NOTE

Huperzia squarrosa (G. Forst.) Trev. (Lycopodiaceae) in Manipur: Taxonomy and Biological Aspects

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ABSTRACT: The tassel fern, *Huperzia squarrosa* (G. Forst.) Trev. is a rare epiphytic fern ally found in specific localities of the state Manipur, North East India. Regarded as a sacred plant, it has been traded extensively from ages in local markets. The species has great potential in terms of sustainability and exploited for traditional and horticultural purposes. To understand the biology of tassels, a detailed study on taxonomy is provided along with data on ecology, life cycle, reproduction, propagation techniques and current conservation status of the species. Significant harvesting guidelines and recommendations along with features that could enhance their conservation were also highlighted.

KEY WORDS: Biology, conservation, *Huperzia squarrosa*, Manipur, taxonomy.

INTRODUCTION

Huperzia Bernh. is a genus of Lycophyte plants, commonly known as tassel ferns, or sometimes as the fir mosses or fir club mosses. These species have traditionally been included in the genus *Lycopodium* L., but the trend in recent treatments has been to place them in a separate genus (Holub, 1985; 1991). They are epiphytes found in moist forests and rainforests at high altitudes in and amongst mosses and other angiospermic epiphytes. Sometimes, it also grows in terrestrial and lithophytic condition including moist infertile soils in and around swamps and depressions. As many as 415 species are reported from all over the world from the tropics to the Artic and Sub - Antarctic zones and from sea level to alpine environments (Singh and Singh, 2010). About 20 species along with two varieties of tassel ferns are reported from India (Ghosh *et al.*, 2004). Tassel ferns are regarded as one of the most valued ornamental species all over the world and make stunning specimen hanging in lawns, corridors, verandas, glass houses, *etc.* Because of their grace and beauty, they are collected mercilessly from the wild habitat mainly for horticultural trade. In both traditional medicine and pharmaceutical product forms, *Huperzia* has already established a long track record of safety and efficacy in Chinese herbalism for fever, as a diuretic, for blood loss, and for irregular menstruation. Huperzine alkaloid extracted from tassels has also been shown to enhance memory in adolescent middle school students and used in several disorders of brain function, including Alzheimer's disease. It also shows anti-oxidant activity and also used as remedies for the treatment of contusions, strains, swellings, schizophrenia and myasthenia gravis (Chang and But, 1987; College, 1985; Ma, 1997; Sun *et al.*, 1999).

Locally known as *Leishang* in Manipur, *Huperzia squarrosa* (G. Forst.) Trev. is considered as a sacred plant by the *Meitei* (plural – *Meiteis*) community of the state. Tassel stems either in dried or fresh form is used in marriage ceremonies, worshipping of local deities and forefathers. Of all the plants associated in socio-religious ceremonies in the state, *Huperzia* proved to be the most expensive and highly demanded item. Plants are collected in wild from the hill districts by the local tribals and marketed again by the womenfolk in the valley districts. These plants were once grown in the valley forest, however today they could not be refound due to heavy exploitation by the people. As the significance of tassels in the Manipur society is enormous, both traditionally and economically, a research programme was initiated with a view to document the factual data on taxonomy, ecology, reproduction, propagation techniques, conservation status, current threats and harvesting guidelines of tassels. The study is first of its kind in Manipur history and nothing has been ever published, nor written or documented on *Huperzia squarrosa.* It is expected that the results of the study will imbibed the spontaneity within the authorities who are concerned with the management of non-timber forest products and promote a better understanding under which the sustainable use of these plants can be achieved in near future.

MATERIALS AND METHODS

Study Site

Manipur is located in the extreme eastern part of India and separates the country from Myanmar (Fig. 1). Though small in size with a total geographical area of 22,327 sq. km and lying at latitude of 23°83'N - 25°68' N and longitude of 93°03'E - 94°78'E, the topographic structures are very unique from others. It has an oval-shaped green valley of about 1, 843 sq. km surrounded by mountains. The hills cover $9/10^{th}$ of the total area of the state. The Manipur hills have peaks rising to 2,590 m and are mainly covered in jungle. The valley region houses about 70 % of the total population of Manipur. According to 2001 census report, the total population of the state is 2,388,634 and distributed in nine administrative districts, *viz*., Imphal East, Imphal West, Thoubal, Bishenpur, Senapati, Churachandpur, Ukhrul, Tamenglong and Chandel. Among these, Imphal East, Imphal West, Thoubal and Bishenpur are valley districts while the remaining are hill districts. Three indigenous main communities dwell in the state, Hindu *Meiteis* in the valley districts, Christian *Nagas* and *Kukis* in the hill districts. Other traces of population are contributed by *Manipuri Muslims*, *Nepalis* and *Biharis*.

Taxonomic studies

Relevant data were collected and analysed through repeated field trips conducted during 2006-2010. A number of group and individual meetings were conducted to discuss the mode of uses, species availability, harvesting methodology and other data related to tassels. Classification and identification of tassel is done based on the morphological structures and arrangement of reproductive parts of the sporophytic body. Referred literatures are Baker (1887), Wardlaw (1957), Ollgaard (1975), Baishya and Rao (1982), Bir *et al*. (1989), Cody and Britton (1989), Wagner and Beitel (1992) and Ghosh *et al.* (2004). The specimen was also compared with the herbarium at Botanical Survey of India (BSI), Central National Herbarium, Howrah, Kolkata and BSI (Eastern Circle), Shillong. Spore samples were studied through Scanning Electron Microscopy (SEM). Size of spores was recorded by taking the mean average calculated from a minimum of 10 readings. Spore size is expressed as polar diameter × equatorial diameter. Descriptive terminology by Harris (1955), Wilce (1972) and Devi (1977) is followed in the present study. Specimen was collected in triplicates for herbarium and deposited in Herbarium, Department of Life Sciences, Manipur University, India.

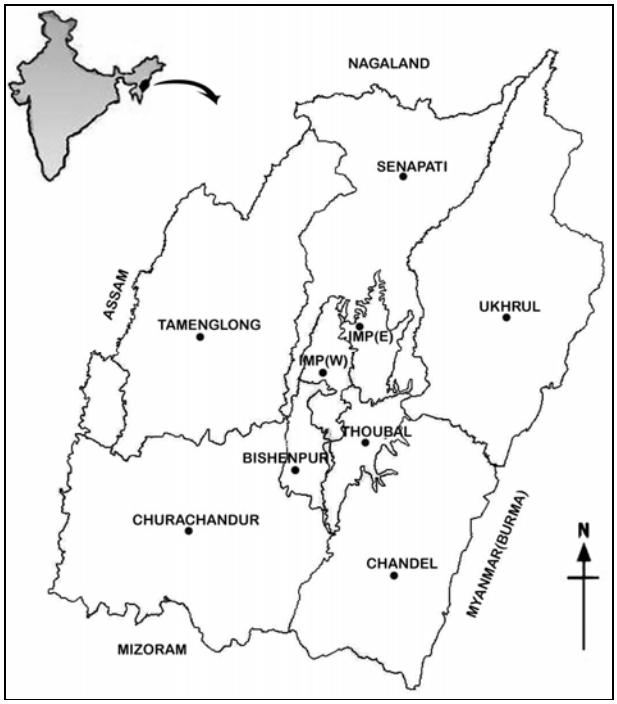

Fig. 1. Map of Manipur showing the nine districts

RESULTS AND DISCUSSION

Taxonomy and species description

Huperzia squarrosa (G. Forst.) Trevisan, Atti Soc. Ital. Sci. Nat. 17: 247 (1874).

Lycopodium squarrosum G. Forst., Fl. Ins. Austr. 479 (1786). *Huperzia epiceifolia* (Desv. *ex* Poiret) Trevisan, Atti Soc. Ital. Sci. Nat. 17: 248 (1874). *Huperzia squarrosa* (G. Forst.) Rothm., Repert. Spec. Nov. Regn. Veg. 54: 62 (1944). *Lycopodium epiceifolium* Desv. *ex* Poiret, Encycl. suppl. 3: 559 (1813 [1814]). *Lycopodium forsteri* Poiret, Encycl. suppl. 3: 545 (1813 [1814]) [nom. superfl.]. *Lycopodium gnidioides* Blanco, Fl. Filip. 824. 1837, ed. 2.569. 1845, ed. 3 (3): 239 (1879). *Lycopodium madagascariense* Desv., [nom. inval. (Spring 1842: 34)]. *Lycopodium pseudo-squarrosum* Pampan., Bull. R. Soc. Tosc. Orticult. 111, 13: 99, t.2 (1908). *Phlegmariurus squarrosus* (G. Forst.) Love & Love, Taxon 26: 324 (1977). *Plananthus squarrosa* (Sw.) P. Beauv., Prodr. Aetheog. 112 (1805). *Urostachys epiceaefolius* (Desv.) Herter *ex* Nessel. Die Barlappgewachse 202 (1939). *Urostachys epiceaefolius* var. *acutifolius* (Desv.) Herter *ex* Nessel, Die Barlappgewachse 292 (1939). *Urostachys epiceifolius* (Poiret) Herter *ex* Nessel, Barlappgewachse 202 (1939). *Urostachys madagascariensis* (Desv. *ex* Nessel) Herter, Estudios Botanicos en la Region Uruguaya 20: 68 (1949), Alt. title: Index Lycopodiarum 68 (1949). *Urostachys madagascariensis* (Nessel) Herter, Index Lyc. 68 (1949). *Urostachys squarrosus* (G. Forst.) Herter, Botanisches Archiv 3: 14 (1923). *Urostachys squarrosus* (G. Forst.) Herter var. *prolifer* (Blume) Nessel f. *madagascariensis* Nessel, Repert. Spec. Nov. Regn. Veg. 36: 189, t.175 (1934). *Urostachys squarrosus* var. *proliferus* (Blume) Herter *ex* Nessel, Barlappgewachse 200 (1939).

Plant differentiated into rhizomes and aerial stem, rhizomes extension of stem performing the function of root for attachment to substrate and acquisition of nutrients, water and retain moisture, true roots present in aerial stem portion only; stem pendulous, upto 60 cm long, branching regularly dichotomous and isotomous; Leaves microphyllous, crowded, spirally arranged, not imbricated, monomorphic, linear, upto 1.5 × 0.1 cm, juvenile leaves mostly larger than mature leaves, sharply acute, entire, rigid, coriaceous, shining, veins simple with no branches or unions, extended to middle portion (Fig. 2 A & B).

Sporophylls nearly isomorphous with vegetative leaves; strobili 5-15 cm, sessile, terminal, sporangium exposed, distinguishable into stalk and capsule, uni-locular, raised on a short stalk, reniform in shaped, loosely arranged in whorl of 5 or 6 in number, borne individually at adaxial base of un-modified leaves, every sporophyll supplied from the stele of stem by single trace, dehiscence of sporangium preceded by elongation of internodes of central axis, sporo-phylls spreading out and exposing sporangia, dehiscing along a transverse slit on upper surface, disseminated by wind (Fig. 2 E, F, G).

Spores uni-cellular, enclosed within a thick spore wall, light yellow in colour, average size of 29 × 30 µm, trilete in nature, sub-triangular in outline with rounded angles, proximal surfaces unornamented, distal surface with foveolate ornamentation, germination of spore taking place usually after 3 - 8 years after liberation from the sporangium, swelling up under the presence of moist leading to the rupture of sporal wall and growing into prothallus, antheridia and archegonia developed in mature prothallus (Fig. 2 H & I).

Specimens examined: Amatala, Kameng, F.D (N.E.F.A), INDIA, 8/5/1958, *G. Panigrahi, 15169*, ASSAM (8972); Flora of Assam, INDIA, 26/4/1942, *G.K. Deka, 21206*, ASSAM (36933).

Distribution

Tropical regions of India (West Bengal, Sikkim, Meghalaya, Assam, Nagaland, Manipur, Arunachal Pradesh, Kerala, Andaman and Nicobar Islands), Nepal, Burma, Bangladesh, Sri Lanka, Malesia, Philippines, Polynesia, Seychelles, Mascarenes, Tahiti, peninsular Malaysia, Borneo, China, Taiwan, Queensland, Madagascar, Mauritius, Java, Thailand.

Tassels as a source of Huperzine A

Members of *Huperzia* possess alkaloids such as Huperzine A (Hup A), Huperzine B (Hup B), N-methyl-huperzine B, Huperzinine, Lycoporine A, Carina-tumine A *etc.* These alkaloids are used for treating various diseases including Alzheimer Disease, vascular dementia and myasthenia gravis by Chinese scientists. Hup A has also shown to be a potential clinical benefit in attenuating the progression of Parkinson's disease (Singh and Singh, 2010). Earlier *Huperzia serrata* was regarded as the potent producer of Hup A, however other members of *Huperzia* also yield significant quantity of Huperzine. Some of Indian species reported to contain Hup A are *H. cancellata* (Spring.) Trev. (358.44 ± 0.16) µg/g, *H. carinata* (Desv. ex Poir) Trev. (560.46 ± 0.21) µg/g, *H. fordi* (Bak.) Dixit (376.18 ± 0.23) µg/g, *H. herteriana* (Kumm.) Sen et Sen (254.58 ± 0.22) µg/g, *H. phlegmaria* (L.) Rothm. (345.23 ± 0.18) µg/g, *H. pulcherrima* (Hook. & Grev.) Pichi-Serm. (342.57 ± 0.20) µg/g, *H. selago* (L.) Bernh. ex Schrank et Mart. (114.58 ± 0.24) µg/g, *H. serrata* (Thunb. ex Murray) Trev. (80.16 ± 0.17) µg/g and *H. squarrosa* (G. Forst.) Trev. (378.83 ± 0.33) µg/g (Singh and Singh, 2010).

Biology relevant to species existence

The tassels, a member of lycopods are one of the most primitive plants alive on earth 200 million years ago and have survived extreme climatic conditions. Over the years, they have adapted to different habitats ranging from epiphytic to mesophytic condition. However, very little is known about the general biology related to growing conditions, epiphytic adaptations, reflection of morphological elements in reproductive biology and propagation of *Huperzia squarrosa.* Below we present some important notes on tassel ferns.

Habit and growing conditions: Tassels are naturally epiphytes but not entirely obligate in nature. It grows as epiphyte on tree laden with humus, soil and mosses. Repository host tree includes many angiospermic plants like *Alnus nepalensis* D. Don., *Callistemon lanceolatus* DC., *Castanopsis cuspidata* (Thub.) Schott., *Cinnamomum camphora* (L.) Nees & Eberm., *Delonix regia* (Hook.) Raf., *Dipterocarpus tuberculatus* Roxb., *Eugenia jambolena* Lam., *Ficus benghalensis* L., *F. religiosa* L., *Gmelina arborea* L., *Mangifera indica* L., *Mimusops elengi* L., *Phoenix sylvestris* Roxb., *Plumeria acuminata* Ait., *Stereospermum personatum* (Hassk.) DC., *Tamarindus indica* L., *Toona ciliata* Roem., *Kigelia pinnata* DC., *Myristica fragans* Houtt., *Shorea robusta* Roth., *Shima wallichii* (DC.) Korth. *etc.* Common associated epiphytic plants are wild orchids, *Davallia trichomanoides* Blume, *Nephrolepis cordifolia* (L.) Presl., *N. biserrata* (Sw.) Schott,

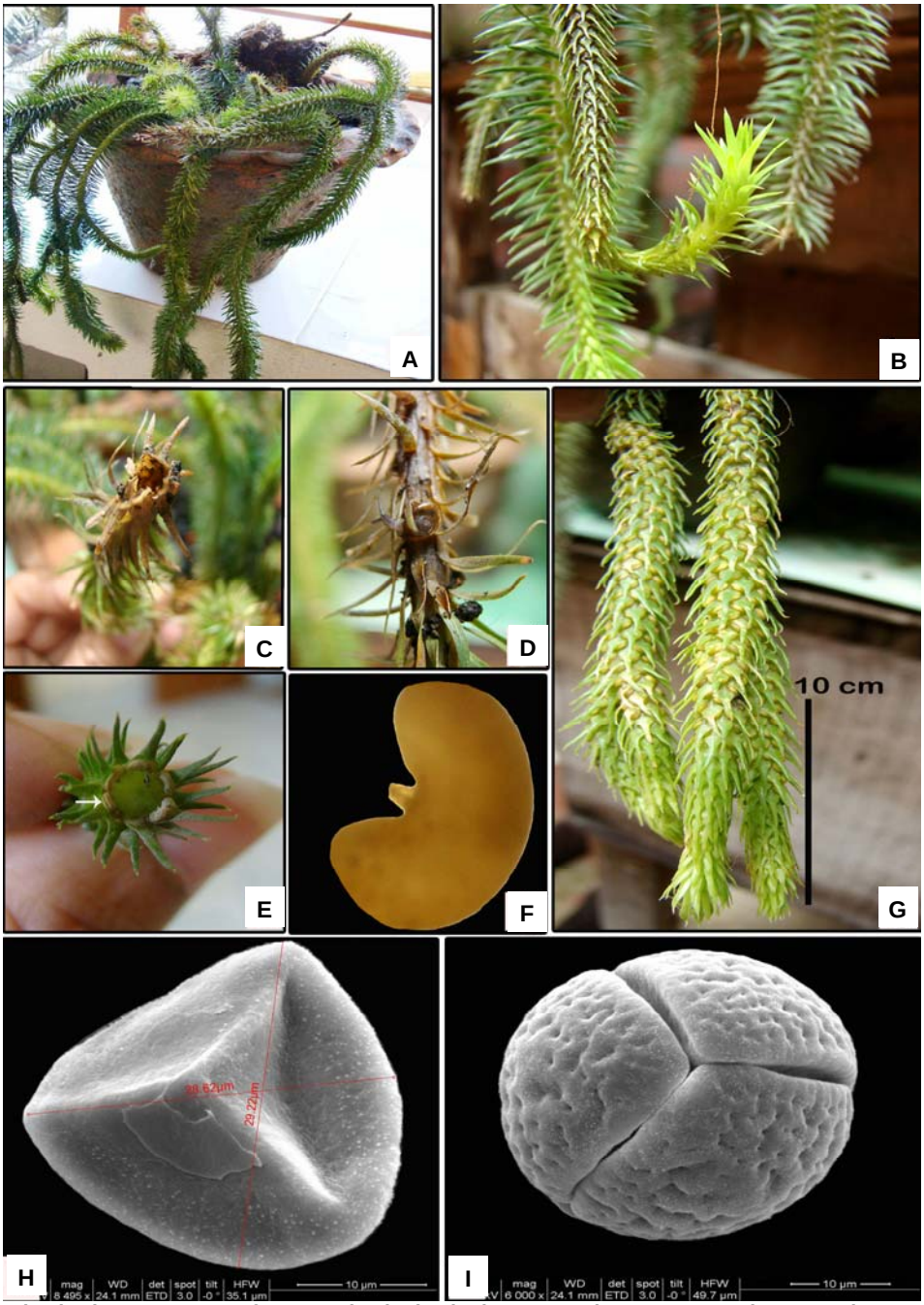

Fig. 2. Morphological structures of sporophytic body in *Huperzia squarrosa* (G. Forst.) Trev. a: Habit. b: Upward turned stem. C: Rotting in stem. D: Infestation by snail. E: Stem with transverse dehiscence. F: Sporangium, 100X. G: Fertile sporophyll. H: Proximal surface of spore. I: Distal surface of spore.

Phymatosorus cuspidatus (Don) Pic. and common polypodiaceous ferns (*Drynaria quercifolia* (L.) J. Sm., *D. propinqua* (Wall. ex Mett.) J. Sm., *Lepisorus subconfluens* Ching, *Microsorium punctatum* (L.) Copel., *Pyrrosia lanceolata* (L.) Farewell). Sometimes, they are seen growing as lithophytes in crevices and ledges of collapse wall, caves entrance, boulders and taluses, which are usually rich in humus deposition. They proved to be highly successful when planted on pots and other suitable media. They are moisture loving plants with considerable low intensity of light and can be grown in a wide range of artificial growing conditions.

Epiphytic adaptations in *Huperzia squarrosa*: Epiphytes have unique ecological characteristics that enable them to survive in the forest canopy. Some of the characteristic adaptations in tassels include:

1. Firm holdfast rhizome for adhering to the bark and branches of trees, so that wind or other forces do not knock them down;
2. Vegetative leaves are thick, rough and coriaceous in texture thus resistant to drying out in dry canopy, other wear and tear elements and even distract herbivores pest for it is too tough to be chewed;
3. Crowded spiral arrangement of leaves allow collection of water and dust to form a nutrient pool for the rhizomes;
4. Sporophylls are dichotomous, terminal in position and pendent to enable free movement of strobili for effective spore dispersal;
5. Each sporangium is over protected by sporophylls and retains potential spores from further damages. This also enable the tassels in production of quality spores;
6. Spores small in size, can lodge in tiny crevices in tree bark and remain viable for many years.

Reproductive biology: Reproduction method takes place both by asexual and sexual methods. Main asexual methods are rhizome division and formation of bulbils. Natural rhizome division is a very slow process and can be performed artificially. Another unique feature of utmost significance is the periodic formation of specific vegetative propagules which are called bulbils or gemmae. These are vegetative buds usually formed towards the apex of the stem. The presence of bulbils, whether dormant or active, is important taxonomically. Stem of mature plants turned upright and produced roots at the curved junction (Fig. 2B). These are miniature preformed plantlets ready for individual survival (Gola, 2008). They detach off from mother plant and have the potential to grow into new genetically identical plant. This type of vegetative propagation is a quick process, and resultant clonal plantlets have the genotype that has already been environmentally tested. Sexual method of reproduction is carried out through spores. All ferns and fern-allies produce spores and experience an alternation in generations, the sporophytic phase being the prominent one. It is a means of dividing, segregating and recombining chromosomes. In general, mature stem of tassels started producing potential sporophylls after many years. However, it is greatly altered by local growing conditions. Spores responsible for sexual reproduction are formed in sporangia which are borne on leaves termed as sporophylls. If a cluster of sporophylls terminate a shoot, this is termed as strobilus. A special architecture developed for increase production of sporangia is by dichotomous branching in parts bearing sporangia. The spores may remain viable for many years and could take up to 7 or more years to develop into a gametophyte (Cobb, 1963). A gametophyte bears the sexual mechanism for reproduction and, given optimal conditions, may result in a new, genetically unique sporophytic plant that would be recognized as a Tassel. Spore germination in natural environment is a very long process and varies in where they germinate. A complete life cycle from spore to gametophyte to sporophyte may take up to 10 years in most of the lycopods.

Notes on propagation: Tassel ferns can be grown successfully in lawns, verandas and greenhouses (Kaur and Chandra, 1972). Media for potting can either be natural or artificial. Some effective natural media are slightly decomposed coconut fiber, dead wood of tree ferns (*Cyathea* Sm.), charcoal dust, sphagnum moss, mixture of dried *Azolla* Lam. and sawdust, coarse stone chips mixed with fertile soil *etc.* Propagation media like perlite and vermiculite also proved as a favourite media for lycopods. Media should be well drained with plenty supply of aeration and should have good moisture holding capacity. To enhance aeration to the rhizome, pieces of charcoal and brick can be used. Ingredients which are prone to rapid decomposition should be used sparingly. A regular supply of water is a must all throughout the year. It is more effective if grown in greenhouses. Weak natural liquid fertilizer solutions such as fish emulsion and diluted cow-dung solution can be applied once a month. During winter season, watering frequency is reduced to check excess of water. Humidity is an important factor and unimpeded air movement is also vital. Synthetic growth hormones are also recommended to enhance growth. Care should be taken to prevent from seasonal pest. Some common pests include snails, molds, slugs, spiders *etc.* Main symptoms of infestations are initial browning followed by rotting of the tissues (Fig. 2 C & D). The basal portion of stem near the rhizome is commonly prone to rotting as it is more exposed to water. To prevent infestation of stems and roots, sterilization of the rooting media is preferred. Some propagation techniques include stem cutting, rhizome division, layering *etc.* For cutting purposes, upward turning stems in mature plants often make an ideal propagation material (Jones, 1987). Rhizome division is one of the most successful types of propagation. We recorded 100% survival rate in this method. Another method is air layering in aerial stem region. Though one needs lots of concentration and expertise, it is one of the options. Spore culture in laboratories is of course feasible, however it takes many years.

Current species listings

The tassel fern is listed as endangered or rare species by many independent organizations of the world. Though not listed under the Red Data by International Union for Conservation of Nature and Natural Resources (IUCN), it is evident from various literatures that this very species needed worldwide attention for probable conservation strategies. Some of the current listings are as follows:

1. as Endangered Species under Commonwealth Endangered Species Protection Act, 1992 (Australia: State of Environment, 1996),
2. as Endangered Species in Pteridophytes of conservation significance in Australia (ANZECC, 1999),
3. as Endangered taxa from the Western Ghats (Benniamin *et al.*, 2008),
4. as Rare and Endangered Species of India (Bir, 1987) and
5. as Rare and Endangered Species of Kamaun Himalaya (Pande and Bir, 1994).

Threats in Manipur

The main identified threats to tassel ferns are collecting pressure exerted mainly by the *Meitei* community of Manipur, compounded with soaring market value for horticultural trade. According to 2001 census report, 40.04 % of the rural populations in hills live below poverty line. This is one of the key factors for unauthorized collection in hilly areas in search for alternative livelihood by local inhabitants. The state has been witnessing limited employment opportunities also compounded again with unstable law and order situation. People are unrest, lack farmland and compelled the illiterate tribals to practice jhuming and the result is loss of forest area. Many unrecorded collection of rare plants including orchids has prevailed for years and legal actions are not initiated to check the plant trafficking to bordering countries. The marketing mechanism also lack management without alternative means of stock supply, but rather adhering to easy extraction of raw materials from the wild forest. Other potential threats to the viability of *Huperzia squarrosa* include habitat loss and competition from invasive species. Forest loss in the valley districts has caused total elimination of the species from the entire plain region.

Guidelines and recommendations on tassel harvesting

In Manipur, there are no formal rules or procedures for harvesting tassel ferns or any other wild plants. This is the ugly truth for plant environmentalist. Many wild rare orchids, bulb of lilies, rhizomes of Zingiberales *etc.* are collected and trafficked through the porous bordering states and countries. Among the wild plants so far exploited in Manipur, *Huperzia* are the most heavily exploited groups next to orchids. All the harvesting in deep jungles is done by illiterate tribesmen and this has severely affected the surviving condition of *Huperzia*. Some of the guidelines and recommendation for harvesting tassel are as follows:

1. Uprooting of plants along with healthy rhizomes to ensure survival and avoid collection if plant bears fertile sporophyll;
2. Use of sharp tools instead of direct uprooting as it lessens the impact of tearing;
3. To develop an indigenous easy handling propagation protocol for common laymen;
4. To increase the duration of harvesting in a particular site;
5. Bulbils should be preferred while selecting materials for planting;
6. Awareness programme on the status of tassels mainly to the women folk;
7. Enact state and regional laws for strengthening the conservation strategies.

CONCLUSION

Huperzia squarrosa (G. Forst.) Trev. is a highly explored fern ally. From centuries, the Chinese have been exploiting the medicinal properties of *Huperzia*. So far, no one has reported on the medicinal practices of this high profile species from India. In the *Manipuri* society, the tassels are considered as an indispensible plant both religiously and economically. From ages, trading of tassels in Manipur is running very successfully. As for the time being, there is no alternative source for extraction of raw materials other than the forest in hill districts. This over exploitation has depleted the natural resources over the years. Their status in Manipur never crossed our mind, nor given any interest by any authorities. The International Union for Conservation of Nature and Natural Resources (IUCN) has been playing an important role in listing out endangered species or those species that are on the verge of extinction. They have listed 770 species of pteridophytes from all over the world as threatened species in 1998 (Subhash *et al.*, 2008). India, which comes under world biodiversity region, accounts only 7.7 % of IUCN Red List of threatened vascular plants, while a high figure is recorded in countries like USA, Australia, South Africa (Benniamin *et al.*, 2008). This is mainly because efforts have not yet been undertaken to fully assess the flora

within its political boundary. Though *Huperzia squarrosa* (G. Forst.) Trev. is not listed under the Red Data List by IUCN, this fern ally is aggressively exploited in most parts of the world. In Manipur, it has totally vanished from the valley region and now confined only in five hill districts. It seems improbable that such a highly acclaimed species will never be able to spread naturally even after artificial introduction in the wild because of extreme habitat destruction in the valley. The population status occurring in hills is not yet accessed. However, we know our plants here and seen how they are exploited discriminately. Many species has declined from the historic memory of today's generation, and *Huperzia squarrosa* is one of them. Many countries has developed and enacted different legislation to conserve tassels. Likewise, we can also set our own state policy towards conservation of our own natural resources. It is high time to understand plants around us and assess them at high level for conservation strategies, or else a part of heritage and tradition will also loss along with the extinction of certain plants. Last but not the least, it is crucial that the traditional knowledge of tassels be preserved and integrated into management policies by developing effective propagation methods, systematic commercialization and even take up the tassel trade at international level as they are highly demanded globally by pharmacological companies.

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印度曼尼普爾邦（Manipur）的杉葉石松（*Huperzia squarrosa*），其分類與生物學研究

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摘要：在印度東北部曼尼普爾邦(Manipur) 杉葉石松(*Huperzia squarrosa*)是一種稀有的附生擬蕨類，僅出現於特定的某些地方。他被視為神聖的植物，並已經廣泛地在當地市場交易多年。這個物種在傳統和園藝用途上具有很大的可持續性利用潛力。為了解石松的生物學，本研究提供一個詳細的分類研究，包含該物種的生態、生命週期、繁殖、人工繁殖技術和目前的保護現狀等資料；並特別強調石松的重要採收準則以及如何保育等等的建議。

關鍵詞：生物學，保育，杉葉石松，曼尼普爾邦(Manipur)，分類學。