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Original Contribution

COMPARATIVE ANATOMICAL STUDY OF FIVE SPECIES OF GENUS *ASPARAGUS* IN BULGARIA

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ABSTRACT

The current study represents investigation on morphological and anatomical characters of five native species of genus *Asparagaceae* - *A. tenuifolius*, *A. acutifolius*, *A. maritimus*, *A. verticillatus* and *A. officinalis*. Have been prepared anatomical features of phylloclade and stems of the taxa to evaluate the importance of anatomical parameters as a tool for the taxonomy. Anatomical features of phylloclade are species-specific and can be effectively used in taxonomy of the members of family *Asparagaceae*. The number of palisade parenchyma cells and the number of vascular bundles are conservative and constant as indicators of species-level, and making them reliable and convenient for taxonomic purposes. The structure of the stems in the studied Bulgarian species corresponds to their ecological specialization, but missed histological, discrete quantitative and qualitative differences are therefore a low informative for taxonomy of the genus *Asparagus*. The evaluated anatomical characters are used to build a determination key.

Kew words: anatomy, phylloclade, stem, determination key, taxonomy value.

INTRODUCTION

Genus *Asparagus* L. consists about 160-300 monoecious and bisexual species, distributed in the temperate and tropical regions of Europe, Asia and Africa (1). Seven species are noticed in the flora of Bulgaria (2, 3). Genus *Asparagus* is one of the problematic genera in Bulgarian flora, with complicated taxonomical relationships. The major taxonomical study is published in volume 2 of Flora of Republic of Bulgaria (4).

The macromorphologial characters for division of the species in genus *Asparagus* are few, and they are not selective in the borders of the Bulgarian representatives. This causes difficulties in the determination of the taxa. The phyloclades represent unusual polymorphism, which leads to difficult delimitation in the different taxa (5-7). The facts above, together with the short blooming period, cause problems in the building of determination keys in the floristic literature.

Comparative anatomical studies are known for the stems and phyloclades of species from Asia and Africa. The results show the anatomical profiles of the species as good diagnostic characters (6, 8). Anatomical data about the species, distributed in Bulgaria was not found in the literature.

The aim of this study is to compare the anatomical structure of the phyloclades and stems of five wild species in Bulgaria, and to value the anatomical characters as taxonomic markers for objective determination.

MATERIALS AND METHOD

Samples of genus *Asparagus* are collected by the authors due the terrain studies in the period 2002-2010 from natural populations. The studied taxa and their localities are represented in **Table 1**. For the anatomical work is used fresh material, conserved in 75 % ethanol.

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Table 1. *Voucher specimens of the examined Asparagus species*

Species	**Floristic region, MGRS coordinates, altitude, locality, date of collection**
A. officinalis L.	Tracian plain, 35TKG99, 285 m, oak forest near Pyasachnik dam lake, 20.10.2002 Danube plain, 34TPF64, 122 m, the marshes near Slivata, 08.07.2007
A. maritimus (L.) Miller	Danube plain, 34TPF64, 110 m, bushes, near the marshes near Slivata, 08.07.2007
A. acutifolius L.	Northern Greece, near Nea Peramo, 26.04.2010 Black Sea Region (south) 35TNG85, 40 m, xerophylous oak forest near Sinemorets, 06.06.2006
A. verticillatus L.	Black Sea Regiom (north), 35TPJ21, 50 m, stony terrains, in the bush, Yailata reserve, 17.05.2010 Tracian plain, 35TKG95, 252 m, between Krichim and Brestovitsa, 28.05.2008
A. tenuifolius Lam.	Sredna gora (west), 35TKH71, 1025 m, Panagyurski-Kolonii, loc. Bogdanitsa, 20.05.2002 Stedna gora (east) 35TLH21, 353 m, conifer timber woodland near Vedrare, 16.07.2006

The cross-sections are taken from the middle part of the phyloclades and stems. The samples are taken from the 3th node of the stem's length. The microphotograps are taken in magnification 10×16 using a microscope Amplival (Carl Zeiss – Jena) with digital photo camera Samsung V5 (3.2 Mpix) mounted on the binocular head.

RESULT AND DISCUSSION

Phyloclades (Figure 1)

The cross-sections of the phyloclades from the five studied species have shown in general a homogenous structure. In other side, they can be delimited by diameter, as well as by shape – circular or 3-4 to 5 angular. Oval or circular shape have the sections of the phyloclades in *A. tenuifolius* Lam. and *A. acutifolius* L. Polymorphism in the shape is represented in *A. maritimus* (L.) Mill., *A. verticillatus* L. and *A. officinalis* L. The shape in these species varies from irregular elliptic to stellar with unclear angles, stellar and 4-5 angular. Because of the similar morphological characters of the vegetative parts, *A. maritimus* and *A. officinalis* are wrongly determinated too often. The determinations of the anatomical characters are the shape of the cross-section of the phyloclades. *Asparagus maritimus* had oval to irregularly angular cross-sections. The cross-sections of *A. officinalis* have 3 or 4 angles (**Figure 1**).

The epidermal cells are rectangular, with more or less thickened external cell walls, covered, by solid cuticle. Some species have short trichomes on the protuberant parts of the angles. Under the epidermis are situates 2-3 lines of palisade chlorenhymatose cells with different length in each species. Morphologically this tissue corresponds to the pallisade tissue in the leaves of the outstanding families, as noticed (4). The highest level of secialisation is observed in the species with phyloclades, organized to increase the volume of the assimilation tissue and to decrease the volume of the vascular tissue. These characters approach to the structure of the typical leaf.

In *A. verticillatus* and *A. acutifolius* is displayed differentiation of the mesophyl as a tissue similar to the typical pallisade tissue, as one character for the typical leaf. This fact is regular in relation to the lack of normally developed leaves. The longest pallisade cells and respectively the biggest count of cells are observed in *A. verticillatus*. Three to four rows of pallisade cells are observed in A. verticillatus and *A. acutifolius* but the second species has approximately isodiametric cells. In *A. tenuifolius* the assimilation parenchyma is represented by 2 rows of slightly prolonged cells. In other case, *A. maritimus* and *A. officinalis* have also 2 rows but more prolonged cells.

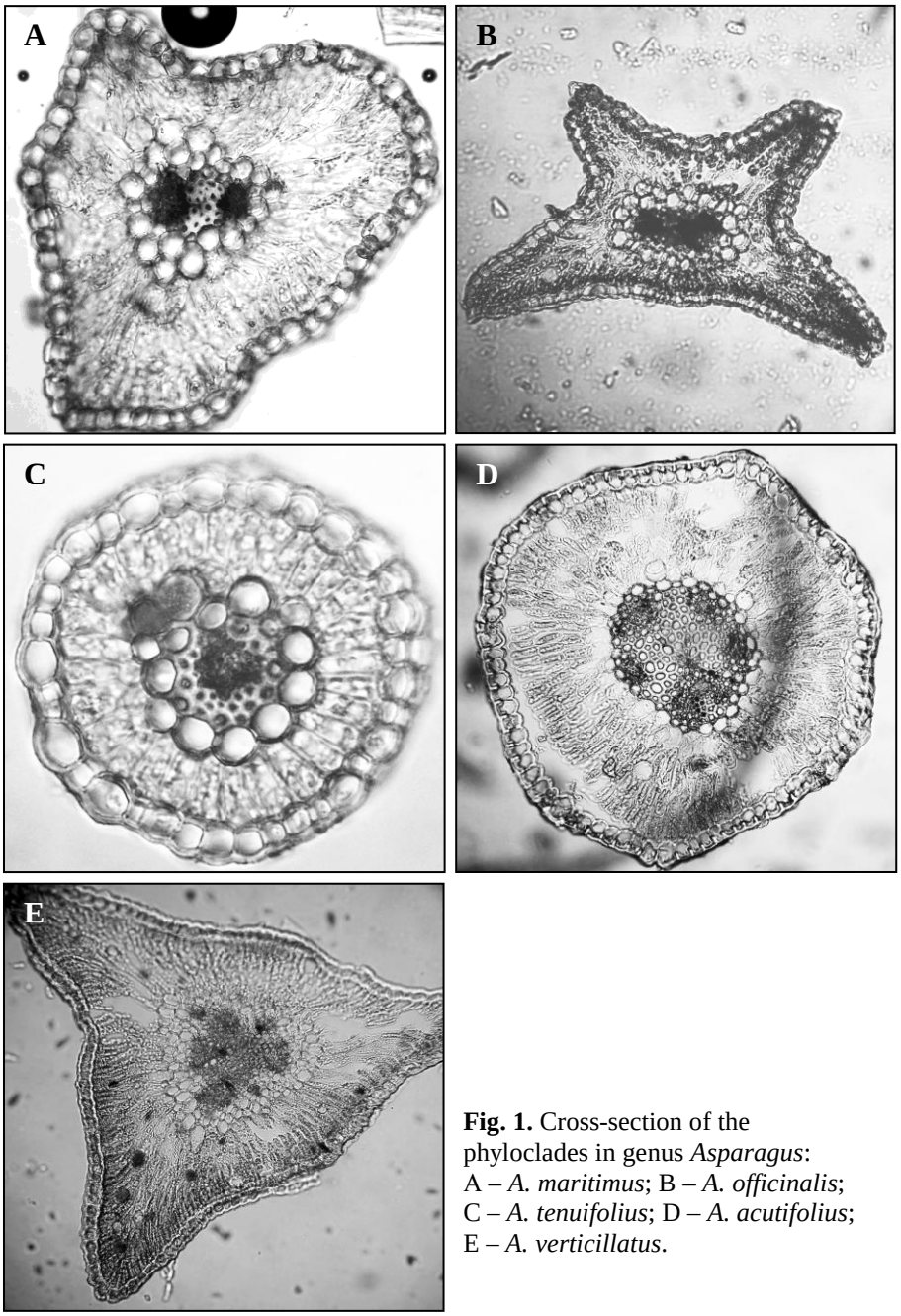

Fig. 1. Cross-section of the phyloclades in genus *Asparagus*: A – *A. maritimus*; B – *A. officinalis*; C – *A. tenuifolius*; D – *A. acutifolius*; E – *A. verticillatus*.

Between the assimilation tissue and the vascular tissue is situated endodermis of parenchimatouse cells. It is represented by one row in *A. tenuifolius* and *A. acutifolius*, and 2 to 4 rows in all other species.

The phyloclades have developed vascular system, with cyllindric or flat shape, detoured by sclerenchym fibres and parenchyma. The vascular system has, delimited from the assimilation parenchyma by one row. The vascular bundles are colateral. The count of the vascular bundles and the rows of parenchymatous cells in the phyloclades is species - dependent. The phyloclades of *A. verticillatus* have 6 vascular bundles (3 thin and 3 thick). In *A. acutifolius* they are 5, with equally developed vascular cells. All other species have 2 bundles with central position.

The pith of the phyloclades is built by lignificated cells, with highest amount in *A. acutifolius*. This fact corresponds to the xerophytic habitats of this species.

The anatomical characters of the phyloclades are slightly inluenced by the environmental factors. The data above suggest that the anatomical characters of the phyloclades are useful for the taxonomical purposes and they give an option for hopeful identification of morphologically similar species in genus *Asparagus*.

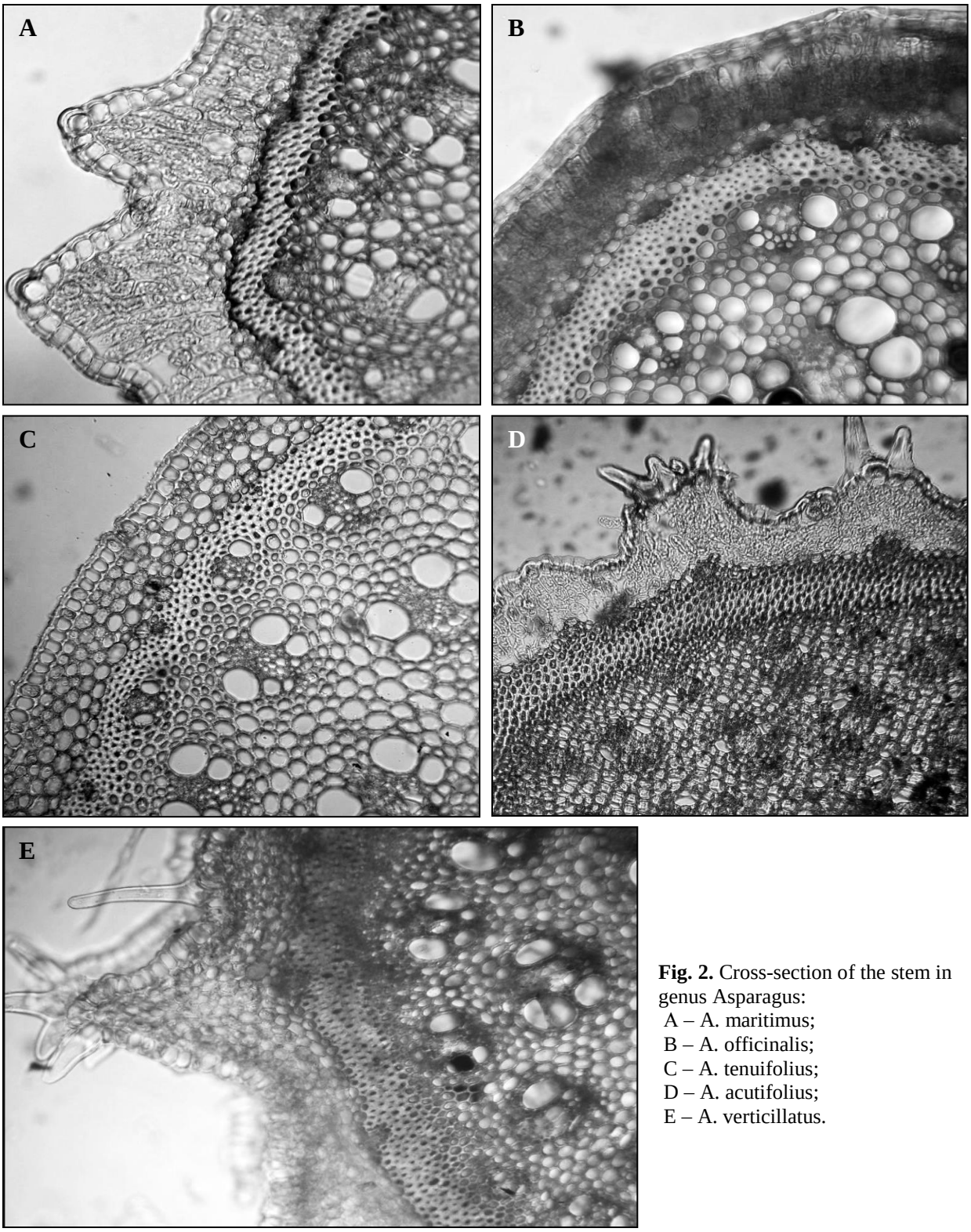

Fig. 2. Cross-section of the stem in genus Asparagus:
A – A. maritimus;
B – A. officinalis;
C – A. tenuifolius;
D – A. acutifolius;
E – A. verticillatus.

***Stem* (Figure 2)**

The stem anatomy in the examined species displayed variation in the quantity of mechanical and parenchymatous tissues. The epidermis in all examined species is one-layered, with thick cutin layer. This layer is the thickest in *A. acutifolius* and *A. maritimus*. This fact corresponds to the more xerophytes habitats of the both species. Under the epidermis are located 2-3 rows chlorenchym, represented by rounded cells with little intercellular spaces. The quantity of the sclerenchyma is correlated to the ecological specialization of the studied species. *Asparagus acutifolius* and *A. verticillatus* distributed on open rocky xerophytous terrains have the most developed sclerenchymatous ring of 5-6 cell rows. The quantity of the mechanical tissue of the other species is less because they are xeromesophytes, mainly distributed in the border of the forest phytocenoses. The stem of *A. officinalis* and *A. tenuifolius* has oval section. All other species have more or less represented ribs **(Figure 2)**.

In the phyloclades and stems of all observed species are observed idioblasts with raphides, located between the pallisade cells.

CONCLUSION

The anatomy of the vegetative epigeous organs of the investigated *Asparagus* species displayed some taxonomically important characters. The cross-sections of the phyloclades show discrete quantitative and qualitative differences. The cell count of the pallisade parenchyma and the count of the vascular bundles are conservative and constant as characters on species level. They are slightly dependent by the environmental factors. The exposed above suggests them as useful for taxonomical purposes. The stem structure in the studied Bulgarian species corresponds to the ecological specialisation but there is lack of quantitative or qualitative differences in histological aspect, and in this case they can't provide information for the taxonomy. The anatomical characters of the investigated species are useful as criteria for delimitation. On this basis is built a determination key.

Key for anatomical determination of the investigated species

1. Assimilation parenchyma of 3-4 cell rows. Vascular bundles 2 2
1*. Assimilation parenchyma of 2 cell rows. Vascular bundles more than 2 3
2. Phyloclades with oval cross-section. Vascular bundles 5, with similar size. Assimilation parenchyma with short cells, almost isodiametric (**Figure 1-D**) .....*A. acutifolius*

2*. Phyloclades with irregular cross-section, with unclear ribs, to to stellar or 4-5 angular. Vascular bundles 6, alternate 3 small and 3 big. Assimilation parenchyma with long, pallisade cells (**Figure 1-F**). ..... *A. verticillatus*

3. Endodermis of 1 cell row. Assimilation parenchyma with slightly prolonged cells (**Figure 1-C**) ..... *A. tenuifolius*

3*. Endodermis of 2-3 cell rows. Assimilation parenchyma with clearly prolonged cells 4

4. Phyloclades with 3-4 angular cross-section (**Figure 1-B**). Stem with oval cross-section. ..... *A. officinalis*

4*.Phyloclades with oval to irregularly ribbed cross-section (**Figure 1-A**). Stem with ribbed cross-section ..... *A. maritimus*

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