Pak. J. Bot., 42(6): 3723-3728, 2010.

THE FIRST ANATOMICAL REPORT AND MORPHOLOGICAL REEXAMINATION OF *PSILOTUM NUDUM* L., IN IRAN

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Abstract

Psilotum nudum L., is of considerable importance in palaeobotany considering its age of evolution i.e. Devonian. It has also been recognized as a medicinal herb. In 2003, one sample of this species was identified just by morphological research and introduced as a new record. This research aims at introducing other samples discovered through other anatomical research besides morphology. *P. nudum* grows on the trunks of old trees of *Alnus glutinosa* (L.) Gaertn., in the form of epiphyte, as suspended, in North Iran. The plant is 30 cm long and aerial shoot has dichotomic branches. There are prominent longitudinal lines, tiny scaly leaves with spiral phyllotaxy and bifid bracts of synangia on aerial shoot. Synangia are configured spirally on the stem and each contains three connected sporangia. Stele in *P. nudum* L., is protostele. In rhizome it is of actinostele type. However, it is of siphonostele type in aerial shoot.

Introduction

Fossils of ferns indicate age of their depository layers. Having the first vessels, they can be regarded as an evidence of evolution of plants. Since a great number of them have also medicinal properties, it seems essential to conduct a research in this connection mainly because *Psilotum nudum* L., has similar antigens to insulin (Silva *et al*., 2002), which are of medicinal importance. Hawaiian use spores of *P. nudum* as a substitute for talcum powder (Foster & Gifford, 1974).

P. nudum is also known as Whisk Fern (Qiu & Palmer, 1999). According to researchers of Biology Department of UCC, this denomination is due to its broom-like appearance. "psilotum" means naked in Greek and "nudum" means naked in Latin. Seemingly, this denomination results from lack of clear big leaves.

Pteridophyta amounts to 13000 living species of which, 3 species are wisk ferns (*Psilotum*), about 300 species are club mosses (*Lycopodium*), about 700 species are spike mosses (*Selaginella*), about 65 species are quillworts (*Isoetaceae*), 25 species are horsetails (*Equisetum*) and about 12000 species are ferns (Jones & Luchsinger, 1986). Plants variety was rapidly developed in Devonian by emergence of vascular plants. There is a small group among vascular plants with an undeveloped structure and reproductive system including two genera of *Psilotum* with two or three species and *Tmesipteris* with ten species. (Ray *et al*., 1983)

Psilopsida is the oldest and most primitive vascular plants including extinct taxa of Silurian, Devonian and contemporary taxa. *Psilotum* is indigenous plant of tropical and subtropical zones. These small plants do not have any root and real leaves and grow as epiphyte on stem of plants such as tree ferns or on soil surface or beside trees and rocks. *Psilotum nudum* L. can grow in greenhouses and are cultivated in botanical gardens as well.

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Systematic condition of *Psilotum* (Singh *et al*., 2003)

Class: Psilotopsida
Order: Psilotales
Family: Psilotaceae
Genus: *Psilotum*

Sporophytical generation of *Psilotum*: Cytological observations indicate interaction of fungus and host in sporophyte of *Psilotum* that is autotroph and its gametophyte that is heterotroph. These observations have revealed that Psilophytes are exploitative of the fungus in both generations (Jeffrey *et al*., 2005).

Close similarities between the water-and food-conducting elements in both generations viz., vessel elements with scalariform perforation plates and sieve cells with refractive spherules and lacking callose at all stages in their development, add support to the homologous theory of the "alternations of generations" (Jeffrey *et al*., 2005).

There is controversy on different species of *Psilotum*. All of botanists approve *P. nudum* L., but no agreements have been reached on other species. According to Singh *et al*., (2003), *Psilotum* L. consists of two species including *P. nudum* L., and P. *flaccidum* Wall. The former grows in tropical and subtropical zones while the latter lives as epiphyte hung from trunk of the trees and grows in Jamaica, Hawaii, Florida, Mexico, and some of the Pacific Ocean islands. Only *P. nudum* has been identified in India. *P.nudum* has very small aerial shoots with 15 to 20 cm long that reach to 90 cm high in *P. flaccidum*.

According to Qiu & Palmer (1999), two species of *P. nudum* L. and *P. complanatum* Sw., with an intermediate species have been identified. The recent molecular genetics studies together with morphological evidence support this idea that *Psilotum* may be fern.

This research aims at final approval or rejection of the above species through anatomy besides morphology in ecosystem of Iran.

Materials and Methods

A population of *P. nudum* in the rainy forest of Chaloos (Hyrcanian Province) was encountered. The entire population was observed on the trunk of *Alnus glutinosa* (L.) Gaertn., toward the south. This region is humid. A number of photos were taken in natural habitat of the above plant. The plant was collected intact from trunk of the trees with a little soil around the rhizome. It was, then, planted in a flower pot and cultivated in a moist place by spraying water with indirect sun light in 25°C. The research was continued in a laboratory. Some of the samples were carefully dried that are kept in the Herbarium of High Education Centre of Agriculture at present. Morphological studies were carried out through examination of morphological properties of the plant by stereo microscope and its comparison with the existing authentic sources (Singh *et al*., 2003).

Anatomical studies were carried out through manual exact cross-sectioning of rhizome, stem, synangium and sporangium. Double staining was then performed to identify types of steles and tissues better. Several slides were prepared and pictures taken from cross-sections were observed by Image Analyser microscope. Finally they were compared with world-known *P. nudum* L., *P. flaccidum*, *P. complanatum*, and *P. triquetrum* species. The existing authentic sources (Jones & Luchsinger, 1986; Jeffrey *et al*., 2005; Gifford & Foster, 1988) were used for such comparison.

Results

Morphological and anatomic studies in this research show that the intended plant is of *Psilotum nudum* L., species and the anatomic studies are reported subsequently. Morphologic studies were reconducted on this plant and the following results were achieved.

The plant lives as epiphyte on the trunk of *Alnus glutinosa* (L.) Gaertn., trees in rainy forests in the vicinity of Chaloos, Mazandaran Province (Fig. 1). The branches have dichotomic forks (Fig. 2). The plant is 25-35 cm long. A dichotomous bract is around synangium (Fig. 3). Scaly leaves are observed on the stems (Fig. 3). Longitudinal parallel lines twisted slowly around the stem are observed (Fig. 3). Synangium is composed of three connected sporangia separated by three longitudinal and arched depressions (Figs. 3 and 7).Yellow spores are in the sporangia.

Rhizome has a broad cortex in the cross-section (Fig. 4). Sclerenchymatous pericycle is surrounded by endodermis in rhizome (Fig. 4). A number of cortical cells include brown reserves in rhizome (Fig. 4). Star-shaped vessel tissue in rhizome is surrounded by phloem (Fig. 4). Therefore type of stele in rhizome of the anatomized plant is protostele and is of actinostele type.

In aerial shoot, epidermis cells are externally cutinized (Fig. 5). In aerial shoot, cortical cells are divided into three parts including outer cortex, middle cortex and inner cortex (Fig. 5). Middle cortex having 4-5 layers includes sclerenchyma cells (Fig. 5). Outer cortex includes parenchyma cells with thin and compressed wall (Fig. 5). In aerial shoot, stele is surrounded by clear layer of endodermis (Fig. 5). In aerial shoot, star-shaped vessel tissue is surrounded by phloem (Figs. 5 and 6). Stem pith is wooden and sclerenchymatous (Figs. 5 and 6). Therefore, stele in anatomized aerial shoot is of siphonostele type.

Discussion and Conclusion

Collected samples of *Psilotum nudum* L., were studied through anatomical research. The results of these studies are published in Iran for the first time. Protostele in rhizome of *Psilotum* has been introduced as haplostele and actinostele yet. As Singh *et al.* (2003) proved to protostele of rhizome is of haplostele type i.e., non-differentiated tracheids to protoxylem and metaxylem in the pith are surrounded by phloem.

Our research revealed that the concerned species in Iran has protostele, of actinostele type in rhizome since the radial- or star-shaped xylem is placed on exarch pattern surrounded by phloem (Fig. 4). Stele in the stem is of siphonostele type and is solenostele among different types of siphonostele (Fig. 5), since stem pith has become wooden and sclerenchymatous and is not parenchyma such as dictyostele and eostele. The results of our research conform to the research made by Gifford & Foster (1988).

In our research, which is in conformity with Singh *et al*., (2003), middle cortex is multi-layered and sclerenchymatous with thick walls. They also reported that these layers are responsible for mechanical protection of the plant. In our observations we found out that outer cortex is parenchymatous with thin walls, that was compressed and rather darker than middle cortex. Singh *et al*., (2003) have also introduced outer cortex as parenchymatous and rich in starch. It seems abundant starch in the cells results in darkness of this part.

Fig. 1. *Psilotum nudum* L., leaves as epiphyte on the trunk of *Alnus glutinosa*.

Fig. 2. *Psilotum nudum* L., the branches have dichotomic forks.

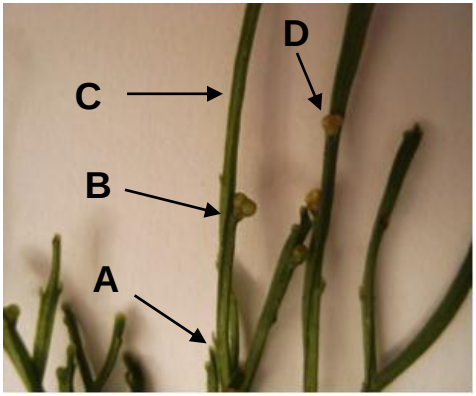

Fig. 3. *Psilotum nudum* L., A. Scaly leaves, B. Dichotomous bracts, C. Longitudinal parallel lines twisted slowly around the stem, D. Synangium with trilobed structure.

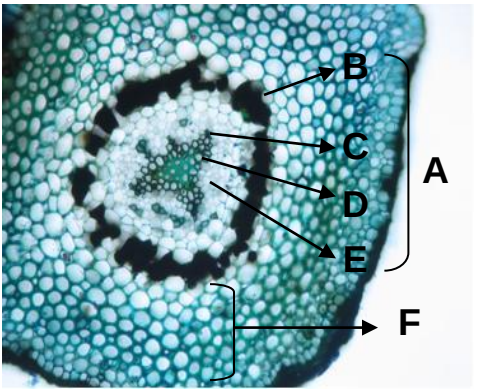

Fig. 4. *Psilotum nudum* L., Transverse section of rhizome. A. Stele region, B. Endodermis cells with conspicuous casparian strips on radial walls, C. Protoxylem, D. Metaxylem, E. Phloem, F. Cortex (X400).

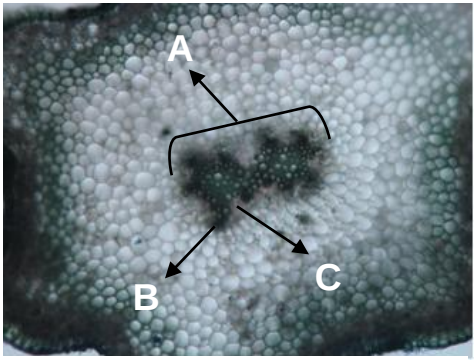

Fig. 5. *Psilotum nudum* L., Transverse section of aerial shoot. A. Stele region, B. Xylem, C. Phloem (X400).

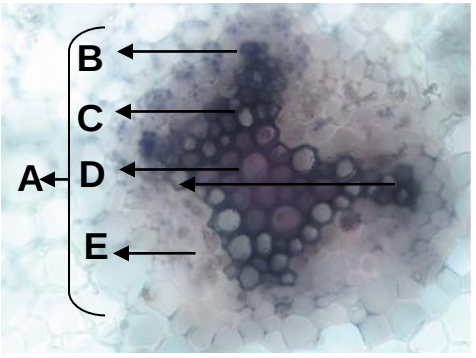

Fig. 6. *Psilotum nudum* L., A. Stele region, B. Protoxylem, C. Metaxylem, D. Sclerenchymatous pith, E. Phloem (X 1000).

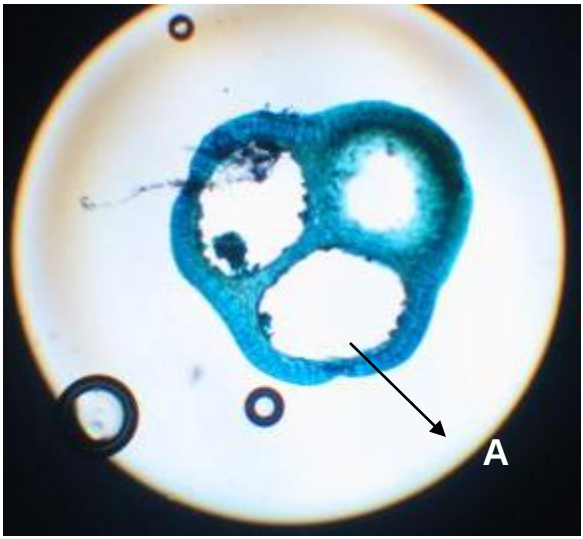

Fig. 7. *Psilotum nudum* L., Transvers section of synangium with trilobed structure. A. Sporangium (X 400).

Today the researchers seek for finding evidence in order to attribute *Psilotum* to ferns and prove their phylogeny derived from *Psilotum*. In our research, *P. nudum* in rhizome has a tracheid which has been developed to protoxylem and metaxylem and this may be one of the reasons that makes *Psilotum* close to ferns and explains how ferns are evolved.

Comparison of our anatomical and morphological studies on *P. nudum* with other researches carried out on *P. triquetrum*, *P. flaccidum* and *P. complanatum* indicates apparent differences and ensures soundness of *P. nudum*. According to the research carried out by Morgan (1962), *P. triquetrum* has been found in rock fissure through which water is flowing and shows all normal appearances of the stem and its angles. However, *P. nudum* is epiphyte and grows on the trunk of trees. Leaves and synangia are not seen in the stem angles but are emerged by spiral phyllotaxy. In another report by Singh *et al*., (2003), *P. flaccidum* is 90 cm long and the leaves are in opposite position on the edges of plane branches. But, *P. nudum* is 40 cm long and the leaves are placed in alternate position. Finally Qiu & Palmer (1999) found out that stem in the cross-section is triangular. However, in our subject plant, *P. nudum*, the stem in the cross-section was not triangular but was marginally dentate.

According to the report by Huss & Bergman (1990) *Alnus glutinosa* (L.) Gaertn., which is introduced as host in our observations, has a symbiotic relation with mycorrhiza in its root. This paves the way for symbiosis of mycorrhiza with *P. nudum* since mycorrhiza lives as endophyte in outer cortex cells (Singh *et al*., 2003).

A. glutinosa was observed and introduced as host for *P. nudum* in this research, however, in the former research (Rezaei, 2003) *Parrotia persica* (DC.) C. A. Mey played the host role. *Alnus glutinosa* grows in Iran to a broad distribution, so *P. nudum* may be found in other parts of Hyrcanian Province. Therefore, it seems appropriate to present more documentary, comprehensive evolutionary and phylogenic discussions on plants found in Iran.

At the end it can be said that once it was thought that *P. nudum* is indigenous to the U.S. and a number of other regions. However, it has been found in Asia and Iran now and anatomical and morphological research proves the same vascular shapes in rhizome and aerial shoot as those found in some other parts of the world. *P. nudum* dates back to Devonian i.e., 400 million years ago. This may broaden our views to our natural environments and broaden new horizons in connection with palaeobotany.

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(Received for publication 29 July 2009)