

## The morphological and anatomical reinvestigation of the *Psilotum nudum*, in Hyrcanian forests, N Iran

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### Abstract

*Psilotum nudum* is an ancestral epiphytic of the monilophytes which has been recently reported from Hyrcanian forests. It was already introduced as an epiphytic species from some patchy habitats in the lowland parts of Hyrcanian forests. Moreover, *Psilotum nudum* was reviewed using morphological and anatomical characteristics in two different populations in central parts of Hyrcanian zone. The results showed that there were no significant differences between morphological and anatomical characteristics. The aerial stems of the plant were linear, chlorophyllous, and dichotomously branched. Leaves were much reduced, simple, scale-like, and without ligules. Synangium was composed of 3-locular capsule-like sporangia. Also, stele type in apex aerial stem and aerial stem was actinostele; and it was haplostele type in rhizome. In this paper, a new locality of this species growing on the rocky sites was reported in Ata-kuh of Guilan.

**Key words:** Anatomy, Morphology, Forest rock, Submountain, Hyrcanian forest, *Psilotum nudum*, Iran

### Introduction

Psilotaceae, as a family of Psilotales, comprises two genera (*Psilotum* Sw., *Tmesipteris* Bernh.) and about 15 species of fern-like plants (Kenrick, 2000). The genus *Psilotum* consists of two species, *P. nudum* (L.) P. Beauv. (= *P. triquetrum* Sw) and *P. flaccidum* Hook. & Grev. (= *P. complanatum* Sw.). Most commonly, it grows erectly on the ground or in crevices among rocks, but it may also grow as an epiphyte on tree-ferns or among other epiphytes on tree branches (Sporne, 1962). It is sometimes found in botanical gardens and in greenhouses (Nazarian *et al.*, 2010). *Psilotum nudum* is fairly common in tropical and subtropical parts of both hemispheres (Singh *et al.*, 2010).

This genus is often grouped with the extinct Psilophytes, the Rhyniales and Zosterophyllales dating from the Devonian some 400 million years ago (Roux, 2003). Among the vascular plants, they considered as one of the oldest and the simplest. The word *Psilotum* is derived from the Greek word Psilos which means naked or bare which is in regard to its lack of true leaves on the stem (Amanda, 2010). *Psilotum nudum* is more

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commonly known as the Whisk Fern; this name comes from the likeness of the branched stems to whisks and alludes to its use in the past as a small broom.

Recently, study of its morphology and anatomy suggests that the ancestral features present in living Psilophytes represent a reduction from a more typical modern fern plant. Likewise, molecular evidence strongly confirms that *Psilotum* is a fern (Smith *et al.*, 2006).

The genus *Psilotum* is characterized by dichotomously branched stems, the absence of roots, a relatively simple vascular structure, small reduced leaves and thick-walled, homosporous synangia (Roux, 2003). These small plants consist of a brown underground rhizome which is likewise much branched. *Psilotum* gametophytes apparently have a lipid coating (Bierhorst, 1971), and they are also subterranean, mycorrhizal, and not ephemeral. Likewise, it has two cytological races, a diploid with a chromosome number  $2n = 52$ ; and a tetraploid with  $4n = 104$  (Khullar, 2008).

So far, only a few anatomical and morphological studies have been published on the genus *Psilotum* (Ford, 1904; Stiles, 1910; Sporne, 1962; Schulte *et al.*, 1987; Khoshravesh *et al.*, 2009; Nazarian *et al.*, 2010; Singh *et al.*, 2010). However, the current study can relate these data to other available morphological and anatomical data of this genus and will certainly enrich the botanical information on this species.

In the present study, firstly we report a new locality with a new habitat in the Hyrcanian area. Then, we systematically examined the morphology and anatomy of aerial stem and rhizome in *Psilotum* species growing in two lowland forests (Ata-Kuh and Ramsar), in order to determine the presence of *Psilotum nudum* in forest ecosystem of Guilan.

## Materials and Methods

Plant samples were collected from natural habitats and the voucher specimens were deposited in the Herbarium of Guilan University. For each sample, replicates were collected from two populations. Morphological data evaluated based on 10-15 plant specimens. Anatomical studies carried out on the samples conserved in a solution of alcohol-water-glycerine. The cross sections were stained with methylene blue and congo red and mounted with glycerine jelly to make permanent slides (Vardar, 1987). Well-stained sections of the aerial stem, apex aerial stem and rhizome were studied under the light microscope (using ocular and stage micrometer) and photographed by camera. The aerial stem was placed in a tube filled with 70 % lactic acid for 4-5 days. The sections were then prepared by a sharp scalpel or by hand.

## Results

### Habitat preferences and morphological evidences

*Psilotum nudum* is a perennial plant which grows as an epiphyte on the trunk of *Parrotia persica* C.A.May. in the Ramsar riparian forest or river bank (586 m a.s.l.) and as a lithophyte in crevices among rocks near streams in the Ata-Kuh forest (207 m a.s.l.) (Figure 1A, B). Aerial stems are 26 cm (Ramsar population) to 31 cm (Ata-Kuh population) long, and 2 mm in diameter, repeatedly dichotomously branched above with 20-36 branches, often pentagonal towards the first dichotomy and in the most distal portions triangular, erect, glabrous and chlorophyllous, with longitudinal parallel lines and dichotomous bract. Sporangia are 2.5 mm in diameter, large, with walls two cells thick, two or three sporangia fused to form a synangium, and orange-brown when mature. Leaves are 1.5-2 mm long, with spiral phyllotaxy on stem, lanceolate to ovate in outline, scale-like, pointed, with no stomata, lacking a midrib and without ligules (Figure 1C, D). Rhizomes are 3-4 cm (Ata-Kuh) and 2-4

cm (Ramsar) long, to 1.5 mm in diameter, cylindrical, lack chlorophyll (non-photosynthetic), subterranean, rootless, with short and long rhizoids and mycorrhizal. This is the first report of the species in the Hyrcanian forests of Guilan (Figure 2).

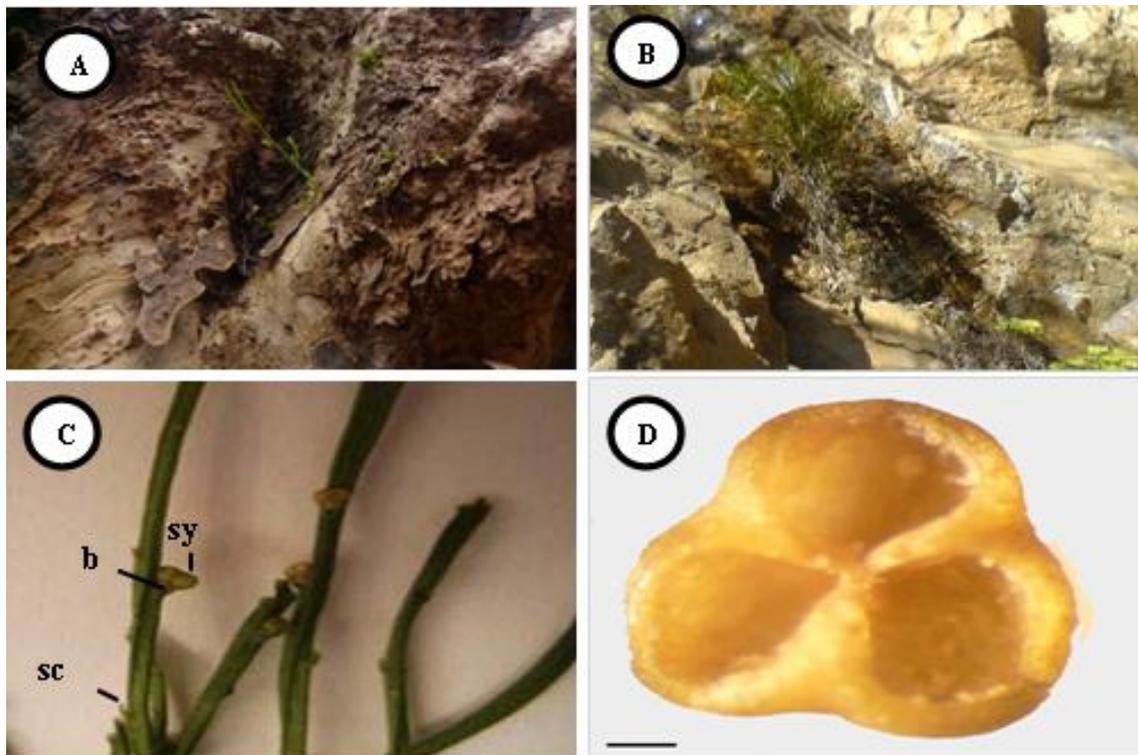


Figure 1. *Psilotum nudum* (L.) P. Beauv. A and B. Habit and Habitat (Ramsar on *Parrotia persica* C.A.Mey.); C. Synangia (b. Bracts; Sc. Scaly leaves; Sy. Synangium); D. Close up of synangium. Scale Bar D = 0.1 mm

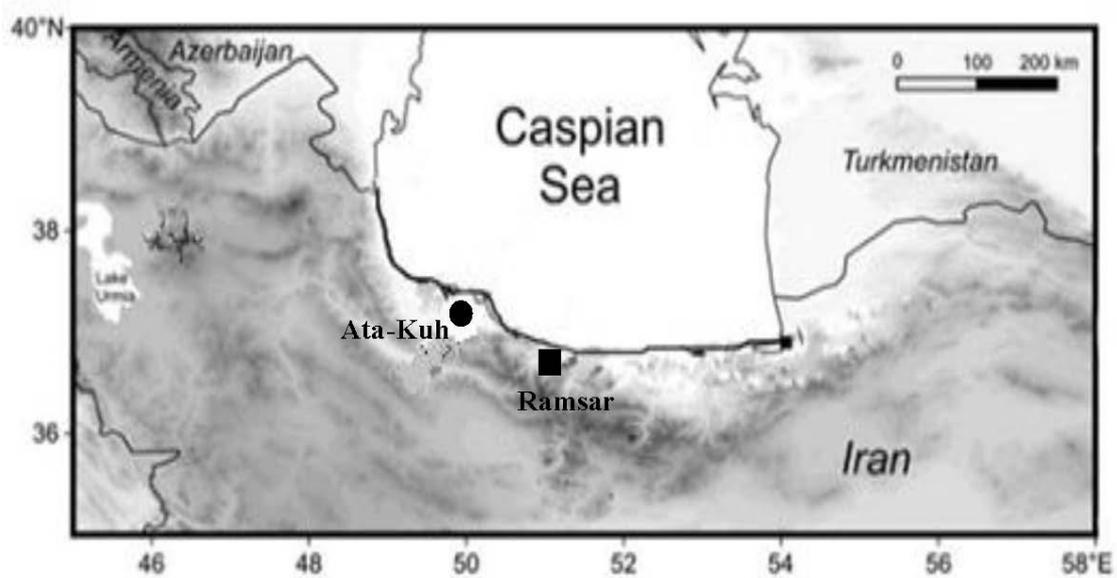


Figure 2. Map showing the distribution of the sampled population

## Anatomical studies

### Aerial stem

Cross sections of the aerial stem were irregular in outline (pentagonal in Ramsar samples and star-like in Ata-Kuh samples) due to the presence of grooves and ridges. Epidermis was composed of oblong cells that were heavily cutinized and interrupted by large numbers of stomata. Stomata were of the anomocytic type (Figure 3). Each stoma had two dumbbell shaped guard cells that are somewhat sunk below the level of the other epidermal cells and had heavily cutinized walls like those of epidermal cells (Figure 4D, J). The cortex was differentiated into three zones (I) outer cortex consisting of 2-4 layers of chlorophyllous cells (since the leaves did not perform their photosynthetic function, the green stem capture this activity), (II) middle cortex comprising 3-6 layers of sclerenchymatous cells, and (III) inner cortex, including thin-walled parenchymatous cells (Figure 4B, H). The stele was a protostele (actinostele) with xylem rays (star-like and pentarch to octarch) and surrounded by distinct layers of endodermis with characteristic casparian bands on the radial walls of its cells. The center of the stele was occupied by sclerenchymatous pith (Figure 4C, I). The characteristics of the aerial stem, apex aerial stem and rhizome anatomy of *Psilotum* species of 2 localities were given in Table 1.

### Apex aerial stem

In cross section, apex aerial stem was triangular. Epidermal cells are quadrangular. Cuticle was present on the epidermis. Stomata were of the anomocytic type. The cortex was undifferentiated and the stele was a protostele (actinostele) that was surrounded by a clear layer of endodermis. In the apical part there were only 2-3 radiating rays (diarch or triarch) (Figure 4E, F, K and L).

### Rhizome

Cross sections of the rhizome are elliptic-circle in outline. Rhizome epidermis is composed of quadrangular cells that are cutinized. Beneath this layer is a cortex that is differentiated into three zones. The outer and middle cortex includes parenchymatous cells. Inner cortex is dark brown in colour because of the presence of phlobaphene. Endodermis is distinguishable. It is followed by single layered pericycles which encircle the stele; the stellar type is a protostele (haplostele) (Figure 5).

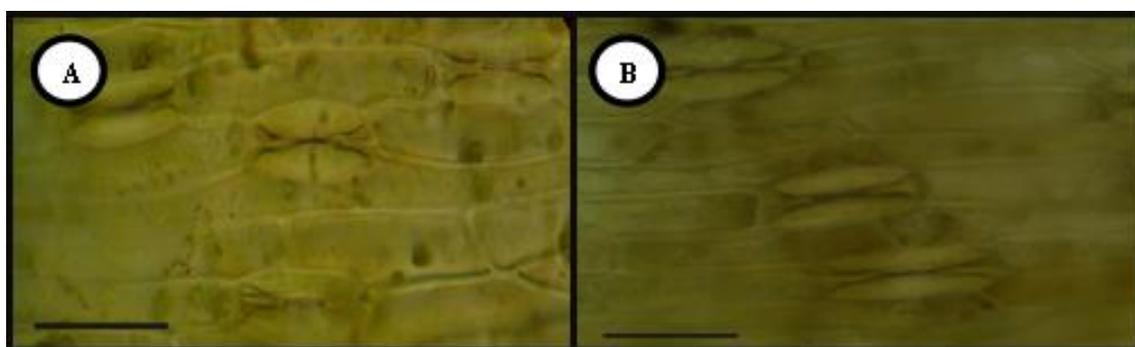


Figure 3. Stomatal type in the aerial stem in two studied localities (A. Ata-Kuh and B. Ramsar samples). Scale Bar = 0.1 mm.

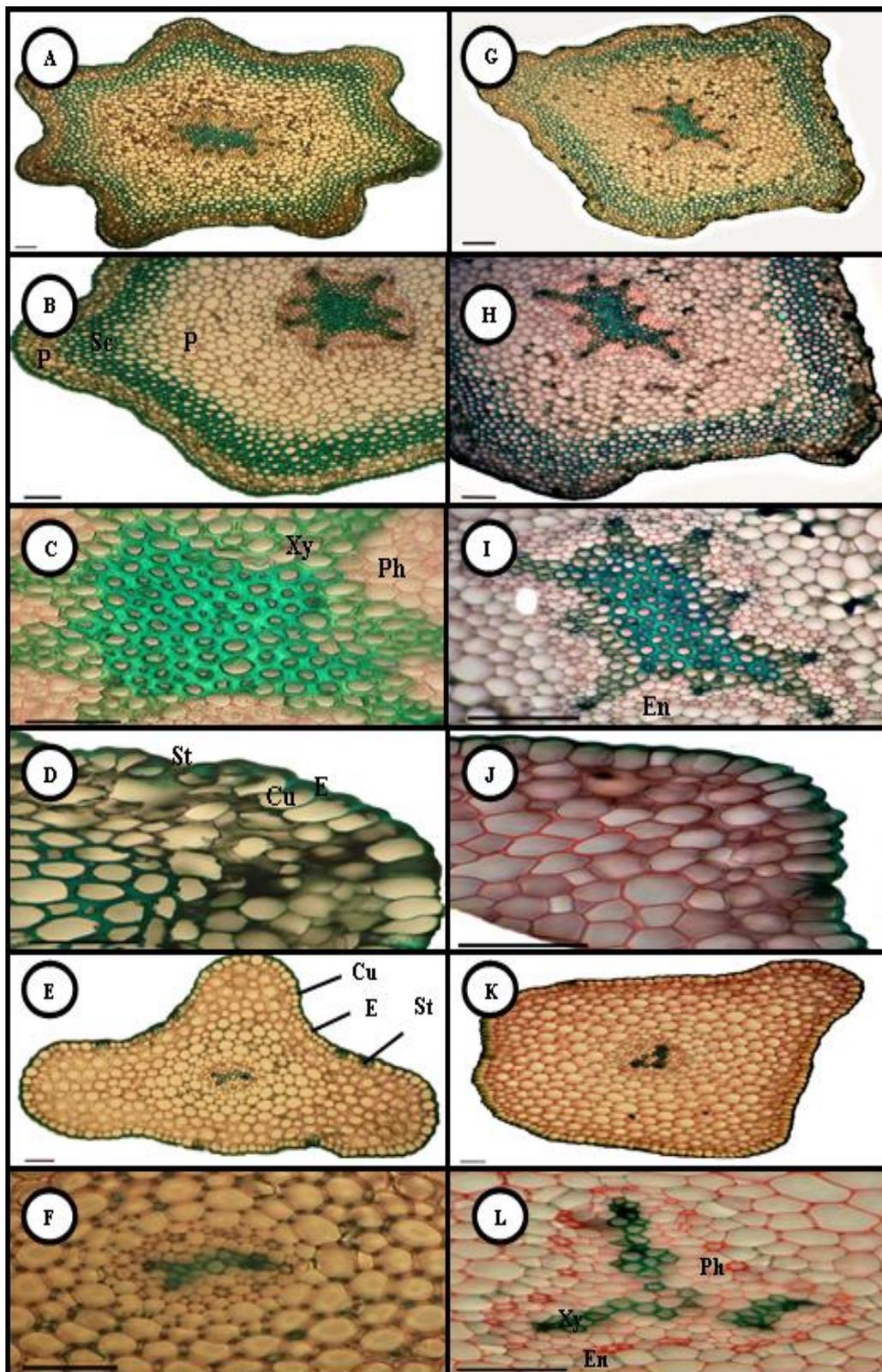


Figure 4. Transverse sections of the aerial stem (A-D. Ata-Kuh; G-J. Ramsar samples): A and D. General aspect; B and H. A part of aerial stem; C and I. Stele region. D and J. Stoma cell with cutinized walls. Transverse sections of the apex aerial stem (E-F. Ata-Kuh; K-I. Ramsar samples): E and K. General aspect; F and I. Stele region. Abbreviations: E. Epidermis; C. Cuticle; St. Stomata; Ph. Phloem; Xy. Xylem; P. Parenchyma; Sc. Sclerenchyma; En. Endodermis. Scale bar = 0.1 mm

Table 1. Anatomical characters of *Psilotum nudum* in Ata-Kuh and Ramsar forests

|                  | Characters                                  | <i>P. nudum</i> (Ata-Kuh) | <i>P. nudum</i> (Ramsar) |
|------------------|---|---------------------------|--------------------------|
| Aerial stem      | Cross section shape                         | Starlike                  | Pentagonal               |
|                  | Cuticle diameter ( $\mu\text{m}$ )          | 12.5                      | 11                       |
|                  | Epidermal cell shape                        | Oblong                    | Oblong                   |
|                  | Epidermis diameter ( $\mu\text{m}$ )        | 23                        | 24                       |
|                  | Stomata type                                | Anomocytic                | Anomocytic               |
|                  | Outer parenchyma layer number               | 2-4                       | 1-3                      |
|                  | Outer parenchyma diameter ( $\mu\text{m}$ ) | 32                        | 31.5                     |
|                  | Sclerenchyma layer number                   | 4-6                       | 3-6                      |
|                  | Sclerenchyma diameter ( $\mu\text{m}$ )     | 100                       | 98                       |
|                  | Inner parenchyma layer number               | 8-12                      | 10-12                    |
|                  | Inner parenchyma diameter ( $\mu\text{m}$ ) | 131                       | 132                      |
|                  | Endodermis cells                            | +                         | +                        |
|                  | Stele type                                  | Protostele (Actinostele)  | Protostele (Actinostele) |
| Apex aerial stem | Cross section shape                         | Triangular                | Triangular               |
|                  | Cuticle diameter ( $\mu\text{m}$ )          | 7.5                       | 7.5                      |
|                  | Epidermal cell shape                        | Quadrangular              | Quadrangular             |
|                  | Epidermis diameter ( $\mu\text{m}$ )        | 25                        | 23.5                     |
|                  | Stomata type                                | Anomocytic                | Anomocytic               |
|                  | Cortex diameter ( $\mu\text{m}$ )           | 179                       | 181                      |
|                  | Endodermis cells                            | +                         | +                        |
|                  | Stele type                                  | Protostele (Actinostele)  | Protostele (Actinostele) |
| Rhizome          | Cross section shape                         | Elliptic-Circle           | Elliptic-Circle          |
|                  | Cuticle diameter ( $\mu\text{m}$ )          | 7.5                       | 7.5                      |
|                  | Epidermal cell shape                        | Quadrangular              | Quadrangular             |
|                  | Epidermis diameter ( $\mu\text{m}$ )        | 40                        | 37                       |
|                  | Cortex diameter ( $\mu\text{m}$ )           | 475                       | 468                      |
|                  | Phlophaphn                                  | +                         | +                        |
|                  | Endodermis cells                            | +                         | +                        |
|                  | Pericycle                                   | +                         | +                        |
|                  | Stele type                                  | Haplostele                | Haplostele               |

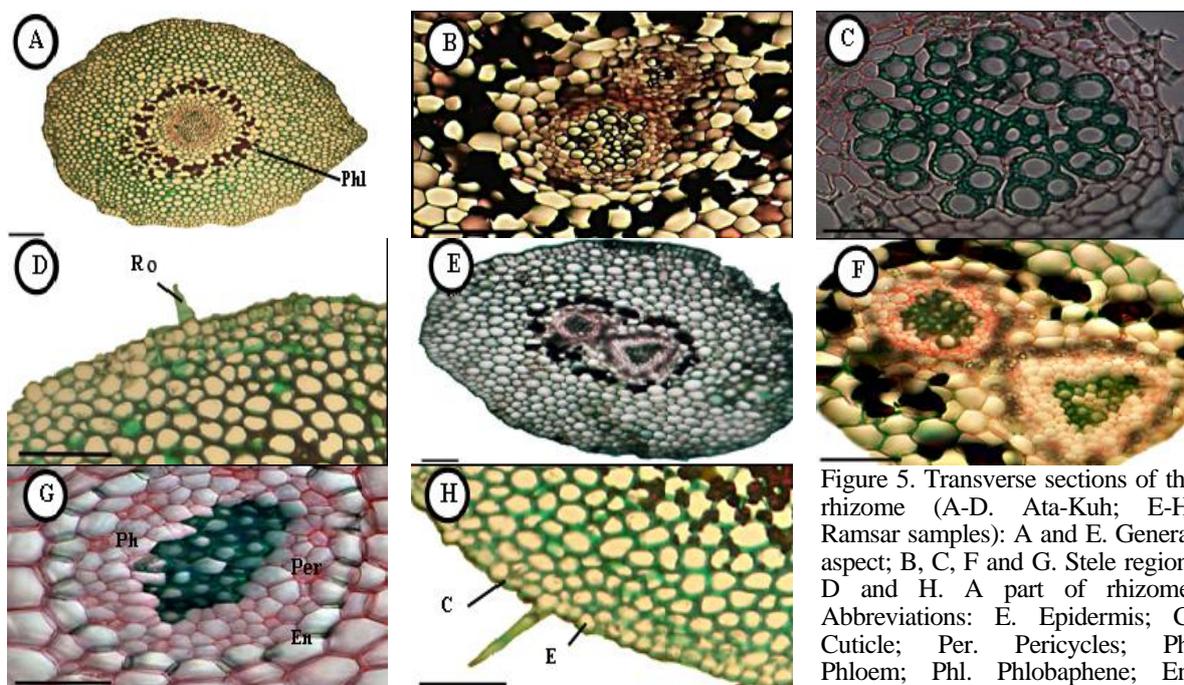


Figure 5. Transverse sections of the rhizome (A-D. Ata-Kuh; E-H. Ramsar samples): A and E. General aspect; B, C, F and G. Stele region; D and H. A part of rhizome. Abbreviations: E. Epidermis; C. Cuticle; Per. Pericycles; Ph. Phloem; Phl. Phlophaphn; En. Endodermis; Ro. Root hair. Scale Bar = 0.1 mm

## Discussion

### Habitat preferences

*Psilotum nudum* was discovered in a lowland Hyrcanian forest of Ramsar in 2003 (Rezaei, 2003). It was mainly growing on the trunks slots and roughness of *Parrotia persica* trees in a very shady habitat. During last ten years after the first discovery of the species, this species was also reported elsewhere as epiphyte on some other species e.g. *Alnus glutinosa* (L.) Gaertn. in Chalus valley (Nazarian *et al.*, 2010), Nowshahr (Dr. Habib Zare, personal communication) and in Ramak valley in Ramsar (Naqinezhad and Zarrei, in preparation). In all mentioned habitats, *P. nudum* was found as epiphyte on lowland trees. Moreover, Khullar (2008) stated that it grew on the ground or in rock crevices, and Morgan (1962) reported that *P. triquetrum* had been found in rock fissure through which water is flowing. In the current study, a new different habitat, rocks, was found for this species in Ata-Kuh forest and also, as an epiphyte on *Parrotia persica*, in the Ramsar forest, confirming previous findings.

These habitats are under the threat of damage and destruction by road building and human activities. Therefore, they should be regarded as threatened species according to IUCN categories and criteria.

### Anatomical and morphological reexamination

We decided to investigate *Psilotum nudum* morphologically and anatomically in detail. Our morphological findings of *P. nudum* were consistent with the morphological description of the taxon, of Ford (1904), Stiles (1910), Sporne (1962), Khoshravesh *et al.* (2009), Nazarian *et al.* (2010), and Singh *et al.* (2010).

According to Singh *et al.* (2010) *P. flaccidum* was 90 cm long and the leaves were in opposite position on the edges of plane branches, while *P. nudum* was 25-35 cm long and the leaves were placed in alternate position. Another comparable feature with respect to *P. nudum* and *P. flaccidum* in stem morphology was in the form of the aerial stem. In *P. flaccidum* the lower part was triangular and the upper part flattened in one plane, while in *P. triquetrum* this is multiangular in the lower parts and triangular in the ultimate branches (Stiles, 1910). Our results showed that the cross section of *P. nudum* stem supports this idea. Moreover, in another report by Sporne (1962), *P. nudum* had upright habit and *P. flaccidum* had pendulous habit.

Our results, like those of Ford (1904), Sporne (1962), Khullar (2008), Khoshravesh *et al.* (2009), Nazarian *et al.* (2010), and Singh *et al.* (2010), showed that the cortex in the stem was differentiated into three zones (outer photosynthetic, middle sclerenchymatous and inner parenchymatous) and the sclerenchymatous layers were responsible for mechanical protection of the plant. The result of this study showed that stele in the aerial stem was protostele (actinostele). The stele has been interpreted as a protostele (actinostele) by Schulte *et al.* (1987), Khoshravesh *et al.* (2009), and Singh *et al.* (2010) and as a siphonostele by Nazarian *et al.* (2010) and Gifford and Foster (1988). Khullar (2008) also mentioned that if the pith was considered as composed of sclerenchymatous cells, then the stele was a siphonostele, but if these sclerenchymatous cells belonged to the xylem, then it was a protostele. Also, our results showed that the cortex in the rhizome was differentiated into three zones (outer, middle and inner); this finding was in agreement with results obtained by Sporne (1962) and Ford (1904). Also, we found that stele in the rhizome was a protostele (haplostele), since non-differentiated tracheids to protoxylem and metaxylem in the pith are surrounded by phloem. Nazarian *et al.* (2010) have reported the stele is protostele (actinostele) in the rhizome. However, Singh *et al.* (2010) have reported that the

stele was a protostele (haplostele), which supported our observations in this research.

The size and shape of the stomata are taxonomically important characters (Tahir and Rajput, 2009). In our study, stomata type was anomocytic; this is in agreement with observations reported by Roux (2003) and Mickle (2012).

### Conclusion

In the current investigation, the morphological and anatomical studies on *P. nudum* indicate similarity between the samples collected from the two habitats, and a comparison of our results with other researcher's studies on *Psilotum* species indicates apparent differences with the *P. flaccidum* species and confirms the existence of *P. nudum* in forest ecosystem of Guilan.

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### References

- Amanda, M. (2012) General description of *Psilotum* species. Retrieved from [http:// www. sciences 360.com](http://www.sciences360.com). On: 7 February 2012.
- Bierhorst, D. W. (1971) Morphology of vascular plants. Macmillan Publishing, New York.
- Ford, S. O. (1904) The Anatomy of *Psilotum triquetrum*. Annals of Botany 18: 589-608.
- Gifford, E. M. and Foster, A. (1988) Morphology and evolution of vascular plants. Freeman, W. H. and Company, New York.
- Kenrick, P. (2000) The relationships of vascular plants. Philosophical Transactions of the Royal Society B 355: 847-855.
- Khoshravesh, R., Akhiani, H., Eskandari, M. and Greuter, W. (2009) Ferns and fern allies of Iran. Rostaniha 10: 1-132.
- Khullar, S. P. (2008) Pteridophyta. In: Diversity of microbes and cryptogams. (Eds. Singh, V., Pande, P. C. and Jain, D. K.). Rastogi Publications, Meerut.
- Mickle, J. E. (2012) Stomatal development in aerial axes of *Psilotum nudum* (Psilotaceae). Journal of the North Carolina Academy of Science 128: 95-99.
- Morgan, D. (1962) *Psilotum triquetrum* Swartz in Basutoland. Nature 195: 1121.
- Nazarian, H., Taghavizad, R. and Khosravii, E. (2010) The first anatomical report and morphological reexamination of *Psilotum nudum* L., in Iran. Pakistan Journal of Botany 42(6): 3723-3728.
- Rezaei, A. (2003) *Psilotum nudum* (L.) P. Beaut. (Psilotaceae), A new record for the flora of Iran. Iranian Journal of Biology 10(1): 1-3.
- Roux, J. P. (2003) Swaziland ferns and fern allies. Southern African Botanical Diversity Network, Pretoria.
- Schulte, P. J., Gibson, A. C. and Nobel, P. S. (1987) Xylem anatomy and hydraulic conductance of *Psilotum nudum*. American Journal of Botany 74: 1438-1445.
- Singh, V., Pande, P. C. and Jain D. K. (2010) Diversity of microbes and cryptogams. Rastigo Publication, India.
- Smith, A. R., Pryer, K. M., Schuettpelz, E., Korall, P., Schneider, H. and Wolf, P. G. (2006) A classification for extant ferns. Taxonomy 53(3): 705-731.

- Sporne, K. R. (1962) The morphology of pteridophytes. Hutchinson and Company, New York.
- Stiles, M. A. (1910) The structure of the aerial shoots of *Psilotum flaccidum*, wall. Annals of Botany 24: 373-392.
- Tahir, S. S. and Rajput, M. T. M. (2009) S.E.M. structure distribution and taxonomic significance of foliar stomata in *Sibbaldia* L. species (Rosaceae). Pakistan Journal of Botany 41: 2137-2143.
- Vardar, Y. (1987) Botanikte preparasyon tekniđi. Ege Üniversitesi Fen Fakültesi Kitaplar, Uygulama Kitab, Bornova-İzmir, Turkey (in Turkish).



## بررسی مجدد ریخت‌شناسی و تشریحی *Psilotum nudum* در جنگل‌های پست خزری، شمال ایران

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### چکیده

*Psilotum nudum* یک نهانزاد اپی‌فیت ابتدایی است که اخیراً از جنگل‌های هیرکانی گزارش شده است. در مطالعات پیشین این گیاه به عنوان گونه‌ای اپی‌فیت از زیستگاه‌های متعدد در ناحیه پست خزری گزارش شده بود. به علاوه، ویژگی‌های ریخت‌شناسی و تشریحی *P. nudum* در دو جمعیت از این گونه در بخش مرکزی ناحیه هیرکانی بازنگری شد. نتایج مطالعه حاضر گویای این است که هیچ اختلاف معنی‌داری در ویژگی‌های ریخت‌شناسی و تشریحی مشاهده نشده است. ساقه هوایی گیاه خطی، سبز با انشعابات دو شاخه‌ای است. برگ‌ها تقلیل یافته، ساده، فلس‌مانند بدون زبانک هستند. سینانژیوم از اتحاد سه اسپورانژیای کپسول‌مانند تشکیل یافته است. همچنین، نوع استوانه آوندی در رأس ساقه هوایی و ساقه هوایی اکتینواستل و در ریزوم هاپلواستل است. در بررسی حاضر، بستر رشد جدیدی از این گونه روی تخته سنگ، از جنگل عطاکوه در گیلان گزارش شده است.

**واژه‌های کلیدی:** تشریح، ریخت‌شناسی، تخته سنگ جنگلی، جنگل نیمه کوهستانی عطاکوه، *Psilotum nudum*  
ایران