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MORPHOLOGICAL VARIATIONS OF FERTILE SPIKE IN *HELMINTHOSTACHYS ZEYLANICA* (L.) HOOK

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Abstract

The evolutionary history of *Ophioglossaceae* is enigmatic mainly because fossils of the family trace back only from the earliest Tertiary. Phylogenetic analyses indicate that *Helminthostachys* is sister to the broadly defined *Botrychium*. Generally the sporophore of *Botrychium* is a pinnately compound, whereas it is simple in *Helminthostachys*. Here examples of different *Helminthoistachys* are represented which show double or triple spikes with some variations. Plants showing variations in their spike morphology are also grown normally. Variations of *Helminthostachys* spike morphology indicate a tendency to form a compound sporophore structure and in that way have a strong relationship with *Botrychium*.

Key words: *Helminthostachys zeylanica*, pteridophyte, *Ophioglossaceae*, fertile spike.

Izvleček:

Evolucijski razvoj družine kačjejezikovk (*Ophioglossaceae*) je precej skrivnosten, saj prve fosilne ostanke predstavnikov družine najdemo šele iz zgodnjega terciarja. Filogenetske raziskave kažejo, da je monotipični rod *Helminthostachys* sestrski sicer širše definiranemu rodu mladomesečin (*Botrychium*). Na splošno je fertilni ali plodni del sporotrofofila pri rodu *Botrychium* pernato deljen, medtem ko je pri rodu *Helminthostachys* valjast in enostaven. V študiji so predstavljeni različni osebki vrste *Helminthostachys zeylanica* z enim ali več plodnimi izrastki. Osebki, ki kažejo na variacijo v obliki trosnih izrastkov rastejo v naravnem okolju.

Različna morfologija plodnega-trosnega dela sporotrofofila pri rodu *Helminthostachys* nakazuje tendenco k tvorbi pernato deljenih trosnih izrastkov, kar je znak sorodnosti omenjenega rodu z rodom *Botrychium*.

Ključne besede: *Helminthostachys zeylanica,* praprotnice*, Ophioglossaceae,* plodni izrastek.

1. INTRODUCTION

The plants of the family *Ophioglossaceae* are ancient and primitive pteridophytes (Bower 1926, Sporne 1966, Wagner 1990). This group of plants are represented by about eighty living species (Sporne 1966). The non-fern nature of this group was first demonstrated by Sen (1968). The peculiar fertile spike, absence of circinate vernation, absence of sclerenchyma, presence of oval or circular bordered pit in metaxylem trachied, hairless mycorrhizal roots and presence of subterranean gametophyte with indefinite apical growth are among the most important features of this group (Sen 1968). In *Ophioglossaceae* there are genera including *Ophioglossum* L., *Botrychium* Sw. and *Helminthostachys* Kaulf. (Bower 1926, Sporne 1966, Sen 1968, Wagner 1990). Another genus *Mankyua* B.–Y. Sun, M. H. Kim and C. H. Kim has also been described (Sun et al. 2001).

Helminthostachys zeylanica (L.) Hook, a monotypic terrestrial pteridophyte, is distributed in Indo-Malaysia and Australian regions (Beddome 1883, Sporne 1966, Wagner 1990, Khullar 1994). In India the plants are found in South India, North India, South Bengal, Darjeeling Hills, Ma-

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nipur, Assam and Cachar (Beddome 1883, Khullar 1994). The plants are used as vegetables and their medicinal properties are also known (Beddome 1883, Dixit & Vohra 1984, Suja et al. 2004).

Bower (1926) suggested that *Helminthostachys* is comparatively isolated from other members of *Ophioglossaceae*. Clausen (1938) recognized *Helminthostachys* as nearly intermediate between *Ophioglossum* and *Botrychium*. Evolutionary relationships among the members of *Ophioglossaceae* are generally based on morphological characters such as leaf venation, degree of leaf dissection, sporangia structure, venation, and gametophyte construction (Bower 1926, Clausen 1938, Hauk et al. 2003). In all the genera of this group, the fertile fronds have two distinct structures – the fertile part in the form of spike and sterile lamina (Sporne 1966, Sun et al. 2001). Morphologically the fertile spike, a spore bearing structure of Ophioglossaceae, is unique among other pteridophytes. In this communication morphological variations of the fertile spike of *Helminthostachys zeylanica* are reported and the evolutionary significance of these variations is discussed among the members of *Ophioglossaceae*.

2. MATERIALS

The investigation for fern flora was started in the year 2001 at Raghunathpur Forest of Dakshin Dinajpur district in West Bengal, India. This district lies between 26° 35' 15" and 26° 10' 15" north latitude and 89° 30' and 87° 48' 37" east longitude and is situated in the north of West Bengal. The forest area was regularly visited and surveyed up to the year 2008.

Ecological data such as temperature, rainfall, soil type, soil pH, soil moisture and vegetation type were collected.

Photographs of *H. zeylanica* population and individual members showing variations in their fertile spike morphology were taken from time to time. The length of the common stipe, sporophore and fertile spike of different individual plants were measured. All the voucher specimens were deposited at the Herbarium of Balurghat College. Spike morphology was studied using a simple microscope and spores were examined using a binocular compound research microscope.

During this study some rhizomes of *H. zeylanica* plants were uprooted carefully and planted to other areas in the forest for conservation.

3. RESULTS

Ecology

The temperature of the region reaches up to 40–42 °C in summer and comes down to 5–6 °C in winter. Annual rainfall is 1700 mm. Occasional showers in winter are not uncommon. The study area containing clay type soil is slightly acidic.

Short description of *H. zeylanica*

The mature perennial, terrestrial *H. zeylanica* plants are 10–75 cm long. Rhizomes are creeping with numerous fleshy roots. The fronds of the plants consist of a long common stipe bearing sterile (trophophore) and fertile (sporophore) segments. Trophophore lamina is palmately divided into three distinct parts. Each part is petiolate and again giving rise to two lateral pinnae and a terminal one. The sporophore, arising from top of a common stipe, is composed of a stalk and a fertile spike. The fertile spike is solitary and unbranched (Figure 1b). Sporangia are borne in clusters on short lateral branches (sporangiophores) and are arranged in several rows on the axis of the fertile spike. The sporangial dehiscence is of longitudinal type. Spores are yellowish brown.

Variations of fertile spike

The forest area was regularly surveyed and the *H. zeylanica* population was carefully observed in the study area (Figure 1a). During investigation the authors came across the following types of morphological variations in the fertile spikes of *Helminthostachys* (Table 1). Normally *H. zeylanica* bears a single, unbranched fertile spike. Interestingly a growing branch was observed from the lower part of the fertile spike (Figure 1c). A bifurcation at the upper part of the fertile spike was also observed (Figure 1d). A very deep bifurcation of the fertile spike was not uncommon (Figure 1e). Two distinct fertile spikes of a plant were also found among the *H. zeylanica* population (Figure 1f). Incomplete trifurcation of a fertile spike was also observed. In the latter instance the tip of the spike was bifurcated first and then one of the branches again bifurcated, which led to the formation of a trifurcated fertile spike (Fig-

ure 1g). Besides these morphological variations among the population three complete fertile spikes of a single plant were also recorded in the forest (Figure 1h). The plant shown in Figure 1h was developed from a rhizome having a normal spike bearing plant.

Relationship between vegetative growth and sporangial development

Branching of the fertile spike does not indicate any irregularity in their sporangial development. Spore structures do not show any difference compared to the normal one. The vegetative growth of these plants was normal.

4. DISCUSSION

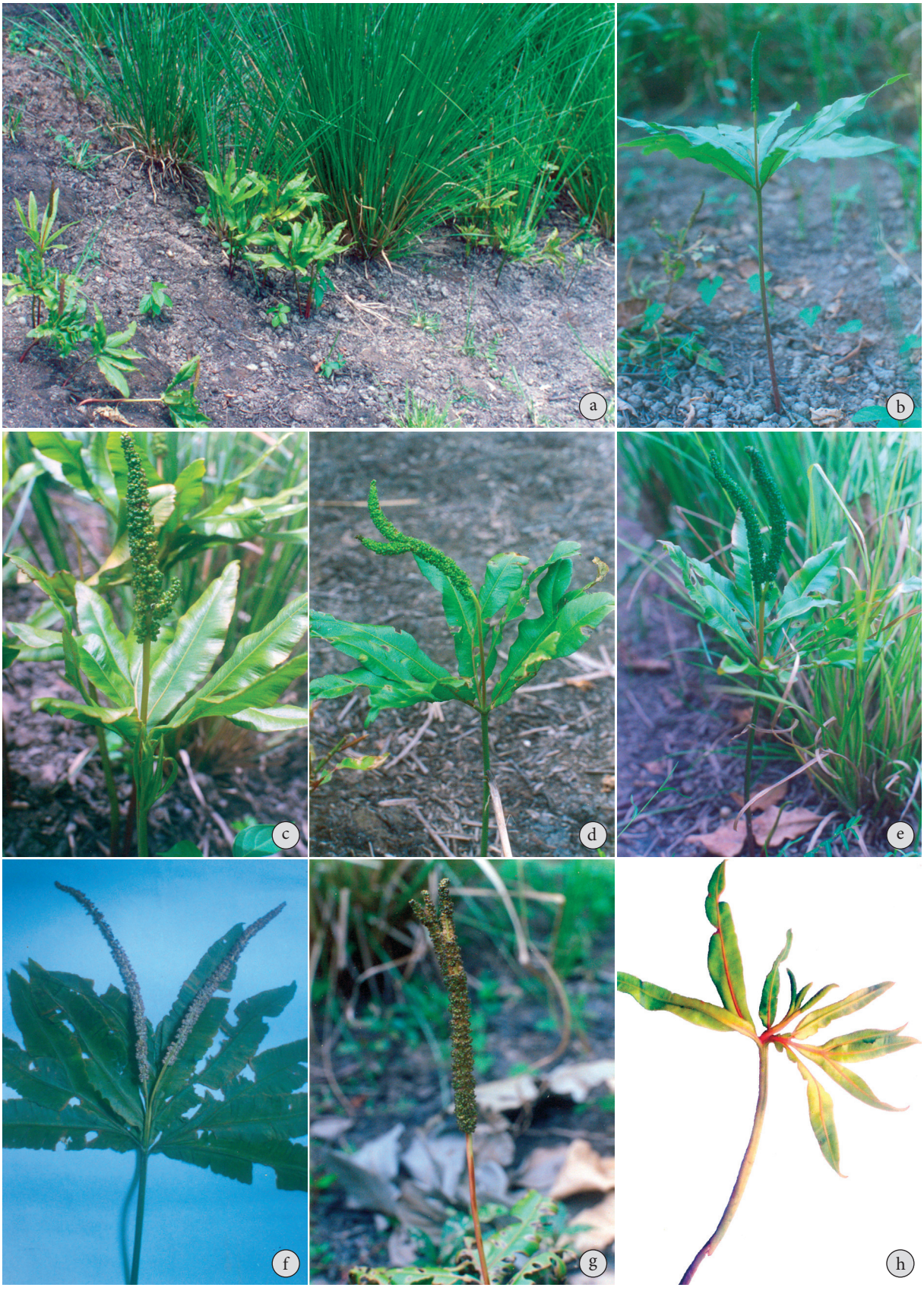
The evolutionary history of *Ophioglossaceae* is enigmatic mainly because fossils of the family trace back only from earliest Tertiary (Rothwell & Stockey 1989). According to Bower (1926), the spike of *Helminthostachys* is often subjected to accessory branching and these may be combined with correlative vegetative growth where sporangia are absent. Bifurcation at the middle of the spike (Banerjee 1951, Sharma et al. 1966) and at the terminal end of fertile spike (Rao 1960) in *H. zeylanica* are also reported earlier. Bower (1926) referred that there is a balance between the vegetative and sporangial development. But all the specimens under consideration (Figure 1b–1g) show quite normal sporangia with spores in them. The plant having triple fertile spikes (Figure 1h) was not mature enough when collected because this plant in the natural habitat faced a mechanical injury at its common stipe region. Although the sporangial development in this particular plant was started as normally, unlike Bower (1926) our observations are supported by earlier workers (Banerjee 1951, Rao 1960).

Both the genus *Ophioglossum* and *Botrychium* show transverse sporangial dehiscence (Sporne 1966, Sun et al. 2001). We support the view of Sporne (1966), Sharma et al. (1966) and Sun et al. (2001) regarding the longitudinal sporangial dehiscence in *Helminthostachys*, unlike Hauk et al. (2003) who considered transverse sporangial dehiscence. On the basis of fertile spike morphology it is known that *Botrychium* shows a branched spike, whereas as *Ophioglossum* bears an unbranched fertile spike (Bower 1926, Clausen 1938, Sporne 1966, Hauk et al. 2003). Sporangia are borne either on sporangiophore in *Botrychium* and *Helminthoistachys* or are directly attached to rachis of the spike as in *Ophioglossum* (Bower 1926, Clausen 1938, Sporne 1966, Hauk et al. 2003). The sporophore of *Botrychium* is a pinnately compound, whereas it is simple in *Helminthoistachys*. The sporophore of *Helminthoistachys* bears numerous sporangiophores (Sporne 1966, Gifford & Foster 1989) which may represent a reduced form of pinnate branching (Hauk et al. 2003). The tendency to form a compound nature of sporophore in *Helminthostachys* is shown in Table 1 and in Figure 1b–1h. Development of triple spikes from a rhizome having a normal spike bearing plant indicate that the compound nature of the sporophore was dormant in the gene pool of a normal plant. Phylogenetically *Helminthostachys* was placed with the same clade of *Botrychium* sensu lato (s.l.) when DNA sequences were studied (Hauk et al. 2003). Within the *Ophioglossaceae* the botrichioid clade is not closely aligned to *Ophioglossum*. Though Hauk et al. (2003) did not consider the *Helminthostachys* plants as having

Table 1: Morphological variations in *Helminthostachys zeylanica.*
Tabela 1: Morfološka variabilnost vrste *Helminthostachys zeylanica.*

Fertile Spike Morphology	Common Stipe Length	Sporophore Length	Fertile Spike Length	Voucher/ Herbarium
1. Normal, unbranched	29.0	20.0	10.5	Chakraborty 10/ BLGC
2. Branch from lower part	26.0	19.0	12.5	Chakraborty 11/ BLGC
3. Bifurcation from upper part	25.0	20.0	9.5	Chakraborty 12/ BLGC
4. Deep bifurcation	29.0	16.5	10.1	Chakraborty 13/ BLGC
5. Double Spikes	31.0	19.0	13.2, 14.5	Chakraborty 14/ BLGC
6. Incomplete trifurcation	27.5	25.0	13.5	Chakraborty 15/ BLGC
7. Triple Spikes	12.0	3.4	0.8, 1.3, 1.7	Chakraborty 16/ BLGC

Length is measured in cm


a
b
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a branched fertile spike while studying phylogeny of Ophioglossaceae. The different branched spike morphology of *H. zeylanica* might be another indication of the strong relationship between *Botrychium* s.l. and *Helminthostachys*.

Therefore, all these morphological evidences support the conclusion that *Helminthostachys* having spike variations are a close associate of botrychioid clade and not closely aligned to *Ophioglossum*. While considering the evolutionary hiatus between normal *H. zeylanica* and *Botrychium* s.l., these individuals (Figure 1c–1h) may accordingly be treated as intermediate link. Molecular studies of *Helminthostachys* having variable spike morphologies may throw further light on this issue.

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Figure1: Morphological variations of the fertile spike in *Helminthostachys zeylanica.*

a) habitat of *H. zeylanica*, b) plant with usual single spike, c) spike showing branch at lower part, d) spike showing bifurcation at upper part, e) plant with deeply bifurcated spike, f) plant with double spikes, g) plant with incompletely trifurcated spike, h) plant showing triple spikes.

Slika 1: Morfološka variabilnost plodnih izrastkov pri vrsti *Helminthostachys zeylanica.*

a) rastišče *H. zeylanica*, b) rastlina z običajnim izrastkom, c) izrastek z razvejanjem v spodnjem delu, d izrastek z razvejanjem v zgornjem delu, e) rastlina z močno razvejanim plodnim izrastkom, f) rastlina z dvojnimi plodnimi izrastki, g) rastlina z nepopolno trojni razvejanimi izrastki, h) rastlina s trojnimi plodnimi izrastki.

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