 mm long.

Seeds (Fig. 1 o, p) 1-5, black, broadly ellipsoid, 4.75-5.14 mm × 2.06-2.46 mm, sharp longitudinal ridged; base surrounded by lobed and white, hollow papery aril c. 7.22 mm high, funicle long.

The flowers of *Stichoneuron* are monocarpellary and rarely functionally unisexual, as a prefiguration of some dimorphism (Duyfjes 1991; Kubitzki 1998; Rudall *et al.* 2005), whereas it was also assumed that hermaphroditic-looking flowers are either functionally female or male in *S. bognerianum, S. caudatum* and *S. halabalensis* (Duyfjes 1993). The description and drawing provided by Hooker involved a functionally female flower with patent reduced stamens, a larger ovary (c. 1.5 mm across) than in the other known species and possibly two stigmas on the top of the conical style (Inthachub *et al.* 2009). But we recorded its ovary as only 0.33 -0.62 mm long × 0.47-0.56 mm diameter, which is very near the known range of ovary diameter i.e. c. 0.50 mm across recorded for other *Stichoneuron* species. However, its functional male flower was also recorded much smaller than in all other *Stichoneuron* species (Tanaka *et al.* 2007); and these ones exhibit a less pronounced functionally male and female dimorphism, based on the small number of flowers seen (Inthachub *et al.* 2009). Thus, hermaphroditic flowers of *S. membranaceum* have pedicel c. 10 mm long and corolla c. 4 mm in diameter, whereas in male flower pedicel is 3-6 mm long and corolla c. 3 mm in diameter only. Dimorphic flowers could be recognized in the field by bright orange dorsifixed thecae (2) on top of the filament in slightly smaller staminate (male) flower, while pale-yellow thecae (3) separated on the top of the filament in pistillate (female) flower with addition of two white glands. Nevertheless, Inthachub *et al.* (2009) also suggested that the flowers of *S. membranaceum* are protandrous with initially forward directed protruding stamens with orange-yellow pollen and minute ovary, and ovary possibly develops later when the stamens with empty anthers are laterally pushed away.

The availability and sexual differences in population may be due to seasonal and microhabitat preferences; which are generally high in herbs due to more allocation of energy to reproduction (Bawa & Opler 1975) and easily perceptible within the population than between different microsites (Meagher 1980). However, competition between male for mates might increased the male flowering population of *S. membranaceum* in both habitats. We also observed that female plants were growing larger, with larger leaves and stem,

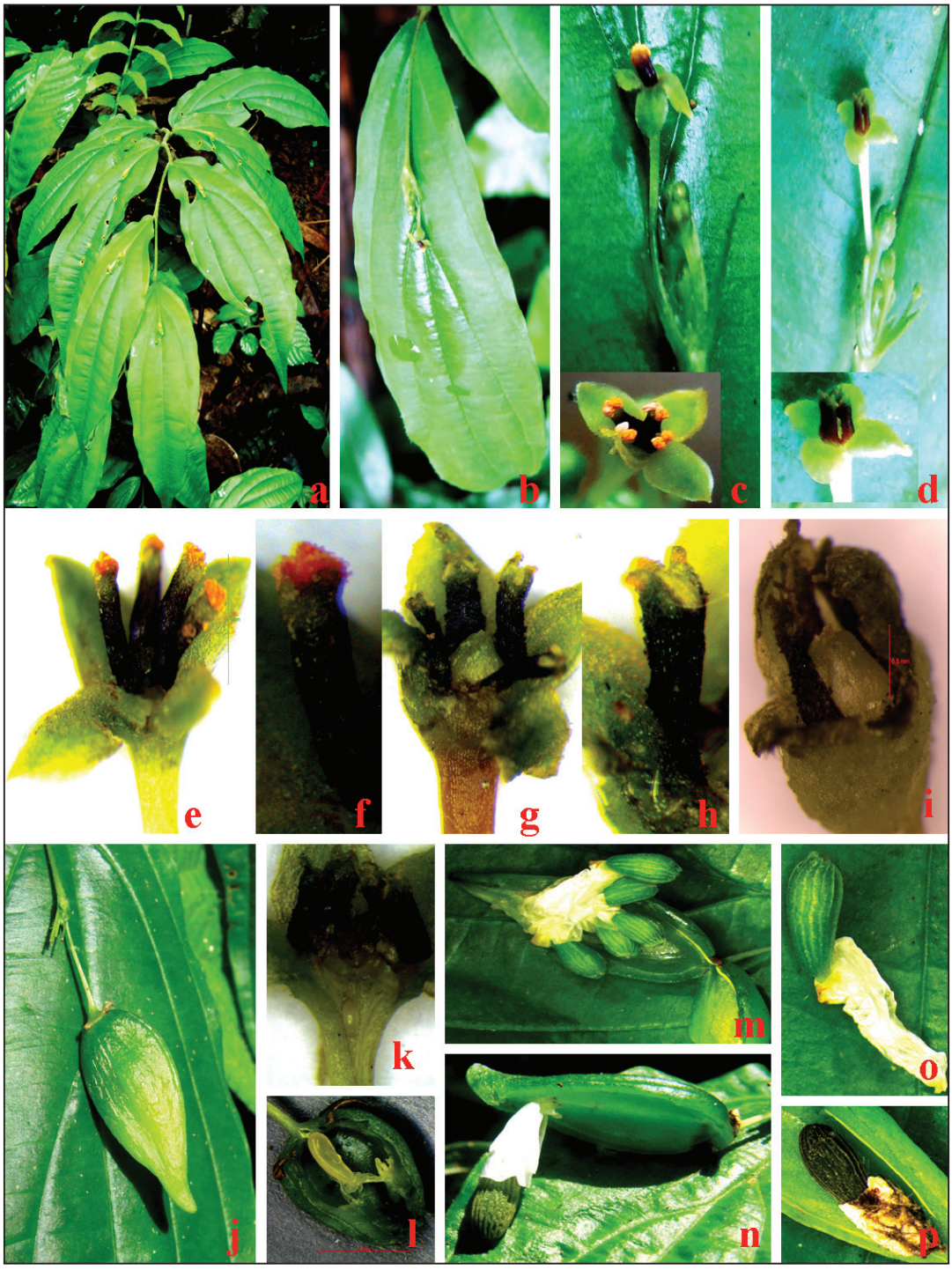

Fig. 1. *Stichoneuron membranaceum*: **a** – habit; **b** – inflorescence along the midrib of the leaf; **c** – male inflorescence and functional male flower before anther dehiscence (inset); **d** – hermaphrodite inflorescence and functional female flower (inset); **e** – male flower after anther dehiscence; **f** – stamen of the functional male flower; **g** – functional female flower; **h** – stamen of the functional female flower; **i** – ovary; **j** – mature fruit; **k** – ovule; **l** – immature seed; **m** – 5-seeded capsule; **n** – single-seeded capsule; **o** – seed with papery aril; **p** – mature seed. See text for sizes.

population was much less frequent, flowering later than the males especially at the end of the growing season. The females produced smaller quantity of flowers slightly larger than the male flowers. These differences in secondary sex characteristics in *S. membranaceum* may have been due to the cost of seed production. Because females contribute more to fruit production and embryo development, the cost of producing an offspring is assumed to be greater for females (Cavigelli *et al.* 1986).

Pollination in *S. membranaceum* is entomophilous and might be enhanced mostly by Diptera species. Although flowers are small in size, but the special position of inflorescences lying on midrib of the leaf may provide enough support for landing of relatively larger insects like bees and butterflies etc. We observed that the same habitats were tremendously frequented by several pollinating insects especially butterflies. This may result from the uniqueness of marginal vegetation along the streams, diversity of seasonal foraging plants, random sunlight penetration, shadiness, lower temperature and availability of water and mud minerals etc. facilitating an ideal habitat for greater pollinator diversity. However the attractive orange-yellow anther of *S. membranaceum* may have enough roles in cross-pollination. Because hermaphroditism result the benefits of some features for pollinator attraction and dual roles of promoting both male and female floral functions (Charnov 1976; Givnish 1980). Seed may disperse by rain and dry papery aril with long funicle may help during floating and carrying on long distance. However, being the stream marginal habitat and water current during monsoonal season at most may limit seeds to deposit on the suitable substratum or preferred habitat and easy germination in water logging condition. Hence, many seeds may simply washout with stream water and excessive moisture of crevices in rock bed may restricts its easy regeneration. *S. membranaceum* also has short rhizome, with crowded roots lacking tubers supporting both older and newer branches for several years; possibly they have rhizomatous multiplication potentiality. It will be very imperative if the seed viability, dispersal, germination, seedling growth and regeneration status would be investigated in more details.

S. membranaceum generally prefer to grow along the banks of seasonal streams, sometime on the rock beds. Both the localities were recorded under this study situated in two different districts, which are quite different in terms of geography, altitude, soil characteristic and other biological associations of the microhabitats. The association of *S. membranaceum* was dominated by the species mostly belonging to Urticaceae, Begoniaceae and Agavaceae etc. However, *Tacca integrifolia* Ker-Gawl., *Polygonum strigosum* R. Br., *Elatostema platyphyllum* Wedd., *Boehmeria platyphylla* D. Don., *Pilea glaberrima* (Bl.) Bl., *Begonia surculigera* Kurz., *Pleomele spicata* (Roxb.) N.E., *Pleomele angustifolia* (Roxb.) N.E., *Brassaiopsis glomerulata* (Bl.) Regel., etc. and several fern species were also observed.

Although, we do not have any information about pollen morphology, production, viability, sireing ability of pollen and other aspects of pollination biology both from hermaphrodite and staminate individuals. The present population of *S. membranaceum* was androdioecious, where both hermaphrodite (fewer) and androecious (dominant) populations were present and such androdioecy is extremely rare among angiosperms (Mayer & Charlesworth 1991; Pannell 1997; Manicacci & Després 2001). Because male exhibits more than a twofold advantage in survival and (or) male fitness compared with hermaphrodites to be maintained in natural populations (Lloyd 1975; Fetscher 2001). It is also assumed that the present trend may turn in the line of evolutionary biology from hermaphroditism to dioecy and possibly through different transient phases. Where morphological hermaphrodites have no or very low male function as a result of indehiscent anthers and (or) nonfunctional, often inaperturate pollen (Mayer & Charlesworth 1991), and the sex ratio may be close to a typical dioecious 1:1 value (Manicacci & Després 2001).

Conclusions

It will be very noteworthy to determine the quantitative population in terms of sex ratio and spatial association of males and females and to compare male and female size and age class distributions. Our preliminary data are a prelude for any further study in pollination, reproduction and evolutionary biology of *S. membranaceum*. Long-term investigation both in natural and controlled conditions would allow a better understanding of those unknown aspects.

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